

The University of Manitoba

The effect of heat stress on the estrous
cycle and hormone concentrations
in sheep

by

Mirghani Abbas Sheikheldin

A Thesis

submitted to the Faculty of Graduate Studies
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Department of Animal Science

Winnipeg, Manitoba

1987



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THE EFFECT OF HEAT STRESS ON THE ESTROUS CYCLE AND
HORMONE CONCENTRATIONS IN SHEEP

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MIRGHANI ABBAS SHEIKHELDIN

A thesis submitted to the Faculty of Graduate Studies of
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ABSTRACT

A thermoneutral temperature (NT) of $18 \pm 1^\circ\text{C}$ (constant) and a 12 h cyclic heat stress (CHS) of 18°C - 35°C - 18°C was employed in a series of experiments to test the hypothesis that the reduction in the fertility of livestock during exposure to CHS might result from depressed function of the hypothalamo-pituitary-gonadal axis. Several endocrine systems were investigated using a total of 22 adult ewes which were shorn and fed at 2.5 times the maintenance ration. The peripheral concentrations of progesterone (P_4), cortisol, triiodothyronine (T_3), luteinizing hormone (LH), follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH) and prolactin (PRL) were estimated using radioimmunoassay procedures.

The results indicated that CHS raised the rectal temperature ($p < 0.001$), increased the respiration rate ($p < 0.001$), elevated serum PRL ($p < 0.001$), and lowered mean cortisol ($p < 0.05$), but had no effect on either the 24-h rhythm or the progressive fall of cortisol values over time, in response to the stress of sampling ($p < 0.05$).

In the cycling ewe, CHS tended to increase area under the P_4 curve and elevated mean P_4 at 36 h before the preovulatory LH surge peak ($p < 0.05$). Cyclic heat stress also lowered the LH surge peak ($p < 0.05$) and reduced the area under the FSH surge curve ($p < 0.05$). Cyclic heat stress had no effect on either the onset or the magnitude of the second FSH surge. In the cycling ewe in which PRL release was inhibited with bromocryptine (CB-154), the area under the P_4 curve was also greater during CHS ($p < 0.05$). Bromocryptine treatment during CHS, increased the magnitude of the area under LH surge curve ($p < 0.01$), but had no effect

on the first or the second FSH surges. Following the complete regression of the corpus luteum (CL) of the cycle, P_4 concentration remained basal until the formation of the new CL.

Cyclic heat stress tended to increase the adrenal secretion of P_4 and to depress that of cortisol. The injection of an acute single dose of adrenocorticotrophic hormone (ACTH) into ovariectomized (ovx) ewes, resulted in an abrupt, and prolonged, rise in P_4 and cortisol concentrations, irrespective of the temperature ($r=0.75$, $p<0.001$). However, the concentration of P_4 during CHS or in response to ACTH was comparable to the basal values observed in the cycling ewe during the preovulatory period. In the P_4 -primed ovx ewe, the pituitary response to an acute single dose of exogenous GnRH was not significantly influenced by temperature, P_4 concentration or CB-154 treatment. Also, the response of the pituitary to CB-154 treatment in the ovx ewe seemed to depend upon the prevalent ambient temperature.

The results further indicate that in the cycling ewe, the CL is the main source of the elevated P_4 during CHS. PRL had no significant role in the function of the ovine CL. The magnitude of the preovulatory gonadotropin surges is positively related to the duration of the interval from basal P_4 to the onset of the surges. Data also suggest that CHS depresses the concentration of GnRH.

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ABBREVIATIONS

SYMBOL	NAME
ACTH	Adrenocorticotropic Hormone
bLH	Bovine Luteinizing Hormone
BPA	Bovine Plasma Albumin
BSA	Bovine Serum Albumin
CHS	Cyclic Heat Stress (18°C-35°C-18°C)
°C	Degree Centigrade or Celsius
cAMP	Cyclic Adenosine Monophosphate
CB-154	Bromocryptine
C.F.%	Crude Fibre Percent
C.P.%	Crude Protein Percent
Ci	Curie(s)
cm	Centimeter
cpm	Counts per Minute
Cortisol	Hydrocortisone
C.V.	Coefficient of Variation (%)
D	Day
da	Dalton (Molecular Mass)
db-cAMP	Dibutyryl Cyclic Adenosine Monophosphate
DF	Degrees of Freedom
dpm	Disintegration per Minute
Expt(s)	Experiment(s)
EW	Egg White
E ₂	Estradiol-17β
FSH	Follicle-Stimulating Hormone
g	Gram(s)
GnRH	Gonadotropin-Releasing Hormone (LHRH)
>	Greater Than
h	Hour
hCG	Human Chorionic Gonadotropin
hFSH	Human Follicle-Stimulating Hormone
HSA	Human Serum Albumin
ht	Height
³ H	Tritium
I.D.	Inside Diameter
i.m.	Intramuscular(ly)
I.U.	International Unit
¹²⁵ I	¹²⁵ Iodine
kg	Kilogram(s)
L	Litre(s)
l	Length
<	Less Than
LH	Luteinizing Hormone
m	Metre(s)
MBH	Median Basal Hypothalamus
min	Minute(s)

SYMBOL	NAME
mL	Millilitre(s)
mo	month
MS	Mean Square
μ g	Microgram(s) ($g \times 10^{-6}$)
μ L	Microlitre(s) ($L \times 10^{-6}$)
ng	Nanogram(s) ($g \times 10^{-9}$)
n	Number
NS	not significant
NSB	Nonspecific Binding
NT	Thermoneutral Temperature (18°C)
oFSH	Ovine Follicle-Stimulating Hormone
oLH	Ovine Luteinizing Hormone
oPRL	Ovine Prolactin
ovx	Ovariectomized
P	Probability
PBS	Phosphate Buffer Saline
pg	Picogram(s) ($g \times 10^{-12}$)
$\text{PGF}_{2\alpha}$	Prostaglandin $\text{F}_{2\alpha}$
PMSG	Pregnant Mare Serum Gonadotropin
P_4	Progesterone
q.s.	Quantum Sufficient
%	Percent
r	Correlation Coefficient
RR	Respiration Rate
REP	Replicate (rep)
RIA	Radioimmunoassay
rpm	Revolutions per Minute
rT ₃	Reverse Triiodothyronine
s.c.	Subcutaneous(ly)
SD	Standard Deviation
SEM	Standard Error of Mean
T	Testosterone
Tamb	Ambient Temperature
TB	Total Binding
Trec	Rectal Temperature
TRH	Thyrotropin-Releasing Hormone
TSH	Thyroid-Stimulating Hormone
T-1/2	Half-life
T ₃	Triiodothyronine (Liothyronine)
T ₄	Tetraiodothyronine
w	Width

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1. INTRODUCTION

In recent times livestock producers in the tropical and subtropical countries have been faced with many challenges. In most areas the excellent improvement in veterinary services that led to an enormous buildup of livestock populations has not been paralleled by improvement in nutrition. Even when nutritional adequacy was achieved, low productivity of the indigenous breeds has hampered any progress. Alternatively, many countries have adopted policies to introduce breeds from temperate regions to save time and reduce costs. It is here that the dilemmas begin, especially where cattle and sheep are involved, because the technologies of maintaining these breeds in their temperate homelands are not as easily transferrable as in the case of swine and poultry. In addition to feeding, environmental constraints raise management costs to phenomenal levels. As a result some vital aspects like reproduction are neglected because implementing management programs would lead to prohibitive costs.

Eventually the underlying management costs of adopting routine estrous synchronization, artificial insemination, ova transfer and other techniques to improve the reproductive processes depend upon the ease of adoption, absence of risks and the existence of favorable environmental conditions that would allow the animal to express its true potential. The ability of the animal to respond to any of these techniques, in turn, is also related to how flexible its homeostatic mechanisms are in sensing the changes in the internal and external environments and making the necessary adjustment to accommodate them. Two of the major environmental constraints on livestock reproduction are ambient

temperature and relative humidity.

To overcome these constraints, different livestock breeds have evolved their homeostatic control systems to function within specific boundaries of thermocomfort zones. As long as the animal is undisturbed in its thermocomfort zone, its body functions are biochemically, physiologically and behaviorally maintained at norm. Hence heat stress, as it will be referred to hereafter, is the combination of both ambient temperature and relative humidity above the upper limit of the animal's thermocomfort zone, that would induce an estimable degree of disruption to its homeostatic systems. The effect of heat stress is more distressing in tropical and subtropical areas where elevated temperatures last for several months annually. Though the reproductive activities of sheep in these areas are not strictly seasonal, extended periods of infertility are not uncommon, presumably because of poor nutrition and heat stress. In cattle, Johle (1972), compared the fertility in several breeds of tropical cattle kept under improved management systems in tropical countries including Mexico, Guatemala and The Niger, and concluded that around 68% of the variation in conceptions could be explained by temperature-related factors.

The effect of heat stress on the estrous cycle of several domestic species has been thoroughly investigated. In cycling female laboratory and farm animals, heat stress has been blamed for extended estrous cycles (Stott and Williams 1962; Madan and Johnson 1973; Sawyer et al. 1979), silent heat (Agrawal et al. 1972; Abilay et al. 1975a; Hill and Alliston 1981) and shorter manifestation of estrual behavior (Monty and Wolf 1974; Gwazdauskas et al. 1981). Increased embryonic loss (Yeates

1953; Dutt 1964) and reduced birth weights (Cartwright and Thwaites 1976; Brown et al. 1977) have been noticed in ewes kept in heated rooms.

Endocrine studies to correlate hormone concentrations with observed reproductive wastage have been conducted in laboratory and farm species. Existing evidence indicates elevated progesterone during heat-stress cycling ewes (Sawyer et al. 1979) and cows (Stott and Wiersma 1973; Abilay et al. 1975a). Heat stress is also associated with high concentration of prolactin (Tucker and Wettemann 1976; Hooley et al. 1979; Hill and Alliston 1981) and catecholamines (Alvarez and Johnson 1973; Rosak et al. 1980). Thus, either progesterone through its negative feedback or prolactin by its antigonadal effect could interfere with gonadotropic function. Depressed Luteinizing Hormone (LH) release has been cited during heat stress in ovariectomized ewes (Schillo et al. 1978; Hill et al. 1980) and during the preovulatory surge in cycling cows (Madan and Johnson 1973; Miller and Alliston 1974b). However, others (Vaught et al. 1977; Hooley et al. 1979) could not detect significant differences in basal or the preovulatory surge of LH during heat stress. There are no indications that the corpus luteum secretes more progesterone during heat stress, nor evidence that elevated prolactin concentration interferes with pituitary function in heat-stressed females. On the contrary, cattle breeds that are believed to be heat tolerant (B. indicus) have been reported to have smaller corpora lutea that secreted less progesterone when compared to B. taurus breeds (Irvin et al. 1978). The literature reveals little support for a significant role of prolactin during the ovine estrous cycle. In spite of claims made by Kann and Denamur (1974) that daily infusions of prolactin alone (but not LH) into cycling ewes hypophysectomized on D2

of the cycle, has maintained the corpus luteum for 12 days, short-term inhibition of prolactin release had no effect on the preovulatory gonadotropin concentration (Niswender 1972; McNeilly and Land 1979) nor on the subsequent estrous cycle (Louw et al. 1974). Nonetheless, other workers (Jackson 1977) have shown that the inhibition of prolactin facilitated LH release.

Other endocrine dysfunctions reported during heat stress were depressed secretion of thyroid (Collier et al. 1982; Scott et al. 1983; Epstein et al. 1979) and adrenocorticoid (Lee et al. 1971; Christison and Johnson 1972) hormones and the indication that the adrenals secreted more progesterone during heat stress (Stott et al. 1967).

Thus, while the literature shows a consensus on the effect of heat stress on behavioral estrus, attempts to correlate it with endocrine events have led to some contradictory conclusions. Most of the results have been drawn from studies using short-term or constant exposure to elevated temperatures which were dissimilar to the normal fluctuations encountered by animals. Also, endocrine investigations have covered only certain phases rather than the whole estrous cycle. Thus, conclusions drawn from such experiments are difficult to interpret and incorporate into reproductive management programs. Moreover, perhaps because of technical difficulties, the effect of heat stress on the hypothalamo-pituitary axis has not been investigated previously. There are many shortcomings in our knowledge of hormone relationships in heat-stressed animals. Considering recent trends in livestock breeding programs and the technologies that are rapidly becoming available to the producers, there is even more urgent need to pursue research in this

direction.

Therefore, the purpose of the series of experiments to be described here were to investigate several endocrine models in the ewe under conditions of ambient temperature and relative humidity that was programmed to simulate the diurnal fluctuations of a typical hot dry tropical day.

2. LITERATURE REVIEW

2.1 THE ENDOCRINE CONTROL OF THE OVINE ESTROUS CYCLE:

2.1.1 THE ROLE OF PROGESTERONE (P_4) DURING THE ESTROUS CYCLE:

The ewe normally has a 17-day estrous cycle (Hafez 1952; Bjersing et al. 1972; Yuthasastrakosol et al. 1975; El-Fouly et al. 1977) that can arbitrarily be divided into four phases (follicular, early-luteal, mid-luteal and late-luteal) based on morphological and endocrine changes that occur in the ovary. These changes are closely correlated to patterns of hormone release from the hypothalamo-pituitary axis. Since progesterone (P_4) is the dominant hormone of the cycle it is possible to follow the cycle by estimating P_4 changes in peripheral blood, milk or even saliva.

During the follicular phase (D0-D4), serum P_4 concentration is less than 1.0 ng mL^{-1} . It rises gradually throughout the early-luteal phase (D5-D8) to a peak of $3-6 \text{ ng mL}^{-1}$ during the mid-luteal (D9-D13) phase (Thorburn et al. 1969; Thorburn and Mattner 1971; Bjersing et al. 1972; Baird et al. 1976; Karsch et al. 1980). Late-luteal phase P_4 concentrations are characterized by an initial gradual decline to $1.5-2.5 \text{ ng mL}^{-1}$ (D14-D15), followed by an abrupt fall in the next 48 to 36 hours. It reaches nadir values of less than 0.5 ng mL^{-1} at about 6-12 hr after the preovulatory LH surge.

The changes in serum P_4 are paralleled by morphological (Deane et al. 1966; Gemmell et al. 1976) and functional (Moor et al. 1975) changes which include degeneration and the eventual loss of steroidogenic ability of the corpus luteum (CL). Recent evidence suggests that

shortly prior to the degenerative stage, the association between the small and large luteal cells (Fitz et al. 1982; Rodgers et al. 1985; Schwall et al. 1986) make them more responsive to the luteolytic effects of the rising concentration of prostaglandin $F_2\alpha$ (Baird et al. 1976; Baird and Scaramuzzi 1976a; Nett et al. 1976; Stacy et al. 1976; Ottobre et al. 1980) from the uterus (Mapletoft and Ginther 1975; Scaramuzzi et al. 1977).

The main source of P_4 from the ovine ovary is the small luteal cells (O'Shea et al. 1979; Fitz et al. 1982; Rodgers et al. 1983, 1985) of the corpus luteum (Bjersing et al. 1972), which specifically bind LH and respond to LH stimulation (Fitz et al. 1982; Rodgers and O'Shea 1982; Hoyer et al. 1984). In addition, a small amount of P_4 is also secreted by the atretic follicles (Moor et al. 1978; Ireland and Roche 1983). Progesterone secretion patterns have been described in cattle as pulsatile secretion (Schallenger et al. 1985) that follow LH peaks. However, such correlations could not be defined in sheep (Hauger et al. 1977).

2.1.2 THE NEGATIVE FEEDBACK OF PROGESTERONE ON GONADOTROPINS:

As the cycle advances, rising P_4 concentration in the presence of a low concentration of estradiol- 17β (E_2), exerts a negative feedback by suppressing the pulsatile peaks of LH (Scaramuzzi et al. 1971; Yuthasastrakosol et al. 1975; Goodman and Karsch 1980; Rawlings et al. 1984; Hanker et al. 1985; Tamanini et al. 1986) and as a consequence, LH basal concentration is progressively depressed (Foster et al. 1976; Pant et al. 1977). Progesterone alone has no effect on gonadotropin secretion in ovariectomized ewes (Scaramuzzi et al. 1971; Howland et al. 1978a;

Goodman et al. 1981b; Rawlings et al. 1984; Clarke and Cummins 1984). Even at high dosage, P₄ suppression of the post-castration rise of LH in the ewe is either transient (Foster and Karsch 1976) or produces inconsistent effects (Howland et al. 1978c; Martin et al. 1983; Rawlings et al. 1984; Clarke and Cummins 1984). Progesterone withdrawal associated with the regression of the corpus luteum (CL) during the late-luteal phase, or after implant removal in ovariectomized ewes is accompanied by a sustained elevation of LH (Hauger et al. 1977; Karsch et al. 1979; Jeffcoate et al. 1984; Haresign 1985). In these animals, reinstatement of mid-luteal phase P₄ concentration either inhibits (Baird and Scaramuzzi 1976a; Goodman et al. 1981b) or delays (Haresign 1985) the occurrence of the LH surge. Elevated P₄ concentration in ovariectomized ewes has been reported (Howland et al. 1978a, 1978c; Karsch et al. 1979, 1980; Jeffcoate et al. 1984) to block an E₂-induced LH surge. Despite the P₄ negative feedback on gonadotropin release, P₄ secretion by the functional CL does not seem to interfere with local development and maturation of the follicles on the ipsilateral ovary (Baird et al. 1975).

How P₄, alone or in association with E₂ and other factors, induces inhibitory effects on gonadotropin release has been investigated in several species. Progesterone receptors have been identified in the anterior pituitary cells, the hypothalamus and the preoptic areas of the rat brain (Parsons et al. 1982; Attard 1984). Based on comparable LH responses to gonadotropin-releasing hormone (GnRH) in ovariectomized ewes, Tamanini et al. (1986) have concluded that P₄ action on LH was due to its modulation of the release of hypothalamic GnRH. These findings are also confirmed by studies using bovine pituitary cells. Padmanabhan

and Convey (1981) have reported that perfusion of bovine pituitary cells for 12 h with serial doses of P_4 that ranged between 3.1 and 31 ng mL⁻¹ had no effect on subsequent GnRH-induced LH release; rather, it completely blocked the E_2 -induced LH release.

Activation of P_4 receptors on the anterior pituitary cells (Attard 1984), seems to modify the responses to GnRH or E_2 stimulation. For example, in pituitary cells from anestrous or cycling ewes during the breeding season, prior exposure to P_4 significantly reduced LH response to GnRH (Batra and Miller 1985). According to Padmanabhan and Convey (1981), long-term (>12 h) perfusion of bovine pituitary cells with P_4 inhibited any LH response due to GnRH.

Thus, the literature indicates that the CL is the major source of circulating P_4 in the ewe and that P_4 in association with other ovarian factors reduce both basal and pulsatile secretion of LH. This process is brought about through binding of P_4 to receptors which are mainly in the hypothalamus, but in the pituitary as well. It follows that during the preovulatory period elevated P_4 concentration resulting from a delay in CL regression, atretic follicles or adrenal secretion (see below, 2.1.4) would possibly lead to failure of a LH surge and consequently of ovulation. The inhibitory effects of P_4 on gonadotropin release are opposite to its action on the brain where it synergistically intensifies E_2 -induced estrual behavior.

2.1.3 EFFECT OF HEAT STRESS ON PROGESTERONE (P_4) SECRETION:

2.1.3.1 PROGESTERONE CONCENTRATION IN CYCLING ANIMALS:

As previously mentioned, elevated concentration of (P_4) in the presence of low concentration of (E_2) depress gonadotropin release. Therefore, failure of P_4 concentration to fall during the late-luteal phase would not only interfere with the final stages of follicular maturation, but also would alter the length of the estrous cycle.

That the P_4 concentration is elevated during heat stress in cycling animals is well established in livestock. Abimny et al. (1975a) have shown that P_4 concentration during the cycle was higher when cows were kept at 33.5°C as compared to 18.2°C . Elevated P_4 concentration was also reported in cows during the hot summer months (Roussel et al. 1977) and were associated with reduced conceptions (Vaught et al. 1977). Miller and Alliston (1974a), however, reported no change in P_4 concentration in cycling heifers subjected to a fluctuating heat cycle that provided some cooling (21°C - 34°C - 21°C vs 17°C - 21°C - 17°C). In contrast, others (Stott and Wiersma 1973; Rosenberg et al. 1977, 1982) have reported that both P_4 concentration and fertility were lower in cows during summer than winter.

In sheep, Sawyer et al. (1979) have shown that the preovulatory decline in P_4 was delayed in heat-stressed ewes, leading to extended estrous cycles. The preovulatory LH surge was either absent or depressed in heat-stressed ewes (Hill and Alliston 1981).

2.1.3.2 BREED DIFFERENCES IN RESPONSE TO HEAT STRESS:

As mentioned previously, for many livestock producers in warm climates improved productivity can be easily achieved by direct transfer

of genes from temperate breeds, rather than by selection from indigenous breeds. Thus, there is a need to compare breeds in their response to the constraints of the tropical temperatures. Current data from cattle suggest that Zebu cows (B. indicus) have smaller CL which secrete less P_4 than B. taurus cows (Irvin et al. 1978; Adeyemo and Heath 1980). Randel (1980a) have reported comparable P_4 values in tropical and temperate cattle breeds during the 48 h preceding, and up to 24 h after the preovulatory LH surge.

2.1.4 ADRENAL P_4 SECRETION:

The existence of two steroidogenic organs (the gonads and the adrenal cortex) has stimulated extensive investigations to elucidate the role of either in the increased P_4 concentration during heat stress. As previously cited, P_4 can be synthesized in the gonads as a final end product or for further steroid synthesis, depending on the predominant functional structure. In the adrenal cortex P_4 is an important intermediary in the steroidogenic pathways through its two main metabolites, 17α -hydroxprogesterone which leads to hydrocortisone (cortisol), and deoxycorticosterone which gives rise to corticosterone. Balfour et al. (1957) have measured adrenal arterio-venous difference in P_4 concentration in ovariectomized and castrate cattle, sheep and pigs. Their results showed that P_4 concentration in the adrenal vein was 10-100 times higher than in the adrenal artery. Hence, it is not surprising that P_4 concentration in peripheral blood may increase in animals subjected to stress.

The sheep pituitary secretes exclusively the large adrenocorticotropin (ACTH1-39) while in cattle it secretes a mixture of

ACTH1-39 and ACTH1-24 (Coslovsky and Yalow 1974). This may explain why the adrenal cortex of sheep secretes cortisol as the predominant glucocorticoid (>90%), while in cattle both cortisol and corticosterone are secreted in ratio of 1:1.

The infusion of either human chorionic gonadotropin (hCG) or LH in ovariectomized rats (Resko 1969), and prolactin (PRL) in hypophysectomized ovariectomized ewes (De Silva et al. 1983) had no effect on the adrenal release of P_4 . Several data generated from cycling (Wagner et al. 1972; Watson and Munro 1984) or adrenalectomized cycling cows (da Rosa and Wagner 1981) and in sheep (De Silva et al. 1983; Benhaj and Cooke 1985) have shown that exogenous ACTH increased circulating P_4 concentration. The significance of this elevation in P_4 remains equivocal because the concentration in peripheral blood following the rise has been shown to be comparable to concentrations observed during the preovulatory LH surge.

Recently, Wendorf et al. (1983) have studied the adrenal contribution to P_4 in pregnant cows (Day 215 of gestation). When cows were either left as intact controls, bilaterally adrenalectomized or simultaneously ovariectomized and adrenalectomized, only the last group failed to successfully complete pregnancy. Since the placenta does not significantly contribute to the circulating P_4 in the cow (Donaldson et al. 1970), these results indicated that P_4 from the adrenal maintained the pregnancy in the absence of the gonads. In the ewe, the placenta contributes significant amounts of P_4 after D80 of pregnancy (Bassett et al. 1969). Hence, studies in pregnant ewes similar to those done with cattle would be difficult to interpret.

Therefore, in summary, the literature indicates that elevated P_4 concentration (ovarian or adrenal) in heat-stressed animals is likely to depress gonadotropin release and fertility. Heat-tolerant Zebu cows have smaller CL which secrete less P_4 . The adrenals secrete considerable amounts of P_4 in response to ACTH alone. Neither PRL nor LH influences the adrenal secretion of P_4 . Available data show that P_4 release from the adrenal increases following ovariectomy in normothermic cattle (Wendorf et al. 1983); however no parallel data exist during exposure to heat stress. If P_4 secretion by the adrenals increases during chronic heat stress despite a general decline in glucocorticoid secretion, this then suggests that either ACTH differentially or some other factor interferes with the progression of the steroidogenic pathway leading from P_4 to the production of glucocorticoids.

2.1.5 ESTROGENS:

2.1.5.1 CIRCULATING ESTRADIOL-17 β (E_2)

DURING THE ESTROUS CYCLE:

Estradiol-17 β (E_2) is the main estrogen secreted by the cycling ewe, and it is also the most biologically potent. One of the earliest functions of E_2 to be reported was its vital role in the manifestation of estrual behavior. Ball (1941) has shown that injection of E_2 alone into hypophysectomized rats induced behavioral estrus. A combination of E_2 and P_4 produced more intense signs (Astwood and Dempsey 1941). Other functions of E_2 include its induction of the preovulatory gonadotropin surge, its role in follicular growth and its involvement in growth of the secondary sex organs and bone tissue.

Compared to P₄, normal circulating E₂ concentration is quite low in the cycling ewe. The highest concentrations occur during the preovulatory period prior to the LH surge peak. It has been reported (Bjersing et al 1972; Baird and Scaramuzzi 1976b) that luteal phase E₂ concentration in the ovarian vein ipsilateral to the largest follicle was greater than 5 times that in the contralateral vein; by D15 of the cycle the difference had grown to more than 15-fold. Hauger et al. (1977) have mentioned two waves of elevated E₂ during the cycle in the ewe: a large rise 72-48 h before the onset of the preovulatory surge and a second smaller rise on D4-D6 of the cycle. It has been postulated that the second rise is from follicles recruited to a pool from which the next preovulatory follicle(s) could be selected. There is currently no evidence to substantiate this hypothesis (Ireland and Roche 1983).

2.1.5.2. BREED DIFFERENCES IN E₂ SECRETION:

Peripheral E₂ in different breeds of cattle was evaluated during the preovulatory period (Randel 1980b). The maximum preovulatory rise in E₂ occurred 24 h before estrus in Brahman (Zebu) as compared to 16 h in Brahman crosses with Hereford and 8 h in pure Hereford cows. BY 24 h after estrum, E₂ was lowest in the Brahman, intermediate in the crosses and highest in the Hereford cows. Breed comparison in the response to E₂ injection (Randel and Rhodes 1980) has shown that, in addition to the highest minimum dose of E₂ to induce behavioral estrus in Brahman cows, this breed had the longest period from E₂ injection to standing heat. Because of the short duration of estrus in Brahman cows, all breeds had a comparable period from E₂ injection to ovulation.

2.1.5.3 ESTRADIOL-17 β DURING THE GONADOTROPINS SURGES:

The injection of exogenous E_2 results in an initial decline in LH (Radford et al. 1969; Goding et al. 1970; Jonas et al. 1973; Karsch et al. 1973; Kesner et al. 1981) and follicle-stimulating hormone (FSH) (Pant et al. 1977). Subsequent to this initial depression the release of both LH and FSH is enhanced to culminate into concomitant surges (Jonas et al. 1973; Reeves et al. 1974; Pant et al. 1977; Howland et al. 1978b). The initial E_2 -induced decline in the FSH secretion from ovine pituitary cells has been reported (Miller et al. 1983) to result from decreased synthesis of entire FSH as well as of the FSH β -subunit (Miller et al. 1983).

The effect of E_2 -priming on the pituitary response to GnRH has also been investigated. Data from Beck and Convey (1977) and Deaver and Dailey (1984) showed that E_2 delayed a GnRH-induced gonadotropin release, and the amount released varied according to the interval from E_2 treatment to GnRH stimulation.

In addition to the direct influence on the pituitary, E_2 -implants have been shown to increase GnRH concentration in the pituitary stalk blood of ovariectomized rats (Speight et al. 1981) suggesting that E_2 influenced GnRH release as well. Tobias et al. (1983) reported that whereas treatment with E_2 alone decreased LH release, there was an increase in GnRH content in the median basal hypothalamus (MBH) of the rat. The inhibition of norepinephrine and dopamine in E_2 -primed rats, on the other hand, resulted in decreased GnRH content in MBH and partial reversal of LH inhibition, indicating that the initial decline after E_2 treatment is due to action not only at the pituitary level but also at the MBH.

To summarize, the literature indicates that behavioral estrus is related to the effect of E_2 on the brain, without the involvement of gonadotropins. The growing follicles are the major source of circulating E_2 . The concentration of E_2 is low during the luteal phase. Estradiol- 17β exerts negative and positive feedback mechanisms on the secretion of gonadotropins. The inhibitory mechanism is associated with the tendency to reduce the release of GnRH from the MBH and also to decrease the pituitary synthesis of gonadotropins. The stimulatory mechanism, on the other hand, seems to increase the secretion of GnRH and to potentiate the effects of GnRH on the pituitary. Temperate and Zebu breeds of cattle seem to differ in the minimum amount of circulating E_2 which is associated with the manifestation of behavioral estrus. The occurrence of the preovulatory gonadotropin surge results in the termination of ovarian steroidogenesis.

2.1.6 THE EFFECTS OF HEAT STRESS ON THE ESTROUS CYCLE:

2.1.6.1 CYCLE LENGTH AND BEHAVIORAL ESTRUS IN HEAT-STRESSED EWES:

Extended estrous cycle length, absence of behavioral estrus and reduced conception rates during the hot summer months, despite nutritional adequacy (Yenikoye et al. 1982), are some of the infertility problems encountered by livestock producers in the warm areas of the world. Yeates (1953) reported that only 1/8 of the ewes lambled when they were kept in heated rooms from service to two months after breeding. Sawyer et al. (1979) studied successive estrous cycles in ewes maintained at temperatures of 23-24°C or 41-42°C. Their results showed that the cycle length was extended and that behavioral estrus was

either absent or of a shorter duration in the ewes held at the high temperature. In addition, Hill and Alliston (1981) and Yenikoye et al. (1982) also have reported that a higher percentage of ewes failed to show behavioral estrus during heat stress. The effects of shade and shearing on the estrous cycle during heat stress were studied in Barki ewes (subtropical) and their Merino crosses in Egypt (Mokhatr et al. 1983). Results revealed that the estrous cycle length was longer in unshorn-unshaded ewes irrespective of breed.

Sheep breeds have been reported to differ in their response to heat stress. In Egyptian indigenous Ossimi and Rahmani sheep breeds (El-Fouley et al. 1977), estrous activity was higher in summer than spring and there was no seasonal variation in the estrous cycle length. Moreover, Darbeida and Brudieux (1980) have reported maximum testosterone (T) concentrations in rams in the indigenous Algerian sheep breeds during summer months (Jun.-Aug.). According to Galil and Galil (1982) semen quality of Sudan Desert Sheep rams was not adversely affected by the extreme elevation of summer temperatures. Contrary to that observed in tropical and subtropical sheep breeds, imported pure Suffolk and their crosses with the indigenous Egyptian Ossimi sheep were reported (Aboul-Naga 1978) to be completely anestrus during summer (May-Jun.).

2.1.6.2 CYCLE LENGTH AND BEHAVIORAL ESTRUS IN HEAT-STRESSED COWS:

Data drawn from heat-stressed cattle also support the findings in sheep. Estrous cycle length was longer in cows exposed to elevated temperatures (Madan and Johnson 1973; Abilay et al. 1975a) and during summer months (Poston et al. 1962; Stott and Williams 1962; Lewis et al.

1984). Heat stress was also associated with a high incidence of 'repeat breeders' among Holstein cows imported to Iraq (Ali et al. 1983). Also, the duration of behavioral estrus has been reported to be shorter in heat-stressed cows (Agrawal et al. 1972; Madan and Johnson 1973; Monty and Wolf 1974; Abilay et al. 1975a). Nevertheless, luteal regression following an injection with prostaglandin $F_{2\alpha}$ was comparable between heat-stressed and normothermic cows (Gwazdauskas et al. 1981). Breed differences have also been reported in cattle. Rhodes and Randel (1978) have reported that the duration of estrous behavior was shorter in Brahman cows (B. indicus) than in Hereford or Brahman x Hereford cows. When exogenous E_2 was used to induce behavioral estrus, the minimum effective dose was higher for Brahman cows, indicating that this breed might have an endocrine relationship which differed from the Hereford.

2.1.6.3 CYCLE LENGTH AND ESTROUS BEHAVIOR IN HEAT-STRESSED SOWS:

Few studies have examined the effect of heat stress on the swine estrous cycle. Paterson et al. (1978) have analyzed five-year records of commercial Australian piggeries and concluded that as temperature rose from 20°C to above 32°C, the percentage of sows returning to estrus after mating increased. In another study (Hurtgen and Lemon 1980), the percentage of sows and gilts that farrowed was lowest when mated during summer months (Jul.-Sept.).

2.1.6.4 RELATIVE VULNERABILITY OF REPRODUCTIVE PROCESSES TO HEAT STRESS:

The main purpose of the estrous cycle is to yield healthy ova which will be fertilized successfully and subsequently give rise to normal offspring. In sheep, as in pigs, there seems to be a critical stage

during which fertility is most vulnerable to heat stress. For example, when ewes were exposed to 24 h of heat stress on the day of estrus (D0), fertilization was adversely affected (Dutt 1964). In contrast, exposure on D1 of the cycle had no effect on the number of fertilized ova recovered, but was severely detrimental to subsequent development (Alliston et al. 1961; Dutt 1963; Thwaites 1970). These observations were also described in heat-stressed gilts (Omtvedt et al. 1971).

Heat stress also results in reduced birth weights in sheep (Yeates 1953; Shelton and Huston 1968; Brown et al. 1977; Hopkin et al. 1980) and pigs (Omtvedt et al. 1971). Cartwright and Thwaites (1976) have reported that decreased lamb birth weights of heat-stressed ewes was not due to undernutrition, because intake-restricted control ewes, fed a ration similar in amount to the quantity eaten by the heat-stressed ewes, delivered lambs of comparable birth weight to those delivered by ad libitum fed control ewes.

The reduction in birth weight associated with heat stress could be due to reduced uterine blood flow (Roman-Ponce et al. 1978). It has been shown that in heat-stressed ewes the percentage reduction in uterine blood flow was significantly greater than the reduction in umbilical blood flow (Oake et al. 1976). In ovariectomized ewes E_2 has been implicated with increased uterine blood flow (Roman-Ponce et al. 1978), while P_4 alone had no effect (Caton et al. 1974). Roman-Ponce et al. (1978) have reported that either heat stress or the injection of epinephrine caused a reduction of E_2 -induced uterine blood flow. Since heat stress has been reported (Alvarez and Johnson 1973; Rosak et al. 1980) to elevate catecholamines, it is more likely that the reduced

uterine blood flow during elevated temperature is mediated by catecholamines. Therefore, it is conceivable that during heat stress, dissipation of excess heat load by evaporation is brought about by catecholamines which facilitate peripheral vasodilation at the expense of reduced blood flow to the internal organs. However, in sheep, the bulk of the evaporative heat loss takes place through the respiratory tract (polypnea) rather than from the skin.

In summary, the literature indicates that heat stress causes extended estrous cycles, suppresses the intensity and reduces the duration of behavioral estrus, and depresses fertility. Also, a difference in heat tolerance between tropical and temperate breeds exists. Heat stress does not seem to inhibit fertilization but is detrimental on embryonic development. The literature suggests that catecholamines mediate the effect of heat stress by reducing blood flow to the uterus.

2.1.7 THE ROLE OF LUTEINIZING HORMONE (LH):

2.1.7.1 BIOCHEMICAL CHARACTERISTICS OF LH:

The LH is a glycoprotein hormone secreted by the pituitary gonadotrophs in response to a specific stimulation by GnRH. In sheep the molecular weight is about 30,000 da, of which 12-13% is carbohydrate. The polypeptide, which is composed of two peptide chains (α - and β -subunits), has only 1-2 sialic acid residues and a half-life of 30 min in peripheral blood. In addition, the biological specificity is solely related to the β -subunit (Strickland and Puett 1981; Moudgal

and Li 1982) which is solely comprised of amino acids. The α -subunit of LH is similar to that of FSH and thyroid stimulating hormone (TSH), and contains 90-110 amino acid residues. Luteinizing hormone H binds to its receptors on follicular and luteal cells and is internalized (Mock and Niswender 1983). The rate of internalization of LH has been reported (Mock et al. 1983) to depend upon the β -subunit. In rat gonadotrophs the process of LH secretion following GnRH stimulation revealed (Naor et al. 1982) the existence of calcium ion-dependent and several time-related releasable pools.

2.1.7.2 LUTEINIZING HORMONE FUNCTION AND PATTERNS OF SECRETION:

Our understanding of the role of LH in reproduction exceeds that of the other pituitary hormones. Luteinizing hormone is luteotrophic in the ewe and hypophysectomy on the day of estrus (D0) results in failure of CL development (Kaltenbach et al. 1968). In heifers, Donaldson and Hansel (1965) have shown that the lifespan of the CL could be extended by infusion of pituitary extracts. Bolt and Hawk (1970) have reported that a combination of LH and FSH or hCG protected the CL from E_2 -induced regression when given to ewes on D0 or D10 of the cycle. Though the infusion of LH prolonged the lifespan of the CL when given before D13 of the cycle (Karsch et al. 1971), treatment after this day failed to save the CL, perhaps because the CL was already regressing. Recently, De Silva et al. (1986b) have shown that large preovulatory follicles were absent in the ovaries of cows immunized against bLH, despite normal concentration of FSH, suggesting that LH is also involved in the growth of the follicle.

The LH is released in a pulsatile fashion (Nankin and Troen 1971; Butler et al. 1972; Yuthasastrakosol et al. 1975; Baird et al. 1976); characterized by abrupt elevations followed by a gradual decline (Geschwind and Dewey 1968; Carr 1972). The frequency of the pulses is higher during the follicular phase, which represents an inverse relationship to the concentration of circulating P_4 . Luteinizing hormone reaches nadir values during the mid-luteal phase (Bjersing et al. 1972; Foster et al. 1975; Pant et al. 1977) when P_4 is at a peak. Early-luteal phase patterns of LH release in cows reflect low amplitudes and high pulse frequencies, while opposite patterns of release prevailed during the mid-luteal phase. (Rahe et al. 1980).

The rapid decline in CL function which occurs at 72-48 h prior to the next estrus is characterized by a sustained rise in tonic and pulsatile release of LH. In the ewe, each pulsatile peak of LH is followed by a corresponding rapid rise and a gradual fall in E_2 (Baird et al. 1976; Baird 1978; McNeilly et al. 1982). This dependency of E_2 on LH release has also been confirmed using exogenous LH pulses during the preovulatory period in normally cycling ewes (Goodman et al. 1981b) or in ewes passively immunized against GnRH (McNeilly et al. 1984); as well as in ovaries from P_4 -primed rats (Peluso et al. 1984). McNatty et al. (1981a) were able to induce estrous behavior in ewes using pulsatile injections of LH.

2.1.7.3 LUTEINIZING HORMONE AND FOLLICULAR DEVELOPMENT:

Because the functional integrity of the ovarian follicles are maintained by a close association between the theca and granulosa cells, there are numerous investigations on this aspect of the LH role.

Earlier in vitro studies by McIntosh and Moor (1973) showed that incubation of ovine follicles in the presence of LH led to a steady release of cyclic adenosine monophosphate (cAMP), E_2 , T and P_4 . Subsequently, substantial work in this area (Richards et al. 1980) led to the development of the Two-Cell Hypothesis for the control of ovarian steroidogenesis; whereby androgens and progestins synthesized by the theca cells were converted to E_2 by the granulosa cell aromatases. According to results by Richards et al. (1980), the incubation of P_4 -primed rat ovaries with LH enhanced the accumulation of E_2 in the Graafian follicles and this response was paralleled by a marked increase in LH receptors in both theca and granulosa cells. In the cycling rat, these events coincided with a sustained rise in serum E_2 which subsequently triggered the gonadotropin surge, ovulation and luteinization of the ruptured follicle.

Small antral follicles from rat ovaries (Bogovich and Richards 1982), predominantly released P_4 in response to cAMP, hCG or human follicle-stimulating hormone (FSH) but failed to convert 3H - P_4 to T or E_2 . In contrast, preovulatory follicles secreted large quantities of P_4 , T and E_2 when incubated with cAMP, hCG or hFSH, and were capable of converting significant amounts of 3H - P_4 to T or E_2 . Parallel work from sheep (England et al. 1981; Webb and England 1982) have indicated that, follicles with the highest number of LH receptors in the theca and granulosa cells were responsible for the enhanced E_2 secretion during the preovulatory period in ewes ovariectomized on D0 of the cycle. McNatty et al. (1981b) have shown that during the preovulatory period, LH concentration rose dramatically in the preovulatory follicle(s) and that follicles with the highest FSH also contained higher concentrations

of E_2 . The steroid contents of the large non-atretic follicle(s) in the ewe have been estimated (Moor et al. 1978) to be 80%, 13% and 7%, for E_2 , T and P_4 , respectively. Recently, in an attempt to distinguish the functional characteristics of theca cells in bovine follicles collected between D(-4) and D0 of the cycle, McNatty et al. (1985b) have identified three types of cells (I, II, and III). Though all follicles with different theca cell types bound LH equally, they differed in their subsequent response. Follicles with type I cells responded to LH stimulation by an increased accumulation of cAMP and androstenedione. Follicles with type II cells responded by increased cAMP accumulation only, while those having type III cells failed to respond, and were presumed to be atretic. These results suggested that the fate of follicles to grow either to enter the final stages of maturation or regress depended on their post-receptor response.

2.1.7.4 THE RELATIONSHIP BETWEEN LH SURGE PEAK, ESTROUS BEHAVIOR AND THE TERMINATION OF FOLLICULAR STEROIDOGENESIS:

As a result of increasing E_2 secretion by the preovulatory follicle, the ewe is induced to exhibit a period of behavioral estrous. However the duration of estrus and its intensity have been observed to be associated with age and breed as well as environment and management stresses. The duration has been reported (Hafez 1952; Smith et al. 1976) to be longer in adult (range 30-40 h) than in maiden ewes (range 19-30 h). Tropical sheep breeds (Ammar-Khodja and Brudieux 1982; Aboul-Naga et al. 1985) have a shorter duration of estrous behavior.

Although results from ovariectomized ewes have indicated that the LH response to E_2 is characterized by an initial depression followed by a

progressive rise to a surge (Brown et al. 1972; Karsch et al. 1983), there is no evidence that an initial depression occurs in the intact cyclic ewe.

The occurrence of the preovulatory LH surge in the ewe has been described as a rapid, steep rise to a peak (ascending limb), immediately followed by an acute precipitous decline (descending limb). The duration of the surge has been estimated (Goding et al. 1969; Crighton et al. 1973; McNatty et al. 1981a) to last 8-10 h in the ewe. Data from cows sampled at a frequency of 6 min for 24 h during the preovulatory LH surge (Rahe et al. 1980), have revealed the existence of LH pulses in both the ascending and descending limbs of the surge. However, the amplitudes were higher during the ascending than the descending limb. Reports from the ewe also agree with the presence of pulsatile peaks on both the ascending and the descending limbs of the preovulatory surge curve of LH (Rawlings et al. 1986).

The period from the onset of estrus to the LH surge peak has been reported to vary from 4 h to 15 h in the ewe (Geschwind et al. 1968; Goding et al. 1969; Bjersing et al. 1972; Evans and Robinson 1980). Despite the massive amounts of LH released during the preovulatory period, results using exogenous LH in female hamsters treated with anti-GnRH serum have shown that only 11% of the circulating concentration of LH was required to induce ovulation (De La Cruz et al. 1976). A major shift in ovarian steroidogenesis occurs during the first few hours following the preovulatory LH surge. Steroidogenic activity has been described as lowest in the 12 h after the LH peak (Baird et al. 1981; Murdoch and Dunn 1982; Webb and England 1982). Moor (1974) has reported

that in cycling ewes the infusion of LH after lutectomy inhibited E₂ release by the Graafian follicle(s). Nevertheless, the termination of steroid secretion following the LH surge occurs gradually; perhaps the initial signs of inhibition start during the ascending limb of the peak. According to McNeilly et al. (1982), during the induction of cyclicity in anestrus ewes using LH pulse injections, E₂ secretion which followed these pulses began to decline rapidly when LH concentrations reached greater than 5.0 ng mL⁻¹. Ovulation in the cycling ewe occurred at 24-48 h after the LH surge (Murdoch and Dunn 1982). The corresponding period in the cow has been reported to be 20 h (Carr 1972).

In summary, the literature indicates that LH secretory patterns are interrelated with those of E₂. The recent literature also shows that LH plays a more important role in follicular development than has been previously assumed. Through its receptors which already exist on the follicular theca cells, as well as those acquired in the course of development by the granulosa cells, LH is involved not only in defining the fate of the ovarian follicles but also is essential for the sustained rise in E₂ which triggers the preovulatory gonadotropin surge. The onset of the preovulatory surge of LH is associated with the termination of steroidogenesis in all ovarian structures.

2.1.7.5 EFFECTS OF HEAT STRESS ON LH DURING THE ESTROUS CYCLE:

This review has previously noted that LH (in association with FSH) is luteotropic and is involved in the final stages of follicular growth and maturation in the ewe. Heat stress has been shown to over-ride two of the most important functions of LH, stimulation of the sustained rise in

E₂ and the induction of ovulation. Under natural conditions these two functions are inseparable time-related events. The period between these two events is vital for the final maturation of the ovum. Thus, elevated P₄ concentration (Baird and Scaramuzzi 1976a; Goodman et al. 1981b) or heat stress (Dutt 1964) during this period interferes with both functions.

Madan and Johnson (1973) have reported low basal LH and reduced peaks in cows kept for one cycle at 33.5°C. The preovulatory LH surge curve was described (Miller and Alliston 1974b) to be lower in Angus heifers subjected to a fluctuating heat stress. However, other workers have investigated cows exposed to either constant (Gwazdauskas et al. 1981), fluctuating (Fuquay et al. 1980) or seasonal (Vaught et al. 1977; Rosenberg et al. 1982) heat stress and have reported that neither basal concentration nor the preovulatory LH surge curve was different from values observed in normothermic cows. An important observation in these studies was that because of the heterogeneous release patterns of LH and the wide variation between animals, more frequent sampling would have been required to reflect differences in concentration and trends between treatment groups. Luteinizing hormone concentration was also compared between B. indicus (Brahman) and B. taurus (Hereford) and their crosses. Griffin and Randel (1978) have reported that basal concentration, number of peaks, and the preovulatory LH surge maximum were lower in the Hereford than the Brahman cows. However, later data from the same laboratory (Randel and Moseley 1980) and others (Post and Bindon 1983) showed no difference in LH release characteristics between B. indicus and B. taurus cows. In addition, the former group have reported no breed difference in the response to exogenous GnRH.

The findings on the response of LH to heat stress in sheep are in agreement with data from cattle. In ovariectomized ewes kept at constant temperatures of 21°C or 35°C (Schillo et al. 1978), basal LH concentration was lower at 35°C, but there was no change in the pulsatile LH release. Sawyer et al. (1979) have reported fewer LH peaks in ewes followed for several cycles in heated rooms. Hill and Alliston (1981) have used temperature settings similar to those described by Schillo et al. (1978) and reported that whereas basal LH concentration was unaffected, peak values were lower at 35°C. Data from Hooley et al. (1979) showed that in Merino ewes exposed daily to 46°C for 10 h, neither basal LH nor its response to GnRH was affected as compared to the response at a control temperature.

To summarize, there is inconsistency in the data related to the influence of heat stress in cattle and sheep on the characteristics of LH release. Despite the fact that in most studies LH concentration was either depressed or showed no change, three observations emerge. Firstly, because of the inherent nature of pulsatile LH release, treatment differences are likely to be hidden by large coefficients of variations in studies where sampling was infrequent. Secondly, most of the studies were carried out in heated rooms at constant temperature settings. Thirdly, the hormone response to heat stress depends upon the degree of acclimatization as well as on thermal liability which depends on physical characteristics.

2.1.8 FOLLICLE-STIMULATING HORMONE (FSH):

2.1.8.1 BIOCHEMICAL CHARACTERISTICS OF FSH:

Follicle-stimulating hormone is a glycoprotein hormone composed of two unidentical subunits (α - and β -) secreted by the gonadotrophs of the anterior pituitary. Despite the controversy about a separate FSH release factor, GnRH appears to be the major releasing hormone for both gonadotropins. Ovine FSH has a molecular weight of 32,000 da, of which 25% is carbohydrate. In contrast to LH, FSH has a larger amount of sialic acid residues (5%) and its half-life in circulating blood has been estimated to be 2 h. Another divergence from LH is that the α -subunit of FSH in sheep is comprised of 96 amino acids and contains sialic acid residues (Sairam 1981). The biologically active β -subunit contains 111 amino acids (Sairam et al. 1981).

2.1.8.2 FOLLICLE-STIMULATING HORMONE CONCENTRATION DURING THE ESTROUS CYCLE:

Numerous data from in vivo experiments have examined the FSH release patterns and surges. Unlike the discrete pulsatile release of LH, FSH secretion is characterized by minor fluctuations of heterogeneous amplitude and duration. Nevertheless, several major surges have been described. The first major surge to be identified overlaps with the preovulatory LH surge (Pant et al. 1977; Bister and Paquay 1983; Lahlou-Kassi et al. 1984). Surges similar in magnitude and duration to the preovulatory one have been induced in intact or ovariectomized females using either E_2 (Reeves et al. 1974; Howland et al. 1978b), GnRH (Jonas et al. 1973) or both (Kesner and Convey 1982; Kesner et al. 1982).

Other transient FSH surges have also been described in the ewe. A second surge has been reported (Pant et al. 1977; Bister and Paquay 1983; McNeilly et al. 1982; Lahlou-Kassi et al. 1984) to occur at 12-36 h after the preovulatory LH peak, while a third surge has been described to occur on D5-D6 of the cycle (Miller et al. 1981; Bister and Paquay 1983; Lahlou-Kassi et al. 1984).

2.1.8.3 EFFECTS OF FSH ON THE OVARY:

The abrupt rise in FSH accompanying the preovulatory LH surge is caused by the acute stimulation of the gonadotrophs by GnRH. An explanation for the function of the preovulatory FSH surge would be to synergize with LH during the final stages of development of the preovulatory follicle(s). It has been postulated that the other FSH surges function to select and to set the pace of growth of follicles (from the preantral pools) destined to mature in succeeding cycles. Nonetheless, available data strongly argue against this hypothesis. In the ewe, the largest two follicles ovulated when lutectomy was performed on D14 of the cycle but not on D10 (Bherer et al. 1977). The injection of LH and FSH on D10 led to a significant rise in the ovulation rate of marked follicles. Further, results from cattle (Ireland and Roche 1983) have suggested the existence of cyclical waves in the pattern of follicular growth. All follicles identified during the early stages of the luteal phase were atretic and could not be identified as E₂-active after D7 of the cycle. The preovulatory follicle(s) destined to ovulate was from the group that has been identified as E₂-active on D13 of the estrous cycle.

Experimental evidence drawn from many species, but mostly the rat (Richards and Midgley 1976; Richards 1980) as indicated above has established the importance of FSH in the selection and early growth of ovarian follicles. In heifers treated with pregnant mare's serum gonadotropin (PMSG), as a FSH source, there was a dramatic increase in the number of preantral follicles without a change in the number of large antral follicles (Monniaux et al. 1984). McNatty et al. (1985a) have reported a significant increase in the number of medium and large size non-atretic follicles in cycling ewes given FSH between D8 and D10 of the cycle. These researchers claimed that ovulation rate was positively correlated with FSH concentration during the 48 h preceding luteal regression.

It is believed that FSH induces its effect by binding to receptors on the follicular theca and granulosa cells. How the subsequent actions correlate with FSH concentration has been the subject of various investigations. According to Rani et al. (1981) the infusion of rat granulosa cells with FSH led to a dramatic increase in their capacity to bind labelled hCG (^{125}I -hCG), suggesting that FSH increased LH receptors. Additional evidence for the role of FSH also came from work with porcine granulosa cells incubated in steroid-free medium (Baranao and Hammond 1986). In these cells, FSH increased the incorporation of ^{14}C -acetate into sterols and cholesterol, and this effect could not be inhibited by the blockage of cholesterol side-chain cleavage.

To date, the growing follicles have been shown to exhibit major functional changes in their binding characteristics to gonadotropins. Some of these changes have been mentioned before (LH section) and

involve the acquisition of LH binding ability by the granulosa cells of the growing follicles (Richards et al. 1980; England et al. 1981). Subsequently, there is a progressive rise in the appearance of aromatase enzymes (Bogovich and Richards 1982) which convert androgens and P_4 to E_2 . Therefore, it is not surprising that the follicles with the highest FSH in the antral fluids not only have the highest concentration of E_2 , but also tend to be the largest. As the follicles grow larger in size, the granulosa cell binding to FSH decreases (Carson et al. 1979).

2.1.8.4 CONTROL OF FSH SECRETION: THE EFFECTS OF E_2 AND P_4 :

The literature reveals different opinions regarding the negative feedback of ovarian steroids on the pituitary. Whereas a combination of E_2 and P_4 were effective in suppressing the amplitude and the pulsatile release of LH (Goodman et al. 1981a; Karsch et al. 1983), the effect on FSH release has been inconsistent. In ovariectomized ewes injected with E_2 alone, FSH concentration was depressed by 13% (Goodman et al. 1981a). The corresponding value for using P_4 alone was 30%. Because a higher dose of E_2 was required to effectively suppress FSH than LH following ovariectomy, and that the inhibition of P_4 on FSH is transient, a non-steroidal ovarian factor has been suggested to be involved in the negative feedback control of FSH.

Recent evidence indicates that treatment with steroid-free follicular fluid suppressed FSH concentration (>50%) in ovariectomized heifers (Ireland et al. 1983) and ewes (Findlay et al. 1985), without affecting LH concentration or the ovulation rate (McNeilly 1985). This non-steroidal factor has been identified as an inhibin-like molecule (Vale et al. 1986). Antibodies against this molecule (Henderson et al. 1984)

were capable of reversing the FSH suppressive activity without influencing LH secretion. The replacement of FSH in animals infused with follicular fluid led to increased ovulations (McNeilly 1985; McNatty et al. 1985a).

In summary, there is firstly strong evidence emerging from the literature to indicate that the sole function of FSH could be the early selection of oocytes and the development of preantral follicles to acquire granulosa cell LH receptors that would determine both their pace of growth as well as their subsequent destiny. Secondly, in vivo studies have described several FSH surges during the cycle, yet the function of none is fully understood. Thirdly, ovarian steroids are only partially effective in suppressing FSH release. Recent evidence points to the fact that the negative feedback loop on FSH release could be controlled by ovarian steroids in addition to inhibin-like factors from the preovulatory follicles.

Data on the effect of heat stress on FSH concentration and release pattern is currently unavailable in the literature.

2.1.9 THE ROLE OF GONADOTROPIN-RELEASING HORMONE (GNRH):

2.1.9.1 SOURCES AND RELEASE PATTERNS OF GNRH:

GnRH is a decapeptide (Schally et al. 1973) secreted by the hypothalamic neurons. Studies in sheep have revealed that the contribution of neurons originating from the retrochiasmatic and median basal hypothalamus (MBH) were involved in maintaining normal basal LH

concentration, while those originating from the suprachiasmatic and preoptic nuclei were necessary for the preovulatory surge (Jackson et al. 1978; Domanski et al. 1980). Accumulating evidence suggests that the mechanisms of stimulation or inhibition of GnRH release from these neurons, both at the secretory and delivery terminal at the median eminence, are controlled by complex adrenergic, dopaminergic and possibly serotonergic systems (Jackson 1977; Tobias et al. 1983; Moore 1986).

Subsequent to its release from neuronal terminals, GnRH is carried by the portal blood to its target cells, gonadotrophs, in the anterior pituitary gland. GnRH is the major hypothalamic factor that stimulates the release of both LH and FSH. Data using either passive or active immunization against GnRH in various species, are strongly in favor of this hypothesis. The preovulatory gonadotropin surge was absent in rats (Arimura et al. 1974) and ewes (Narayana and Dobson 1979) immunized against GnRH. When ovariectomized ewes were immunized against GnRH, the E₂-induced LH surge was blocked (Fraser et al. 1981). In addition, the rise in gonadotropins which normally follows ovariectomy could be blunted in gilts immunized against GnRH (Esbenshade and Britt 1985). In studies where passive immunization was associated with incomplete neutralization of the effects of endogenous GnRH, FSH concentration was less affected (Popkin et al. 1983; McNeilly et al. 1984). The secretory response of FSH to GnRH differs from that of LH. According to Childs et al. (1983) in the rat 60%, 23% and 18% of the gonadotrophs contained either LH + FSH, FSH alone or LH alone, respectively. These workers further classified the gonadotrophs into small, medium and large and estimated their contents. A higher population of large gonadotrophs

(78%) contained LH + FSH than either FSH (12%) or LH (10%) alone. In contrast, only 15-22% of the small cells contained both LH + FSH while 30-40% and 46-50% contained either LH or FSH alone. These studies suggested the sequestered nature of the releasable pools of gonadotropins which would partially explain the variance in responses. Recent evidence indicates that there is another endogenous gonadal factor that mimics the effects of GnRH on FSH release without influencing LH concentration. Vale et al. (1986) and Ling et al. (1986) have reported the isolation of FSH releasing protein from porcine follicular fluid. This protein is composed of the two β -subunits of inhibins (A and B) linked by a disulphide bridge. It differs from GnRH in its stimulation of the gonadotrophs in that it does not require GnRH receptor binding and also the induced response requires a longer latent period. It is more potent than GnRH and stimulation of release is not followed by desensitization, since it stimulates FSH synthesis at a faster pace than its depletion rate.

2.1.9.2 THE MODE OF ACTION OF GNRH ON ITS TARGET CELLS:

Unlike the rat in which receptors for GnRH have been reported in both the pituitary and the gonads, in the ewe as well as in the cow and the sow GnRH receptors seem to be confined to the brain and the pituitary cells (Brown and Reeves 1983). Autoradiographic studies have shown the GnRH binds to the plasma membranes of the gonadotrophs (Pelletier et al. 1982) and the rise in binding parallels the increase in gonadotropin release (Katt et al. 1985). GnRH has been shown to potentiate its own receptors (Reeves et al. 1982). Other studies have described the binding as biphasic. At physiological concentrations, GnRH stimulated

its receptor binding in rat gonadotrophs, but at pharmacological concentration binding was markedly reduced (Papavasiliou et al. 1986). Using Scatchard plot analysis Loumaye and Catt (1982) have concluded that the GnRH-induced rise in receptor binding was due to an increase in receptor numbers rather than changes in the binding affinity.

2.1.9.3 POST-RECEPTOR EVENTS OF GNRH STIMULATION:

GnRH binding to its receptors is followed by internalization (Pelletier et al. 1982) and stimulation of post-receptor events. The significance of the binding has been extensively investigated in rat pituitary cells. Young et al. (1985) have reported that, when given separately, GnRH, dibutyl-8-bromo-cyclic adenosine monophosphate (db-cAMP) and the low Ca^{2+} ionophore, A23187, have increased GnRH receptors on the gonadotrophs by 70-100% and subsequently induced desensitization (in terms of response to further stimulation). Nonetheless, it is only in the case of GnRH that desensitization has been coupled with the depletion of LH reserves, suggesting that the stimulation of gonadotrophs is associated with post-receptor events and not with receptor binding.

These post-receptor events have been reported to be related to intracellular changes in Ca^{2+} . Depletion of intracellular Ca^{2+} significantly depressed GnRH-induced LH release (Liu and Jackson 1985). It is believed that Ca^{2+} mobilization is important not only for the release of the arachidonates but also the release of protein kinase C which is a crucial part to the response stimulus. According to Chang et al. (1986), the incubation of protein kinase C inhibitor (retinol) or the lipoygenase inhibitor (norhydroguaiaretic acid), with rat pituitary

cell culture have led to a reduction in the GnRH-induced LH release. The addition of both factors simultaneously has completely blocked the LH response to GnRH.

Further studies also included the effects of post-receptor events on gonadotropin release. A recent report by Papavasiliou et al. (1986) has described an increase in LH β -subunit mRNA synthesis and LH secretion, paralleling the rise in GnRH receptor concentration in rat gonadotrophs. In addition, the increase in α -subunit also showed a linear relationship to the concentration of GnRH. Similar studies (Starzec et al. 1986) have demonstrated the ability of GnRH to enhance the incorporation of ^{35}S -methionine into both α - and β -subunits of LH and that this process could be blunted by the addition of the protein synthesis inhibitor, cycloheximide.

2.1.9.4 EFFECTS OF STEROIDS ON GNRH STIMULATION:

The response of the pituitary to GnRH is dependent on the stage of the estrous cycle, which in turn is defined in terms of the prevalent steroid milieu. This area of endocrinology has prompted numerous in vitro and in vivo investigations. Preliminary studies involved the use of single injections which were sufficient in inducing the pituitary response. Because GnRH potentiates its own receptors over time, the response to a single injection has been reported to be smaller than the preovulatory surge seen at a normal estrus (Foster and Crighton 1974).

In contrast, the pulsatile release of LH and its dependence on endogenous GnRH stimulation suggested that results would be more creditable if done by simulating the endogenous GnRH release pulses.

When anestrus ewes were injected with 500 ng GnRH at 2 h intervals for 40-80 days (McNatty et al. 1982) the induced estrous cycles were comparable in magnitude and duration of P_4 concentration to cycles observed during the normal breeding season. In cycling ewes (Adams et al. 1975) and in hamsters passively immunized against GnRH (De La Cruz et al. 1976) prior treatment with E_2 enhanced the LH response to GnRH. Estradiol- 17β has been shown to inhibit GnRH stimulation initially, but it later enhances the response (Kesner and Convey 1982).

Hausler and Malven (1976) studied the effect of P_4 on GnRH-induced LH response in ovariectomized heifers during the inhibitory and the facilitatory phase of E_2 . Their results indicated that during the inhibitory phase (up to 12 h) P_4 either decreased or inhibited the pituitary response to GnRH. Progesterone had no effect on LH response to GnRH during the facilitatory phase of E_2 priming (>16 h post-treatment with E_2). Receptor studies in ovariectomized ewes have shown that E_2 increased pituitary GnRH receptors (Moss et al. 1981). In contrast P_4 alone had no effect on pituitary GnRH receptors, but in combination with E_2 was detrimental.

2.1.9.5 CIRCULATING LEVELS OF GNRH:

Since GnRH is the main hypothalamic factor that controls the secretion of both LH and FSH, knowing the circulating concentration and patterns of release of GnRH would be extremely valuable in the pursuit to understand reproductive processes. Laboratory estimation of this hormone is plagued with several problems that need to be considered. Firstly, GnRH is secreted in minute amounts and subsequently subjected to enormous dilution in peripheral blood. Secondly, the molecule is

easily degradable in blood with an estimated half-life of 4-7 min (Redding et al. 1973; Nett et al. 1973; Wheaton 1982). Thirdly, serum samples contain not only aminopeptidases which enhance the breakdown of GnRH (McDermott et al. 1981), but also contain binding factors that could lead to the overestimation of concentration (Nett et al. 1974; Nett and Adams 1977). Fourthly, aminopeptidases have been reported (McDermott et al. 1981) to be present in commercial bovine serum (BSA) and bovine plasma (BPA) albumins used in assay buffers.

Therefore it is not surprising that earlier investigators were less successful in correlating peripheral concentration of GnRH with release patterns of gonadotropins (Crighton et al. 1973; Jonas et al. 1975; Foster et al. 1976). Nett et al. (1974) could not detect parallel changes in GnRH and gonadotropins' concentration, in the serum samples drawn from cycling, anestrus and ovariectomized ewes. Moreover, in ewes given 500 ng GnRH boluses hourly, the maximum response estimated in acidified plasma samples varied between 50-131 pg mL⁻¹ while the range of the basal concentration taken 30 min after GnRH was 9.5-16.1 pg mL⁻¹ (Clarke and Cummins 1984). These basal concentrations were considerably lower than the 71-128 pg mL⁻¹ range reported by Nett et al. (1973) in serum from ewes and rams. Unlike peripheral concentration, GnRH concentration in hypophyseal portal blood has been shown to correlate significantly with pulsatile LH concentration in jugular blood (Clarke and Cummins 1982).

Recent studies have used pulsatile LH release to characterize the pulsatile nature as well as the differential gonadotropins' responses to GnRH stimulation. Using the technique of push-pull canula to flush the

third ventricle with artificial cerebrospinal fluid, Levine et al. (1982) have estimated the GnRH pulse frequency to be 1 pulse every 30-40 min. These pulses either coincided with or were shortly followed by LH peaks. In ovariectomized ewes with hypothalamic deafferentation, Clarke et al. (1984) increased LH basal concentration, peak values and pulse amplitude without a change in FSH, by raising the dose of exogenous GnRH from an amplitude of 250 ng to 500 ng. A reduction in GnRH frequency from 2 h to 4 h increased LH pulse amplitude and reduced its basal concentration Clarke et al. 1984). In comparison to LH, basal FSH increased when GnRH pulse frequency was decreased from 2 h to 4 h, but there was no change when it was reduced to 1 h from 2 h. Results from perfused sheep pituitary cells revealed that reduced GnRH pulse frequency increased LH amplitudes and response (McIntosh and McIntosh 1985). Taken together, these data emphasize that peripheral variation in the patterns of LH and FSH release cannot be explained in terms of differences in their half-life alone, and that GnRH pulse amplitudes and frequencies contribute significantly to the observed fluctuations in peripheral concentration of gonadotropins.

In summary, the literature indicates that, whereas the pituitary secretion of LH is solely under hypothalamic GnRH control, that of FSH seems to be dually controlled by both GnRH and an inhibin-like peptide secreted by the preovulatory follicles. However, it is not clear whether both act simultaneously on all releasable gonadotropic pools or have differential actions. The literature also indicates that GnRH probably does not have any extra-pituitary influence in domestic animals. Also, GnRH potentiates its own receptors in the pituitary by increasing the receptor population rather than by increasing the binding

affinity. Receptor binding, which is also expressed by several other molecules, does not seem to be as important as compared to post-receptor activities in the case of GnRH action. These activities are characterized by the mobilization of intracellular Ca^{2+} , protein kinase C and enzymes of the lipoxgenase pathway of arachidonate metabolism. Subsequently, GnRH stimulates the release of gonadotropins and the synthesis of their respective α - and β -subunit mRNAs. GnRH is secreted in a pulsatile fashion. Estradiol-17 β exerts a biphasic (an initial inhibitory followed by a facilitatory phase) effect on LH and FSH response to GnRH. Progesterone precipitates the initial inhibitory effect, but has no influence on the subsequent enhancement phase. Low concentrations and rapid degradability of GnRH in peripheral blood and serum samples has led to a difficulty in correlating circulating concentration of GnRH with those of the gonadotropins. No data is available on the effect of heat stress on endogenous basal concentration and release patterns of GnRH in livestock. However, as previously mentioned, the LH response to GnRH was not affected during heat stress in ewes (Hooley et al. 1977) and cows (Gwazdauskas et al. 1981).

2.1.10 PROLACTIN (PRL):

2.1.10.1 NATURE AND MECHANISM OF PROLACTIN RELEASE:

Prolactin (PRL) is a polypeptide hormone with a wide range of biological functions. In sheep it is composed of 198 amino acids arranged in a single chain that contains three disulfide bridges. Prolactin has a molecular weight of 22,500 da. Its release can be triggered by various psychological, physiological and pharmacological

stimuli. It has been estimated that PRL constituted 15% of the total proteins synthesized in the bovine anterior pituitary gland (Baxter and Gorski 1981). The neuroendocrine control of PRL is considered important because a disturbance in this axis leading to elevated PRL secretion has been associated with infertility in man, primates and laboratory animals (Bohnet et al. 1975; McNeilly 1980; Smith 1980). In livestock elevated PRL concentration occur under several situations including extended photoperiods (Hart 1975), lactation (Rhind et al. 1980), various stresses (Raud et al. 1971; Lamming et al. 1974; De Silva et al. 1986a) and at the preovulatory period in cycling animals (Kann and Denamur 1974).

The control of PRL secretion from the lactotrophs involve both release and inhibition factors. Though no claims have been made for the identification of the former, a few agents are commonly used by researchers to induce PRL release. They include thyrotropin release hormone (TRH) (Tashjian et al. 1971; Schally et al. 1973), metoclopramide (Fitzgerald and Cunningham 1982) and β -endorphin (Ferland et al. 1978; Van Loon et al. 1980). Whereas, TRH and metoclopramide reverse the usual inhibitory mechanism on PRL blood concentrations by increasing the synthesis and release (Potter et al. 1981; Braley et al. 1983), β -endorphin increases PRL release by inhibiting the hypothalamic release of PRL release-inhibiting factor (Fitzgerald and Cunningham 1982). According to Vician et al. (1979) and Franks (1983), E_2 stimulates the synthesis and release of PRL by the lactotrophs.

Despite recent reference to the identification of a PRL release-inhibiting factor which exists within the precursor of human GnRH

(Nikolics et al. 1985), dopamine remains the major known biologically active hypothalamic PRL inhibiting factor (Creese 1982; Gallardo et al. 1984). Dopamine and its agonist, bromocryptine (CB-154), have been used extensively to suppress PRL concentration because of their specificity and potency.

There is a general agreement in the literature to indicate that even though dopamine or CB-154, alone, is capable of depressing basal concentration as well as the pulsatile release of PRL, neither is effective in completely inhibiting its secretion. In vitro data using pituitaries from sheep (Ray and Wallis 1982) and rats (Delbeke and Dannies 1985) have shown that dopamine impeded PRL processing by inhibiting the intracellular release of both Ca^{2+} , and cyclic adenosine monophosphate (cAMP).

2.1.10.2 PROLACTIN RELEASE PATTERNS:

Available data indicate that PRL has a circannual release pattern which is positively correlated with fluctuations in photoperiod and ambient temperatures. Thus, concentration are elevated during the months of increasing day length and ambient temperatures, but are lower during the short and cool days of winter. With a half-life of 7 min in peripheral blood, PRL release patterns have been described as pulsatile with a considerable variation in amplitudes, but lack specific ultradian or circadian trends. These observations were based on results drawn from intensive and frequent sampling of cattle (Swanson and Hafs 1971; Fulkerson et al. 1980), sheep (Butler et al. 1972; Lamming et al. 1974; Kann and Denamur 1974) and man (Bohnet et al. 1975). Furthermore, PRL increases concurrently with LH and FSH during the preovulatory period

(Cumming et al. 1972; Louw et al. 1974; Lamming et al. 1974). There are no indications that the resultant preovulatory surge of PRL is triggered by GnRH (Lamming et al. 1974; Wright et al. 1981), but is rather caused by the rising E_2 concentration from the preovulatory follicle.

Estrogens have been shown to induce PRL release in in vivo and in vitro studies. In anestrous, pregnant and lactating ewes (Fell et al. 1972) an increase in PRL has been detectable five min after the onset of E_2 infusion. Howland et al. (1984) have also shown that in ovariectomized ewes aromatizable androgens (testosterone, testosterone propionate) were also capable of increasing PRL concentration. Similar effects of E_2 on PRL release have also been reported from in vitro studies using pituitary slices from sheep (Shupnik et al. 1979) and rats (Maurer 1982). This enhancement by E_2 involves not only release of PRL by the lactotrophs, but also is associated with the synthesis of pre-PRL mRNA (Vician et al. 1979). There is some species variation. Baxter and Gorski (1981) have reported that E_2 had no effect on PRL synthesis in pituitary slices from cattle.

2.1.10.3 PROLACTIN FUNCTION DURING THE ESTROUS CYCLE:

Significant amounts of PRL are secreted by the mammalian pituitary (Miller et al. 1982). In most species the main role of PRL is related to the establishment of lactation. Other reproductive functions of PRL especially during the estrous cycle remain vague. An exception to this generalization is the laboratory rat, in which this hormone functions as a part of the luteotropic complex (beside LH and FSH). In pigs PRL has been shown to be involved in the secretion of non-steroidal follicular factors that block the oocyte maturation process (Baker and Hunter 1978;

Channing and Evans 1982). It has been claimed that in lactating sows, the suppression of PRL leads to an increase in LH release (Bever et al. 1983).

In contrast, data from ruminants suggest that PRL has little role to play during the estrous cycle. The only positive report came from Kann and Denamur (1974), who claimed that four daily doses of PRL (250 I.U.) given immediately following hypophysectomy in ewes maintained the CL for 12 days, while LH at a daily dose of 5 mg or higher failed to do so.

So far the role of PRL in the ovine estrous cycle has been thoroughly investigated for several reasons. Firstly, because elevated PRL concentrations parallel the onset of seasonal anestrus. Secondly, for less known reasons, a surge in PRL, perhaps stimulated by E_2 , coincides with the preovulatory LH and FSH surges. Thirdly, as mentioned before, PRL indirectly inhibits the oocyte maturation process in some species.

2.1.10.4 EFFECT OF INHIBITING PRL RELEASE ON THE ESTROUS CYCLE:

In cycling ewes, twice-daily injections of CB-154 (1-2 mg) is effective in blunting PRL concentration without affecting the cycle length, behavioral estrus (Niswender 1972; Kann and Danamur 1974), or the magnitude and duration of the preovulatory surges of LH and FSH (Louw et al. 1974; McNeilly and Land 1979). Deaver and Daily (1983) have tested the influence of the method of P_4 withdrawal during dopamine infusion in ewes which were either ovariectomized only, lutectomized only, or ovariectomized and treated with E_2 . They concluded that dopamine inhibited PRL release but had no effect on the occurrence of an LH surge; irrespective of the method of P_4 withdrawal. Similarly, Riggs

and Malven (1974) have reported an inconsistent effect of dopamine on LH concentration; even when given intraventricularly at a dosage range of 2.5, 10, 25, 50 and 150 $\mu\text{g animal}^{-1}$. The inhibition of PRL with CB-154 had no effect on LH and FSH response to exogenous GnRH (McNeilly and Land 1979).

Low doses of dopamine have been reported (Vijayan and McCann 1978) to enhance pulsatile LH release in rats. The perfusion of pituitary cells from ovariectomized rats with low dosage of CB-154 (Pieper et al. 1984; Duncan et al. 1986) was shown to augment GnRH-induced GnRH receptor binding, but had no effect on LH release. In contrast to low concentration of treatment, dopamine at pharmacological doses, (Vijayan and McCann 1978) or CB-154 (Owens et al. 1980) impaired LH secretion.

2.1.10.5 EFFECT OF HYPERPROLACTINEMIA ON THE ESTROUS CYCLE:

As mentioned before, PRL concentrations in sheep are elevated in nursing ewes and during seasonal anestrus. In several studies (either short or long), elevated PRL concentration was simulated by the infusion of TRH or metoclopramide. In normothermic ewes repeatedly injected with TRH or PRL during the preovulatory period, hyperprolactinemia had no effect on the onset of estrus, duration of estrus, the preovulatory LH surge, or LH response to exogenous GnRH (Kann et al. 1976; McNeilly and Baird 1977; Chamley 1978). In these ewes LH pulse amplitudes were low and the pulsatile rise in E_2 , which always follows LH pulses, was depressed (McNeilly and Baird 1977). However, Kann et al. (1976) have reported that in hyperprolactinemic ewes, E_2 at doses of 12.5, 25 and 50 μg failed to induce an LH surge. Investigations with hyperprolactinemic rats indicated contradictory findings. Nass et al. (1983) used rats

with prolactin-secreting tumors and reported acyclicity associated with low E₂ and LH, but no effect on FSH concentration nor on the response to estradiol benzoate stimulation. In contrast, in rat pituitary cells, prior exposure to PRL inhibited LH response to E₂ (Miyake et al. 1985). The inhibition of LH release paralleled the accumulation of an E₂-receptor complex in the cytoplasm, suggesting that nuclear translocation had been hindered by PRL.

In summary, the literature indicates that PRL release is triggered by a wide range of stimuli. The role of PRL in the estrous cycle is not well known, particularly in ruminants. Although elevated concentration has been observed to coincide with periods of infertility in the ewe, inhibition of PRL release has no effect on the cycle characteristics; perhaps because available agents are not effective in blocking PRL secretion completely. Also E₂ stimulates PRL secretion in ewes.

2.1.10.6 HYPERPROLACTINEMIA DURING HEAT STRESS:

There is general agreement in the literature on the positive correlation between peripheral PRL concentration and ambient temperature. For instance, in cattle circulating PRL concentration increased when the temperature was raised and declined when the temperature was lowered (Tucker and Wettemann 1976; Smith et al. 1977; Mills and Robertshaw 1981; Wettemann et al. 1982). Schams and Reinhardt (1974) have cited a high correlation between PRL concentration and seasonal changes in ambient temperatures in Brown Swiss steers and heifers. In contrast, other studies (Gwazdauskas et al. 1981) found no difference in PRL concentration between heifers kept at 21°C or 32°C.

Prolactin concentration was also elevated in ewes subjected to either short-term (Schillo et al. 1978; Fraser and McNeilly 1980) or long-term (Hooley et al. 1977, 1979; Hill and Alliston 1981) heat stress. Moreover, heat stress enhanced the pituitary responses to exogenous TRH in ewes (Hooley et al. 1977) and heifers (Wettemann et al. 1982).

In summary, the literature reveals that heat stress is associated with elevated PRL concentration. Although the literature suggests that exposure to heat stress enhances the PRL response to exogenous TRH, data on increased concentration of endogenous TRH during heat stress are not available.

2.2 PITUITARY-ADRENAL AXIS AND REPRODUCTION:

2.2.1 HYDROCORTISONE (CORTISOL) SECRETION PATTERNS:

Cortisol secretion has been extensively studied in sheep and cattle subjected to intensive sampling schedules. Frequent sampling revealed the existence of pulsatile patterns as well as 24-h rhythms. Pulsatile secretion of heterogeneous amplitudes have been estimated to occur at frequencies of 0.6-0.9 pulses h^{-1} in sheep (Fulkerson 1978; Fulkerson and Tang 1979; Sheikheldin and Kennedy 1986 - unpublished observation) and 0.6 pulses h^{-1} in cattle (Fulkerson et al. 1980). In addition, the 24-h rhythms were characterized by an early mid-morning peak and lower concentration during the afternoon hours (Butler et al. 1972; McNatty and Thurley 1973; Bassett 1974; Fulkerson et al. 1980; Mesbah and Brudieux 1982). Exaggerated corticosteroid responses have been documented in livestock subjected to various management and stress

conditions. For example, handling and restraint of experimental animals increased glucocorticoid concentration (Wagner and Oxenreider 1972; 1973; De Silva et al. 1986a), and the response was even greater when samples were collected by venipuncture (Bassett and Hinks 1969). As the animal became more acclimatized to the sampling stress, the elevated glucocorticoid response waned over time (McNatty and Thurley 1973). Similarly, when cows were repeatedly given adrenocorticotrophic hormone (ACTH) (Gwazdauskas et al. 1980), the adrenal response decreased over time.

2.2.2 EFFECTS OF PITUITARY-ADRENAL AXIS ON THE ESTROUS CYCLE:

Cortisol secretion patterns during the estrous cycle are not correlated to any of the ovarian hormones, nor to the phases of the cycle (Lee et al. 1971; Roussel et al. 1983; Hennessy and Williamson 1983). The fact that many livestock management practices elevate adrenal activity have stimulated several investigations into the role of this axis in reproduction. In the mid-1950's a paper by Nowell and Jones (1957) described a rise of 400% in the pituitary content of gonadotropins accompanied by a 58% reduction in ACTH reserve, following ovariectomy in rats. Adrenalectomy, on the other hand, led to a 350% rise in pituitary ACTH content. From these results they postulated that an increased secretion of one pituitary hormone might depress the release of another; hence, an increase in ACTH release could lead to depressed fertility.

Since this hypothesis is solely based on hormone events within the pituitary, subsequent investigators have greatly extended our knowledge on the effects of the pituitary-adrenal hormones on reproduction; namely

those of ACTH and glucocorticoids. According to Liptrap (1970) daily injections of ACTH or cortisol into sows from D14 to the onset of estrus delayed the onset of estrus, shortened its duration and also delayed the preovulatory LH surge. Adrenocorticotrophic hormone did not stimulate ovarian P₄ secretion in adrenalectomized cycling heifers (da Rosa and Wagner 1981) but in the adrenal-intact heifers it depressed the height of the P₄ curve. These effects of ACTH on the P₄ curve could be mimicked by exogenous cortisol in adrenalectomized heifers. Thus, the absence of behavioral estrus and failure of LH surge following the infusion of ACTH during the preovulatory period might have resulted from a direct effect of adrenocorticoids on the hormones of the hypothalamo-pituitary-gonadal axes (Liptrap 1970; Moberg et al. 1981; Stoebel and Moberg 1982).

2.2.3 DO GLUCOCORTICOIDS MEDIATE ACTH ACTION ON REPRODUCTIVE HORMONES?

The infusion of cortisol during the estrous cycle has been associated with low conception rates (Howarth and Hawk 1968), behavioral estrus of shorter duration and low intensity (Stoebel and Moberg 1979) and the suppression of the preovulatory LH surge (Barb et al. 1982; Stoebel and Moberg 1982). Nevertheless, recent reports in which GnRH-induced gonadotropin release has been examined in in vivo and in vitro studies using ACTH or glucocorticoids, revealed a rather complicated relationship. For example, Matteri et al. (1986) have reported diminished LH response to GnRH in sheep pretreated with ACTH(1-39) but in cattle (Padmanabhan et al. 1983) the treatment had no effect on basal LH or its response to GnRH. In this latter in vitro study using cattle, cortisol pretreatment had no effect on basal LH. In contrast to the

above reports, Moberg et al. (1981) have reported that LH response to E_2 was enhanced in sheep pretreated with cortisol. In boars, cortisol enhanced LH response to GnRH (Liptrap and Raeside 1983). Studies from the rat indicate that pituitary response after exposure to cortisol might be time-related. Suter and Schwartz (1985a, 1985b) have shown that when pituitaries from female rats were perfused for 48 h with cortisol or corticosterone, LH release was depressed (38-43%), but that of FSH was enhanced (22-64%). In these animals, GnRH-induced LH release was not affected, but that of FSH was enhanced. In pituitaries of male rats, incubation with corticosterone for 48 h had no effect on GnRH-induced LH or FSH release, yet incubation for 96 h enhanced LH release.

2.2.4 PITUITARY-ADRENAL AXIS DURING HEAT STRESS:

2.2.4.1 ADRENAL RESPONSE TO SHORT-TERM EXPOSURE TO HEAT STRESS:

Depending on the severity, duration and the rate of onset, the adrenocortical reaction in heat-stressed animals may be described as a biphasic response. An abrupt rise in glucocorticoids may accompany an acute short-term exposure to heat stress. Data from men acutely exposed to heat stress suggests that the abrupt rise in glucocorticoids following the onset of the the stimulus was associated with increased concentration of ACTH (Rosak et al. 1980). If the stimulus of heat stress continues, glucocorticoids then peak and reach a plateau for several hours before declining gradually to concentration below the normal values encountered prior to the exposure. A gradual exposure to a progressive heat stress can lead to a decline in adrenal activity.

Most of the available data on short-term exposure to heat stress are from experiments with dairy cattle at the University of Missouri (U.S.A.). Christison et al. (1970) reported that glucocorticoids rose sharply and remained elevated until the end of the exposure period in steers heated acutely at 42°C for 4 h. In another study, when cows were put in heated rooms at 35°C, cortisol increased within 10 min peaked 2 h later, plateaued for 4 h and then started to decline gradually (Christison and Johnson 1972). There was no change in the metabolic clearance rate of glucocorticoids, suggesting an increased adrenal secretion rather than a change in metabolism. When nonlactating cows were exposed to either a control temperature of 18°C or heat stressed at 43°C for 3 or 24 days (Alvarez and Johnson 1973), corticosteroids abruptly increased for 4 h, but gradually fell to normal concentration within 48 h and were markedly depressed by D12 of the exposure. In this study, catecholamines remained elevated to the end of the exposure periods (3 or 24 days). Immediate cooling to pre-exposure temperatures (18°C-19°C) led to a dramatic fall in glucocorticoid concentration (Abilay et al. 1975b) and full recovery to pre-exposure values within 10-40 min.

Elevated catecholamines during exposure to acute heat stress has also been reported in healthy men (Rosak et al. 1980).

2.2.4.2 ADRENAL RESPONSE TO LONG-TERM EXPOSURE TO HEAT STRESS:

Since glucocorticoids are calorogenic (Alexander and Bell 1982; Stewart and Thompson 1984), their elevation during heat stress can jeopardize the animal's ability to dissipate excess heat. A depression in glucocorticoids would logically fit into the maintenance of

homeothermy. Thus, in cows subjected to a gradual rise in ambient temperature from 15.5°C to 29.5°C (Lee et al. 1971), cortisol concentration fell linearly. This suggested that the response during acute exposure could be a nonspecific reaction to sympatho-adrenal stimulation. Catecholamines remain elevated for the entire duration of chronic heat exposure as referred to previously (Alvarez and Johnson 1973). The fact that the increase in glucocorticoid release initially accompanied that of catecholamines, but later fell as heat stress continued, indicates a possible dissociation between the activities of the two adrenal components.

Cortisol concentration was depressed in cows kept in heated rooms at a constant temperature of 35°C for two consecutive estrous cycles (Abilay et al. 1975a), as well as in cows during the hot summer months (Roussel et al. 1977; Adeyemo et al. 1981). Guerrini and Bertchinger (1982) have reported that in wethers subjected to heat stress at 29°C-32°C (RH=89-97%) for three weeks, cortisol concentration was 70-96% less than those observed at 18°C-19°C (RH=92-96%).

Short-term relief from heat stress by providing shade (Stott and Wiersma 1976) or water sprinkling (Ansell 1976) have improved fertility in cattle. Even though cortisol concentration might be lower in shaded animals (Ingraham et al. 1979), the response to diurnal fluctuation in ambient temperature which exceeds the upper limits of the thermocomfort zone for extended hours is more likely to interfere with fertility; particularly if the increase coincides with the hormone events that trigger the preovulatory gonadotropin surges.

Depressed adrenal function during long-term heat exposure suggests possible impaired pituitary secretion of ACTH as well. Data from Shayanfar et al. (1975) and Gwazdauskas et al. (1980) showed that adrenal response to ACTH was lower in cows during heat stress. Unfortunately, no attempts have been made to estimate ACTH in heat-stressed animals.

The effect of PRL on adrenal response has also been explored. According to De Silva et al. (1983) the injection of hypophysectomized ovariectomized ewes with exogenous PRL, given alone or in combination with ACTH, had no effect on cortisol secretion nor on its response to ACTH.

In summary, the literature reveals that glucocorticoid secretion is pulsatile with a distinct diurnal rhythm, but is not related to the reproductive hormones nor to the phases of the estrous cycle. Elevated glucocorticoid concentration during exposure to heat stress seems to be associated with increased release of ACTH. The response of catecholamines during heat stress is more dependent on the intensity of the exposure rather than on the duration. Unlike exposure to acute heat stress, long-term exposure to heat stress depresses adrenocorticoid activity. Hence, it is unlikely that glucocorticoids depress the pituitary-gonadal axis activity during chronic heat stress.

2.3. THE THYROID HORMONES:

2.3.1 RELEASE AND FUNCTION OF THE THYROID HORMONES:

Whereas synthesis of thyroid hormones involves the iodination of the tyrosyl units within the thyroglobulin molecule, their release is associated with the breakdown of thyroglobulin into tetraiodothyronine (T_4), triiodothyronine (T_3), reverse triiodothyronine (rT_3) as well as several other mono- and di-iodothyronines. Although circulating T_4 concentration is several fold higher than that of T_3 , the latter is considered the most active biologically. On the other hand, rT_3 is assumed to be metabolically inert. Thyroid activity is directly controlled by thyroid stimulating hormone (TSH) from the anterior pituitary, and hence indirectly under the control of hypothalamic thyrotropin releasing hormone (TRH). Fisher et al. (1972) have studied the thyroid hormones' kinetics in intact and thyroidectomized adult ewes. They reported that the ratio of circulating $T_3:T_4$ was 0.72 and 0.78 in intact and thyroidectomized ewes, respectively, injected with T_4 , suggesting that in sheep 91% of the T_3 is derived from peripheral mono-deiodination of T_4 . In peripheral blood, half-lives of 1.36 days and 5.5 h for T_4 and T_3 , respectively were estimated in this study. Thyroid hormones have a wide range of effects on many body functions. They play important roles in the development and maintenance of the nervous system, growth, basal metabolism and reproduction.

2.3.2 THYROID ACTIVITY DURING HEAT STRESS:

Because thyroid hormones are involved in basal metabolism, it is normal for their secretion to decline during heat stress to reduce heat load on the animal. Johnson and Yousef (1966) have compared the thyroid activity in dairy cows kept at 7°C, 18°C, 29.5°C and 35°C. Their results showed depressed thyroid activity and a faster disappearance

rate of T_4 at 35°C as compared to the lower temperatures. These findings were also confirmed in heat-stressed cattle (Yousef and Johnson 1966; Collier et al. 1982; Scott et al. 1983) and sheep (Ross et al. 1985). The evidence indicates that under certain conditions the depressed thyroid state can be qualitative (in terms of the predominantly circulating iodothyronine) rather than quantitative in its activity. For instance, in men subjected to heat stress, the progressive decline in T_3 concentration was simultaneously accompanied by a rise in the metabolically inactive rT_3 (Epstein et al. 1979). A similar switch in the metabolism of T_4 has been reported in individuals subjected to energy deprivation (Palmlad et al. 1977; Ingram and Ramsden 1981). This suggested that under such conditions the thyroid activity could switch from the release of the calorigenic T_3 to the release of rT_3 . Earlier data by Yousef and Johnson (1966) suggested that in dairy cows, heat stress depressed thyroid activity irrespective of the level of energy intake. In contrast, more recent reports from pigs have shown that high energy intake during exposure to heat stress (35°C vs 10°C), restored the morpho-endocrine function of the thyroid gland (Macari et al. 1983; Dauncey et al. 1984; Dauncey and Ingram 1986).

Heat stress has been shown to depress the thyroid response to TSH in ewes (Ross et al. 1985) and to TRH in steers (Pratt and Wettemann 1986). In addition, serum TSH has been found to be inversely related to rectal temperature in heat-stressed men (O'Malley et al. 1980, 1984b). When the thyroid response to 14 daily injections of TRH was studied in cows subjected to 18.5°C or 35°C , T_3 increased progressively to a peak by day 4 of the treatment, irrespective of the temperature (Vanjonack and Johnson 1975). The decline in T_4 after day 7 was much greater in the

heated cows. Evans and Ingram (1974) have used thermodes to study the effect of cooling (10°C) or warming (25°C) the preoptic hypothalamic area and the cervical spinal cord on plasma T₄ concentration in pigs. Their results showed that plasma T₄ was increased during cooling with no concurrent changes in the secretion rate. They postulated that the increase in plasma T₄ might have resulted from the redistribution of the existing pool, due to increased binding by the plasma proteins. This elevation of plasma proteins occurred as a sequel to increased renal diuresis leading to the shrinkage of the plasma volume.

2.3.3 RELATIONSHIP BETWEEN THYROID HORMONES AND CATECHOLAMINES:

A complex relationship exists between the thyroid hormones and the symphatho-adrenal release of catecholamines. One aspect of this relationship involves the nervous system, thyroid hormones maintain normal brain function, while catecholamines play the role of neurotransmitters and neuroendocrine coordinators. In addition, both hormone systems influence thermogenesis, energy metabolism and reproduction. An early report by Galton (1965) has shown that in thyroid-intact rats treated with radio-labelled T₄, the administration of adrenaline increased the urinary radioactivity; whereas, treatment with reserpine (which depleted the catecholamines from their storage granules) reduced the urinary radio-activity. In addition, the urinary excretion of radio-activity was greater in thyroidectomized rats than in the thyroid-intact group. In these studies, the renal excretion of the infused radio-active iodine per se was not affected by epinephrine, supporting the hypothesis that the increased activity in response to catecholamines after the injection of iodinated T₄ was due to increased

monodeiodination.

Moreover, recent in vitro studies using rat liver cells have shown that, whereas the α -adrenoceptor agonist, norepinephrine, and the β -adrenoceptor agonist, isoproterenol, increased the monodeiodination of T_4 to T_3 , the α -adrenoceptor antagonist, phenoxybenzamine, and the β -adrenoceptor antagonist, propranolol, reduced the monodeiodination of T_4 to T_3 (Wiersinga et al. 1980). In euthyroid and hypothyroid pigs that were either maintained at high or low energy intake, Ingram and Dauncey (1986) have reported that the β -adrenoceptor, propranolol, reduced oxygen consumption, but only in the euthyroid individuals. A more recent report by the same workers (Dauncey and Ingram 1987), has also shown that the injection of propranolol into piglets at 18-24 h after feeding was associated with a decrease in the disappearance rate of T_3 . However, the effect of propranolol on the monodeiodination of T_4 has been reported to be through the inhibition of the 5'-deiodination of all the iodothyronines (Lumboltz et al. 1982).

Although, several studies have shown that catecholamines were elevated in animals during exposure to either cold or warm temperature (Alvarez and Johnson 1973; Rosak et al. 1980; Barrand et al. 1981; O'Malley et al. 1984a), the presumed catecholamine-induced conversion rate of T_4 to T_3 has been found to be greater during the exposure to cold temperatures, irrespective of the level of energy intake (Macari et al. 1983). Paradoxically, such conversion does not occur during fasting where the monodeiodination process is in favor of the metabolically inert rT_3 (Palmlad et al. 1977). Therefore, the general consensus in the literature is that, increased sympatho-adrenal release of

catecholamines is associated with a rise in the conversion of T_4 to the biologically less active rT_3 . To what degree this switch in monodeiodination of T_4 is influenced by the voluntary reduction in energy intake during heat stress is not clear. It would also be of interest to investigate whether the changes in the metabolism of T_4 are accompanied by specific changes in the activity of the thyroid hormone receptor in various tissues.

2.3.4 THE RELATIONSHIPS BETWEEN THE HYPOTHALAMO-PITUITARY-THYROID AXIS AND PROLACTIN (PRL) RELEASE:

It has already been mentioned that TRH is used to stimulate PRL secretion by the lactotrophs. Since thyroid hormones exert an inhibitory effect on the pituitary as well as the hypothalamus, they indirectly reduce PRL concentration. For example, in lambs thyroidectomy was accompanied by a steady rise in PRL up to 42 days (Davis and Borger 1973). This elevation was due to a decrease in PRL metabolic clearance rate coupled with an increase in its secretion rate as well. The acute injection of T_3 in sheep inhibited TRH-induced TSH release and depressed basal PRL concentration (Davis and Anfinson 1976), but chronic daily injections had no effect on basal PRL concentration nor on TRH-induced PRL release (Debeljuk et al. 1973; Davis and Anfinson 1976). Conversely, data from men suggests that the dopamine agonist, bromocryptine, depresses basal secretion of TSH and also abolishes the circadian rhythm of TSH (Sowers et al. 1982). Another important relationship is that thyroid activity has been shown (Stolk et al. 1980) to be inversely related to the activity of the enzyme dopamine- β -hydroxylase which converts dopamine to norepinephrine. If

this is the case, it would then explain the elevated PRL concentration as well as the increased norepinephrine and epinephrine concentrations during heat stress. Thus, PRL concentration rises because of reduced inhibition by dopamine, while the concentrations of the two latter hormones rise as a consequence of increased production. The inconsistency in the literature concerning the relationship between the thyroid hormones and the hypothalamo-prolactin release mechanism might be attributed to the fact that either hormone system plays a permissive rather than a direct role in most of the bioendocrine functions of the body.

2.3.5 THYROID FUNCTION AND REPRODUCTION:

Normal thyroid activity is essential for reproduction and in this review emphasis is given to the reproductive endocrine changes that occur as a result of dysfunctions in the thyroid status. Louvet et al. (1979) have described an anovulatory condition in women associated with hypothyroidism that was non-responsive to gonadotropin therapy, but can be corrected by T_3 therapy. Studies in the rat have shown that hypothyroidism decreased while moderate hyperthyroidism increased gonadotropin concentration (Bruni et al. 1975; Freeman et al. 1975). In hypothyroid rats, daily treatment with physiological or medium doses of T_4 , restored the normal secretory patterns of gonadotropins. Nevertheless, in ovx rats T_4 failed to restore gonadotropins to the concentrations seen in euthyroid individuals (Bruni et al. 1975). It has also been reported that the attenuation of the LH pulse peaks in response to hyperthyroidism was not associated with changes in the metabolic clearance rate of LH (Freeman et al. 1975), indicating a

reduction in LH secretion. Furthermore, when ovx-thyroidectomized rats were given a physiological dose of T_4 , the amount of E_2 required to induce a LH surge was higher than in a similarly treated group, but deprived of T_4 (Freeman et al. 1976). This latter report and others have also shown that in the rat, the response of LH to exogenous GnRH was not influenced by the thyroid status (Freeman et al. 1975; Wang et al. 1987), suggesting that thyroid hormones affect LH release via the hypothalamus.

The effect of altered thyroid function on reproduction has also been investigated in sheep and the results tend to support the above observations in the rat. In the ram, both hypo- and hyperthyroidism have been reported to depress gonadotropin concentration (Davis and Borger 1973; Ponzilius et al. 1981; Chandrasekhar et al. 1985a). In addition, the sheep data reveals that, thyroidectomy reduces basal LH but it either elevates or has no effect on basal FSH concentration (Chandrasekhar et al. 1985b, 1986). Moreover, the sheep data tended to differ from the results in the rat in that, the LH response to exogenous GnRH was depressed in the hypothyroid rams.

With respect to the interaction between thyroid function and heat stress on reproduction, low concentrations of thyroid hormones during summer months are suspected to be the cause of seasonal infertility in sheep. However, data from Bogart and Mayer (1946) have shown that although the testicular degeneration and reduced semen quality in the hypothyroid rams were similar to the changes observed during the hot summer months, treatment with a thyroprotein did not completely restore testicular function. Therefore, low fertility during heat stress can not

be explained in terms of a depressed thyroid function alone.

To summarize, the literature indicates that thyroid function is controlled by TSH, the secretion of which shows an inverse relationship to the hypothalamic temperature. Generally, catecholamines increase the peripheral monodeiodination of T_4 and as a consequence are presumed to potentiate thyroid activity. However, during fasting the monodeiodination of T_4 shifts to the metabolically inert rT_3 and since heat-stressed animals tend to reduce food intake to minimize the body heat load, it is likely that the depression in thyroid function during heat stress is related to the reduction in energy intake. Indeed, high energy intake has been shown to restore some of the effects of heat stress on thyroid hormones. The literature also shows that both hypo- and hyperthyroidism depress gonadotropin secretion. Variation exists in the response of response of gonadotropins to exogenous GnRH in the hypo- or hyperthyroid rat or sheep.

2.4 GENERAL SUMMARY AND CONCLUSION OF THE LITERATURE REVIEW

An attempt has been made in the previous pages to review the endocrine factors involved in the control of the estrous cycle in mammals, but special emphasis has been given to the ewe. In the following passages are brief summaries and wherever possible a conclusion is given.

The main effects of P_4 on LH release during the estrous cycle are on the hypothalamus. Progesterone, alone, is ineffective in suppressing FSH. Progesterone synergizes with E_2 to induce estrual behavior and to intensify the magnitudes of both the negative and the positive feedback

of ovarian steroids on the release of LH and FSH. Heat stress elevates the peripheral concentration of P_4 , but the source remains equivocal. Adrenal P_4 secretion increases during acute psychological stress, also in response to exogenous ACTH and during acute exposure to heat stress. Since P_4 is also elevated in long-term heat-stressed animals in spite of the general depression of the adrenocorticoid concentrations, it can be proposed that there is a differential adrenal steroidogenesis during heat stress. Although heat tolerant breeds of cattle (Zebu) have a smaller CL which secretes less P_4 as compared to the temperate breeds, there is no data to suggest that adrenal P_4 secretion is higher in the Zebu breeds, or that, these breeds would not respond to heat stress by increased P_4 secretion.

The secretion of E_2 is closely associated with the pulse frequency and pulse amplitudes of LH, and the amount secreted by the ovarian follicles is influenced by FSH. The induction of estrual behavior by E_2 does not require an intact pituitary gland. Also E_2 exerts its negative and positive feedback control on gonadotropins by directly acting on both the hypothalamus and the pituitary. During exposure to heat stress, the absence of behavioral estrus is suggestive of an inadequate concentration of E_2 . Temperate and Zebu breeds of cattle differ in the minimum amount of E_2 required to induce estrous behavior. Whether heat stress depresses E_2 secretion from the growing follicles by enhancing the development of atresia requires further investigation.

The literature suggests that the most important roles of LH in the female are the stimulation of E_2 secretion by the ovarian follicles, the induction of ovulation and the maintenance of the CL as a part of the

luteotropic complex (species differences acknowledged). The secretion and the pulsatile release of LH are solely controlled by the interaction of ovarian steroids with GnRH. It seems that during the luteal phase basal LH is related to P_4 secretion, whereas during the preovulatory period LH release is more related to E_2 secretion. Since the occurrence of the preovulatory LH surge is associated with the termination of ovarian steroidogenesis, this endocrine event in cycling animals is considered as an end point of one cycle and the beginning of a succeeding one.

The literature indicates that heat stress depresses LH concentration. Nonetheless, there are many contradictory reports because of: the inherent nature of LH release patterns; differences in the intensity and duration of heat stress; and the failure to take into account the differences between species in the ability to dissipate excessive heat load. Heat stress seems to override two functions of LH in the cycling female; the stimulation of increased E_2 from the Graafian follicles during the preovulatory period and the induction of ovulation. Despite the fact that the heat tolerant breeds of cattle (Zebu) have been reported to have smaller CL which secreted less P_4 and also required more E_2 to induce estrual behavior than do the temperate breeds. The literature indicates that circulating LH during the preovulatory period was comparable between breeds. Whether the susceptibility of the mature ovarian follicle to the luteolytic factors remains the same or changes during heat stress remains unknown.

The literature indicates that FSH, in synergism with LH, controls follicular growth and maturation. Although several FSH surges during

the estrous cycle have been reported, basal release tends to be more stable than LH, and the patterns of secretion during the luteal phase are unlikely to be related to the cyclic P_4 concentration. Instead, they are possibly related to the non-steroidal ovarian factors. Thus, FSH release seems to be dually controlled by hypothalamic GnRH and the ovarian factors. There is no data on the effect of heat stress on FSH. However, based on LH data, one would assume that heat stress should have a similar effect on FSH.

There is a general agreement in the literature that the pulsatile secretion of GnRH (at least in domestic livestock) controls the pituitary synthesis of gonadotropins and their release from the sequestered pools in the gonadotrophs. The GnRH neurons that control basal secretion of gonadotropins are different from the neurons which control the preovulatory surges. Nonetheless, there are species differences in the functional locations of these neurons. The post-receptor actions of GnRH, which involve the mobilization of Ca^{+} , seem to be more important than the receptor occupancy. Additionally, GnRH does not seem to have extra-pituitary action in farm livestock. Ovarian steroids modulate the hypothalamic release of GnRH and also the subsequent action of GnRH on the pituitary. Although the non-steroidal ovarian factors are involved in the pituitary secretion of FSH, their interaction with GnRH at the pituitary level is not understood. Further, the fact that heat stress depresses LH but has no influence on the LH response to exogenous GnRH implies that heat stress reduces the secretion of GnRH.

The role of PRL on the ovine estrous cycle remains controversial

despite the concomitant occurrence of a PRL surge with the preovulatory surges of LH and FSH. In cycling normothermic animals, inhibiting PRL release does not affect the characteristics of the current or the succeeding estrous cycle. Although PRL is elevated during hypothermia, its role as an anti-gonadotropic factor in sheep has not been critically evaluated. Presently, two of the major problems in investigating the role of this hormone on the endocrine control of the estrous cycle are that PRL release is influenced by several endogenously secreted hypothalamic and pituitary peptides and that the inhibitory mechanism of its release involves factors which can directly influence LH and FSH secretion.

The literature indicates that the response of glucocorticoids to heat stress depends upon the intensity and duration of the exposure. Acute heat stress causes an abrupt release of ACTH and an increase in the activity of the sympathetic nervous system. Both responses trigger acute rises in glucocorticoids and catecholamines. Chronic heat stress, on the other hand, depresses glucocorticoids but enhances the release of catecholamines. However, the literature lacks information on the concentration of ACTH in livestock during exposure to heat stress. Although induced elevation of glucocorticoids has been reported to depress the function of the pituitary-gonadal axis, the reduction of the adrenocortical activity during long-term exposure to heat stress (which presumably results from a reduction in circulating ACTH) downplays the possibility that glucocorticoids could directly depress the secretion of gonadotropins during such conditions.

The literature indicates that normal thyroid function is essential

for reproduction. However, because of the complex interrelationships between thyroid hormones and various endocrine systems (especially the catecholamines and the PRL release control mechanism) only clinically detectable thyroid dysfunction can be related to errant morpho-endocrine changes in reproduction. Both hypo- and hyperthyroidism depress gonadotropins. In addition to catecholamines, thyroid function is also dependent on energy intake and environmental temperature. Since heat stress leads to a voluntary reduction in energy intake it is possible that the reported depression of thyroid function in heat-stressed animals could be due to low energy intake.

GENERAL MATERIALS AND METHODS

1. ENVIRONMENTAL CHAMBER (EC):

All investigations were carried out in an environmental chamber, 2.54(l) x 1.88(w) x 2.54(h) m (Coldstream, Fleming-Pedlar Ltd.), fitted with an automatically controlled chart board (Enconaire System Ltd., Winnipeg, MB., Canada). The chamber temperature was maintained by the circulation of glycol solution (one part ethylene glycol:two parts water) in cooling and expansion coils. The latter coils also functioned to control the spray nozzle humidifiers located above the chamber. Changes in the settings of temperature, relative humidity and photoperiod were controlled by automatic timers which were calibrated in 24 one-hour increments. Timers that controlled temperature and humidity were further calibrated to accomodate settings of 5-min intervals. The timer which controlled photoperiod was further calibrated to accomodate settings of 15-min intervals.

Ventilation was provided indirectly by two fans which forced air through a supply duct to two additional fans that blew air directly into the chamber. Air renewal was achieved by exhaust fans which forced the used air into an exhaust duct. In case that the chamber temperature rose above or fell below the the programmed settings, a safety compressor automatically shut off the system, and the door, which was kept closed by an electromagnetic device was dislodged to open. Temperature and humidity settings were additionally checked using a dry bulb thermometer and a hygrometer placed inside the chamber.

3.2 INTACT EWES:

3.2.1 MANAGEMENT:

Nine adult Finnish Landrace- and Suffolk-cross ewes (age greater than 15 m) were used in investigations during the normal breeding season (Sept.-Feb.). The first investigation consisted of two replicate studies using 4 (year 1) and 5 (year 2) ewes, to evaluate the effect of temperature, alone, on the estrous cycle and hormonal relationships. In the second investigation, prolactin concentration was blunted with bromocryptine (CB-154) (5 Finnish Landrace-cross ewes) and the same parameters were studied. Prior to every investigation ewes were observed for two estrous cycles using vasectomized rams. The ewes were then shorn to a fleece length of less than 0.5 cm. Estrus was synchronized with two injections of the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue (Cloprostenol, ICI Pharma, Mississauga, Ontario, Canada), given 10 days apart. Each injection (250 μ g) was split into two doses (125 μ g/dose) given intramuscularly (i.m.) at 700h and 1700h on the same day. The first $PGF_{2\alpha}$ injection was given while the ewes were in a closed barn under natural photoperiod. The second injection was given 48 h after the ewes moved into the chamber where they were studied for two consecutive cycles.

During the first estrous cycle (thermoneutral, NT) the chamber temperature was programmed to a constant temperature of $18.0^{\circ}\text{C}\pm 1.0^{\circ}\text{C}$. In the second estrous cycle the chamber temperature was allowed to fluctuate on a 12 h circadian cycle of $18^{\circ}\text{C}-35^{\circ}\text{C}-18^{\circ}\text{C}$ (cyclic heat stress, CHS) (to simulate a typical hot tropical day). During both

estrous cycles, relative humidity ($55.0 \pm 2.0\%$) and photoperiod (14D:10L) were maintained constant. The change of the chamber temperature from NT to CHS setting was done at 1200h on D1 of the next estrous cycle. Similarly, the temperature was switched back to the NT setting at the same day and time of the second cycle.

The ewes were left to roam freely in the chamber and were fed in the same manger. All ewes were given a daily individual feed allowance of 900 g of crushed barley, 1200 g alfalfa pellets (CP>16%; CF<26%; Alfalfa Product Ltd., Fort Whyte, MB., Canada) and 1000 g of hay. This ration was given in two equal portions at 730h and 1530h. Animals had free access to water and mineral salt licks. No attempt was made to measure either food intake or water consumption. However, during the heat-stressed cycle cool water was provided more frequently.

3.2.2 PARAMETER RECORDING AND SAMPLING PROTOCOLS:

Twice (at 700h and 1500h) daily observations were recorded for ambient and rectal temperatures as well as for respiration rates, during the NT cycle. During the CHS cycle these observations were recorded three times daily (at 700h, 1200h and 1500h). Additionally, because it was assumed that the chamber temperature remained within the programmed settings throughout the investigations, diurnal temperature was calculated from the hourly changes between D14 and D18 of the estrous cycle. These records were used to composite the diurnal fluctuations in ambient temperatures. Since neither the rectal temperature nor the respiration rate was recorded continuously, only daytime values which were taken during both cycles were used. Records were always made prior to feeding. Rectal temperature (to the nearest 0.01°C) was taken using

a clinical thermometer inserted at least 10 cm into the rectum for a minimum of two minutes. Respiration rate (min^{-1}) was recorded from visual counting of the flank movements to the nearest round number; the average of two counts taken within 10 min was recorded at each observation.

Blood samples were collected by venipuncture using 7.0 mL vacutainers (Becton and Dickinson Canada, Mississauga, ONT., Canada). Daily (D1-D13, day of estrus as D0) samples were collected at 1100h. In addition, from 700h of D14 to 700h of D18 of each cycle, hourly samples were collected. Samples were cooled at 4°C for a minimum of 2 h, and the sera were harvested and stored at -20°C until hormone analyses were made.

3.3 OVARIECTOMIZED (OVX) EWES:

3.3.1 MANAGEMENT:

Two groups of adult ewes ($n=4$ per group) of the same breeds as the previously described were used to investigate the effect of heat stress on two endocrine axes: the pituitary-adrenocortical axis and the hypothalamo-pituitary axis. Ewes were observed for cyclicity using vasectomized rams prior to ovariectomy. Ovariectomies (done 5-6 months prior to each investigation) were performed by midventral incisions. Ewes were maintained in wooden metabolic crates inside the chamber and were individually fed a daily ration similar to the one described above. All other management conditions and chamber maintenance procedures were as described for the previous groups.

3.3.2 SAMPLING PROCEDURES:

Injections and samplings were performed through jugular polyethylene catheters (I.D.=1.14 mm) that were fitted 24 h prior to every intensive sampling session. Catheters were maintained by flushing with heparinized saline (5 I.U. mL⁻¹).

3.4 LABORATORY ANALYSIS:

Chemicals and analytical reagents used in hormone assays, unless specified, were all of radioimmunoassay (RIA) grade. Ingredients for assay buffers were weighed beforehand. Buffers were prepared and used fresh, or kept at 4.0°C and changed every 10 days. The hormone standards and the unknowns were run in triplicate and duplicate, respectively. Prior to every assay, both the standards and the unknowns were thawed overnight at 4.0°C. For each hormone, a single preparation of the standard curve was used to estimate the concentrations in the unknowns. With the exception of triiodothyronine assay, all samples from an individual ewe were estimated in the same run.

Cortisol and progesterone (P₄) assay tubes were counted for 4.0 min in a liquid scintillation counter (Rackbeta #1217, LKB, Wallac OY, Finland). Those for luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (PRL) were counted for 1.0 min in a gamma counter (CompuGamma #1282-802, Universal Gamma Counter, LKB, Wallac OY, Finland). Both counters were fitted with built-in microcomputers that were programmed to calculate and extrapolate the unknown concentrations from the standard curves using spline-functions (Wold 1974; Marschner et al. 1974).

3.5 STATISTICAL ANALYSIS:

3.5.1 CALCULATION OF RADIOIMMUNOASSAY (RIA) DATA:

Non-specific binding (NSB) and total binding (TB) were calculated according to Rodbard et al. (1970) and Abraham (1974). The sensitivity of the assay (Abraham 1974; Ekins 1974) was calculated as amount tube⁻¹ at the 95% binding point. The intra- and inter-assay coefficients of variation were calculated as described by Rodbard et al. (1968). The standard curves for GnRH and triiodothyronine assays were constructed according to Rodbard and Lewald (1970). The standard curves for progesterone, cortisol, LH, FSH and PRL assays were calculated using the spline functions (Wold 1974; Marschner et al. 1974). The onset of a hormone surge was calculated as defined by: a sustained increase (for more than 3 consecutive points) in the hormone concentrations which is above the mean of the preceding nadir points (n=6) by 3 times the SD of that mean. Areas under LH and FSH curves were calculated using an on-line computer program (written by Dr. F.S. Chebib, Faculty of Dentistry, Univ. of Manitoba, Winnipeg, MB., Canada) as reported by Howland et al. (1978b).

3.5.2 PULSAR ANALYSIS OF HORMONE PROFILES:

All estimates of hormone concentrations made on frequently collected samples were initially subjected to analysis by the Pulsar program (Merriam and Wachter 1982; Veldhuis et al. 1984), which identifies baselines, amplitudes, peaks, frequencies, duration of peaks and

interpeak intervals. This program eliminates circadian and long-term trends by using Robust Locally Weighted Regression Analysis (Cleveland 1979), through specified window settings (related to the time sequence of the original data collection), to generate a smoothed data series. Each point of this smoothed series is then subtracted from its respective raw data point, and the residuals are used to identify the peaks. The latter step is based on the value of the amplitude from the immediately preceding nadir point (Santen and Bardin 1973; Cliften and Steiner 1983).

Because the smoothing process is achieved by moving the window settings along the raw data series, sufficient intervals were provided to yield smoothing that should simulate endogenous hormone release patterns. Hence, for estimates made from samples collected more frequently (15-20 min intervals) up to a 12 h period, the window settings were specified at 50% of the entire sampling period. The window settings for samples collected at hourly intervals over 5-day periods was 12 h. These arrangements gave smooth curves that were visually indistinguishable from manually constructed ones using the corresponding raw data series.

In addition to the assay detection limits (sensitivity), the program also uses the multiples of the intra-assay standard deviations (referred to as the 'G values') as cutoffs to identify peaks. To reduce the number of false peaks, an inverse-related calculation was adopted between the number of points within a peak (duration) and the multiples of the intra-assay standard deviations (G values). Thus, a peak would be 5.5, 4.5, 4.0, 3.5 and 3.0 times the intra-assay standard deviation

above the preceding nadir point, if it contained 1,2,3,4, and 5 or more points, respectively. However, Because pulse peaks are identified based on how high their amplitude rises above the preceding point, the (G values) are only effective in helping to identify pulse peaks in a data series that reflects a stable pattern of hormone profiles. Thus, the Pulsar program fails to identify all the pulse peaks in a rapidly changing hormone profile, as during the preovulatory surge curve.

For the intra-assay standard deviations we initially selected 6-7 ranges of concentrations read from the standard curves of every hormone. The standard deviations (SD) of these concentrations were used to generate constant, linear and quadratic functions which were then included in the analysis. However, we were unsuccessful in utilizing these functions in our analysis due to the existence of negative relationships in the quadratic function of some hormone assays (cortisol, GnRH and PRL). Since there were no significant differences between the means of the intra-assay SD of the selected range of concentrations in any of the hormone assays, the overall mean SD for each hormone assay was used as a constant function in the analysis. Zero values were entered for the other two functions in the program.

3.5.3 GENERAL STATISTICAL ANALYSIS:

Experimental data, including the Pulsar program-generated results as well as calculated areas under hormone curves, were analyzed by computer program using the Statistical Analysis System (SAS Institute Inc. 1982) packages. Analysis of variance (ANOVA) was adopted uniformly over all data. All means are expressed as (mean \pm SEM). Due to the large standard deviations in the Pulsar program-generated means for basal

concentration, pulse amplitude, duration of pulse peak and the interpeak interval, the SD for each parameter was incorporated as a weight factor in the statistical analysis of the respective parameter. During the estrous cycle, Tamb, Trec, RR and hormone concentrations were analysed by arbitrarily dividing the cycle into 4 phases: follicular (D1-D4), early-luteal (D5-D8), mid-luteal (D9-D12), D12) and late-luteal (D13-D16). Specific statistical analysis is explained in each manuscript. Analysis of variance of T₃ concentration in these

Manuscript #1

PHYSIOLOGICAL AND ENDOCRINE RELATIONSHIPS IN LONG-TERM
HEAT-STRESSED SHEEP

4.1 ABSTRACT:

Adult (60.3 ± 2.8 kg liveweight), estrous synchronized, cycling ewes treated with or without bromocryptine (CB-154) (1.0 mg twice daily) during the breeding season (Sept.-Feb.) were used to investigate the relationships between physiological responses and hormone changes in response to heat stress (CHS). Utilizing a temperature-controlled environmental chamber, the first of the two consecutive estrous cycles was studied at a constant thermoneutral temperature (NT) of $18 \pm 1^\circ\text{C}$, while in the second, the chamber temperature was allowed to fluctuate on a 12 h cycle of 18°C - 35°C - 18°C (cyclic heat stress, CHS). Relative humidity ($55.0 \pm 2.0\%$) and photoperiod (14D:10L) were kept constant. The ewes were fed a ration which was calculated at 2.5 times maintenance. The daily rectal temperature (T_{rec}) and respiration rate (RR) were recorded twice and three times during NT and CHS, respectively. Daily (D1-D13) and hourly (D14-D18) blood was drawn by venipuncture to estimate cortisol, triiodothyronine (T_3) and prolactin (PRL) using RIA procedures.

During NT RR was positively correlated ($p < 0.001$) to T_{rec} . None of the hormones estimated showed significant relationship to either physiological parameter. Cyclic heat stress elevated ($p < 0.001$) T_{rec} greater than 0.4 - 0.9°C and increased RR greater than 3-5 fold. Bromocryptine treated ewes tended to have lower T_{rec} and higher RR

during CHS. Cyclic heat stress depressed mean daily cortisol ($p < 0.05$). Neither CHS nor CB-154 influenced the progressive decline in cortisol ($p < 0.05$) which was probably due to sampling stress. CB-154, but not CHS, attenuated the 24-h rhythm of cortisol secretion.

Prolactin response to CHS was biphasic. Concentration of PRL rose acutely until T_{amb} was 31.4°C , which corresponded to a T_{rec} of $39.6 \pm 0.14^{\circ}\text{C}$ and a 2.3 fold increase in RR, as compared to values at 18°C . Thereafter, the continued rise in T_{amb} was inversely related to PRL concentration. The exaggerated release of PRL in response to CHS ($p < 0.001$) was due to enhanced release.

These results indicate that different endocrine systems vary in their ability to accommodate transient disruptions of the individual's homeothermic balance. During CHS, both the duration and the intensity of exposure affect the manifestation of the nature of the response. Therefore, unless correlations between the physiological parameters and peripheral hormone concentration were interpreted carefully to exclude overlapping responses, interpretations could lead to contradictory conclusions.

4.2 INTRODUCTION:

The response of livestock to heat stress has been well documented in the literature (Brook and Short 1960; Hales 1969; Johnson 1971; Hooley et al. 1979; Borut et al. 1979; Dmiel and Robertshaw 1983; Ross et al. 1985) and several indices have been used to evaluate the extent of the animal's response (Bianca 1961; Kibler 1964). Peripheral hormone

concentrations and behavior have been extensively used by livestock biometeorologists as indicators of the animal's success or failure to maintain homeothermy. Thus, changes in the concentration of cortisol (Lee et al. 1971; Mesbah and Brudieux 1982; Guerrini and Bertchinger 1982), thyroid hormones (Ingraham et al. 1979; Epstein et al. 1979; Marques et al. 1981; Guerrini and Bertchinger 1983; Pratt and Wettemann 1986) and prolactin, PRL (Hill et al. 1980) have been taken to reflect heat stress-induced disturbances in endocrine homeostasis.

However, the release of these hormones in heat-stressed sheep and cattle depends upon the severity and duration of the exposure. For example, the response of cortisol to heat stress has been reported as biphasic; a dramatic rise occur during acute exposures (Christison and Johnson 1972; Abilay et al. 1975b), but concentration is depressed when CHS is imposed either gradually (Lee et al. 1971) or for durations longer than 48 h (Alvarez and Johnson 1973). Other workers (Fuquay et al. 1980) could not detect differences in cortisol concentration in cattle subjected to 24-h cycles of heat stress.

Though earlier data from cattle (Yousef and Johnson 1966) have shown that thyroid activity was depressed during exposure to heat stress, irrespective of the level of energy intake, recent reports on heat-stressed young pigs (Macari et al. 1983; Dauncey et al. 1984; Dauncey and Ingram 1986) suggested that high energy intake counteracted the effects of elevated temperatures on the release and metabolism of thyroid hormones. In contrast to the thyroid hormone response, an exaggerated PRL release following acute exposure to heat stress has been reported in sheep (Schillo et al. 1978; Hill et al. 1980), cattle

(Wettemann et al. 1982) and man (Mills and Robertshaw 1981). There have been few reports in the literature (Hooley et al. 1979) on the concentration of PRL during long-term heat stress. In addition, little is known about the function of elevated PRL concentration during heat stress, especially in ruminants. Recently, Becker et al. (1985) suggested a possible involvement of PRL in renal hemodynamics in cattle. As well, claims have been made (Faichney and Barry 1986) that inhibition of PRL release in heat-stressed sheep led to failure of maintaining homeothermy.

Therefore, the purpose of this study was: 1) to examine effects of ambient temperature on rectal temperature and respiration rate, and their relationships to peripheral concentrations of cortisol, T_3 , and PRL during NT and CHS in cycling ewes; 2) to determine if blunting PRL release (using bromocryptine, CB-154) influenced some of the parameters examined (as has been claimed recently in the literature).

4.3 MATERIALS AND METHODS:

4.3.1 ANIMALS:

Fourteen adult (60.3 ± 2.8 kg liveweight) Finnish Landrace- and Suffolk-cross cycling ewes were used in two experiments during the breeding season (Sept.-Feb.). The first experiment (Expt. #1) was comprised of two groups of ewes ($n=9$) in two replicates (Rep.). Rep. #1 (4 FinnX ewes) was done during the first year, while Rep. #2 (5 SuffolkX ewes) was completed during the breeding season of the following year. In Expt #2, five Finnish Landrace ewes were each given twice daily (at 1100h and 2300h) injections of 1.0 mg CB-154 (i.m.), throughout the

investigation period. Prior to each investigation, estrus was synchronized using two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Each ewe was studied for two consecutive cycles. The first cycle was at a thermoneutral temperature (NT) of $18\pm 1^{\circ}\text{C}$, while the second was at a cyclic heat stress (CHS) of a 12 h cycle of fluctuating temperature of 18°C - 35°C - 18°C . Ewes were managed and fed as described in the General Materials and Methods section.

4.3.2 TEMPERATURE SETTINGS:

All investigations were carried out in a temperature-controlled environmental chamber. The chamber size and description, the maintenance of temperature, relative humidity and photoperiod were as referred to in the General Materials and Methods.

4.3.3 PARAMETER RECORDING AND SAMPLING TECHNIQUES:

Ambient temperature, relative humidity (during Expt. #1 only), rectal temperature and respiration rate were recorded twice daily (at 700h and 1500h) during NT and three times daily (at 700h, 1200h and 1500h) during CHS. Blood sampling schedule were as mentioned in the General Materials and Methods.

4.3.4 HORMONE ANALYSIS:

Cortisol, T_3 and PRL were analyzed by radioimmunoassay (RIA) procedures. PRL concentrations were estimated on all daily samples during the cycle and on hourly samples collected from D14 through D18 of the cycle. Cortisol concentration was estimated in the daily samples during the cycle for all the ewes, but the hourly concentrations during

the preovulatory periods (D14-D18) were estimated for two ewes from each replicate in Expt. #1 and for two ewes from Expt. #2. T_3 concentration was estimated on all daily samples but only on 6-h pooled samples collected on D14 through D18 of the cycle. The protocols for the estimations of hormone concentration for the individual ewes, and the chemicals used in the assays were as described in the General Materials and Methods.

4.3.4.1 RIA OF CORTISOL:

4.3.4.1.1 ASSAY REAGENTS:

The cortisol RIA was a modification of the assay described by Rawlings and Ward (1978) using an assay buffer, phosphate buffer saline (PBS) (Yuthasastrakosol et al. 1974). Cortisol standard (Hydrocortisone, Steraloid Inc., Wilton, N.H., U.S.A.) was prepared in twice charcoal-stripped ovx ewe serum (Abraham 1974) and was run as follows: 0.00, 0.025, 0.05, 0.10, 0.25, 0.50, 1.0, 2.5, 5.0, 10.0 and 50.0 ng tube⁻¹. An additional set of tubes was also included to account for nonspecific binding (NSB). Labelled cortisol (³H-hydrocortisone, New England Nuclear, Boston, MA., U.S.A.) was prepared in the assay buffer to give 28,000 dpm (6000 cpm) in a total volume of 200 μ L (approximately 60 pg tube⁻¹).

The anti-serum (Cortisol #4, N.C. Rawlings, Dept. of Vet. Physiol. Sciences, W.C.V.M., Univ. of Saskatchewan, Saskatoon, SK, Canada) was developed in sheep against (4-pregnen-11 β ,17 α ,21-triol-3,20-dion-3-CMO:BSA) and cross-reacted at 10% with progesterone, but at <1.0% with either dehydroepiandrosterone, pregnenolone, testosterone, estradiol-17 β , estradiol-17 α or cholesterol. The anti-serum was diluted

in the assay buffer and used at a titre of 1:2500.

4.3.4.1.2 ASSAY PROTOCOL:

Prior to cortisol assay, P_4 was extracted from all samples with petroleum ether (Grade #20-40°C, J.T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.) because of elevated progesterone (P_4) concentration in cycling ewes. All extraction steps were performed at 4°C. 1.0 mL of petroleum ether was added to (12x75) culture tubes containing 200 μ L of either the standard (triplicates) or the unknowns (duplicates). The tubes were stoppered, vortexed gently (20 sec) and kept at -20°C for 1.0 h. The supernatant (containing P_4) was then aspirated and discarded. Subsequently, 1.5 mL of absolute alcohol were added to all tubes. After vortexing (60 sec) and centrifugation (at 2000 rpm) for 10 min, the supernatant layer was decanted into a new set of (12x75) tubes. Alcohol was evaporated in a water bath (at 60°C) under a gentle flow of nitrogen (18 min). Following this final extraction, 200 μ L of cortisol anti-serum were pipetted to all tubes, except the (NSB) tubes into which an equivalent volume of buffer was pipetted. 200 μ L of the cortisol trace were added to all tubes, including the (NSB) tubes. All tubes were then vortexed (60 sec), covered with parafilm and incubated at 4°C, for a minimum of 16 h. Separation was carried out at 4°C and involved the addition of 0.5 ml of charcoal (Activated Norit A, Matheson Coleman and Bell, Norwood, OH., U.S.A.) solution (375 mg of washed charcoal per 100 mL of the assay buffer) to all tubes. Following the addition of charcoal, the tubes were vortexed (5 sec), incubated at 4°C for 10 min and finally centrifuged (at 2000 rpm) for 10 min. The supernatant was decanted into plastic minivials and 4.0 mL of the cocktail fluid (Scinti-Verse II, Fisher Scientific Co., Lawn, N.J., U.S.A.) was added.

The minivials were then capped, shaken well and left on the lab bench to equilibrate (4-6 h). Triplicate minivials containing 200 μ L of buffer, 200 μ of trace and 4.0 mL of cocktail were included to account for total radioactivity.

The efficiency of P_4 removal by petroleum ether was evaluated using three procedures: first, equivalent volumes of twice charcoal-stripped ovx ewe serum pools, to which known amounts of labelled P_4 ($^3\text{H}-P_4$) was added, were extracted with petroleum ether and both the supernatants and the precipitants were counted. This procedure removed 85.5% of the added $^3\text{H}-P_4$. Second, when the petroleum ether extracts of known amounts of P_4 were included in the cortisol assay (Table 1), the mean recovery rate was 6.1% (n=4). Third, P_4 inhibition curves (n=3) were determined in which the binding of the anti-serum was tested in the presence of equivalent amounts of P_4 on each point of the cortisol standard used. These curves were then compared to the cortisol standard curve alone, or to petroleum ether-extracted P_4 standard curve alone (Appendix Fig. 1). They revealed no significant interference by P_4 on the estimation of cortisol. The amount of cortisol loss resulting from extraction with petroleum ether was $3.0 \pm 0.9\%$ (n=6).

Total binding of the anti-serum was 24.0% (n=23) with (NSB) of 0.8%. The sensitivity of the assay at 95% binding was 46.5 pg tube⁻¹. The intra-assay coefficients of variations (C.V.) of ewe serum pools containing 10.7, 20.5 and 32.3 ng cortisol mL⁻¹ were 9.3%, 6.2% and 5.7%, respectively. The corresponding values for the inter-assay C.V. were 11.1%, 7.6% and 11.7%, respectively.

4.3.4.2 RIA OF TRIIODOTHYRONINE (T_3):

Because of technical difficulties T_3 was only estimated on the samples from ewes of Rep. #1 in Expt. #1. Samples from an individual ewe were quantified in five separate runs. Both the standard and the unknowns were run in duplicate assay tubes.

T_3 was estimated using a procedure previously described by Brown and Eales (1977). However, EDTA-azide buffer (12.0 g dibasic sodium phosphate, anhydrous, 6.0 g disodium ethylene diamine tetraacetic acid, EDTA, 1.0 g sodium azide and q.s. 1.0 L of deionized distilled water, pH=7.4) was used instead of the barbital buffer. The standard, T_3 (Liothyronine sodium salt) was obtained from Sigma Co. (St. Louis, MO., U.S.A.). Labelled T_3 (^{125}I -liothyronine, Industrial Nuclear, St. Louis, MO., U.S.A.) with an initial activity of 550 uCi mg^{-1} was received in 50% aqueous propylene glycol. The working trace was diluted in 0.1 N NaOH so that $100 \mu\text{L}$ contained 4200-4500 cpm. Lyophilized rabbit anti-serum to T_3 :HSA (Calchemical Laboratory Supplies Ltd., Calgary, Alta., Canada) was diluted in the assay buffer at a titre of 1:10,000. The assay tubes were counted for 1.0 min in an automatic gamma counter (Model #1185, Automatic Gamma System, Searle Analytic Inc., Chicago, IL., U.S.A.).

The standard curve was constructed by plotting $(\text{Bi}/\text{B}_0) \times 100$ against logit-log dose concentration tube^{-1} (Rodbard et al. 1970), where B_0 were counts at the reference (0) tubes and Bi were counts in other points of the standard. Total binding of the anti-serum was 70.6% ($n=21$). The sensitivity of the assay at 95% binding was $19.7 \text{ pg tube}^{-1}$. The intra-

and inter-assay C.V. for a ewe serum pool containing 1.24 ng mL^{-1} ($n=21$) were 6.0% and 16.0%, respectively.

4.3.4.3 RIA OF PROLACTIN (PRL):

Samples were assayed at volumes of 20 to 50 μL in duplicate (10x75) culture tubes. Prolactin assay was as described by Sanford et al. (1978). Sample concentrations were expressed as NIH-PRL-S12 mL^{-1} . Labelled oPRL (^{125}I -oPRL) was prepared using the lactoperoxidase method (Miyachi et al. 1972) as modified by Cheng (1978) and was prepared in the assay buffer to yield 7500-9000 cpm per 100 μL tube $^{-1}$. The anti-serum (Friesen's AF1.6) was developed in rabbits and was prepared at a titre of 1:60,000. Goat anti-rabbit γ -globulin (1:20) was utilized for the separation step. Total binding of the anti-serum was 62.8% ($n=17$) while the (NSB) was 2.5%. The assay sensitivity at 95% binding was 52.8 pg tube $^{-1}$. The intra-assay C.V. of ewe serum pools containing 6.0, 53.5, 75.0 and 124.6 ng mL^{-1} were 3.5%, 2.7%, 3.4% and 4.1%, respectively. The corresponding values for inter-assay C.V. for the same serum pools were 9.5%, 3.8%, 3.6% and 4.0%, respectively.

4.3.5 STATISTICAL ANALYSIS:

4.3.5.1 RIA RESULTS:

Assay results were extrapolated as described in the General Materials and Methods section. RIA results for hourly estimates of cortisol and PRL were initially subjected to the Pulsar program analysis as described in the General Materials and Methods, and the summaries were compared statistically as described below.

4.3.5.2 DATA ANALYSIS:

All data were subjected to analysis of variance (ANOVA) using computer programs according to procedures in the statistical packages provided by Statistical Analysis System (SAS Institute Inc. 1982). Differences between Experiments or Replicates were examined using the split-plot analysis for repeated measurements (Gill and Hafs 1971). Cyclic differences between temperatures were compared by dividing the cycle into 4 arbitrary phases as mentioned in the General Materials and Methods. Although the two replicates of Expt #1 were conducted in 2 separate years, because of the identical experimental design, data from both were pooled into one data-set and the replicate effect was tested. Any significant differences between the two replicates were referred to in the main text and the evidence was provided in the Mean daily values for the ambient temperature (T_{amb}), rectal temperature (T_{rec}) and respiration rate (RR) represent averages of records taken at 700h and 1500h during NT, and at 700h, 1200h and 1500h during CHS.

Means were always expressed as (mean \pm SEM). Since the comparisons between experiments were made using repeated measures designs, 'experiment' was considered as a main effect and ewes within experiments as an error term. Similarly, replicates within Expt. #1 were compared with 'year' as a main effect and ewes within year as an error term. Using this design the effects of cycle, day and hour were tested using the interaction of the respective ewe effects as error terms. Separate analyses for PRL results in Rep. #1 in Expt. #1, and for data in Expt. #2 were carried out with 'ewes' as blocks (Snedecor and Cochran 1967).

Simple correlations (Snedecor and Cochran 1967) were run for all daily estimates during the estrous cycle. Stepwise regression analysis (SAS Institute Inc. 1982) was utilized to examine the % variation in hormone concentration (as a dependent variable) in response to changes in T_{amb} , T_{rec} and RR (as independent variables).

4.4 RESULTS:

4.4.1 AMBIENT TEMPERATURE (T_{amb}):

Mean 24-h temperatures of the environmental chamber during experiment #1 (a) and experiment #2 are shown in Fig. 1. These figures were prepared from hourly observations recorded from D14 to D18 of each estrous cycle. Chamber temperature showed minimum changes during the NT cycles. During CHS, temperature gradually rose from $<22^{\circ}\text{C}$ at 700h, reaching the upper limit of the thermocomfort zone ($27/28^{\circ}\text{C}$) for shorn sheep (Joyce and Blaxter 1964) between 1000-1100h. The chamber temperature was maximum at 1500h. Thereafter, it rapidly (within 3h) fell to $<22^{\circ}\text{C}$ (at 1800h), and remained below this temperature throughout the remaining night hours (1700-600h). Because of technical problems with the chamber's humidifiers, the daily mean relative humidity, which was recorded only for Expt #1, was higher during NT ($55.8 \pm 0.6\%$) than CHS ($51.6 \pm 0.7\%$), Fig. 2a.

Analysis of variance of mean daily values by phase (Table 2) showed significant differences between cycles ($p < 0.001$), which was expected. There was also a significant cycle*experiment interaction ($p < 0.05$), due to the lower temperature attained in the follicular during the CHS in Expt #2 as compared to Expt #1. Means (\pm SEM) are shown in Table 3.

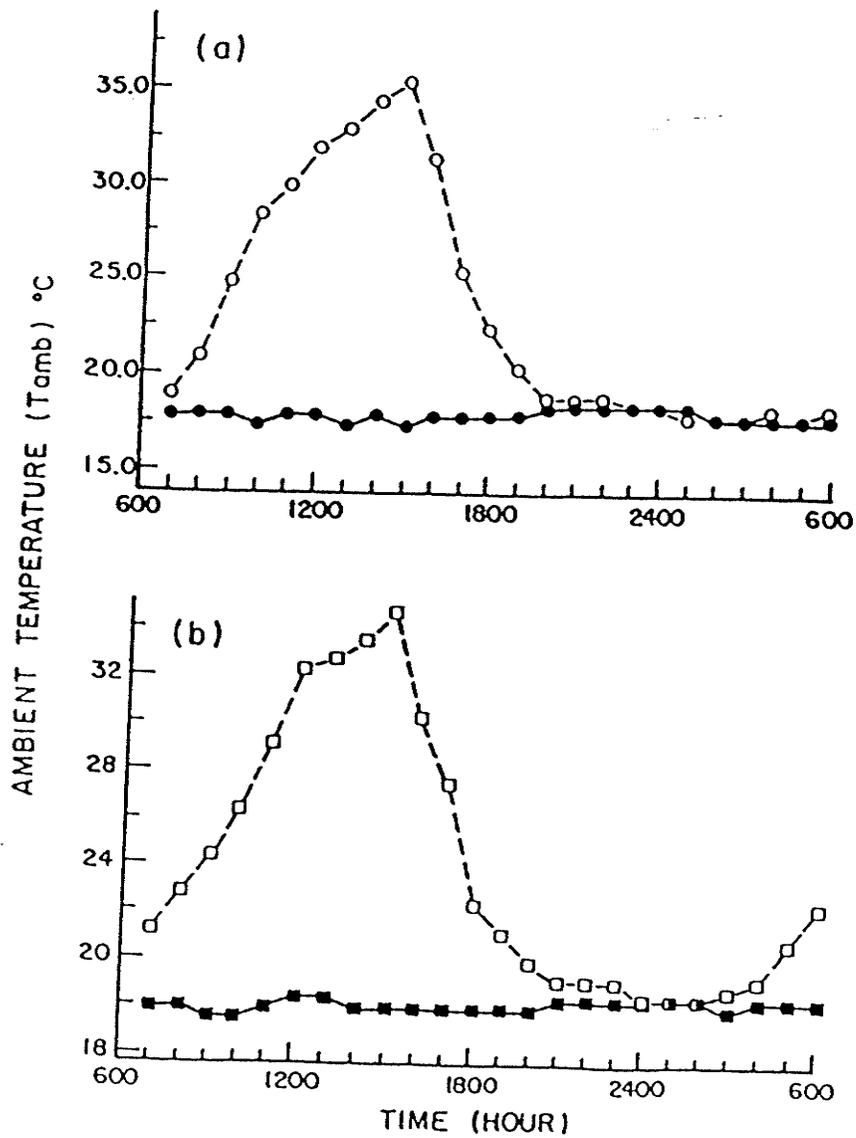


Figure 1: Mean 24-h fluctuation of ambient temperature ($^{\circ}\text{C}$) in Expt. #1 (a) and Expt. #2 (b) at thermoneutral (\bullet , \blacksquare) and at cyclic heat stress (\circ , \square).

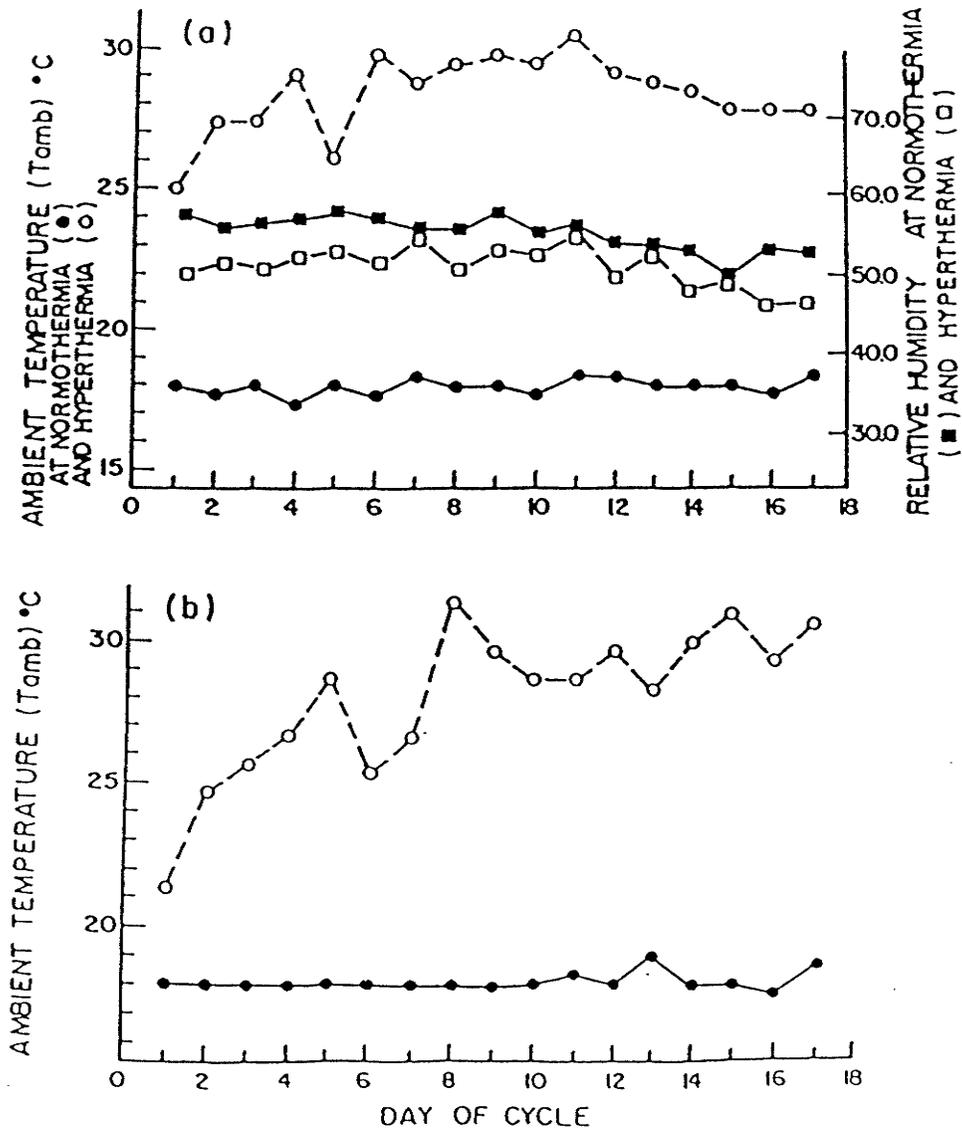


Figure 2: Mean daily ambient temperature (°C) at thermoneutral #1 and (●) and at cyclic heat stress (○) in Expt. #1 (a) and Expt. #2 (b). The Relative Humidity was recorded in Expt. #1 only.

4.4.2 BEHAVIORAL OBSERVATIONS:

During the NT cycle of both experiments, crushed barley was the preferred feed ingredient and alfalfa pellets was the least. The chamber was occupied randomly with no indication of preferential locations for any individuals. However, during the CHS cycle the following observations were recorded: as ambient temperature rose above 26-28°C (1000-1100h), ewes were less inclined to eat or ruminate and tended to huddle at sternal recumbancy in the cooler portion of the room, where air was blown-in directly by the roof fans. Furthermore, after the fourth day of heat exposure, rumination was rarely observed at temperatures above 28°C. Even when new food was offered, it was refused, particularly the was alfalfa pellets. However, the ration was completely eaten during the cooler hours of the day.

In addition, although water intake was was not measured; based on casual observations, water intake was increased as Tamb rose above 25°C. It was noticed that drinking, when attempted, was intermittent, in small quantities, using the tips of the lips. At above 32.5°C (1300-1500h), 2-3 ewes were occasionally seen grouped around the watering trough. They were mostly on sternal recumbancy (70-80% of the time), with necks stretched above the trough, lips were wet and they were panting.

4.4.3 RECTAL TEMPERATURE (Trec):

Mean daily fluctuations in Trec are shown in Fig. 3. Daily variations were more dramatic than changes in Tamb (Fig. 2). Cyclic heat stress raised the mean daily Trec during both experiments. Mean daily Trec was low during the last four days of the CHS cycle in

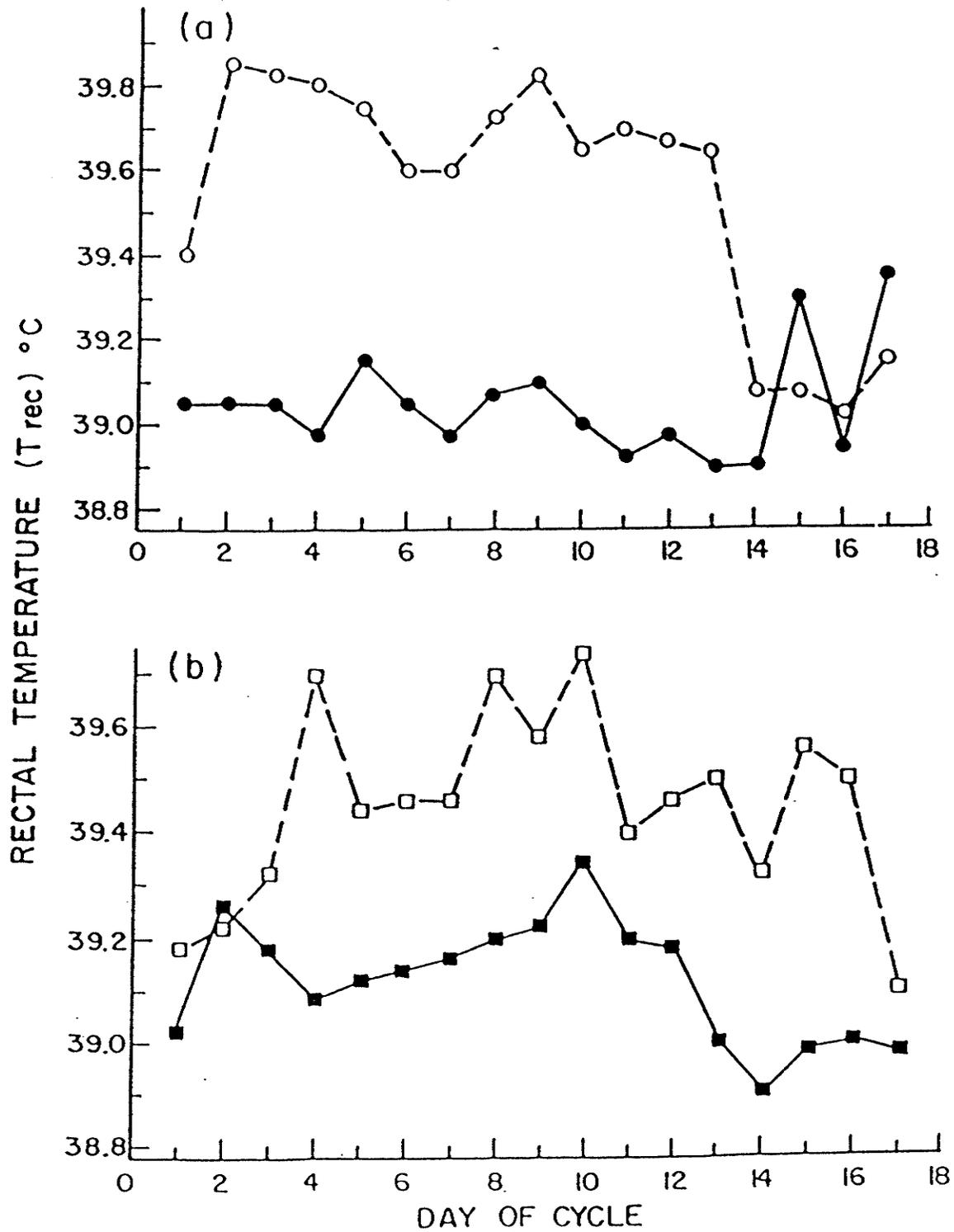


Figure 3: Mean daily rectal temperature (°C) in ewes of Expt. #1 (a) and Expt. 2 (b) at thermoneutral (● , ■) and at cyclic heat stress (○ , □)

Expt #1 despite the elevated values of the corresponding Tamb (Fig. 2a). Similarly, mean daily values were low during the same period in the CB-154 ewes during NT (Fig. 2b). Analysis of variance of mean Trec during the estrous cycle by phase (Table 4), showed significant differences between cycles and phases ($p < 0.001$). The interaction phase*expt was significant due to low Trec in the follicular phase during NT with CB-154. Trec was also low in the late-luteal phase in Expt #1. Means for Trec are given in Table 5). There was no relationship between Trec and Tamb during NT (Table 6), but during CHS (Table 7) the relationship was positive ($r = 0.25$, $p < 0.05$).

Fig. 4 shows Trec values recorded at 700h and 1500h during the normothermic cycle and at 700h, 1200h and 1500h of the hyperthermic cycle. Trec taken during NT were comparable between experiments. Trec values at 700h and 1500h during NT as well as at 700h during CHS were lower than values observed at 1200 and 1500h during CHS. Trec was 0.48°C and 0.79°C higher at 1200h and 1500h, respectively during CHS. During CHS, Trec at 1500h was comparable to that at 1200h. Analysis of variance of the hourly values of Trec showed no differences between experiments, but there were significant differences between cycles ($p < 0.001$) and hours ($p < 0.001$). The interaction of hour*expt was significant ($p < 0.001$) (Table 8).

4.4.4 RESPIRATION RATE (RR):

Normal respiration rates (min^{-1}) as observed during the normothermic cycle (Fig. 5) in the absence of distress or distraction were associated with shallow flank movements and the nostrils were the only routes used for respiration. The analysis of variance of the hourly values of RR

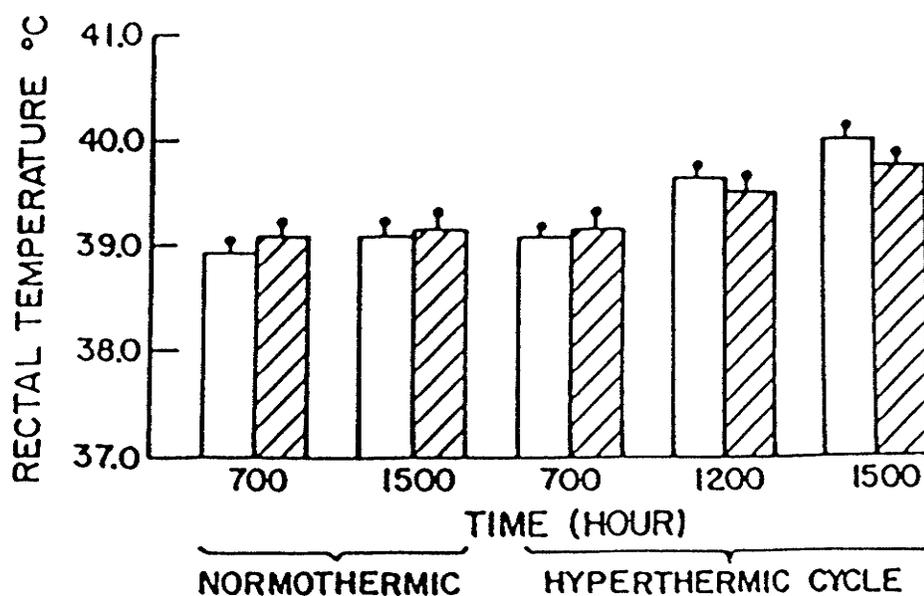


Figure 4: Mean (\pm SEM) diurnal changes in the rectal temperatures ($^{\circ}$ C) in ewes exposed to thermoneutral and cyclic heat stress in Expt. #1 (\square) and Expt. #2 (hatched).

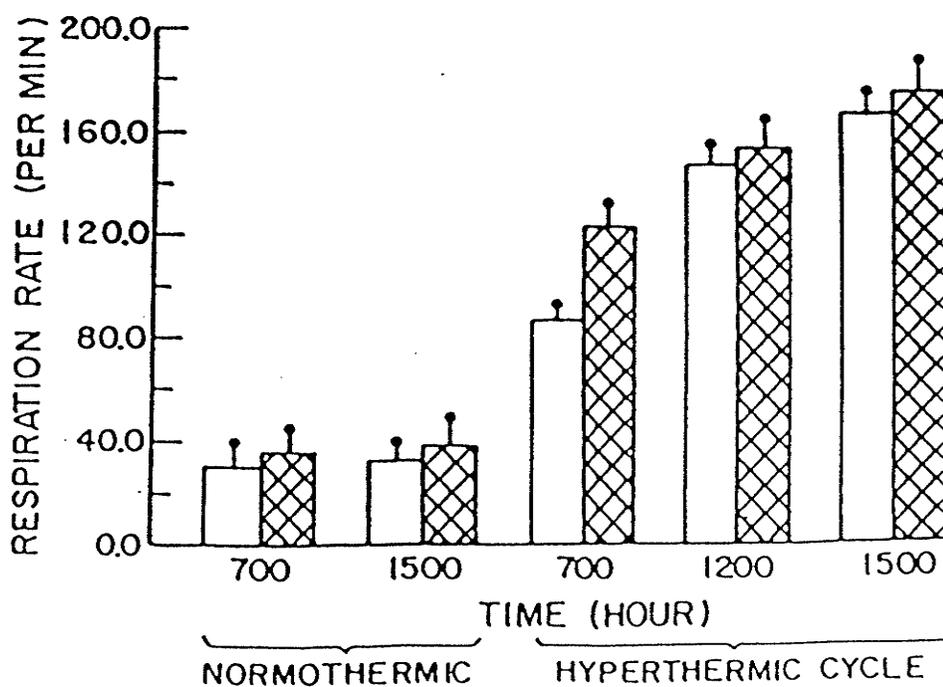


Figure 5: Mean (\pm SEM) diurnal changes in respiration rate (min^{-1}) in ewes exposed to thermoneutral and cyclic heat stress in Expt. #1 (\square) and in Expt. #2 (cross-hatched).

revealed levels of significance similar to those observed for Trec (Table 9). Normal values recorded at 700h and 1500h during NT were below 40 respirations per min. Though respirations at 700h during CHS were higher than NT, they were lower than values recorded at 1200h and 1500h. The respirations during CHS were characterized by rapid shallow flank movements with an open mouth (panting). Deep abdominal breathing was not observed in any of the ewes, even at 35°C. During CHS, respiration rate at 1500h was higher than but not significantly different from values at 1200h. Respirations were consistently elevated in ewes treated with CB-154, irrespective of the cycle or Tamb. However, the difference between experiments was significant ($p < 0.09$) (Table 9).

Mean daily respiration rates are shown in Fig. 6. Like the changes in the mean daily Trec, respirations were lower in Expt #1 during the last four days of CHS. In addition, they showed more stable daily values than Trec. Respiration rate was not correlated to Tamb during NT (Table 6) but was positively correlated to changes in Trec ($r = 0.64$, $p < 0.001$). In contrast, respiration rate was positively correlated to Tamb ($r = 0.41$, $p < 0.001$) and Trec ($r = 0.39$, $p < 0.001$) during CHS (Table 7). Analysis of variance of mean daily respiration rate by phase (Table 10) showed significant difference between cycles ($p < 0.001$) and phases ($p < 0.001$). Because of lower values during the first three days of CHS in Expt #2, and also due to the steep reduction in RR after D13 in Expt #1, there was significant cycle*expt ($p < 0.01$) and cycle*phase ($p < 0.001$) interactions. The reason for the low values of RR in Expt 1 could not be explained since there was no apparent changes in the chamber temperature during the corresponding days. Means (\pm SEM) of RR during the estrous cycle by phase are shown in Table 11.

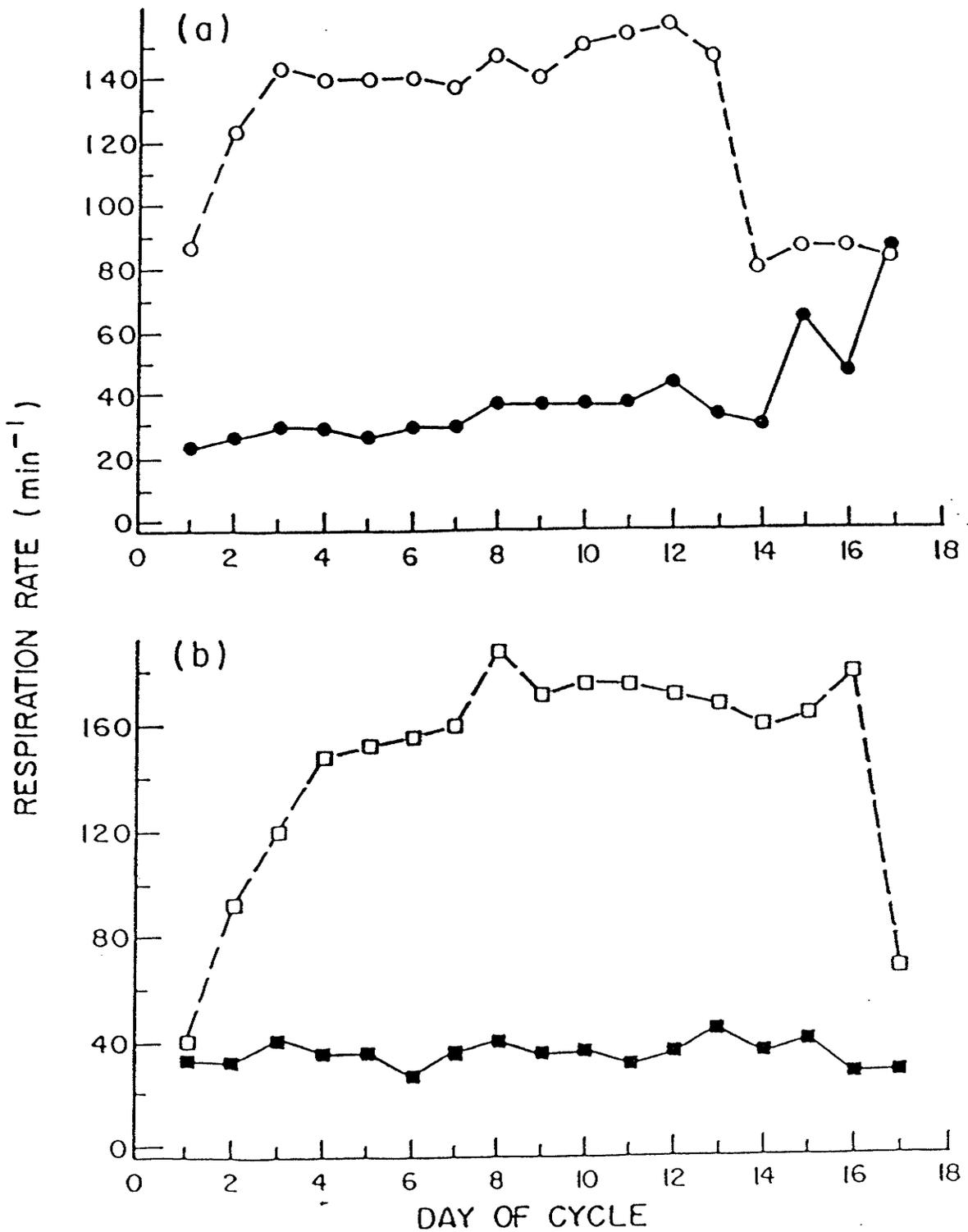


Figure 6: Mean daily respiration rate (min⁻¹) at thermoneutral (●, ■) and cyclic heat stress (○, □) estrous cycles in Expt. #1 (a) and Expt. #2 (b).

4.4.5 CORTISOL:

Since year had no significant effect on mean daily concentration of cortisol (Table 12), cortisol data for Replicates in Expt #1 were pooled together.

4.4.5.1 24-h PROFILES:

The 24-h profiles of cortisol were drawn from hourly samples collected over five days during the preovulatory period, D14-D18, (Fig. 7). The 24-h patterns of cortisol was characterized by peaks of varying amplitudes at comparable frequencies and between cycles in Expt #1. In this experiment, cortisol concentration was slightly elevated in the late morning and the afternoon hours. In contrast, the CB-154-treated ewes failed to show a specific 24-h pattern of cortisol concentration. Analysis of variance for comparisons of cortisol between replicates in Expt #1 showed no difference between replicates or cycles (Table 12). However, the differences between days approached significance ($p < 0.06$) due to the tendency of cortisol concentration to fall over time, presumably in response to the decrease in the stress of sampling.

Further, analysis of variance for cortisol profiles between experiments showed significant differences between experiments ($p < 0.05$), cycles ($p < 0.05$) and days ($p < 0.001$) (Table 13). The differences between experiments were due to higher cortisol in Expt #2, irrespective of the cycle (mean \pm SEM are given in Table 14). Cycle differences were due to the consistent fall in basal cortisol during CHS. Differences between days resulted from the tendency of basal cortisol to fall over time, irrespective of CB-154 treatment or temperature of exposure, and

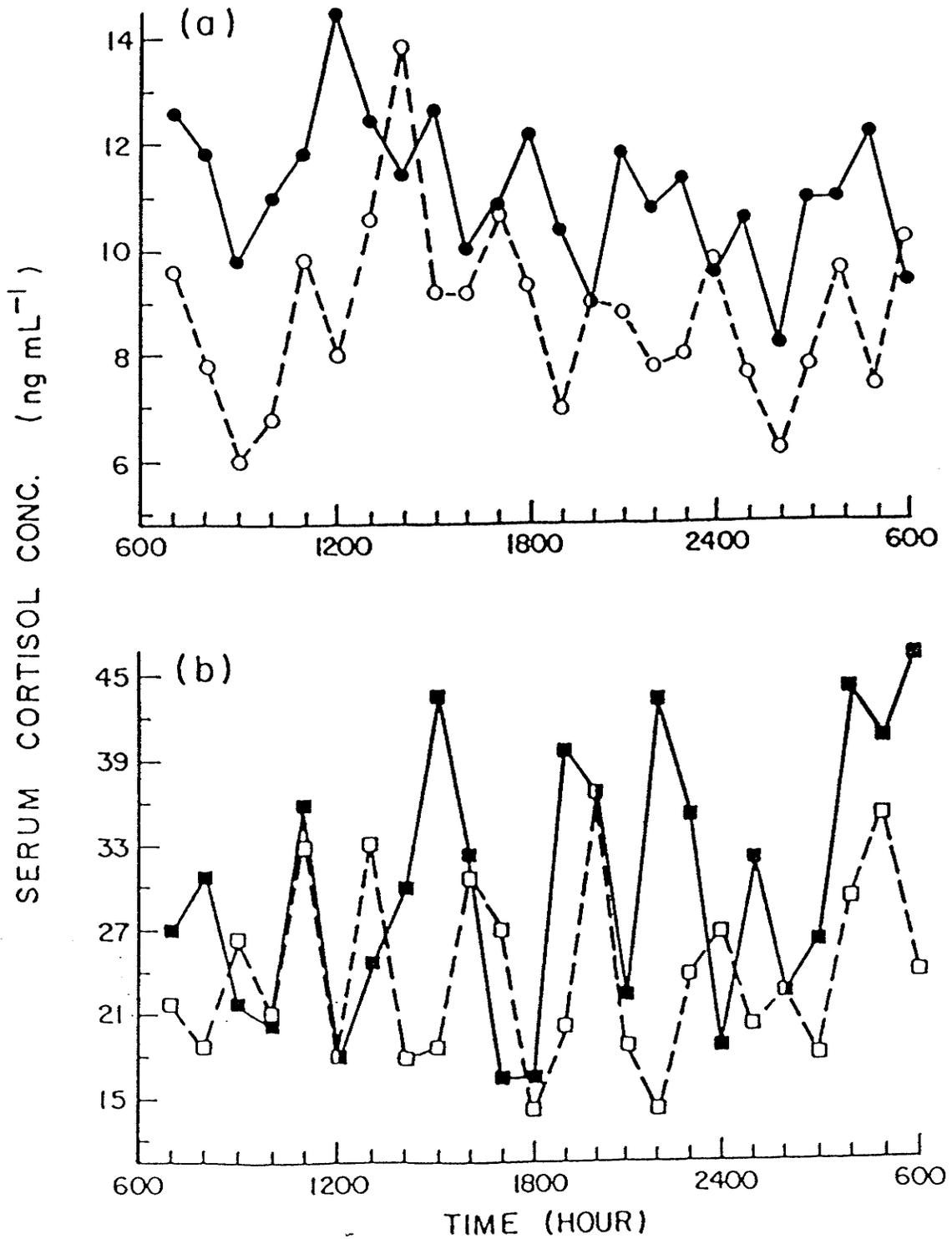


Figure 7: Mean 24-h profiles of serum cortisol in ewes at the thermoneutral (●, ■) and at cyclic heat stress (○, □) in Expt. #1 (a) and Expt. #2 (b)

assumed to be an acclimatization to the intensive sampling. The analysis of variance of the cortisol profiles during the 5-day of intensive sampling showed significant differences between experiments in mean concentrations ($p < 0.05$) (Table 15). Other characteristics were comparable between experiments (mean \pm SEM are given in Table 16).

4.4.5.2 DAILY CORTISOL LEVELS

Fig. 8 depicts mean daily cortisol concentration. Mean concentration during NT resembled those 24-h profiles. Mean concentration was depressed during CHS in Expt #1 (Fig. 8a). Mean daily values in the CB-154 group showed a different trend than those in Expt #1. In this group, during NT and CHS mean concentration of cortisol showed considerable fluctuations (Fig. 8b). Furthermore, during NT cortisol concentration was not correlated to any of the physiological parameters or hormones measured (Table 6). During CHS cortisol was positively correlated to Trec ($p < 0.01$) (Table 7).

Stepwise regression analysis (Tables 17,18) of data in Rep #1 (Expt #1) showed that Tamb, alone or in combination with Trec, accounted for only 6% of the variations in mean daily cortisol values ($p < 0.01$). In Expt #2, Tamb was associated with only 2% of the changes in cortisol ($p < 0.05$).

4.4.6 TRIIODOTHYRONINE (T_3):

4.4.6.1 THE 24-h PROFILES OF T_3 :

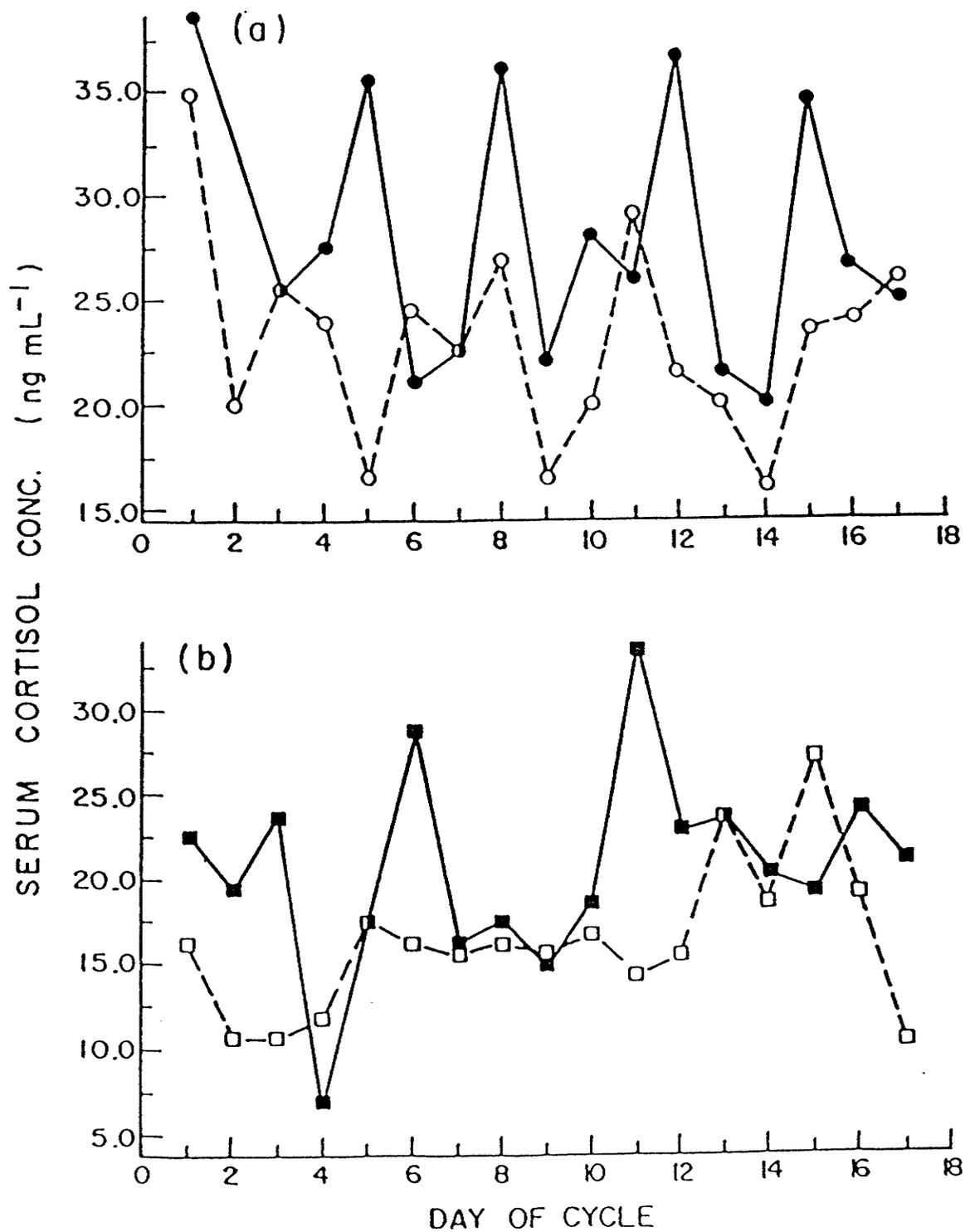


Figure 8: Mean daily concentration of serum cortisol in ewes at the thermoneutral (●, ■) and the cyclic heat stress (○, □) estrous cycle in Expt. #1 (a) and Expt. #2 (b).

The T_3 data represent estimates done on daily samples and on samples collected at 700h, 1200h, 1800h and 2400h between D14-D18 of the cycle in Rep #1 of Expt #1. Analysis of variance of T_3 concentration during the 5-day of intensive sampling showed no differences between cycles (Table 19). Triiodothyronine values during D14-D18 are shown in Table 20. In spite of the comparable T_3 concentration between the cycles, concentration tended to lower at 1200, 1800 and 2400h during CHS. Unlike cortisol, T_3 concentration showed no tendency to fall as a result of repeated sampling over the five-day period (Table 21) day nor hour influenced T_3 concentration during this period.

4.4.6.2 DAILY T_3 CONCENTRATIONS:

The daily patterns of mean T_3 concentration (Fig. 9) were not different between the two temperatures. Analysis of variance of daily T_3 by phase showed no differences between cycles or phases and also no interaction (Table 22).

An attempt to correlate T_3 concentration to T_{amb} , T_{rec} and respiration rate showed no correlation with any of these parameters during NT (Table 6). During CHS, T_3 concentration was negatively correlated to respiration rate ($r=-0.39$, $p<0.001$) (Table 7). Stepwise regression analysis (Table 17) showed that neither T_{amb} nor T_{rec} had a significant influence on T_3 concentration. Respiration rate was associated with only 4% of the variation in daily T_3 concentration ($p<0.05$).

4.4.7 PROLACTIN (PRL):

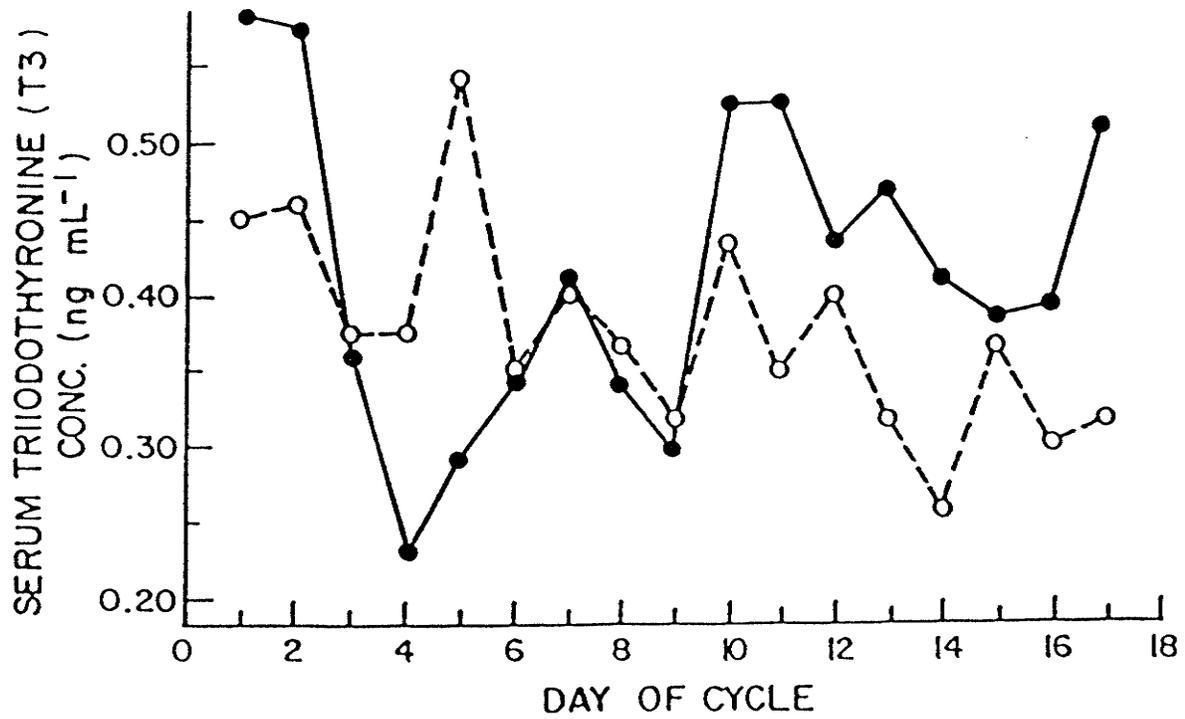


Figure 9: Mean concentration of daily serum triiodothyronine in ewes of Rep. #1, Expt. #1 at thermoneutral (●) and at cyclic heat stress (○) estrous cycles .

Prolactin concentrations in samples from ewes in Rep #2 of Expt #1 were lost during storage and handling. Therefore, estimates reported here represent data on ewes in Rep #1 only. Serum PRL concentration in ewes of Expt #2 (CB-154 group) was below the assay detection limits ($1.06 \pm 0.05 \text{ ng mL}^{-1}$) in all samples irrespective of the temperature treatment.

4.4.7.1 THE 24-h PROFILES OF PRL:

The 24-h profiles of serum PRL and the fluctuations in mean Tamb were presented in Fig. 10. As with cortisol these values represent estimates on hourly samples collected between D14-D18 of the cycle. During NT PRL concentration was low between 1100-1700h and slightly elevated between 1800-300h. In contrast, CHS imposed specific patterns on 24-h profiles of PRL. Starting at 800h, a daily elevation in PRL paralleled the progressive rise in Tamb. The peak of PRL response occurred at 1200h when chamber temperature was 31.4°C . Any subsequent rise in Tamb was unaccompanied by an increase in PRL concentration and PRL concentration began to drop, despite the continuous rise of the Tamb for the next three hours. In comparison to concentration at 1200h, PRL concentration was 5.7%, 5.3% and 14% lower at 1300h (32.3°C), 1400h (33.8°C) and 1500h (35.3°C), respectively. PRL continued to fall as Tamb started to drop, and reached minimum values between 1800-2000h. Cyclic heat stress also delayed the nightly rise of PRL in the rise commenced after 2000h; 3h later than during NT. Prolactin concentration declined during the early morning hours (300-400h) at both temperatures.

The effect of frequent sampling over time on serum PRL profiles was examined during the five-day period. Analysis of variance of the 24-h

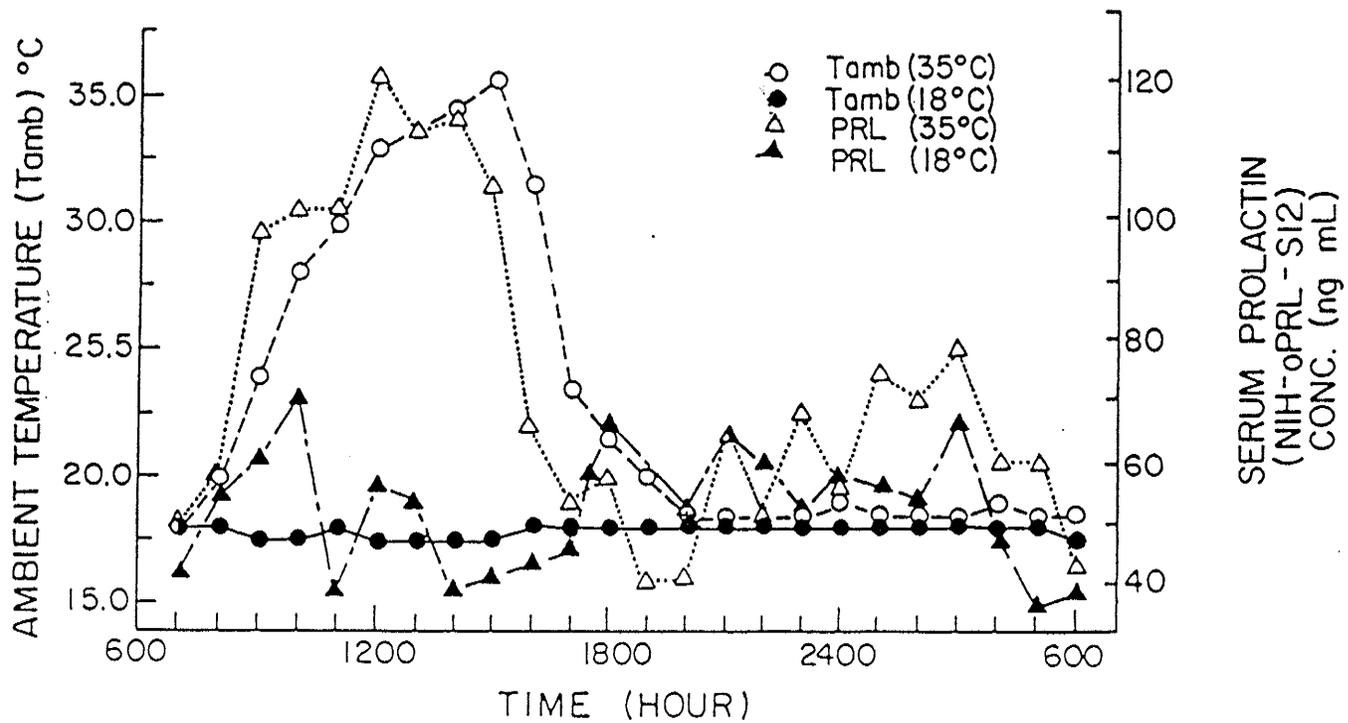


Figure 10: Mean 24-h profiles of serum prolactin in response to changes in the ambient temperature at the preovulatory period in Rep. #1, Expt. #1.

profiles of PRL revealed no differences between cycles and days (appendix Table 23). There was significant hour effect ($p < 0.001$) and also a significant hour*cycle interaction ($p < 0.001$) as a result of the acute rise of PRL during the hot period of the CHS. Also, PRL concentration tended to be elevated in the hours of the late night and the early morning. This consistency in the 24-h patterns was reflected in a nonsignificant day*hour interaction. Means (\pm SEM) of PRL concentration during the 5-day of intensive sampling are shown in Table 24. Analysis of the Pulsar data (Table 25) showed that CHS tended to raise the basal concentration of PRL but had no significant effects on the pulse frequency, pulse amplitudes, the duration of peaks or the interpeak intervals (mean \pm SEM are given in Table 26).

4.4.7.2 DAILY PRL LEVELS:

Fig. 11 illustrates mean daily PRL during the estrous cycles. Elevated mean serum PRL concentrations on D1, D16 and D17 during NT indicated the preovulatory rise. On other days during this cycle PRL concentration was low and stable profiles prevailed between D4-D10. During CHS, mean concentration was low during the first five days of the cycle but sharply rose to a peak on D8-D9. Thereafter, concentration remained elevated until the end of the cycle. The overall mean daily PRL value during CHS ($89.4 \pm 12.4 \text{ ng mL}^{-1}$) was significantly higher ($p < 0.001$) than NT ($25.4 \pm 12.4 \text{ ng mL}^{-1}$). Analysis of variance of daily PRL by phase (Table 27) showed significant differences between cycles ($p < 0.05$) as a result of elevated PRL during CHS. Neither day nor the interaction of cycle*day was significant, which suggested that basal PRL release during

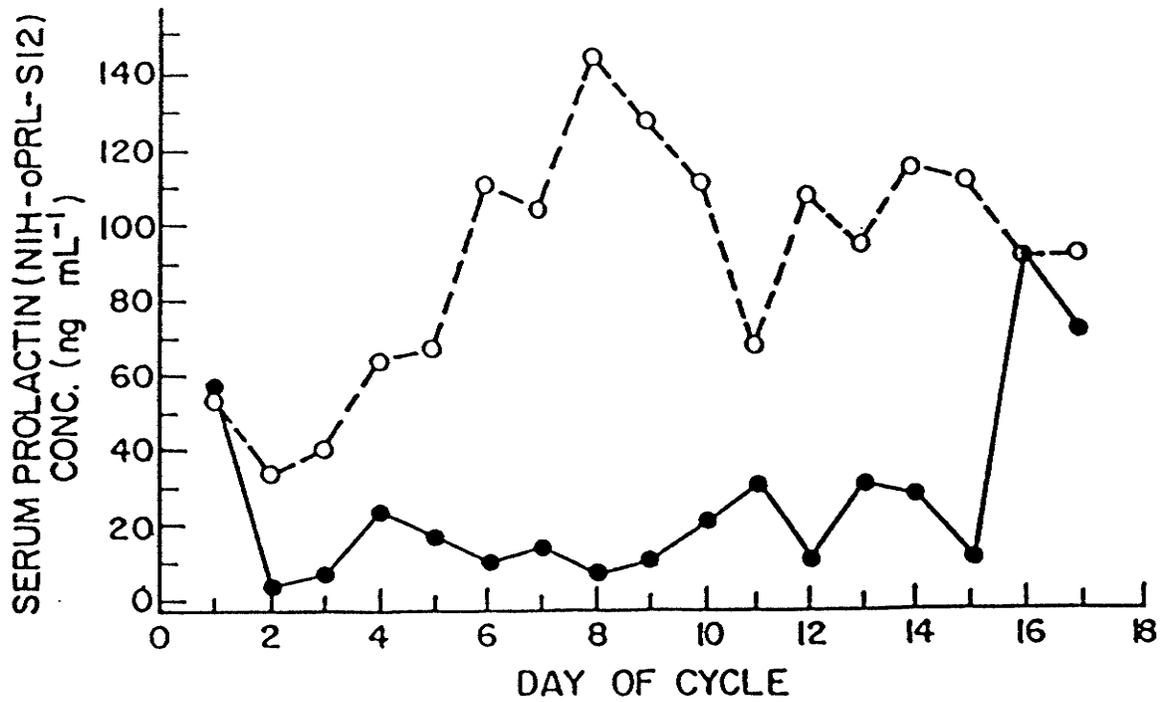


Figure 11: Mean daily concentration of serum prolactin in ewes in Rep. #1, Expt. #1 at thermoneutral (●) and at cyclic heat stress (○) estrous cycles .

the estrous cycle is not related to the ovarian activity in the ewe. Instead temperature played a greater role in PRL release.

Mean daily PRL concentration was not significantly correlated to any of the physiological and hormone parameters at either temperature (Table 7). Stepwise regression analysis (Table 17) showed that T_{amb} alone accounted for 30% of the variability in mean daily PRL concentration ($p < 0.001$), whereas a combination of both T_{amb} and T_{rec} in the model explained only 32% of the variation ($p < 0.05$). Respiration rate showed insignificant effect on PRL concentration.

4.5 DISCUSSION:

Because normal physiological homeostasis of the body's vital functions have limited flexibilities within the animal's homeothermic balance, thermal tolerance can only be explained in terms of the rate by which body temperature drifts towards the prevailing T_{amb} (Hales 1969; Johnson 1971). The T_{amb} and relative humidity settings used during the CHS cycles in this investigation were effective in inducing sufficient heat stress in all ewes to result in significant disruption of normal behavior and changes in the physiological and hormone responses. A rise in T_{amb} to 31.7°C was associated with an elevation of $>0.4^{\circ}\text{C}$ in T_{rec} ($p < 0.001$) and a four-fold increase in respiration rate ($p < 0.001$). When T_{amb} reached maximum (35.0°C), T_{rec} and respiration rate were $0.70\text{--}0.90^{\circ}\text{C}$ and $>5\text{-fold}$, respectively, above values recorded during NT (18.1°C). T_{rec} values observed in this investigation were similar to those reported by Schillo et al. (1978) for post-partum ewes kept at 35°C (RH=78%). The positive correlations between T_{rec} and respiration rate at both temperatures ($p < 0.001$), is in agreement with reports by

Johnson (1971) and Ross et al. (1985).

Increased respiration rate during heat stress (panting) is related to body core temperature (Hales 1969) and under heated room conditions it is the major route for evaporative cooling. Borut et al. (1979) have shown that in goats kept in a heated room at T_{amb} of 40.0°C ($\text{RH}=30\%$), panting contributed up to 66% of the evaporative heat loss, while under similar field conditions this route accounted for only 33% of the loss. Further, panting cools the hypothalamus during CHS (Hales 1969; Dmiel and Robertshaw 1983).

The results of this investigation also showed that although T_{amb} and T_{rec} at 700h of the CHS cycles in either the CB-154 treated and the non-treated ewes were comparable, respiration rate tended to differ ($p<0.09$). Such a situation could have arisen because the mean T_{amb} at 700h during CHS was 21°C for the CB-154 ewes and was 19°C for the other group in Expt #1. Thus it appears that the CB-154 ewes had slightly increased their RR in response to a slightly higher T_{amb} . Thus, even though these findings were in agreement with data from Faichney and Barry (1986) who showed that CB-154 treated ewes kept at mild heat stress had higher respiration rates than a control group kept at the same T_{amb} , present data clearly contradicted their finding that the treated ewes failed to maintain body homeothermy. In contrast, our CB-154 group had consistently lower but comparable T_{rec} values as the non-treated group at 1200h ($T_{amb}=32.5^{\circ}\text{C}$) and at 1500h ($T_{amb}=35.0^{\circ}\text{C}$) of the heat-stressed cycle.

There is always a limit to how effective panting can be as a heat dissipation mechanism during long-term exposure to heat stress. Beside the advantages in evaporative and brain cooling, excessive panting has two main drawbacks. It leads to hypercapnoea and a possible acid base disturbance. It also requires muscle activity which uses energy, generates unwanted heat and may lead to fatigue. Therefore, it is not surprising that when body core temperature continued to rise the animal changes its panting tactics. Hales (1969) has reported that when T_{rec} of sheep reached 40.4-40.8°C, panting changed from shallow rapid costal to deep abdominal breathing. It is at this stage that the survival of the individual is challenged.

Our results indicated complex relationships between the physiological responses to heat stress (T_{rec} and respiration rate) and the endocrine changes that were associated with them. None of the changes in the three hormones estimated during NT expressed significant positive or negative relationship to T_{rec} or RR. In contrast, the 24-h profiles and consequently mean daily concentration of cortisol were depressed during CHS ($p < 0.05$). Lower mean concentration prevailed during the entire exposure periods, including the cooler hours of the day. These results agreed with data from Alvarez and Johnson (1973) where elevated cortisol concentration in chronically heat-stressed cattle returned to normal concentration in a period of 48 h after T_{amb} reached the pre-exposure values.

Since the adrenal cortex is under the direct control of pituitary ACTH, these data suggested that the pulse frequency of ACTH release was

unchanged by CHS, but instead there may have been a reduction in the baseline of the pituitary release of ACTH during each pulse and hence of circulating cortisol. The persistency of a surge-like secretion of cortisol in the late morning and afternoon hours in Expt. 1 during both cycles, suggested that other factors, independent of Tamb, influenced the release of ACTH. The absence of this 24-h rhythm of cortisol profiles in the CB-154-treated ewes tended to suggest its dependence on factors other than Tamb.

The response of cortisol to the presumed sampling stress as judged by the persistence of declining concentration during repeated sampling, irrespective of Tamb or CB-154 treatment, stresses the necessity of acclimatization periods in studies that involve the adrenocortical response. It is worth while to mention that each group of these ewes was handled routinely for 8-9 wks prior to data collection. However, because ewes were left loose and sampled by venipuncture, very intensive interaction with the personnel collecting the data was unavoidable.

Several workers have reported depressed thyroid function in heat-stressed animals. This depression has been interpreted as an attempt by the animal to reduce heat load. Results of this investigation showed no difference in the thyroid hormone concentration as a result of CHS. This could be due to the combined effects of the cool hours and the adequate food intake.

Marques et al. (1983) have studied the effect of cooling or warming the preoptic anterior hypothalamus in adult goats, and reported that changes in temperature of this region of the brain were more closely associated with changes in the peripheral release of TSH than changes in

Trec. Other workers have reported changes in the peripheral monodeiodination of T_4 which led to inflated $T_4:T_3$ ratio (Dauncy and Ingram 1986), perhaps resulting from metabolism of T_4 to the metabolically inert rT_3 (Epstein et al. 1979). Although earlier reports from cattle (Yousef and Johnson 1966) have shown depressed thyroid function during CHS, irrespective of the energy intake, recent evidence from pigs and sheep suggest that peripheral changes in T_3 were energy dependent. In heat-stressed sheep (Guerrini and Berchinger 1983) and young pigs (Dauncey and Ingram 1986) reduced feed intake was associated with higher $T_4:T_3$ ratio and that at high energy intake this ratio was comparable to that at the control T_{amb} . But since during the hours of elevated T_{amb} , feed intake was drastically reduced, we could not completely dissociate the slightly lower T_3 values at 1200h, 1800h and 2400h of the CHS cycle from the influence of the temporary (8 h) reduction of intake.

In an agreement to the no variation in mean daily T_3 concentration, our results indicated no significant changes in the 24-h profiles during the preovulatory period (D14-D18) of the estrous cycle. Since our assay estimated total T_3 , the stable concentration during this period could be due to increased estrogen-induced hepatic synthesis of thyroid hormone binding globulin, prealbumin and albumin. Current investigation also revealed that frequent sampling did not influence the release patterns of T_3 , irrespective of T_{amb} .

Another major finding of this investigation was the nature of the dramatic changes in PRL release under the T_{amb} settings employed.

Prolactin response to CHS was similar to the rise of cortisol following acute exposure to CHS as described in data from cattle (Christison and Johnson 1972; Alvarez and Johnson 1973; Abilay et al. 1975b). Nonetheless, PRL differed from cortisol in that peak concentration was not followed by a plateau. Thus, during CHS the acute diurnal rise in PRL that followed the increasing T_{amb} was transient and was due to enhanced overall secretion rate, particularly basal release. As in cortisol, T_{amb} had no significant effects on the pulse frequency, its duration or the interpeak intervals. Then how did CHS elevate mean PRL concentration without significantly affecting the release characteristics? We speculate that because sampling was carried at an hourly intervals, many secretory peaks of PRL, which have a half-life of 7-10 min could have been missed.

The peak concentration of PRL occurred at T_{amb} of 31.4°C and coincided with a 0.27°C rise in T_{rec} and a 2-3- fold increase in in respiration rate as compared to values recorded at 700h of the same cycle (Table 28). PRL concentration declined when T_{rec} rose above $39.61 \pm 0.14^{\circ}\text{C}$. It continued to fall during the declining phase of T_{amb} and reached minimum concentrations when T_{amb} fell below 21°C . Subsequently, PRL started to rise to values higher but comparable to the concentration observed during NT. Hence, the day time hot hours had no significant residual effects on the nightly rise of PRL during CHS. It follows that because of the variability of PRL response was related to the intensity of CHS, rather than its duration, whereas T_{rec} and RR were related to the duration of the hot temperature, the a consistent relationship between PRL hormone and T_{rec} or RR was not found.

The more frequent drinking which was observed when Tamb rose above 25°C was possibly an attempt by the ewes to reduce body heat load. This behavior also paralleled the acute rise in PRL concentration. Might there be a link between the two events? Mills and Robertshaw (1981) have shown that PRL release in men followed changes in Tamb and were not related to shifts in the fluids or the electrolyte balance. They also reported that during NT PRL concentration was stable within a wide range in relative humidity (32-92%). In contrast, Faichney and Barry (1986) have reported the failure of CB-154 treated anestrous ewes to maintain body temperature when exposed to mild CHS (32°C). They attributed this to the inability of the treated ewes to absorb water from the digestive tract into the circulation where it could function for evaporative heat loss. Our results on changes in Trec contradicted the above interpretation. Other workers have shown that PRL concentration decreased during dehydration (Raud et al. 1971), but the expected response to rehydration produced inconsistent results (Becker et al. 1985).

Further, the response of PRL to repeated sampling during the preovulatory period showed similarity in trend to that of cortisol, but only during NT where values were significantly low on D17 and D18 as compared to D14 or D15 of the cycle. During CHS, on the other hand, mean daily PRL concentration did not reveal a specific trend of increasing or declining, suggesting that the response to CHS was greater than the sampling stress.

In summary, the results of this investigation showed that long-term

heat stress which led to a rise in T_{rec} of 0.4°C and a 4.0 fold increase in respiration rate was associated with significant changes in the secretory patterns and peripheral concentrations of cortisol and PRL. Depressed cortisol concentration was due to low basal secretion, but there was no significant changes in either pulse amplitude, pulse frequency, duration or the interpeak intervals. CB-154 but not CHS attenuated the 24-h pattern of cortisol secretion.

In addition, the imposed CHS tended to depress the mean daily T_3 concentration yet had no significant effect on the 24-h release patterns of this hormone. Mean T_3 concentration was not influenced by the sampling stress.

The results also showed that the response of PRL to CHS was biphasic in nature. An exaggerated release paralleled the progressive rise in T_{amb} until T_{rec} rose above $39.61 \pm 0.14^{\circ}\text{C}$ and respiration rate increased by more than 4-fold above the normal values at 18°C . Thereafter, a further rise in T_{amb} caused PRL to fall. Elevated PRL concentration was caused by the enhanced basal secretion with no change in the other release characteristics.

Therefore, the present results suggest that there are various patterns of hormone secretion that influence basal concentration, 24-h rhythms, etc.. In non-heat-stressed healthy animals at restricted intake, hormone secretion would not necessarily be associated with changes in body temperature or the respiration rate. However, during CHS, the pattern of secretion of each of the endocrine systems examined in our investigation tended to respond in a different fashion.

For instance, CHS lowered basal cortisol but did not modify either the 24-h rhythm or the acclimatization to repeated sampling. Conversely, CHS tended to raise basal PRL and abolished the acclimatization to the response of the stress of sampling. Further, the current results suggest that dopaminergic neurones influence the 24-h secretion patterns of the adrenocorticoids. Because of the depressed adrenocortical activity during CHS, our results tend to downplay any direct significant role for cortisol on the reproduction of heat-stressed animals. Nevertheless, if there is a role to be played by the adrenal cortex of such animals in affecting fertility, it should be through the differential secretion of P_4 . Another finding of this investigation was the elevation in PRL during CHS, which might depress the pituitary-gonadal axis, particularly if the elevation coincides with the onset of the preovulatory gonadotropin surges.

MANUSCRIPT #2

CORPUS LUTEUM FUNCTION AND THE PREOVULATORY RELEASE
CHARACTERISTICS OF LH, FSH AND PROLACTIN DURING A
THERMONEUTRAL AND A CYCLIC HEAT STRESS TEMPERATURE
IN CYCLING EWES

5.1 ABSTRACT:

The effect of heat stress on the estrous cycle, corpus luteum (CL) function, LH, FSH and prolactin (PRL) was investigated in two groups of cycling ewes during the breeding season. Ewes were fed a ration which was calculated to provide 2.5 times maintenance and were allowed to move freely in a temperature-controlled environmental chamber. They were studied for two consecutive cycles. In the first cycle (thermoneutral temperature, NT) the chamber temperature was maintained at a constant $18\pm 1^\circ\text{C}$. During the second cycle (cyclic heat stress, CHS) it fluctuated at a 12 h circadian cycle of 18°C - 35°C - 18°C . The relative humidity ($55\pm 2\%$) and photoperiod (14D:10L) remained constant. Hormone estimates were made on daily (D1-D13) and hourly (D14-D18) serum samples by RIA procedures.

Heat stress elevated mean daily progesterone (P_4) in the early- and mid-luteal phases (D5-D11) and consequently tended to increase the area under the P_4 curve. Progesterone concentration was elevated at 36 h before the preovulatory LH surge during CHS as compared to values observed in the corresponding period during NT ($p < 0.05$). Heat stress had no significant influence on CL function during its development

did not seem to have induced significant fluctuations in the peripheral concentration of P_4 following the regression of the cycle CL. The newly formed CL started to secrete P_4 steadily after 48 h following the preovulatory LH surge.

Heat stress depressed the LH surge peak ($p < 0.05$) and slightly reduced the area under the FSH curve ($p < 0.06$). The second FSH surge occurred between 14-36 h after the preovulatory LH surge in both cycles. Although CHS had no significant influence on LH release characteristics following its preovulatory surge, it depressed the magnitude of FSH concentrations along the ascending limb of its second surge. In addition, the prolactin surge which occurred concurrently with the preovulatory gonadotropin surges was significantly distorted by CHS and was characterized by the presence of several peaks; each of which was equal in magnitude and duration to peak observed during NT cycle.

The results suggest that during CHS the CL is either more resistant to luteolytic factors or that following the withdrawal of the negative feedback effects of ovarian factors, full recovery of the response of the hypothalamo-pituitary axis requires a longer time. Heat stress induced significant changes in the preovulatory release patterns of LH, FSH and prolactin which could not be explained in terms of the negative feedback of gonadal steroids alone. Further investigations are required to substantiate the effect of elevated PRL during CHS on the hypothalamo-pituitary-gonadal axis.

5.2 INTRODUCTION:

Peripheral hormone concentration during the estrous cycle closely

correlates with the morphological and functional endocrine changes that occur in the pituitary-ovarian axis (Bjersing et al. 1972; Trouson and Moore 1974; Yuthasatrakosol et al. 1975; Baird et al. 1976; Baird and Scaramuzzi 1976a; Gemmell et al. 1976; Pant et al. 1977; McNeilly et al. 1977; Baird 1978; Jackson et al. 1978; McNatty et al. 1981a; Jeffcoate et al. 1984; Haresign 1985; McNatty et al. 1985b).

The exposure of cycling females to elevated ambient temperatures has been associated with the disturbance of the estrous cycle and low conception rates in sheep (Sawyer et al. 1979; Hill and Alliston 1981; Yenikoye et al. 1982; Mokhatr et al. 1983), cattle (Abilay et al. 1975a; Gwazdauskas et al. 1981) and swine (Paterson 1978). The reduced fertility during CHS has been reported to be associated with elevated progesterone (P_4) concentration in sheep (Sawyer et al. 1979) and cattle (Miller and Alliston 1974a; Abilay et al. 1975a; Roussel et al. 1977; Vaught et al. 1977). Because exogenous adrenocorticotrophic hormone (ACTH) has been shown to enhance P_4 release in sheep and cattle (da Rosa and Wagner 1981; De Silva et al. 1983; Benhaj and Cooke 1985), there were speculations that adrenal P_4 might be increased in heat-stressed animals.

Hence elevated P_4 concentration in the presence of low concentrations of estrogens (E_2) (Scaramuzzi et al. 1971; Karsch et al. 1979; Clarke and Cummins 1984) could be the cause of the reduced luteinizing hormone (LH) concentration reported in heat-stressed sheep (Schillo et al. 1978; Sawyer et al. 1979; Hill and Alliston 1981) and cattle (Madan and Johnson 1973; Miller and Alliston 1974b). However, there are other reports on heat-stressed cattle that show no significant changes in LH

concentration (Vaught et al. 1977; Fuquay et al. 1980; Gwazdauskas et al. 1981). Further, CHS elevated prolactin (PRL) concentration and enhanced PRL response to exogenous thyrotropin-releasing hormone, TRH (Hill and Alliston 1981; Hooley et al. 1979; Fraser and McNeilly 1980). The fact that elevated PRL concentration was also associated with seasonal and lactational anestrus (Fell et al. 1972; Lamming et al. 1974; Rhind et al. 1980) and was also reported to inhibit estrogen-induced LH release (Kann et al. 1976; McNeilly and Baird 1977) raises the question of possible involvement of the elevated concentration of PRL hormone during CHS in impairing pituitary-ovarian function.

In addition, neither the functional response of the CL during its regression or formation, nor the secretory characteristics of the gonadotropins (Pant et al. 1977; Bister and Paquay 1983; Lahlou-Kassi et al. 1984) in the heat-stressed cycling ewe has been well described. Therefore, this investigation was an attempt to 1) define the patterns of CL response to heat stress during its regression, formation and, full functionality; and 2) to closely follow the concentration and the characteristics of the pulsatile secretion of gonadotropins in the interval from 48 h before to 48 h after the preovulatory LH surge in the heat-stressed ewe.

5.3 MATERIALS AND METHODS:

5.3.1 Animals:

Adult cycling ewes that had been previously mentioned in

Manuscript #1 (Expt 1) were used in two consecutive breeding seasons (Sept.-Feb.). In the first year (Rep. 1), four Finnish Landrace-cross ewes (59.3 ± 4.2 kg liveweight) were studied. In the second year five Suffolk-cross ewes (65.6 ± 1.9 kg liveweight) were studied (Rep. 2). Ewes were checked for cyclicity, and then their cycles were synchronized with two injections of prostaglandin $F_{2\alpha}$. Ewes were studied for two consecutive cycles using a temperature-controlled environmental chamber. The first estrous cycle (thermoneutral temperature, NT) was at $18 \pm 1^\circ\text{C}$, while during the second cycle (cyclic heat stress, CHS) the ambient temperature was fluctuated at a 12 h cycle of 18.0°C - 35.0°C - 18.0°C . Relative humidity ($55.0 \pm 2.0\%$) and photoperiod (14D:10L) were kept constant. Chamber description, maintenance of temperature settings, recording of parameters and physiological responses, and management of the animals were as described in the General Materials and Methods.

5.3.2 SAMPLING TECHNIQUES:

During the estrous cycle, daily (D1-D13) and hourly (from 700h on D14 to 700h on D19 of the same cycle) blood samples were collected by venipuncture. Serum was harvested, stored at -20°C and later assayed for P_4 , LH, follicle-stimulating hormone (FSH) and PRL by radioimmunoassay (RIA) procedures.

5.3.3 HORMONE ANALYSIS:

Progesterone was estimated on daily samples and on 6-h pools of the frequently-collected samples during the preovulatory period (D14-D18) of the estrous cycle. LH, FSH and PRL were estimated on daily and all hourly samples. All samples from an individual ewe were estimated

in the same assay run.

5.3.3.1 RIA OF PROGESTERONE (P_4):

5.3.3.1.1 PROCEDURE FOR P_4 ASSAY:

Progesterone was estimated according to the assay originally validated by Abraham et al. (1971). The assay procedures were identical to those described earlier by Yuthasatrakosol et al. (1974), with the following exceptions: 1), both the standard and the unknowns were simultaneously extracted; 2), during the separation step a straight charcoal solution (375 mg of washed charcoal 100 mL^{-1} of the assay buffer) was used instead of the dextran-coated charcoal; and 3), following the addition of charcoal the assay tubes were incubated at 4°C for 10 min, rather than 20 min.

5.3.3.1.2 REAGENTS FOR P_4 ASSAY:

The P_4 standard (4-pregnen-3,20-dione, Steraloid Inc., Wilton, N.H., U.S.A.) was prepared in ovariectomized (ovx) ewe serum which was twice extracted with washed charcoal (Abraham 1974) and contained $<11.2 \pm 0.1 \text{ pg mL}^{-1}$ ($n=20$ assays). Labelled P_4 (^3H -progesterone, New England Nuclear, Boston, Mass., U.S.A.) was prepared in the assay buffer to yield 34,000-37,000 cpm $100 \mu\text{L}^{-1} \text{ tube}^{-1}$. P_4 anti-serum (Sheep #11, N.C. Rawlings, Dept. Vet. Physiol. Sciences, W.C.V.M., Univ. of Saskatoon, Saskatoon, Sask., Canada) was raised against (4-pregnen-11 α -ol-3-ol-16,17-dione G-COMO:BSA). It crossed-reacted at 0.47%, 0.04%, 0.01% and 0.01% with cholesterol, testosterone, hydrocortisone and estradiol-17 β , respectively. It was used at a titre of 1:5000.

Samples were assayed at volumes of 0.5-1.0 ml tube⁻¹. The efficiency of P₄ extraction was 78.0% (n=26 assays). Total binding of the anti-serum was 41.7% and the nonspecific binding (NSB) was 2.2% (Abraham 1974). The sensitivity of the assay at 95% binding was 15.4 pg tube⁻¹ (Abraham 1974; Ekins 1974). When ewe serum pools containing 0.15, 0.37, 1.50, 2.50, 4.10 and 11.20 ng mL⁻¹ were assayed repeatedly, the intra-assay coefficients of variations, C.V. (Rodbard et al. 1968) were 7.4%, 5.4%, 5.6%, 3.3%, 6.1% and 6.0%, respectively. The corresponding values for the inter-assay C.V. were 15.8%, 7.5%, 6.3%, 4.6%, 7.3% and 6.4%, respectively.

5.3.3.2 RIA OF LUTEINIZING HORMONE (LH):

Samples were assayed at volumes of 200 µl tube⁻¹. LH was estimated in a homologous assay utilizing oLH as a standard, labelled oLH and an anti-oLH serum. The assay procedures were similar to those described by Niswender et al. (1968) as modified by Howland (1972). Concentrations were expressed as ng NIH-oLH-S14 mL⁻¹. NIH-oLH-S14 was labelled (¹²⁵I-oLH) according to Greenwood et al. (1963) and was prepared in the assay buffer to yield 7500-9000 cpm 100 µL⁻¹ tube⁻¹. The anti-serum (GND #15) was used at a titre of 1:100,000. Goat anti-rabbit γ-globulin (1:20) was used to precipitate the anti-serum-bound reactants.

The total binding of the anti-serum was 49.5% and the (NSB) was 3.7% (n=17). The sensitivity of the assay at 95% binding was 31.1 pg tube⁻¹. When serum pools from castrate rams and cycling ewes containing 5.30 and 0.72 ng mL⁻¹, respectively, were assayed repeatedly, the intra-assay C.V. were 2.9% and 4.4%, respectively. The corresponding inter-assay C.V. were 4.1% and 5.5%, respectively.

5.3.3.3 RIA OF FOLLICLE-STIMULATING HORMONE (FSH):

Samples were assayed at volumes of 50-100 μl tube⁻¹. FSH was estimated using a heterologous assay previously described by Cheng et al. (1981). Concentrations were expressed as ng NIH-oFSH-S6 mL⁻¹. Labelled oFSH (¹²⁵I-oFSH) was tagged using the lactoperoxidase method (Miyachi et al. 1972) as modified by Cheng (1978), and was prepared in the assay buffer to yield 16,000-18,000 cpm 100 μL^{-1} tube⁻¹. Rabbit anti-bovine FSH (Cheng's R anti-bFSH) was used at a titre of 1:15,000. Goat anti-rabbit γ -globulin serum (1:10) was used during the separation step.

The total binding of the anti-serum was 43.8% and the (NSB) was 2.6% (n=18). The sensitivity of the assay at 95% binding was 57.2 pg tube⁻¹. The intra-assay C.V. were 13.3%, 9.6% and 8.5% for ewe serum pools containing 1.8, 6.5 and 12.2 ng mL⁻¹, respectively. The corresponding values of the inter-assay C.V. for the same serum pools were 10.5%, 11.5% and 8.5%, respectively.

5.3.3.4 RIA OF PROLACTIN (PRL):

Because of storage problems PRL concentrations in the samples from Rep. 2 were lost. Therefore, values reported here represent only those from ewes in Expt #1. (Rep 1) PRL was estimated according to the procedure described previously (Manuscript 1).

5.3.4 STATISTICAL ANALYSIS:

The calculation of RIA estimates was done as described in the General Materials and Methods. Hourly estimates of LH, FSH and PRL during the

preovulatory period were preliminarily subjected to the Pulsar analysis using the same parameter settings described in the General Materials and Methods. Progesterone estimates were comprised of two data sets. Daily cyclic values were examined by dividing the cycle arbitrarily into four phases: follicular (D1-D4), early-luteal (D5-D8), mid-luteal (D9-D12) and late-luteal (D13-D16). Also CL function was assessed by following the 6-h pooled concentrations of P_4 between 72 h before to 72 h after the preovulatory LH surge. Whenever mentioned, the term 'basal P_4 ' always refers to P_4 concentration of $<0.70 \text{ ng mL}^{-1}$.

Since the estrous cycle was defined as the period between two consecutive preovulatory LH surge peaks, the area under daily cyclic P_4 , as well as those under the preovulatory LH and FSH curves were calculated as mentioned in the General Materials and Methods. The onset of a hormone surge was defined as mentioned in the General Materials and Methods, and used to calculate the area under the curve. Area under the preovulatory gonadotropin surges covered the period of sustained elevations from 12 h before until 12 h after the LH surge. LH and FSH data sets analyzed included daily estimates, preovulatory surges and hourly means from 48 h before to 48 h after the LH surge peak. Daily concentrations of LH and FSH were analysed by dividing the cycle into 4 phases as was done before for P_4 data. This latter data set was analyzed at 6-h periods. Due to the variation between ewes on the onset of the preovulatory gonadotropin surges, complete records for the 48-h interval before the LH surge peak was available for only 5 ewes. Similarly, a complete record for the 48-h interval following the LH surge peak was available for only 6 ewes. Since most PRL data has been reported before (Manuscript 1), only the preovulatory concentration of

this hormone was examined.

All data sets were subjected to analysis of variance using computer programs according to ANOVA procedures provided by Statistical Analysis System (SAS Institute, Inc. 1982). Replicates were analyzed in repeated measurement designs, where 'replicate' was considered as the main effect and 'ewes' within 'replicate' as the error term (Gill and Hafs 1971). Using this design, the interaction of cycle*ewe(replicate) was used to test the effect of cycle and cycle*replicate. Similarly the effects of day, hour and period were tested using the the the respective interactions containing the 'ewe' effect as error terms. The preovulatory PRL concentration in Rep. 1 was analyzed using ewes as blocks (Snedecor and Cochran 1967). Whenever applicable, simple correlations were calculated according to Snedecor and Cochran (1967).

5.4 RESULTS:

Data covering environmental and physiological responses were presented in Manuscript 1.

5.4.1 PROGESTERONE (P₄):

5.4.1.1 DAILY CONCENTRATIONS:

Mean serum P₄ increased progressively during the first 11 days of the cycle (Fig. 12). Progesterone peaked earlier during the hyperthermic cycle (D11, 3.34±0.21 ng mL⁻¹) as compared to the NT cycle (D12, 3.00±0.21 ng mL⁻¹). Despite the precipitous decline of P₄ after each peak, the slope of the curve after D14 was less steep during CHS.

5.4.1.2 CONCENTRATION OF P₄ DURING THE PHASES OF THE CYCLE:

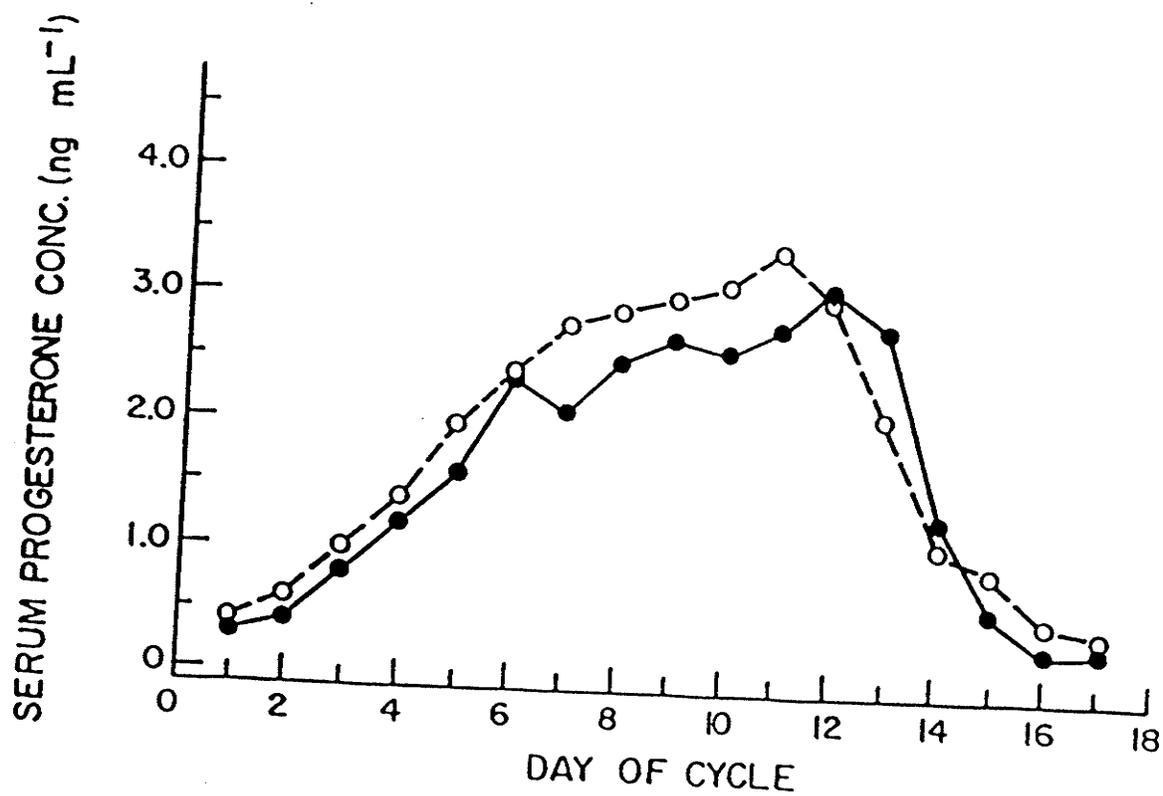


Figure 12: Mean daily concentration of serum progesterone (P₄) in cycling ewes at thermoneutral (●) and at cyclic heat stress (○) estrous cycles.

The estrous cycle was arbitrarily divided into follicular (D1-D4), early-luteal (D5-D8), mid-luteal (D9-D12) and late-luteal (D13-D16) phases. Analysis of variance of phasic P_4 concentrations showed that cycles were comparable (Table 29), indicating that CHS did not induce significant increase in P_4 . Also there was no cycle*phase interaction suggesting that the pattern of P_4 secretion was not influenced by CHS. Mean (\pm SEM) of P_4 concentration by phase is given in Table 30. Means (\pm SEM) of cyclic P_4 concentration by phase for differences between replicates are depicted in (Table 31).

Additionally, analysis of variance of area under the daily P_4 curve, showed no differences between cycles (Table 32).

5.4.1.3 CORPUS LUTEUM (CL) FUNCTION:

CL function was investigated using the 6-h changes in peripheral P_4 concentrations from 72 h before to 72 h after the preovulatory LH surge peak (Fig. 13). Although P_4 concentration started to fall before D14 of both cycles (Fig. 12), values at 72 h prior to the preovulatory LH surge peak were above 1.50 ng mL^{-1} . In the following 24 h, P_4 fell steeply to $<1.0 \text{ ng mL}^{-1}$. During the NT cycle P_4 declined to basal values of $<0.60 \text{ ng mL}^{-1}$ at 48 h before the LH peak and remained low until the new CL started to function (54 h after the the LH surge peak). During CHS, the rapid decline in P_4 after D12 reached a transient plateau between 54-36 h before the LH surge peak. Levels at this plateau were slightly higher than in the NT cycle. Progesterone concentration between the two cycles was comparable from 30 h before until 72 h after the LH surge peak. Analysis of variance of 6-h P_4 profiles at 18-h periods ($n=8$), showed significant differences between cycles ($p<0.06$) (Table 33), but periods

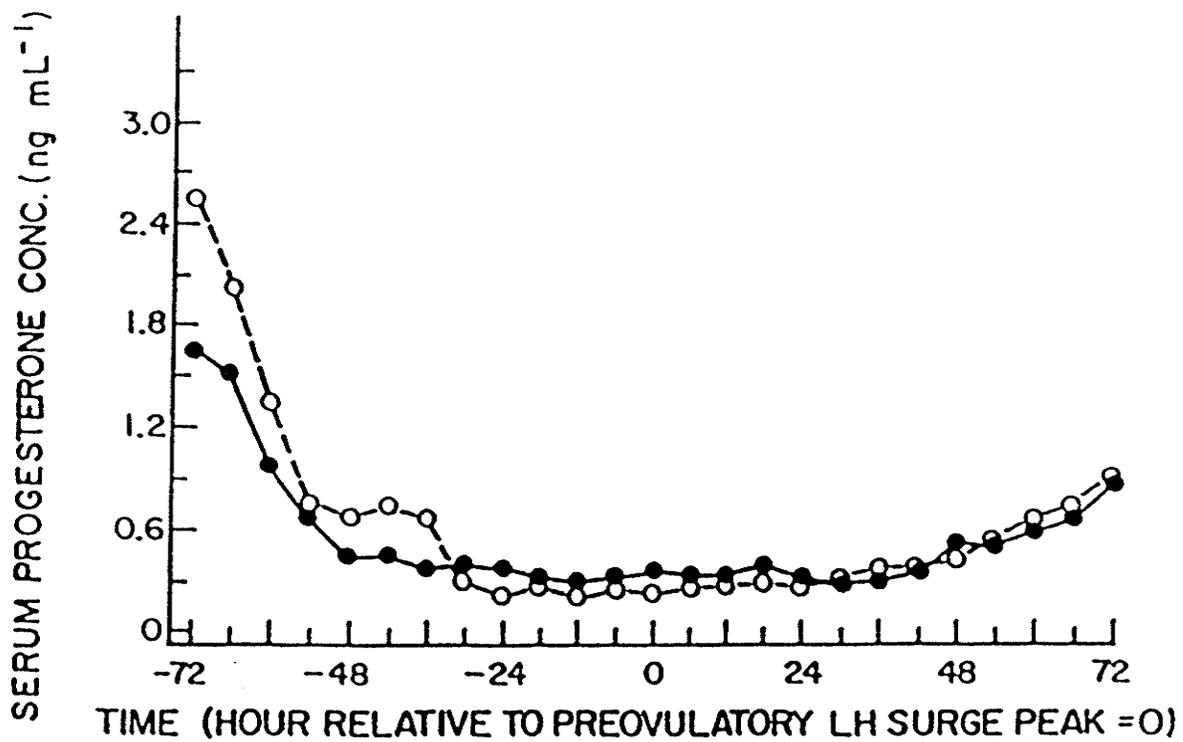


Figure 13: Mean 6-h concentration of serum progesterone (P₄) between 72 h before to 72 h after the preovulatory LH surge peak (time=0) in cycling ewes at thermoneutral (●) and cyclic heat stress (○).

were different ($p < 0.001$). The significant interaction of cycle*period ($p < 0.01$), indicated that the trends of the CL regression was influenced by temperature. Although replicates were different ($p < 0.001$), there was no interaction between replicates and period. Mean (\pm SEM) concentration of P_4 during the regression of the CL is given in Table 34.

5.4.2 THE LENGTH OF THE ESTROUS CYCLE:

The length of the estrous cycle was 16.7 ± 0.1 d and 16.9 ± 0.1 d during NT and CHS, respectively. Analysis of variance of the length of the cycle showed no differences between cycles (Table 35). Means (\pm SEM) of P_4 concentration and the length of the estrous cycle in response to temperature are shown in Table 36.

5.4.3 LUTEINIZING HORMONE (LH):

5.4.3.1 MEAN DAILY PROFILES:

Mean daily cyclic LH concentration is depicted in Fig. 14. The concentration LH during early and mid-luteal phases was lower than those observed during the follicular and late-luteal phases, particularly during CHS. Analysis of variance of daily LH by phase indicated no differences between cycles, suggesting that CHS had no significant effect on basal LH (Table 37). Means (\pm SEM) of cyclic LH by phase are given in Table 38.

Mean daily LH concentration showed an inverse relationship to cyclic P_4 concentration and the correlation coefficient was significant during CHS ($r = -0.65$, $p < 0.01$) but not during NT (Table 39). This indicates that

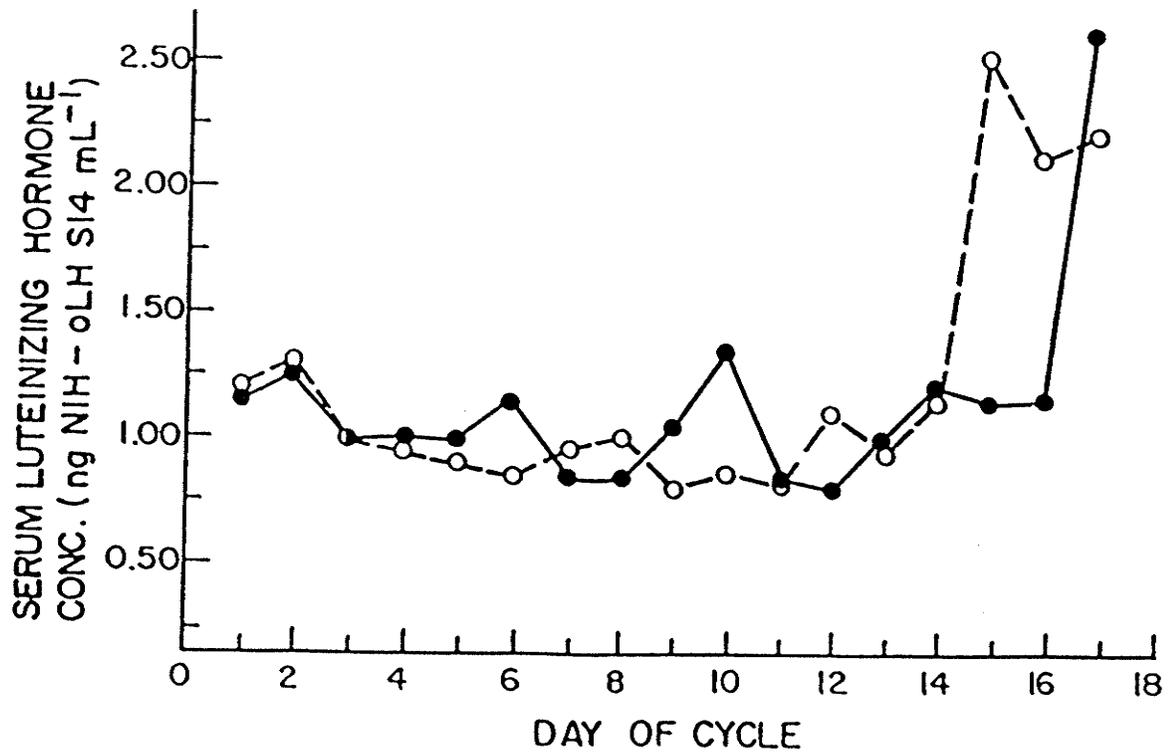


Figure 14: Mean concentration of daily serum LH in cycling ewes at thermoneutral (●) and cyclic heat stress (○).

the effect of the ovarian factors on suppressing basal LH may have been more intense during CHS than NT.

5.4 .3.2 PREOVULATORY LH SURGE:

The preovulatory concentration was analysed as hourly concentrations of LH in all ewes (n=9) between 12 h before to 12 h after its surge peak. As can be seen in Fig. 15, heat stress reduced the height of the preovulatory LH surge curve. The characteristic features of the curve's ascending and descending limbs remained unchanged. Analysis of variance of the area under LH surge curve was comparable between cycles (Table 40). Analysis of variance of the duration of LH surge curve showed no significant differences between replicates or cycles (Table 41). The analysis of variance of the LH surge peak revealed significant differences between cycles ($p < 0.05$) indicating that, CHS depressed LH peak (Table 42). Replicate differences (mean \pm SEM) of the characteristics of the LH surge peak are shown in Table 43. Analysis of variance of the LH release characteristics revealed significant cycle*replicate interactions for the LH pulse amplitude ($p < 0.05$), pulse frequency ($p < 0.05$) and the interpeak interval ($p < 0.01$) (Table 44). Other LH release characteristics were comparable between the two replicates. Means (\pm SEM) of the Pulsar program-generated LH release characteristics are summarized in Table 45.

5.4.3.3 LH RELEASE PATTERNS:

The LH release patterns were examined in two separate intervals relative to the surge peak. The first interval covered hourly

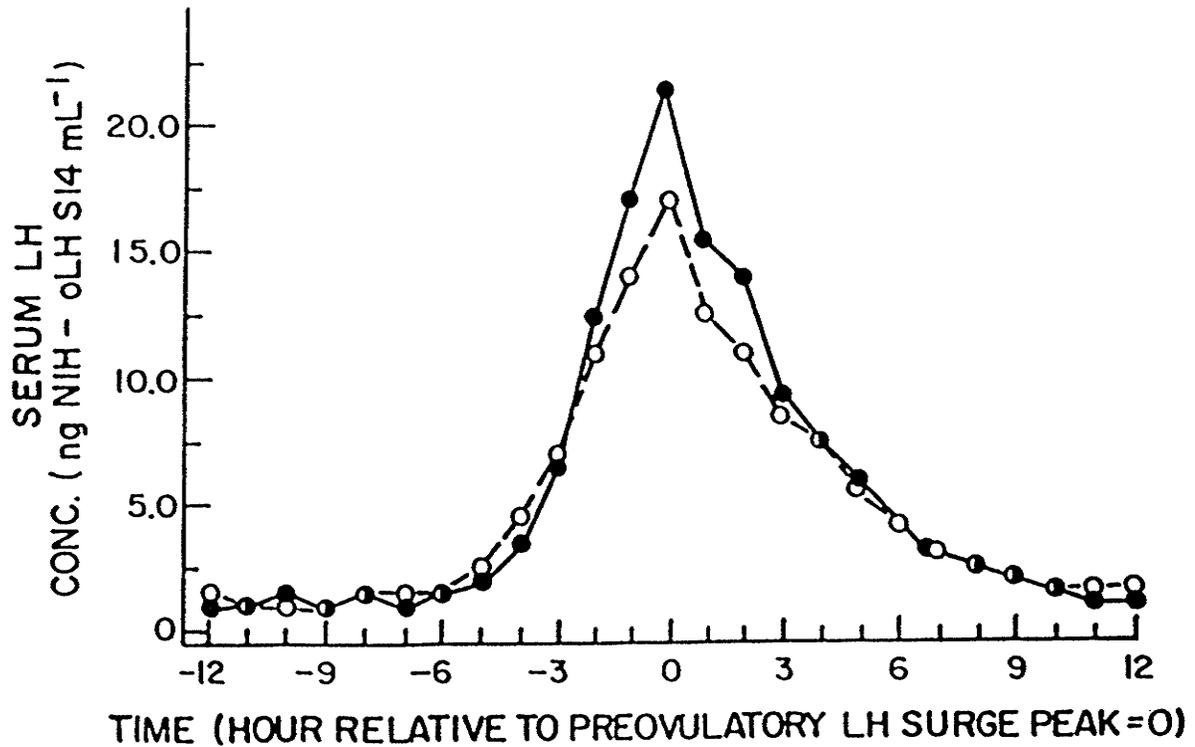


Figure 15: Mean concentration of serum LH at the preovulatory surge in cycling ewes exposed to thermoneutral (●) and cyclic heat stress (○).

concentration during the 48 h before to 12 h after the preovulatory surge. The second interval covered values between 12 h before to 48 h after the surge peak. Data from all ewes was incomplete to cover both intervals, only 5 ewes had complete records for the first interval and 6 ewes for the second.

The hourly mean concentration of LH in the interval between 48 h before to 12 h after the preovulatory surge was subjected to the analysis of variance at 6-h periods (n=10) (Table 46). There were no differences between cycles and no cycle*period interaction, indicating that the LH release pattern was similar between temperatures during this interval.

In Fig. 16, The LH release patterns were examined in the 48-h interval following the surge peak. Subsequent to the onset of the preovulatory surge, mean LH concentration suggested that the LH pulse amplitudes tended to be low between 12-29 h and 15-29 h after the surge peak in the NT and CHS, respectively. However, the comparison of the hourly pulse frequency of LH between 48 h before to 48 h after the LH peak, revealed no differences in the occurrence of the LH pulses before or after the surge (data not shown). Analysis of variance of the hourly LH concentration, 12 h before to 48 h after the surge, at 6-h periods (n=10) showed no differences between cycle and also no cycle*period interaction (Table 47), suggesting that temperature had no effect on the pulsatile activity of LH during the preovulatory period.

5.4.4 FOLLICLE-STIMULATING HORMONE (FSH):

5.4.4.1 DAILY FSH PROFILES:

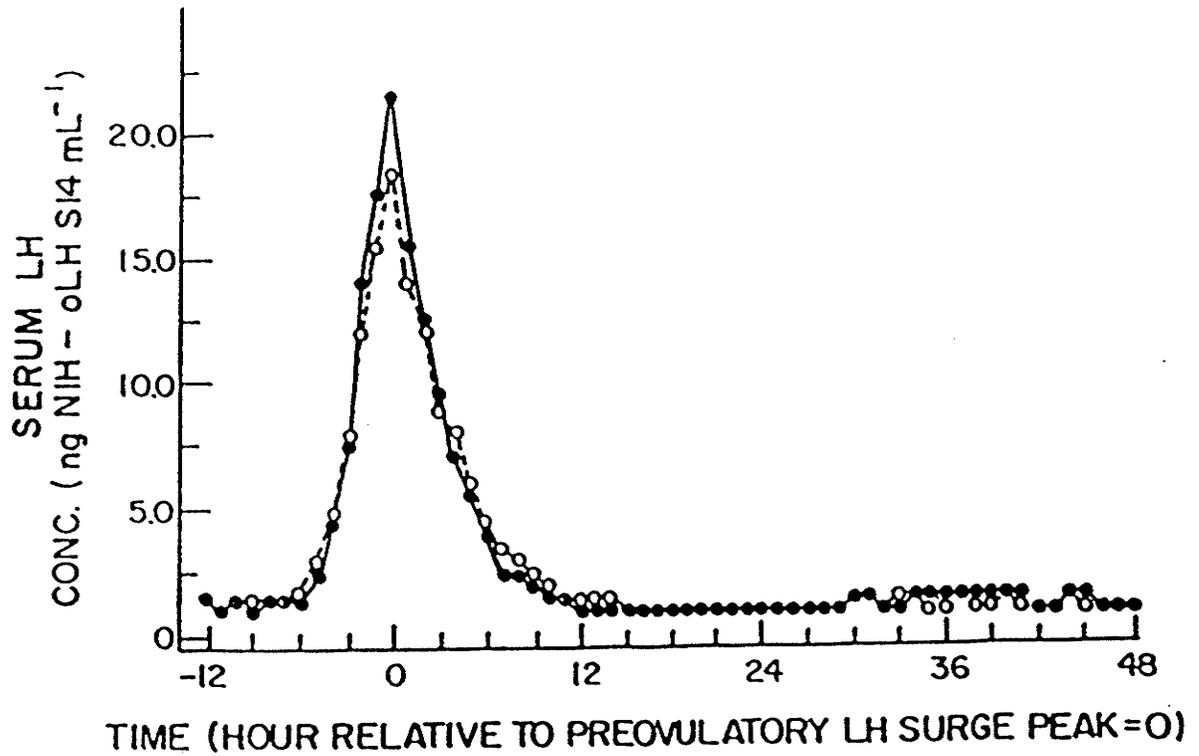


Figure 16: The hourly means of serum LH between 12 h before to 48 h after the preovulatory surge peak (time=0) in cycling ewes (#15,16,18,21,22,23,25) at thermoneutral (●) and cyclic heat stress (○).

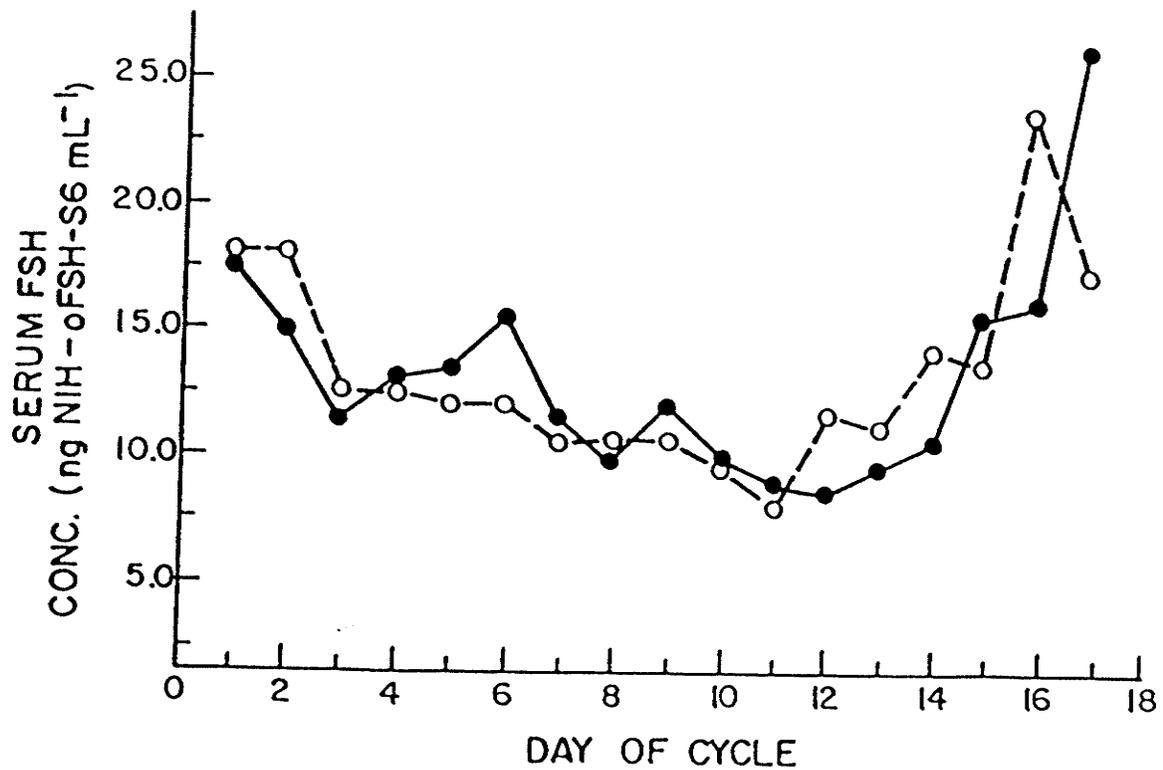


Figure 17: Mean concentration of daily serum FSH in cycling ewes at thermoneutral (●) and cyclic heat stress (○). (SEM=2.05ng mL⁻¹).

Unlike LH, mean daily FSH concentration showed a significant inverse relationship to cyclic P_4 irrespective of the temperature treatment (Fig. 17). Mean daily concentration was lowest on D11 and D12 during the NT and CHS cycles, respectively. Subsequent to these nadir values FSH concentration rose gradually in the late-luteal phase. The correlation coefficients between daily FSH concentration and cyclic P_4 were $r=-0.72$ ($p<0.01$) and $r=-0.82$ ($p<0.001$) during the NT and CHS, respectively (Table 39). Analysis of variance of phasic FSH revealed that cycles were comparable and there was no cycle*phase interaction, suggesting that temperature had no effect (Table 48). Replicates were different ($p<0.05$) because basal FSH was higher in Rep #2 (means \pm SEM shown in Table 49). The significant Rep*phase interaction resulted from the fact that mean FSH in Rep #2 started to rise after a nadir value on D11, whereas in Rep #1, FSH concentration remained low until after D14.

5.4.4.2 PREOVULATORY FSH SURGE:

The preovulatory FSH surge curve is depicted in Fig. 18. The FSH surge curve tended to be smaller during CHS than NT. Nonetheless, CHS did not modify the shape of the surge curve. The analysis of variance for the area under FSH surge curve showed no differences between cycles ($p<0.06$) (Table 50). Further, the analysis of variance of the FSH surge peak indicated no differences between cycles or replicates suggesting neither temperature nor breed had influenced the FSH peak (Table 51). Also, CHS had no effect on basal FSH, the FSH pulse amplitudes, the pulse frequency, the duration of pulse peaks or the interpeak intervals (Tables 52-54).

5.4.4.3 FSH RELEASE PATTERNS:

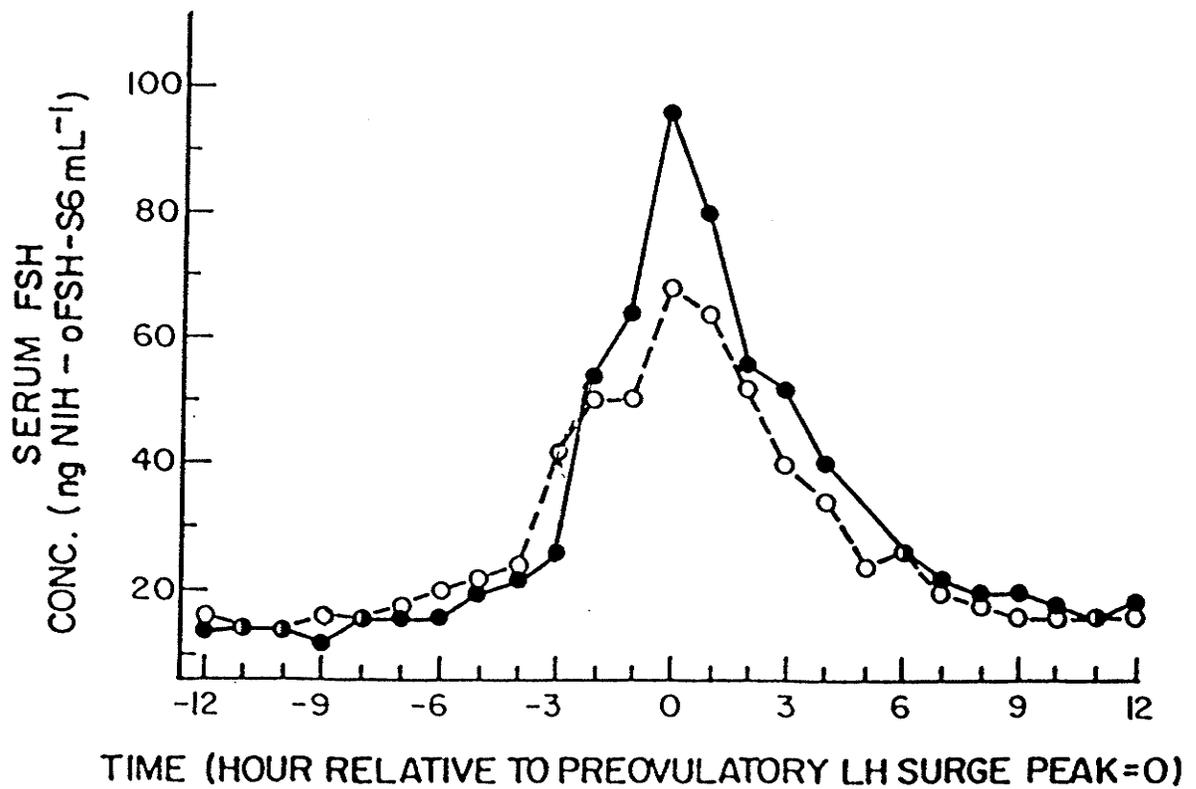


Figure 18: Mean concentration of serum FSH at the preovulatory surge in cycling ewes exposed to thermoneutral (●) and cyclic heat stress (○).

The release pattern of FSH was examined in a similar fashion to that of LH. The hourly FSH concentration in the interval between 48 h before to 12 h after the LH surge peak was subjected to analysis of variance at 6-h periods (n=10) as it was done for LH (Table 55). There were no differences between cycles or replicates, and also no cycle*period or Rep*period interactions, suggesting that the release pattern of FSH was not influenced by temperature or breed during this interval.

FSH release patterns in the interval between 12 h before to 48 h after the preovulatory LH surge peak are depicted in Fig. 19. Following the surge, FSH release patterns showed two major differences from the corresponding patterns of LH release. Firstly, subsequent to the surge peak mean FSH concentration tended to fall and remained low for a short period during which means were low. This transient period of relatively stable means of FSH occurred between 8-14 h and 9-16 h after the LH surge peak in the NT cycle and CHS, respectively. As in the case of LH, the comparison of the hourly FSH pulse frequency during the period with the pulse frequency during the 48-h interval before the LH surge peak showed no change in the mean pulses of FSH (data not shown). Secondly, a second FSH surge followed the first surge. The second FSH surge was of longer duration but of smaller magnitude than the first surge. It occurred between 14-34 h and 16-37 h after LH surge peak during NT and CHS, respectively. The peak of the second surge occurred between 24-30 h in both cycles. During its ascending limb FSH values of the second surge were depressed during CHS so that concentration was lower than in the corresponding limb of second surge curve during the NT cycle. However, analysis of variance of the FSH concentration in the 48-h interval after the LH peak, at 6-h periods (n=10), showed no differences

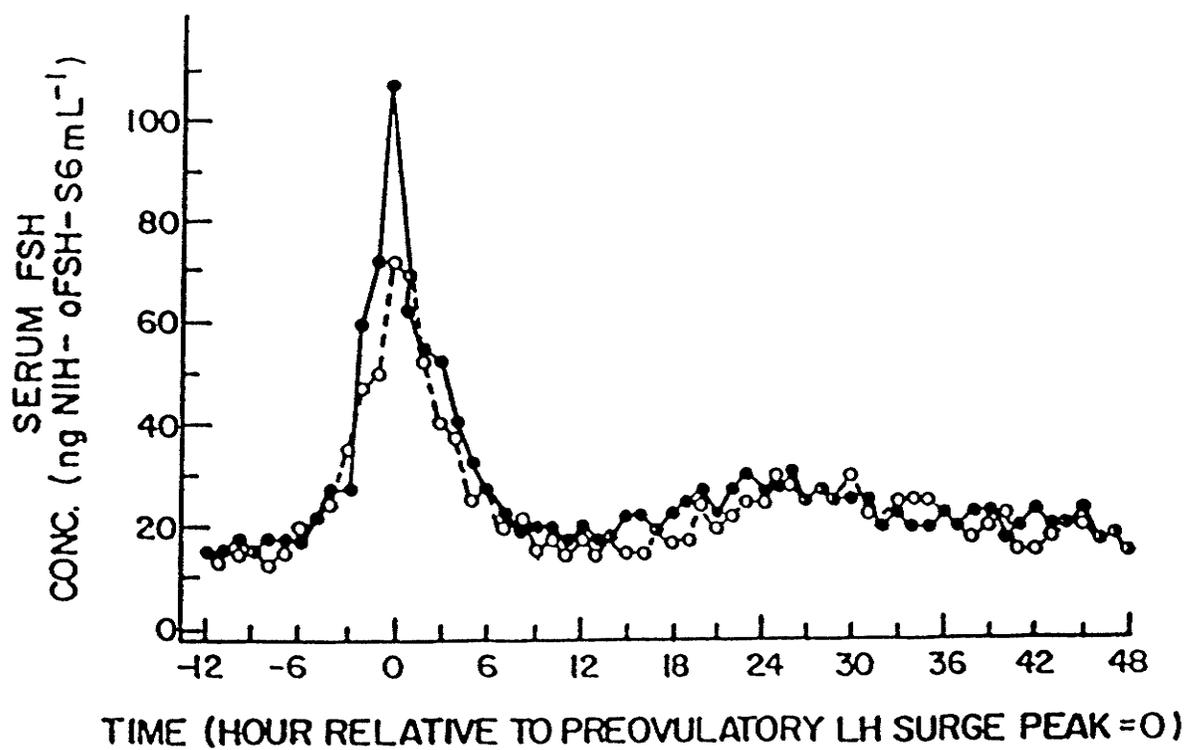


Figure 19: The hourly means of serum FSH between 12 h before to 48 h after the preovulatory LH surge peak (time=0) in cycling ewes (#15,16,18,21,22,25) at thermoneutral (●) and cyclic heat stress (○).

between cycles or replicates and also no cycle*period or replicate*period interactions (Table 56). These findings seem to suggest that CHS, as imposed during this investigation, had no effect on either the first or the second FSH surge.

5.4.5 PROLACTIN (PRL)

Mean daily PRL concentration and the circadian pattern of secretion have already been discussed in Manuscript 1.

Fig. 20 shows the changes in PRL concentration which accompanied the preovulatory gonadotropin surges. In contrast to LH and FSH surge curves which occurred simultaneously with overlapping peaks, the PRL surge curve that accompanied those surges varied in several aspects. Firstly in both cycles the duration of the surge curve was longer than that of the gonadotropins. Secondly, the curve failed to show the steeper rise to a peak followed by a gradual decline, which were characteristic features in the preovulatory gonadotropin surges. Instead, during the NT cycle the peak of PRL which occurred one hour after the LH surge peak, was preceded and followed by inconsistent fluctuations in amplitudes. Thirdly, during CHS, there were four peaks of PRL comparable in their magnitudes and durations to the peak observed in the NT cycle. Also during the CHS, PRL concentration dropped transiently to nadir concentration between 5-6 h before the LH surge curve peak.

Analysis of variance of preovulatory PRL concentration showed no differences between cycles and no cycle*hour interaction (Table 57), suggesting that although CHS appeared to have enhanced the PRL secretion

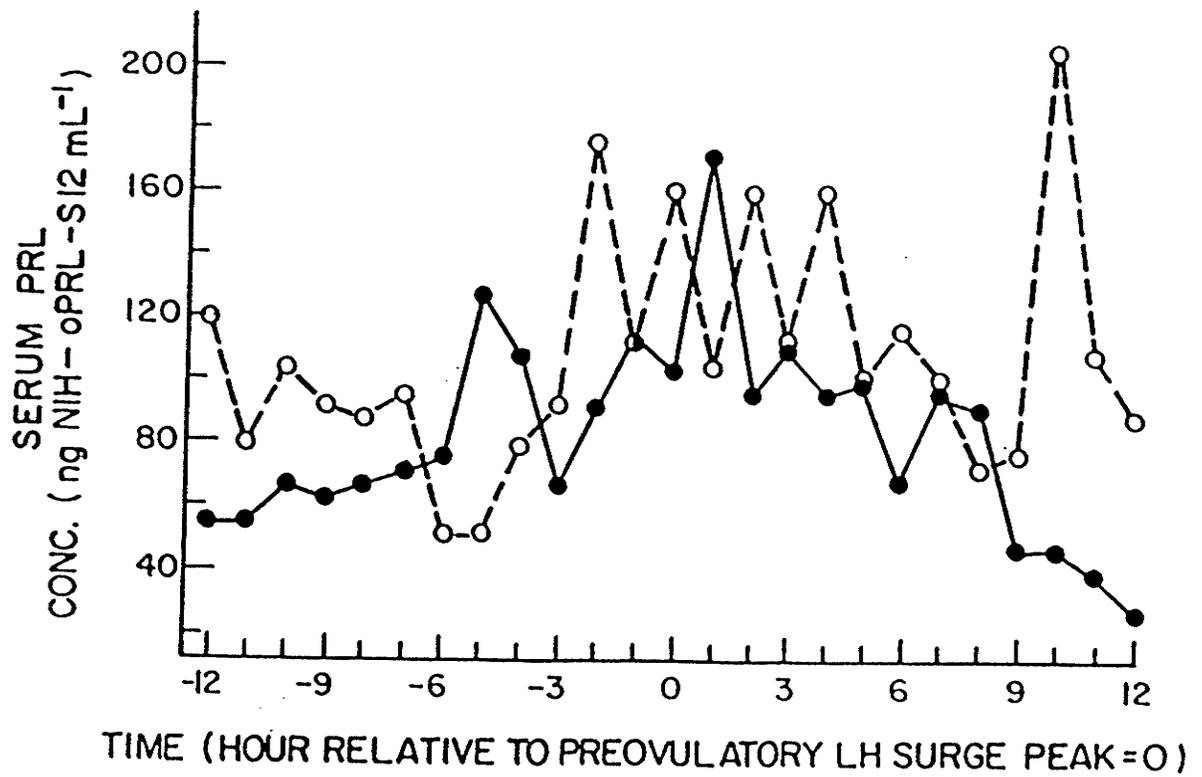


Figure 20: Mean concentration of hourly serum prolactin (PRL) at the preovulatory surge in cycling ewes exposed to thermoneutral (●) and cyclic heat stress (○).

during the surge, the hourly variation in mean PRL tended to be comparable between temperatures.

5.5 DISCUSSION:

Although the use of constant environmental settings to study the effect of heat stress on the fertility of domestic livestock are useful in pointing to the main trends in endocrine responses, they do not reflect natural conditions. Seasonal and daily variations in environmental constraints play an important role in the survival strategies of animals. Seasonal hot weather conditions have been implicated with disturbances in the animal's endocrine homeostasis which would eventually lead to reduced reproductive potential. Therefore, ways to study the effect of environmental heat stress on livestock in warm areas should be adjusted to possibly resemble the natural conditions. However, because of the important role played by radiant heat in contributing to heat stress under such conditions, even results obtained using cyclic heat stress but in heated rooms should be carefully interpreted.

Cyclic heat stress was used in this investigation to simulate the 24-h fluctuations under natural conditions. The nightly cool hours were provided for possible incorporation in management systems and also to assess the ability of the animal to recover from the daytime heat stress. One of the main problems of this design is the subjectiveness of the criteria for the onset of uniform heat stress knowing the tremendous buffering ability of individual animals (Manuscript 1). Heat stress was achieved in these ewes when ambient temperature rose above 31°C and was associated with an increase of 3-5 fold in respiration

rates and a rise of $>0.4^{\circ}\text{C}$ in rectal temperature (Manuscript 1). Thus, taking PRL, since it was the only hormone which closely followed the 24-h fluctuations in ambient temperature, we have observed that even at the peak of its response it did not necessarily correspond with the maximum heat experienced by the ewes.

The results of this investigation showed significant changes in hormone concentration during CHS in spite of the short interval during which the ewes were exposed to elevated temperatures (7-8 h), above the assumed upper limits of their thermocomfort zone of $27-28^{\circ}\text{C}$ (Manuscript 1). Another important observation was the significant replicate effect in most of the parameters examined in this investigation. Such differences could result from variation between breeds (Finn x vs Suffolk x) and also could be due to slight differences in T_{amb} between the two years (Manuscript 1). The patterns of mean daily cyclic P_4 during the NT cycle were similar to those described previously in cycling ewes (Thorburn et al. 1969; Bjersing et al. 1972). During CHS, the area under the cyclic P_4 curve tended to be larger due to elevated concentration in early- and mid-luteal phases of the estrous cycle (D5-D11). Following the early peak (D11) in CHS, elevated concentration was not maintained for a longer duration as has been reported in cycling sheep kept under constant heat stress (Sawyer et al. 1979). Subsequent to the peak, P_4 concentration fell steeply between D12-D14. However, a significant change in the course of this decline occurred after D14, where the fall was slower as compared to the NT cycle. Thus, these results tend to agree with the report by Yenikoye et al. (1982) of prolonged intervals from the onset of the initial decline of P_4 to the preovulatory LH surge peak in cycling tropical sheep in Niger (hot-dry

climate). However, our results suggested that, elevated P_4 in early- and mid-luteal phases of the estrous cycle during exposure to cyclic hyperthermia, does not necessarily lead to the prolongation of the cycle, if the CL starts to regress at the expected time of luteolysis.

A detailed observation of P_4 secretion by the regressing CL during the 72 h preceding the preovulatory LH surge peak showed that concentration was significantly elevated during CHS at 54-36 h before the peak. In contrast, concentration in the NT cycle was basal ($<0.5 \text{ ng mL}^{-1}$) at 48 h before the LH surge peak. The pattern of CL regression during the NT cycle was in agreement with recent data from Haresign (1985) who estimated the interval from basal P_4 to the onset of the preovulatory LH surge in cycling ewes to be 37 ± 2 h and that the duration of LH surge curve to be 13 h.

Although elevated P_4 concentration during the preovulatory period in cycling ewes has been shown to inhibit the gonadotropin surge (Karsch et al. 1979), successful surges have been reported in ewes with circulating P_4 values of 1.0 ng mL^{-1} (Jeffcoate et al. 1984). According to this latter group the interval from P_4 withdrawal to gonadotropin surge was longer in ewes with initially high P_4 values. In our results, the fall of P_4 subsequent to the peak of D11 during the CHS failed to show a progressive drop as it did during the NT cycle. As a result the duration from basal P_4 to LH peak was shorter than in the NT cycle. Since elevated P_4 concentration did not prevail for an extended period, the cycle lengths were comparable in both cycles. Nonetheless, a shorter duration from basal P_4 to the onset of LH surge at CHS could possibly be detrimental to the maturation

of the oocyte (Trouson and Moore 1974).

Moreover, CHS had no significant influence on the development of the new CL. Serum P_4 concentration was comparable between the two cycles from 36 h before to 72 h after the preovulatory LH surge peak. P_4 secretion by the newly formed CL showed a steady rise after 48 h following the LH surge peak. Possible sources of basal P_4 following the the complete regression of the CL could be the atretic follicles and the adrenal cortex. Since P_4 concentration remained basal and did not show significant fluctuations in both cycles, until the formation of the new CL, our investigation could not establish a credible compensatory adrenal P_4 secretion as a result of either the regression of the CL or heat stress in the cycling ewe. Nevertheless, it could be possible that acute exposure to CHS might prompt an exaggerated adrenal response which would lead to an overflow of P_4 into the circulation.

When mean daily concentration of LH was examined the progressive decline as the cycle advanced was more pronounced during CHS, possibly as a result of elevated P_4 concentration. The correlation coefficients between daily cyclic concentration of LH and P_4 were $r=-0.47$ ($p>0.05$) and $r=-0.65$ ($p<0.01$) during NT and CHS, respectively.

The pattern of preovulatory LH release during CHS has been the focus of various reports which reveal many inconsistencies due to differences in the intensities and durations of exposure to heat stress. Also there are major variations between species in their response to elevated temperatures. In cycling ewes and cows subjected to either constant or fluctuating heat stress, basal LH concentration has been reported to be depressed (Madan and Johnson 1973; Miller and Alliston 1974b;

Schillo et al. 1978; Hill and Alliston 1981). Yet Fuquay et al. (1980) and Gwazdauskas et al. (1981) have reported no change in LH release characteristics in cattle exposed to fluctuating heat stress. Our data tended to agree with the findings of the latter group, in which cyclic CHS had no effect on basal LH secretion.

Moreover, during CHS there was a slight reduction in the area under the preovulatory LH surge curve. The tendency of the LH surge curve to be smaller during this cycle despite the low basal P_4 concentration in the 30-hour interval preceding its onset, suggested that following P_4 withdrawal the full recovery of the hypothalamo-pituitary response might be positively related to the interval from basal P_4 to the onset of the surge. Accordingly, this axis might have required the 48 h of basal P_4 , as observed in the NT cycle, to recover from the possible residual effects of the negative feedback.

In agreement with data from cycling ewes (Hill and Alliston 1981) and cows (Miller and Alliston 1974b), CHS significantly depressed the preovulatory LH surge peak ($p < 0.05$) in the present study. The depression might have resulted from the tendency of the pulse amplitudes to be low. Low pulse amplitudes have been reported in ovx ewes primed with a constant concentration of estrogen and where an elevated concentration of P_4 was maintained with implants (Jeffcoate et al. 1984). As mentioned above, since P_4 concentration was basal in our ewes, results could not rule out the existence of other factors beside the negative feedback of steroids, which would have prevented the full expression of the normal pulse amplitudes of LH. In addition, CHS did not influence the duration of the preovulatory LH surge curve.

The duration of the LH surge curve was comparable to intervals reported previously in cycling ewes (McNeilly et al. 1981a; Haresign 1985). Also during the preovulatory period CHS had no significant influence on the pulse frequency of LH, the duration of its pulse peaks or the interpeak intervals.

When the mean elevations of LH were examined in the interval between 48 h before to 48 h after the LH surge peak, it appeared that CHS had no effect on either basal LH or the occurrence of the LH pulse peaks.

However, in the 48-h interval subsequent to the surge, mean LH revealed two features. Firstly, mean LH was relatively low between 12-30 h after the peak. Since there was no apparent decrease in the pulse frequency, there must be a reduction in the pulse amplitudes. Indeed, mean LH during this transient period was slightly lower than the values observed prior to the onset of the preovulatory surge. Thus, our data would tend to agree with a report by Jeffcoate et al. (1984) that the preovulatory LH surge exhausted the releasable pool of this hormone. Secondly, subsequent to the transient period mean LH was comparable between to the values seen prior to the onset of the surge.

In contrast to LH, mean daily FSH concentration showed a progressive decline as the cycle advanced, in a fashion that was inversely related to daily cyclic P_4 during NT ($r=-0.72$, $p<0.01$) and CHS ($r=-0.82$, $p<0.001$). Because the FSH release is presumably a dually controlled by the hypothalamic gonadotropin-releasing hormone (GnRH) and inhibin-like peptides secreted by the growing follicles (Ireland and Roche 1983; Clarke et al. 1984; McNeilly 1985; Vale et al. 1986), the progressive decline of the mean daily cyclic concentration of this hormone could

reflect reduced stimulation by GnRH as well as the increasing suppression by the follicular peptides as the follicles grow.

The effect of CHS on the preovulatory FSH surge was similar to its effect on the LH surge. The area under the FSH surge curve was smaller ($p < 0.05$) during CHS than NT. Nonetheless, unlike LH, the preovulatory surge peaks of FSH were comparable between temperatures, but again as in LH, there was no significant change in basal FSH during the preovulatory period during CHS. Heat stress had no significant influence on either the pulse frequency of FSH release, the duration of its pulse peaks, or the interpeak intervals.

Examination of FSH release patterns in the 48-h interval before the preovulatory LH surge peak also revealed some similarities to LH release during the same interval. Basal FSH did not seem to be affected by CHS. However, in contrast to LH, during both cycles, basal FSH concentration was slightly depressed in the period immediate to the onset of the surge (1-3 h). Therefore, our data indicated that cyclic CHS had no effect on the release characteristics of FSH in the 48-h interval preceding the onset of the preovulatory surge.

Following the preovulatory surge, the release patterns of FSH differed from that of LH. Subsequent to the surge, FSH concentration fell and stabilized for a transient period during which the mean of the pulse amplitudes appeared to be low as in LH. The onset of and the duration of this transient period was similar between cycles. FSH concentration during this period was higher than the values observed prior to the onset of LH surge. Thus, our observations disagree with those of Jeffcoates et al. (1984) who concluded that the preovulatory

FSH surge did not deplete the releasable FSH reserve in ovx ewes. Our results suggested that the duration of this transient period, of relatively low mean pulse amplitudes of FSH, after the FSH surge was not influenced by CHS.

Another major variation between the secretory characteristics of LH and FSH following the preovulatory gonadotropin surges was the occurrence of a second FSH surge between 14-36 h after the LH surge peak. This feature has been previously reported in cycling ewes (Pant et al. 1977; Bister and Paquay 1983; Lahlou-Kassi et al. 1984). Heat stress had no significant influence on the duration of the second FSH surge. The second FSH surge had the following characteristics. Firstly, it was not accompanied by a detectable rise in LH concentration. Secondly, it was longer in duration but of smaller magnitude than the preovulatory FSH surge curve. Thirdly, the magnitude of its ascending limb was depressed so that the mean values were lower than the corresponding limb in the NT cycle. Fourthly, its onset occurred when mean LH was still relatively low suggesting that either the hypothalamic control of pituitary function differentially stimulated FSH secretion at this stage, or that ovarian factors were involved in the second surge.

In Manuscript 1 it was indicated that the 24-h fluctuations in PRL concentration closely followed the daily variations in the Tamb (Manuscript 1) despite the chronic nature of the exposure to heat stress. We have also shown that during the hot interval the response of PRL to CHS overshadowed any daytime release patterns by this hormone.

In this investigation, we have attempted to examine the patterns of PRL release which accompanied the preovulatory gonadotropin surges.

During both cycles PRL surge curves occurred over a longer duration as compared to LH or FSH surge curves. In the NT cycle PRL surge peak occurred an hour later than that of LH. Although the elevated concentration of PRL during the preovulatory period could reflect increased secretion of estrogens (Howland et al. 1984), the factor which causes the preovulatory surge of PRL to occur concurrently with those of gonadotropins is not known, particularly since this hormone is not thought to be luteotropic in the ewe.

The effect of elevated PRL in response to heat stress on the hypothalamo-pituitary axis is not well understood. In cycling ewes rendered hyperprolactinemic by repeated administration of exogenous TRH, the estrogen rise which follows the sustained increase in LH during the preovulatory period has been reported to be reduced (McNeilly and Baird 1977). On the other hand, though such treatment in the ovx ewe had also blunted the estrogen-induced LH surge, it had no effect on GnRH-induced LH surge (Kann et al. 1976), suggesting that hyperprolactinemia interferes with the pituitary response to the direct or indirect stimulation by estrogens.

The present results showed that, PRL remained elevated for an extended period which overlapped with the preovulatory gonadotropin surges. Therefore, the coincidence between elevated PRL and the depression in the magnitudes of the gonadotropin surges during CHS, tends to suggest a cause-effect relationship. Nonetheless, the present investigation falls short of making a direct link between the two endocrine responses to CHS. It could be possible that even though the negative feedback of the ovarian factors was absent for a sufficient

period to allow the full recovery of the hypothalamo-pituitary axis during CHS, elevated PRL might have depressed the function of this axis.

In summary, heat stress tended to increase the area under daily P_4 curve mainly due to elevated P_4 in the early- and mid-luteal phases (D5-D11). During the regression of the CL, the interval from basal P_4 to the preovulatory LH surge peak was shorter in the heat-stressed ewes. Following the regression of the cycle CL, P_4 concentration remained basal, until the CL of the next estrous cycle started to function. Thus, the results provide no evidence of secretion of significant amounts of P_4 from an organ other than the CL during the period when the CL of the cycle regresses until the formation of the next cycle's CL.

Heat stress reduced the area under the preovulatory surge curve of FSH and also depressed the LH peak. Heat stress had no significant effect on the release characteristics of LH and FSH. Also following the preovulatory surges the pulse amplitudes of LH and FSH tended to be low for a transient period. Recovery from this refractory period was earlier for FSH. A second FSH surge occurred between 14-36 h after the preovulatory LH surge. Heat stress did not influence the duration of the second FSH surge but tended to reduce the magnitude of the ascending limb. Furthermore, CHS elevated the PRL surge which accompanied the preovulatory LH and FSH surges.

MANUSCRIPT #3

EFFECT OF INHIBITING PROLACTIN RELEASE ON THE CORPUS LUTEUM
FUNCTION AND THE CIRCULATING PATTERNS OF LH AND FSH IN
CYCLING EWES EXPOSED TO A THERMONEUTRAL AND TO A CYCLIC HEAT
STRESS TEMPERATURE

6.1 ABSTRACT:

The effect of twice daily injections of 1.0 mg Bromocryptine (CB-154) on the response of adult (55.9 ± 2.9 kg liveweight) cycling ewes exposed to a thermoneutral temperature (NT) of $18.0 \pm 1.0^\circ\text{C}$ (constant) and to a cyclic heat stress (CHS) of 18°C - 35°C - 18°C was investigated in two consecutive cycles in a temperature-controlled environmental chamber. Estimates of progesterone (P_4), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (PRL) were made on daily (D1-D13) and hourly (D14-D18) serum samples using radioimmunoassay (RIA) procedures.

Heat stress significantly ($p < 0.05$) increased area under the P_4 curve. The interval from basal P_4 to the onset of LH surge was longer during CHS (42 h) than NT (24 h). Following the regression of the corpus luteum (CL) P_4 concentration remained basal until the newly formed CL started to function. Also CHS enhanced the function of the new CL so that it started to secrete increasing amounts of P_4 at 54 h after the LH surge peak as compared to 66 h during NT.

Results showed that area under the LH surge curve was larger ($p < 0.05$)

during the CHS cycle. FSH surges and peaks were comparable between cycles in CB-154 treated ewes. Nonetheless, during CHS the second FSH surge curve which started immediately after the first tended to have a longer duration (27 h vs 22 h) and a greater magnitude than the corresponding second surge during NT.

Data suggested that during CHS CB-154 treatment failed to modify the tendency of daily cyclic P_4 to be elevated, but it reduced the duration of the interval from the withdrawal of the negative feedback of P_4 to basal P_4 concentration and hence facilitated an ample period for the recovery of the hypothalamo-pituitary positive feedback response.

Results also showed that, in the CHS cycling ewe, the magnitude of the preovulatory LH surge curve was positively related to the duration of the interval from basal P_4 to the onset of the surge.

6.2 INTRODUCTION:

Increased prolactin (PRL) release has been associated with lactational and seasonal anestrus in the ewe (Fell et al. 1972; Lamming et al. 1974; Rhind et al. 1980) and the inhibition of PRL secretion with bromocryptine (CB-154) has been reported to enhance the resumption of cyclicity in post-partum ewes (Fitzgerald and Cunningham 1981). Although such data would seem to suggest that PRL might be involved in reproduction of the ewe, the literature reveals many inconsistencies. For instance, Kann and Denamur (1974) have reported that when luteal phase ewes were given 4-times daily injections of PRL, immediately after hypophysectomy, the corpus luteum (CL) function was maintained 12 days.

In contrast, the elevation of PRL concentration by direct infusion into ovx ewes (Chamley 1978) or by repeated injections of exogenous thyrotropin-releasing hormone (TRH) into cycling (McNeilly and Baird 1977) or ovx (Kann et al. 1976) ewes had no significant influence on luteinizing hormone (LH) concentration or the LH response to exogenous gonadotropin-releasing hormone (GnRH). Furthermore, the treatment of cycling ewes with CB-154 was reported to have had no significant effect on the length of the estrous cycle, behavioral estrus, gonadotropin concentration or the subsequent function of the CL (Niswender 1972; Louw et al. 1974; Rodway et al. 1983).

Elevated PRL concentration has been reported in heat-stressed cycling (Hooley et al. 1979; Fraser and McNeilly 1980; Manuscript 1) and ovx (Schillo et al. 1978) ewes. Evidence from our studies suggested that the increased PRL release during CHS, resulted from enhanced basal secretion (Manuscript 1). Earlier data by Smith et al. (1977) indicated that the elevated PRL in response to CHS, was associated with a reduction in the metabolic clearance rate of this hormone. Therefore, in view of the lack of data on the effect of suppressing PRL release during CHS on the estrous cycle and hormone concentration in the ewe this investigation was undertaken to study the effect of inhibition of PRL secretion with CB-154 on the CL function and the secretory characteristics of gonadotropins during the preovulatory and early follicular periods in cycling ewes exposed to NT and CHS temperatures.

6.3 MATERIALS AND METHODS:

6.3.1 ANIMALS AND TEMPERATURE TREATMENTS:

Mature (55.93 ± 2.90 kg liveweight) cycling Finnish Landrace ewes ($n=5$) were used in the breeding season (Sept.-Feb.) for two consecutive cycles in a temperature-controlled environmental chamber to investigate the response of CB-154 treatment on the reproductive hormones during exposure to a thermoneutral temperature (NT) of $18 \pm 1^\circ\text{C}$ (constant) and to a cyclic heat stress (CHS) of $18^\circ\text{C}-35^\circ\text{C}-18^\circ\text{C}$. The description of the chamber, temperature exposure, relative humidity, photoperiod, synchronization of estrus, management of animals, feeding and the chronology of sampling procedures were as described before in the General Materials and Methods and in Manuscript 1.

6.3.2 BROMOCRYPTINE (CB-154) TREATMENT:

Because estrus was synchronized with two injections of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) 10 days apart, CB-154 treatment was started at 48 h before the second injection (i.e. 4 days before the ewes were moved into the environmental chamber). Each ewe received 1.0 mg of CB-154 (2-bromo- α -ergocryptine methane sulfonate, Sigma Co., St. Louis, Mo., U.S.A.) twice daily (at 1100h and 2300h) in the brisket area (subcutaneously). The treatment was continued until D19 of the cycle following the CHS cycle. CB-154 was initially dissolved in alcohol and then saline was added to produce a final ratio of 95 parts saline to 5 parts alcohol. It was prepared every 15 days in the solvent at a concentration of 1.0 mg mL^{-1} and was kept at 4°C .

6.3.3 SAMPLE HANDLING AND HORMONE ASSAYS:

Daily samples were collected a few minutes before the daytime injections of CB-154 (1050h-1100h). Serum samples were harvested and

stored as described before (General Materials and Methods). P_4 , LH, follicle-stimulating hormone (FSH) and PRL concentrations were estimated according to the radioimmunoassay (RIA) procedures described previously (Manuscript 2). PRL was estimated as described in Manuscript 1.

6.3.4 STATISTICAL ANALYSIS:

The calculations of RIA data, areas under hormone curves and the preliminary summary of the preovulatory concentration of LH and FSH using the Pulsar program analysis was as described in the General Materials and Methods. The length of the estrous cycle was estimated to be the interval (in days) between two consecutive preovulatory LH surge peaks. Data for P_4 was either examined as daily profiles during the estrous cycle, or means were compared by arbitrarily dividing the cycle into follicular (D1-D4), early-luteal (D5-D8), mid-luteal (D9-D12) and late-luteal (D13-D16) phases. In addition, the CL function during its regression and formation was assessed by following the 6-h pools of P_4 between 72 h before to 72 h after the preovulatory LH surge peak (time=0). For statistical analysis this interval was further divided into 18-h periods. As in Manuscript 2, the use of the term 'basal P_4 ' in this investigation always refers to P_4 concentration of $<0.7 \text{ ng mL}^{-1}$.

Mean hourly LH and FSH concentrations were examined using two separate intervals. The 48-h interval before and also the 48-h interval after the preovulatory surge peak of LH. Because of differences between ewes in the time of the occurrence of the LH peak, complete records were available for 3 ewes in either interval. The LH and FSH profiles in these two intervals were analysed at 6-h periods (n=10). Daily and hourly estimates of LH and FSH as well as the Pulsar-generated summaries

were subjected to analysis of variance using ANOVA procedures in the statistical packages provided by Statistical Analysis System (SAS Institute, Inc. 1982). Cycles, days, hours and periods were also compared using ewes as blocks (Snedecor and Cochran 1967). Wherever applicable, simple correlations were calculated according to Snedecor and Cochran (1967).

6.4 RESULTS:

6.4.1 PROLACTIN (PRL):

The assayable volume in the PRL assay was 50 μL . PRL concentration was below the detection limit of the assay ($<1.06 \pm 0.05 \text{ ng mL}^{-1}$) in all ewes, irrespective of the temperature of the exposure.

6.4.2 PROGESTERONE (P_4):

6.4.2.1 DAILY P_4 CONCENTRATION:

Fig. 21 shows mean daily P_4 concentration during the NT and CHS cycles. During the NT cycle P_4 concentration rose steadily from low concentration in the follicular phase reaching a peak on D11. After D12, P_4 declined precipitously to basal values during D16-D17. Progesterone concentration was higher in the follicular and mid-luteal phases during the CHS cycle (D1-D8). Values on D4, D5, D6, D8 and D10 were significantly higher than on the corresponding days during the NT cycle ($p < 0.01$, by paired t-test). During the declining phase towards the preovulatory period, mean daily P_4 concentration was comparable between the cycles. As a consequence of the elevated P_4 during early- and mid-luteal phases of the cycle during CHS, the analysis of variance

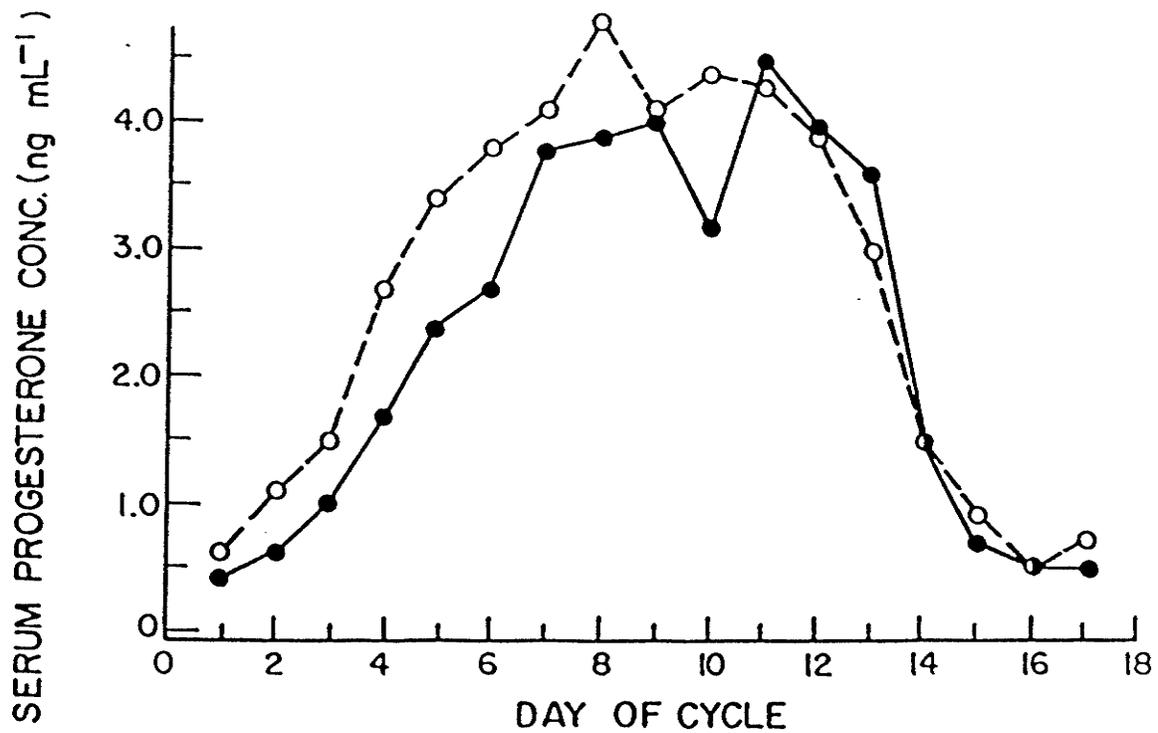


Figure 21: Mean daily concentration of serum progesterone (P_4) in CB-154-treated cycling ewes at the thermoneutral (●) and the cyclic heat stress (○) estrous cycles.

revealed that the area under daily P_4 curve was different between cycles ($p < 0.05$) (Table 58). Also, analysis of variance of phasic concentrations of cyclic P_4 showed significant differences between cycles ($p < 0.01$) (Table 59). There was a significant cycle*phase interaction ($p < 0.05$) due to elevated P_4 in early-luteal phase during CHS. Means (\pm SEM) of phasic P_4 are given in Table 60.

6.4.2.2 CORPUS LUTEUM (CL) FUNCTION:

Fig. 22 depicts the 6-h estimates of P_4 concentration between 72 h before to 72 h after the preovulatory LH surge peak. Prior to 54 h before LH surge peak, P_4 concentration was above 2.0 ng mL^{-1} in both cycles but was significantly higher in the CHS cycle ($p < 0.05$). During NT, P_4 fell steeply from $2.69 \pm 0.6 \text{ ng mL}^{-1}$ at (-72 h) to $1.22 \pm 0.36 \text{ ng mL}^{-1}$ at (-54 h). Subsequently, there was a gradual decline to 0.88 ng mL^{-1} at (-36 h) to reach basal concentration. P_4 concentration during this cycle remained basal until the formation of the new CL.

In contrast to the NT cycle, P_4 concentration during CHS dropped from $2.74 \pm 0.28 \text{ ng mL}^{-1}$ at (-60 h) to basal concentration of P_4 at (-48 h) and remained low until 42 h after the preovulatory LH surge peak. During this interval the serum concentrations of P_4 were stable. The newly formed CL was secreting significant amounts of P_4 by 54 h after the LH surge as indicated by the increase in serum P_4 . Analysis of variance of the 6-h P_4 at 18-h periods ($n=9$), showed no differences between cycles (Table 61). The cycle*period interaction was significant ($p < 0.001$) due to different trends in the response of the CL between cycles, especially in the interval before the LH surge peak. Means (\pm SEM) of P_4 concentration during the preovulatory period was shown in Table 62.

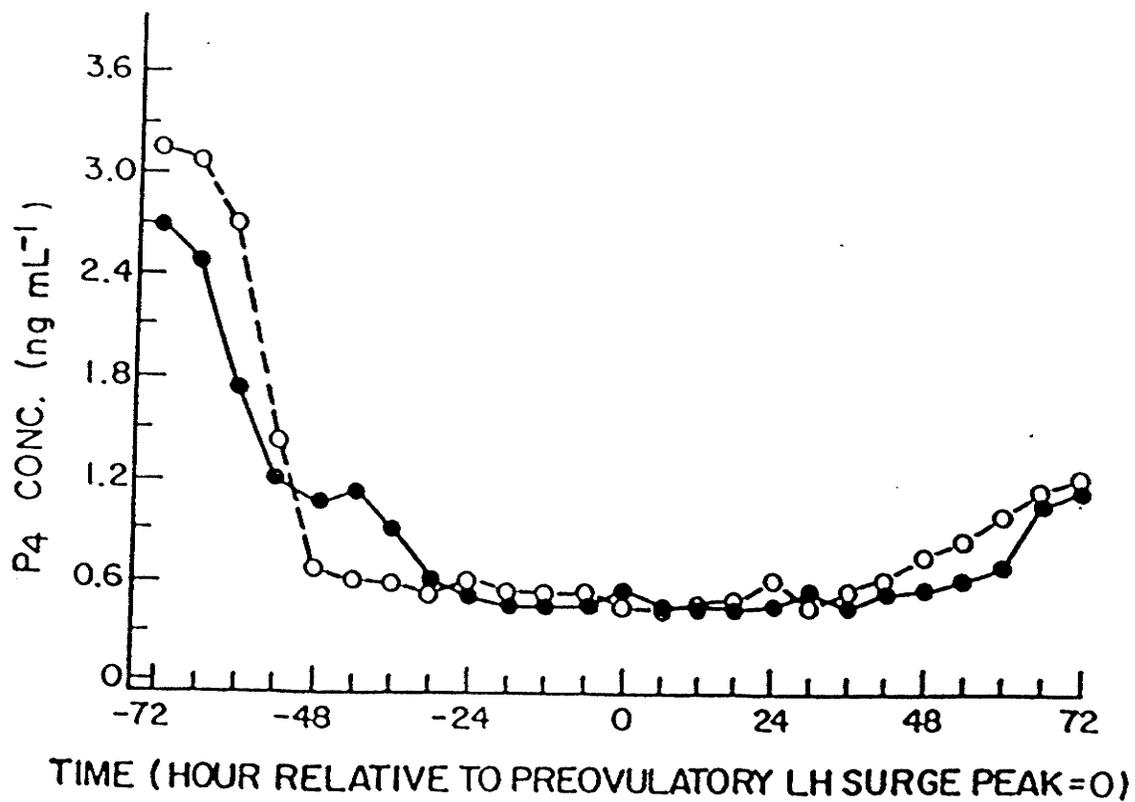


Figure 22: Mean 6-h concentration of serum progesterone (P_4) between 72 h before to 72 h after the preovulatory LH surge peak (time=0) in CB-154-treated cycling ewes at thermoneutral (●) and cyclic heat stress (○).

6.4.3 THE LENGTH OF THE ESTROUS CYCLE:

The length of the estrous cycle was slightly extended during the CHS cycle (16.56 ± 0.78 d) as compared to the NT cycle (15.49 ± 0.78 d). Analysis of variance for the comparisons of the length of the estrous cycles showed no significant differences between cycles (Table 58).

6.4.4 LUTEINIZING HORMONE (LH):

6.4.4.1 MEAN DAILY LH CONCENTRATION:

Mean daily LH concentration is shown in Fig. 23. LH concentration during the NT cycle was slightly higher on D1 and also during the preovulatory period (D16-D17). They showed no significant fluctuations in the early- and the mid-luteal phases. An attempt to correlate daily concentration of LH to those of P_4 during this cycle revealed a significant inverse relationship ($r = -0.78$, $p < 0.001$) (Table 63). During the CHS cycle daily cyclic LH showed inconsistent fluctuations and its inverse relationship with P_4 concentration was not significant. Analysis of variance of cyclic LH concentration by phase showed no differences between cycles or days (Table 64). Means (\pm SEM) of the cyclic LH by phase are shown in Table 65.

6.4.4.2 PREOVULATORY LH SURGE CURVE:

The preovulatory LH surge curves were examined over the interval between 12 h before to 12 h after the surge peak as shown in Fig. 24. The analysis of variance of the area under the LH surge curve showed significant differences between cycles ($p < 0.01$) (Table 66), as a result of a larger area during CHS ($p < 0.01$) (Table 67).

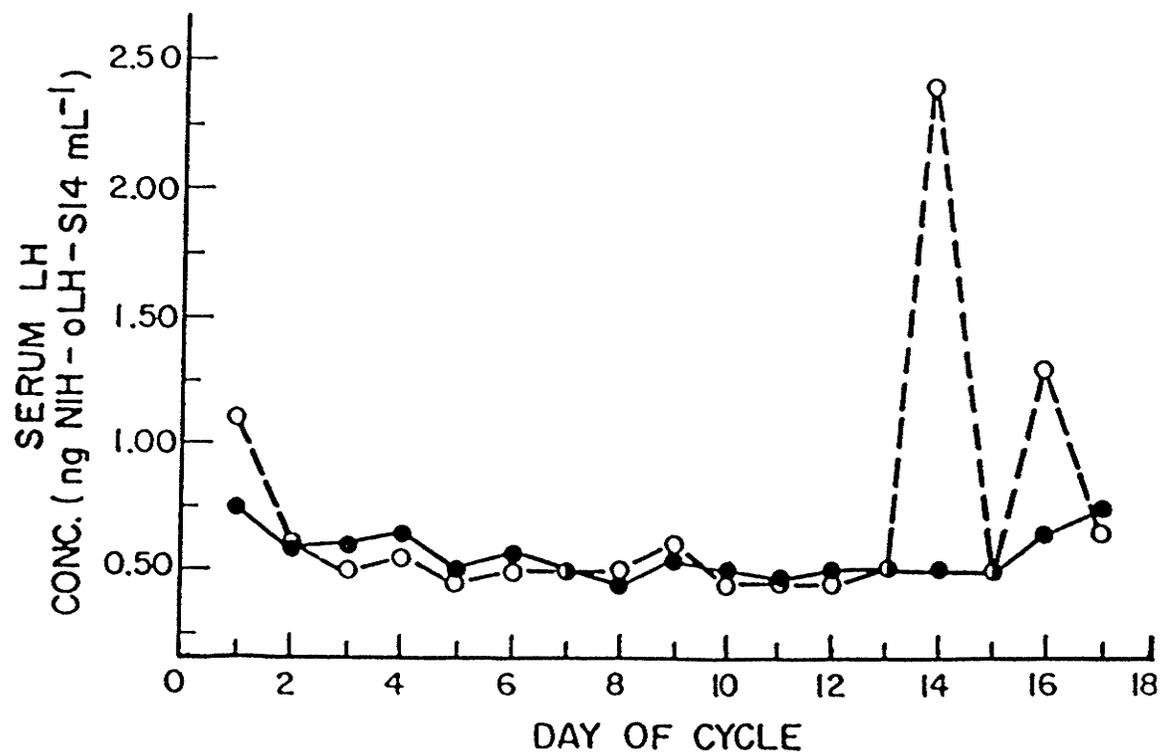


Figure 23: The mean concentration of daily serum LH in CB-154-treated cycling ewes at thermoneutral (●) and cyclic heat stress (○).

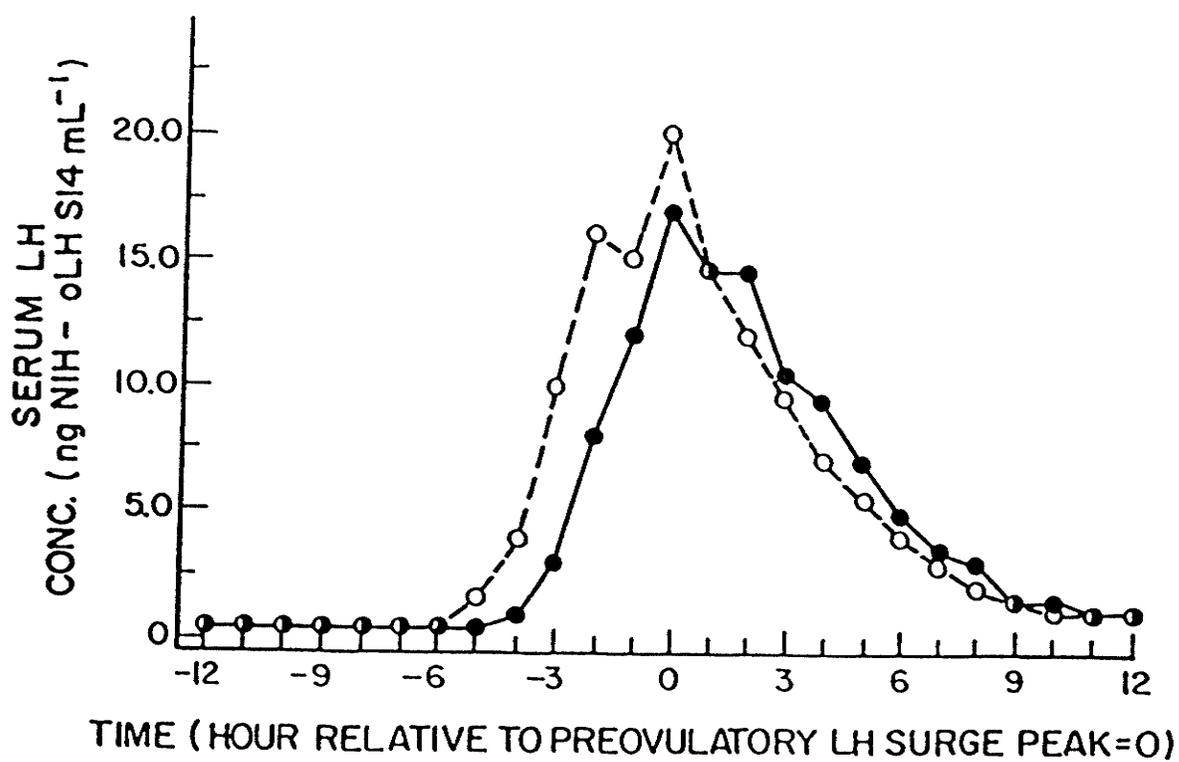


Figure 24: Mean concentration of serum LH at the preovulatory surge in CB-154-treated cycling ewes exposed to thermoneutral (●) and cyclic heat stress (○).

There was no differences between cycles in the duration of the LH surge (Table 66). The analysis of variance of the Pulsar-generated data for the LH release characteristics during the preovulatory period showed that CHS had no significant influence on basal LH, LH pulse amplitudes, frequency, the duration of LH pulse peak or the interpeak intervals (Table 68). Means (\pm SEM) of the release characteristics of the preovulatory surge of LH are depicted in Table 67.

6.4.4.3 LH RELEASE PATTERNS:

The release patterns of LH were examined at an hourly intervals using two data sets. In the first set, data included estimates between 48 h before to 12 h after the LH surge peak. Whereas, the second set of data included estimates between 12 h before to 48 h after the surge peak. Analysis of variance of the hourly LH at a 6-h periods (n=10) revealed no differences between cycles and also no cycle*period interaction (Table 69).

The examination of LH release patterns between 12 h before to 48 h after the preovulatory LH surge peak revealed that mean LH concentration was low in the 48-h interval following LH surge peak (Fig. 25). Analysis of variance of the hourly LH values in the 48-h interval after the surge peak, at 6-h periods (n=10), showed no differences between cycles (Table 70). However, the interaction between cycle*period was significant ($p < 0.05$) due to the an earlier rise of mean LH in the ascending limb of the surge curve during CHS.

6.4.5 FOLLICLE-STIMULATING HORMONE (FSH):

6.4.5.1 DAILY FSH CONCENTRATION:

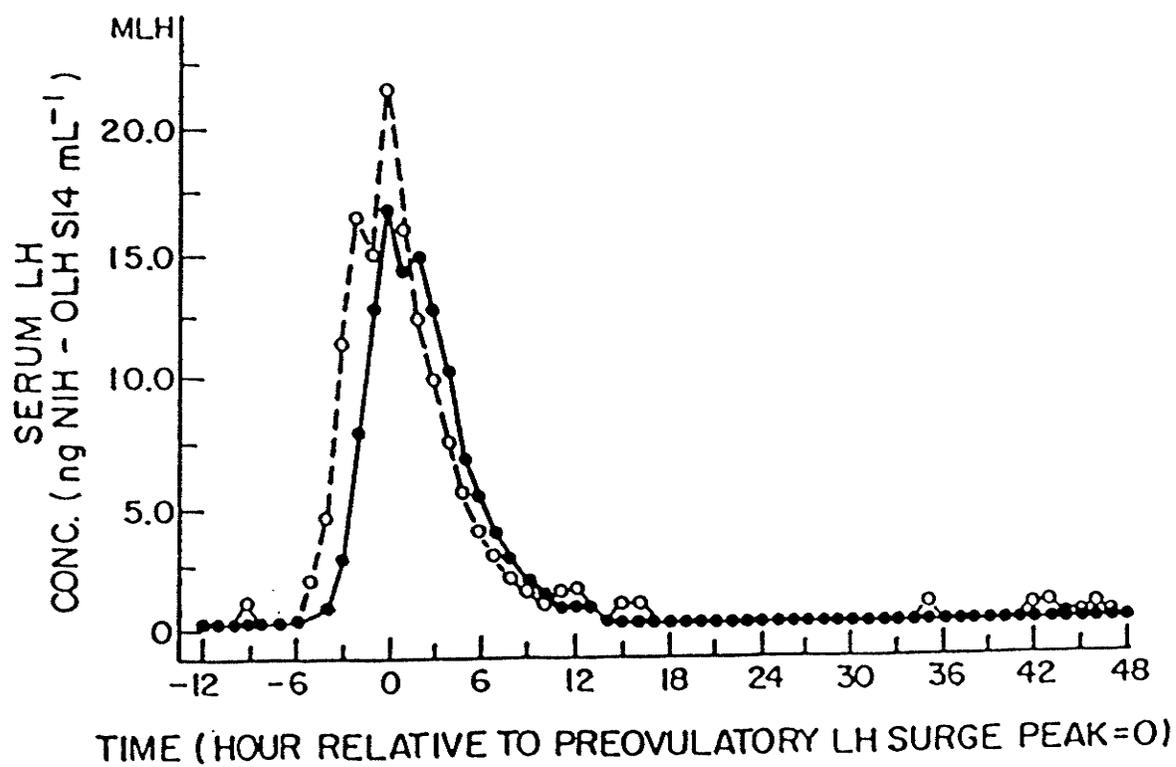


Figure 25: The hourly means of serum LH between 12 h before to 48 h after the preovulatory LH surge peak (time=0) in CB-154-treated cycling ewes (# 38,311,313) at thermoneutral (●) and cyclic heat stress (○).

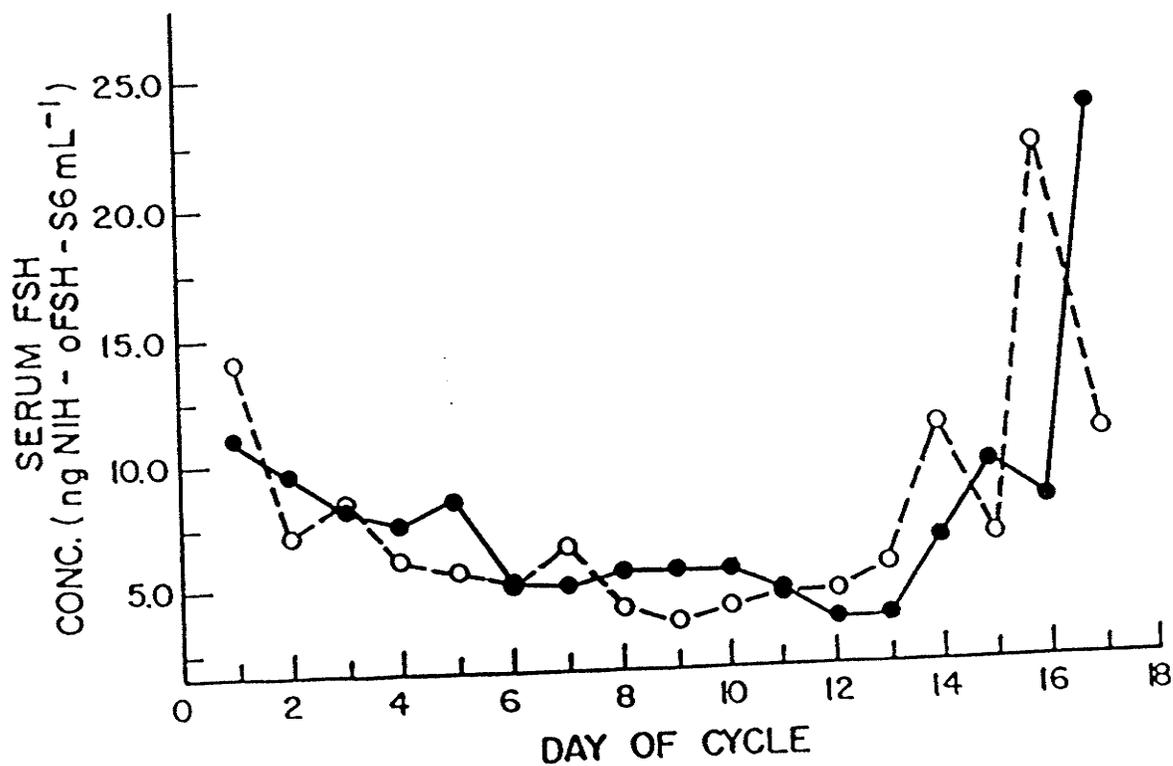


Figure 26: The mean concentration of daily serum FSH in CB-154-treated cycling ewes at thermoneutral (●) and cyclic heat stress (○).

Daily FSH concentrations are shown in Fig. 26. In contrast to daily cyclic LH concentration which showed a significant ($p < 0.001$) inverse relationship to cyclic P_4 only during the NT cycle, daily FSH levels showed significant inverse relationship to cyclic P_4 during NT ($r = -0.67$, $p < 0.01$) and CHS ($r = -0.75$, $p < 0.001$) (Table 63). In addition, mean FSH concentration was lowest in the mid-luteal phase when P_4 concentration was highest. Serum FSH tended to rise in the late-luteal phase (after D13 of either cycles). Analysis of variance of cyclic FSH concentration by phase revealed no differences between cycles (Table 71). Phase was significant ($p < 0.001$) because mean FSH tended to be lower in early- and mid-luteal phases of the cycle. Means (\pm SEM) of the cyclic FSH by phase are shown in Table 72.

6.4.5.2 PREOVULATORY FSH SURGE CURVE:

Fig. 27 shows the preovulatory FSH surge curves. FSH surge curves have been examined during similar intervals to those used for LH data. Both area under FSH surge and the surge peak were comparable between cycles (Table 66). The analysis of variance of the release characteristics of the FSH profiles during the preovulatory period revealed no differences between cycles for any of the parameters examined (Table 73). Means (\pm SEM) of the release characteristics of the FSH profiles are shown in Table 74.

6.4.5.3 FSH RELEASE PATTERNS:

Hourly FSH data was examined in a similar fashion to that of LH. The patterns of FSH release between 48 h before to 12 h after the LH surge peak were analyzed at 6-h periods ($n=10$). Analysis of variance

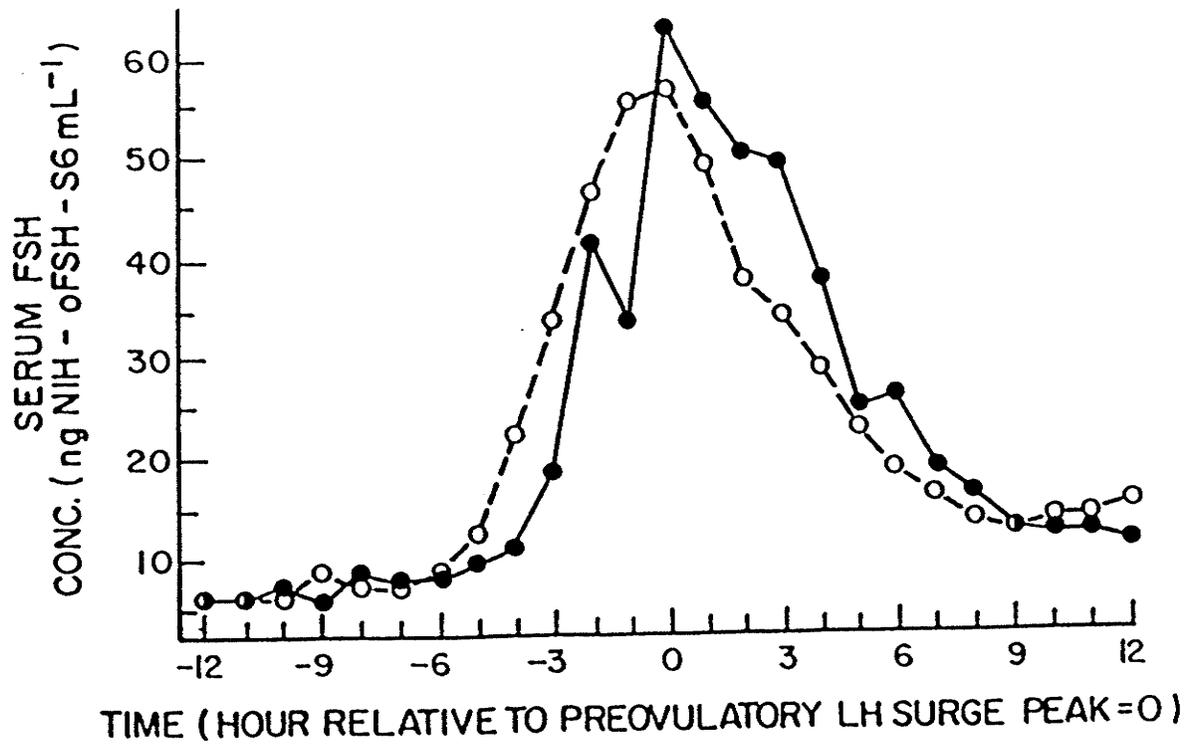


Figure 27: Mean concentration of serum FSH at the preovulatory surge in CB-154-treated cycling ewes exposed to thermoneutral (●) and cyclic heat stress (○).

of FSH values in this interval showed no differences between cycles (Table 75), indicating that temperature had no effect on mean FSH in this interval of the preovulatory period. The significant cycle*period interaction ($p < 0.001$) was due to higher mean FSH in the ascending limb of the surge curve during CHS as compared to NT.

Furthermore, the release patterns of FSH between 12 h before to 48 h after the preovulatory LH surge peak revealed several features that can be seen in Fig. 28. Following the preovulatory surge in either cycle, mean FSH concentration did not fall to values observed prior to that of the surge. During the NT cycle, FSH concentration remained relatively low for a short period (between 11-13 h after LH peak) before rising to a second surge. This second surge occurred between 14-36 h after the LH peak. Thus, the second surge had a longer duration and smaller magnitude than the first. The second peak occurred between 18-24 h after the LH peak. Also, subsequent to the second surge FSH concentration tended to fall to values observed before the onset of its first surge.

During the CHS cycle the steep decline of FSH subsequent to the preovulatory surge was immediately followed by a gradual but inconsistent rise to a second surge between 10-37 h after the LH surge peak. The peak of the second surge occurred between 22-26 h after the LH surge peak. During CHS FSH values in the ascending limb of the second surge tended to be higher than the values in the corresponding limb of the NT cycle. Following the second surge during CHS, FSH means reflected minimal fluctuations but its basal concentration remained higher than values seen prior to the onset of the first surge.

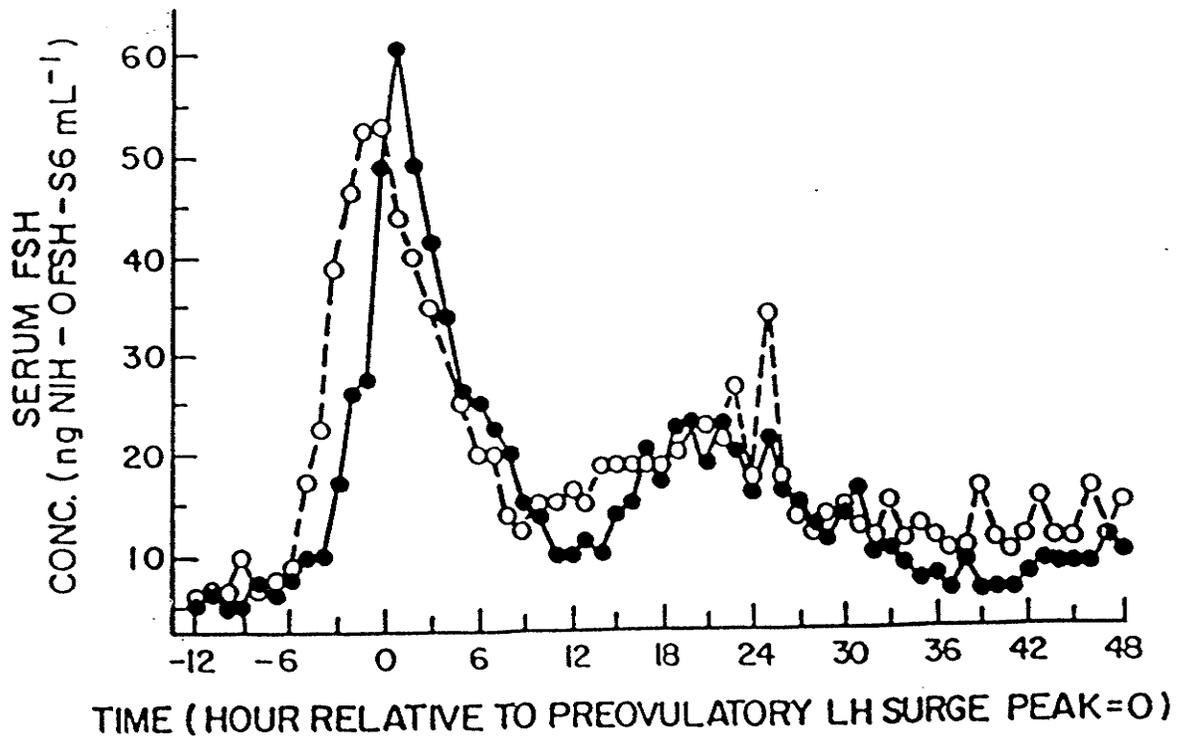


Figure 28: The hourly means of serum FSH between 12 h before to 48 h after the preovulatory LH surge peak (time=0) in CB-154-treated cycling ewes (# 38,311,313) at thermoneutral (●) and cyclic heat stress (○).

Analysis of variance of FSH concentration in the 48-h interval after the LH surge peak showed significant differences between cycles ($p < 0.01$) and also a significant cycle*period interaction ($p < 0.001$) (Table 76). Cycle differences were due to the tendency of mean FSH to be higher in the ascending limb of the first and second surges, and also due to a relatively raised basal FSH following the second surge, during CHS. The significant cycle*period interaction also reflected those trends which are mentioned above.

6.5 DISCUSSION:

The adoption of a reproductive management program to improve livestock fertility in a warm climate necessitates the consideration of two assumptions. Firstly, the main cause of the problem is assumed to be the excessive heat load which results in the disturbance of the normal interrelationships of hormones of the hypothalamo-pituitary-gonadal axis. Secondly, management should attempt to restore normal endocrine functions. We have seen in a previous report (Manuscripts 2 & 1) that despite an extended period of cool hours, CHS significantly enhanced CL function as well as the pituitary release of PRL; and perhaps as a consequence, some of the release characteristics of the gonadotropins were altered.

In the current investigation we have attempted to look further on the effect of reducing PRL release during CHS on pituitary-gonadal function. CB-154 was chosen to blunt PRL despite evidence that at low dose it might not completely block PRL secretion and at high concentration it might reduce pulsatile LH release (Hill et al. 1980). However, the dosage and the schedule used here were based on evidence that CB-154 at

doses of 1.0-2.0 mg given as once or twice daily injections per day had no significant effects on the length of the estrous cycle, behavioral estrus, preovulatory LH concentration or the subsequent CL function (Niswender 1972; Louw et al. 1974; Rodway et al. 1983).

During the investigation CB-154, at the dose of 1.0 mg given twice daily 12 h apart was effective in reducing peripheral PRL concentration to below the detection limit of our assay ($<1.06 \pm 0.05$ ng mL⁻¹). The hourly samples during the last 5 days of the cycle did not reveal any change in the efficacy of the treatment. Results also showed that PRL failed to escape this inhibition during CHS. Since CB-154 is a dopamine agonist, our results suggested that CHS disconnects the PRL inhibitory control on the lactotrophs by interfering with the dopaminergic control mechanism.

The elevated daily cyclic P₄ concentration in the early- and mid-luteal phases during the CHS cycle was in agreement with our previous report (Manuscript 2). Thus CB-154 treatment did not change the response of the CL to CHS. However, the elevated P₄ concentration was not maintained for a long enough period to significantly affect the length of the estrous cycle. Since neither the elevation of P₄ concentration in the early- and mid-luteal phases, nor the onset of P₄ withdrawal following peak values seemed to be significantly influenced by CB-154 treatment, results could not support a positive role by PRL on a fully functional CL in heat-stressed cycling ewes.

The examination of the regression of the CL in the interval before the onset of the preovulatory LH surge showed that the trends of the declining P₄ concentration were initially similar between cycles and

were comparable to those described in Manuscript 2. However, there was a dramatic change during the NT cycle. The steep drop in P_4 values which were seen on D13-D15 changed its course into a more gradual decline between 54-36 h prior to the LH surge peak. During this interval, P_4 concentration was significantly higher than in the corresponding interval of the CHS cycle. Contrary to our previous report (Manuscript 2) in which the interval from the onset of CL regression to basal P_4 was shorter during the NT cycle, current results showed that this interval was shorter during the CHS cycle in CB-154-treated ewes. As a consequence, the interval from basal P_4 to the onset of LH surge (-48 h to -6 h) was longer during CHS (42 h).

Alternatively, the interval from the onset of CL regression to basal P_4 concentration was longer during NT in this investigation and consequently the interval from basal P_4 to the onset of LH surge (-30 h to -6 h) was shorter (24 h). The similarities between the characteristics of CL function during the NT cycle to that observed during the CHS cycle in our previous report (Manuscript 2) and the tendency to enhance the magnitude of the preovulatory LH surge in comparison to their respective corresponding cycles, suggested that the magnitude of a normal preovulatory LH surge is positively related to the duration of the interval from basal P_4 to the onset of the surge.

Results also showed that after the regression of the CL peripheral P_4 concentration remained basal until the formation of the new CL. This finding confirmed our previous observation (Manuscript 2) that in the cycling ewe there was no evidence of significant secretion of P_4 by another endocrine system, following either the normal regression of the

cyclic CL or as a result of exposure to chronic heat stress. The results of this investigation showed that in CB-154-treated ewes exposure to CHS enhanced the function of the newly formed CL. Substantial amounts of P_4 were detected at 54 h after LH surge peak during CHS as compared to after 66 h in the NT cycle. Whether the longer interval from basal P_4 to the onset of LH surge favored the development of several follicles which underwent atresia following the LH surge and hence contributed to circulating P_4 , needs further investigation. Progesterone concentration at 72 h following the preovulatory LH surge peak was comparable between cycles.

Moreover, beside the smaller area under LH surge curve and the slightly lower surge peak in the NT cycle, results suggested that CB-154 treatment had a uniform effect on the other release characteristics of this hormone during the preovulatory period. Unlike our previous report (Manuscript 2) in which mean LH tended to be lower immediately following the preovulatory surge, failure to detect changes in mean LH subsequent to the surge in this investigation seems to imply that CB-154 treatment might have depressed the LH pulse amplitudes. Indeed, compared to our previous observations (Manuscript 2), basal LH tended to be lower in this investigation. This effect of CB-154 was not influenced by the exposure to CHS. Thus, our current results seem to agree with the findings in the literature, that CB-154 depresses LH secretion in the ewe (Hill et al. 1980). The fact that the preovulatory LH surge was enhanced by the CB-154 treatment during CHS, and this was associated with a longer duration of the interval from basal P_4 to the onset of the surge, implies that CB-154 might have no direct effect on the preovulatory surge. By this conclusion, we are tempted

to hypothesize that, elevated PRL release in response to CHS during the preovulatory period might depress the gonadotropin surges by influencing the negative feedback of the ovarian factors on the hypothalamo-pituitary axis. However, more conclusive evidence would be required to qualify this interpretation.

Thus, larger area under the LH surge curve as well as the tendency of the surge peak to be higher could be due to the longer interval from basal P_4 to the onset of the surge which favored a prolonged priming of the hypothalamo-pituitary axis by the sustained rise of estrogens. Indirect support for this interpretation comes from sheep data in which elevated PRL concentration has been shown to reduce the rise in estrogens that closely followed the pulse peaks of LH during the preovulatory period (McNeilly and Baird 1977). In addition, Kann et al. (1976) have reported that in ovx ewes exaggerated PRL secretion produced by repeated injections of thyrotropin-releasing hormone (TRH) inhibited estrogen-induced LH release.

Furthermore, the results of this investigation also showed an inverse relationship between daily cyclic FSH and P_4 concentration ($p < 0.01$), similar to the relationship between these two hormones in our previous report (Manuscript 2). However, basal FSH concentration during the preovulatory period was lower than in the previous report, particularly in the 48-h interval preceding the onset of its surge. Based on the recent literature, we tend to assume that this relationship reflects the correlation between the non-steroidal ovarian factors and basal FSH, rather than between P_4 and basal FSH. It follows that, in the present investigation, neither CB-154 treatment nor the exposure to CHS had

influenced the inverse relationship between the release pattern of basal FSH and the cyclic secretion of the non-steroidal ovarian factors.

In a previous report (Manuscript 2), we have shown that CHS reduced the area under the FSH surge curve and also tended to depress the first FSH surge peak. In the current investigation, the characteristics of the preovulatory surge were comparable between temperatures, suggesting that CB-154 treatment tended to reverse the effects of CHS on the FSH surge. Therefore, though the treatment with CB-154 has a uniform effect on depressing basal secretion of the gonadotropins, it influenced the preovulatory surges of either LH or FSH differently. Conversely, by carefully assessing the FSH data and assuming that the hypothalamic control of FSH release originates from a separate centre for basal secretion and another for the preovulatory surge, CB-154 treatment seems to have the same effect on both centres. This conclusion was drawn from the fact that CHS did not influence the FSH surge, which implied that the surge was already depressed by the CB-154 treatment to the extent that CHS could not depress it further.

The tendency of the second FSH surge to be larger during CHS than in NT is difficult to explain in terms of the relationship to the duration of the interval from basal P_4 to the onset of the first or the second surges. Since the onset of the second FSH surge almost overlapped between temperatures, our data implied that the occurrence of the second FSH surge was not influenced by the first surge. Additionally, even though the duration of the interval from basal P_4 to the onset of the second surge was about 15 h longer during CHS, the simultaneous occurrence of the second surges during both temperatures strongly

implicates the involvement of the non-steroidal ovarian factors in inducing this surge.

In summary, in CB-154-treated cycling ewes CHS elevated the daily cyclic P_4 in the early- and mid-luteal phases. The interval from basal P_4 to the onset of the preovulatory surge was longer during the CHS cycle (42 h) as compared to NT (24 h). After the regression of the cycle CL, P_4 concentration remained basal until the formation of the new CL. Data suggested that, the magnitude of the preovulatory LH surge was positively related to the duration of the interval from basal P_4 to the onset of the surge. The inhibition of PRL release in response to CHS tended to influence the rate of CL regression and hence indirectly affected the magnitude of the LH surge. Further, in ewes treated with CB-154, CHS had no influence on the magnitude of the first FSH surge as well as the onset of the second FSH surge. Also, neither the duration of the interval from basal P_4 nor the first FSH surge seemed to have influenced the onset of the second FSH surge.

The results suggested that in the ovx ewe, 1.0 mg of CB-154 injected twice daily was effective in suppressing PRL release to non detectable concentrations, irrespective of the temperature. However, because of the tendency of CB-154 to influence directly the release of GnRH, we would not recommend the use of this chemical in any investigations that are designed to evaluate the effect of PRL on the gonadotropin release.

MANUSCRIPT #4

PROGESTERONE AND CORTISOL RESPONSE TO AN ACUTE DOSE OF ACTH OR
SALINE IN OVARIECTOMIZED EWES EXPOSED TO A THERMONEUTRAL
AND TO A CYCLIC HEAT STRESS TEMPERATURE

7.1 ABSTRACT:

The response of progesterone (P_4) and cortisol to the injection of an acute single dose of adrenocorticotrophic hormone (ACTH), or saline was investigated in ovx ewes exposed to a thermoneutral temperature (NT) of $18 \pm 1^\circ\text{C}$ and to a cyclic heat stress (CHS) temperature of 18°C - 35°C - 18°C , for 15 days. Ewes were given a ration which was calculated at 2.5 times maintenance. Ewes were frequently bled for 10 h on the 14th day of exposure and serum samples were assayed for P_4 and cortisol using radioimmunoassay procedures.

Results showed that CHS tended to raise mean P_4 and depress mean cortisol concentration. The injection of ACTH significantly enhanced the secretion of cortisol and P_4 ($r=0.75$, $p<0.001$), irrespective of temperature. During CHS, the responses of P_4 and cortisol to ACTH were characterized by an initial acute rise, a transient drop and finally a prolonged elevation. Also following the injection of ACTH significant changes in cortisol and P_4 concentration were detected within 10 min and 15 min, respectively. The P_4 response to ACTH was comparable between temperatures. Since P_4 values in response to either CHS or the ACTH injection were within the range of basal P_4 which was reported in the literature to occur during the preovulatory period in cycling ewes, our

results give no evidence of a direct role for adrenal P_4 , in depressing the pituitary function in long-term heat-stressed ewes.

7.2 INTRODUCTION:

Exposure of cycling ewes and cows to chronic heat stress has been shown to be associated with elevated serum progesterone (P_4) concentration (Abilay et al. 1975a; Roussel et al. 1977; Manuscripts 2,3) and depressed gonadotropin release (Manuscript 2). Since chronic exposure to heat stress also depresses the adrenal activity (Christison and Johnson 1972; Alvarez and Johnson 1973; Shayanfar et al. 1975; Manuscript 1), the source of the elevated P_4 could be the CL itself.

Indeed, in spite of existing reports of increased P_4 secretion following the injection of exogenous ACTH into ovx (Benhaj and Cooke 1985) and ovx-hypophysectomized (De Silva et al. 1983) ewes, we have shown data (Manuscripts 2,3) which revealed that, during the preovulatory period of heat-stressed cycling ewes, P_4 concentration following the CL regression remained basal until the formation of the new CL. In a recent report, Wheeler and Blackshaw (1986), have shown the possible involvement of the increased release of catecholamines during heat stress in the direct enhancement of P_4 secretion from the CL. Therefore, as P_4 is considered a precursor for adrenal synthesis of cortisol, this experiment was initiated to determine: 1) if CHS leads to increased adrenal P_4 secretion in the ovariectomized (ovx) ewe, and 2) whether CHS modifies the adrenal response to an acute exogenous dose of adrenocorticotrophic hormone (ACTH).

7.3 MATERIALS AND METHODS:

7.3.1 ANIMALS:

Adult (85.19 ± 2.15 kg liveweight) ovx Suffolk ewes ($n=4$) were shorn and kept in individual metabolic crates in a temperature-controlled environmental chamber. Ewes were given a ration similar to the one described for the cycling ewes in Manuscript 1, but were fed individually. The description of the chamber, control of temperature, relative humidity, photoperiod, the procedures for ovariectomies and the maintenance of the ewes were as described in the General Materials and Methods. Ewes were twice exposed to two-week periods of thermoneutral temperature (NT) of $18 \pm 1^\circ\text{C}$ (constant) and to a cyclic heat stress (CHS) of 18°C - 35°C - 18°C in an alternate fashion so that during each temperature they either received an acute single injection of saline or ACTH (Porcine-ACTH1-39, Sigma Chemical Co., St. Louis, MO, U.S.A.).

7.3.2 SAMPLING PROCEDURES:

Ewes were subjected to intensive blood sampling sessions between 700h-1700h on D14 of each treatment period, using jugular catheters inserted on D13. On the day of the sampling session, 7 mL of blood was drawn into 10 mL plastic syringes at 15 min intervals for 2 h prior to the injection, starting at 700h. At 900h, ACTH (5 I.U. porcine-ACTH1-39 kg^{-1} liveweight^{0.75}) or saline was injected as a single dose through the jugular catheter (total volume = 5 mL), and samples were collected at 0, 5, 10, 15, 20, 25 and 30 min after the injection. Thereafter, sampling was continued at 15 min intervals until the end of the session at 1700h. Sera were harvested and stored at -20°C until hormone assays. P_4 and cortisol were estimated using radioimmunoassay (RIA) procedures.

7.3.3 HORMONE ASSAYS:

Procedures for hormone assays were as described previously for P₄ (Manuscript 2) and cortisol (Manuscript 1).

7.3.4 STATISTICAL ANALYSIS:

The calculations of RIA results and the preliminary analysis of serum P₄ and cortisol estimates by the Pulsar program were described in the General Materials and Methods. Subsequently, data were subjected to analyses of variance (ANOVA) using computer programs according to the software packages provided by Statistical Analysis Systems (SAS Institute, Inc. 1982). The entire data sets were examined by comparing hormone concentrations in the two hours before the injection to hormone concentration at hourly periods to the end of each sampling session (n=9). Hormone secretion characteristics for the entire data set were compared between temperatures and treatments. In addition, comparisons between treatments and temperatures were made using 2x2x4 factorial analysis (Snedecor and Cochran 1967). The two factors were treatment (ACTH vs saline) and temperature (18°C vs 35°C); ewes were considered as blocks. Wherever necessary, simple correlation coefficients were calculated according to Snedecor and Cochran (1967).

7.4 RESULTS:

7.4.1 AMBIENT TEMPERATURE (T_{amb}):

Fig. 29 shows the 24-h fluctuations of T_{amb} during the investigation. There were minimum fluctuations in the 24-h T_{amb} during exposure to NT (18.29±0.01°C). During exposure to CHS, on the other hand, T_{amb} rose

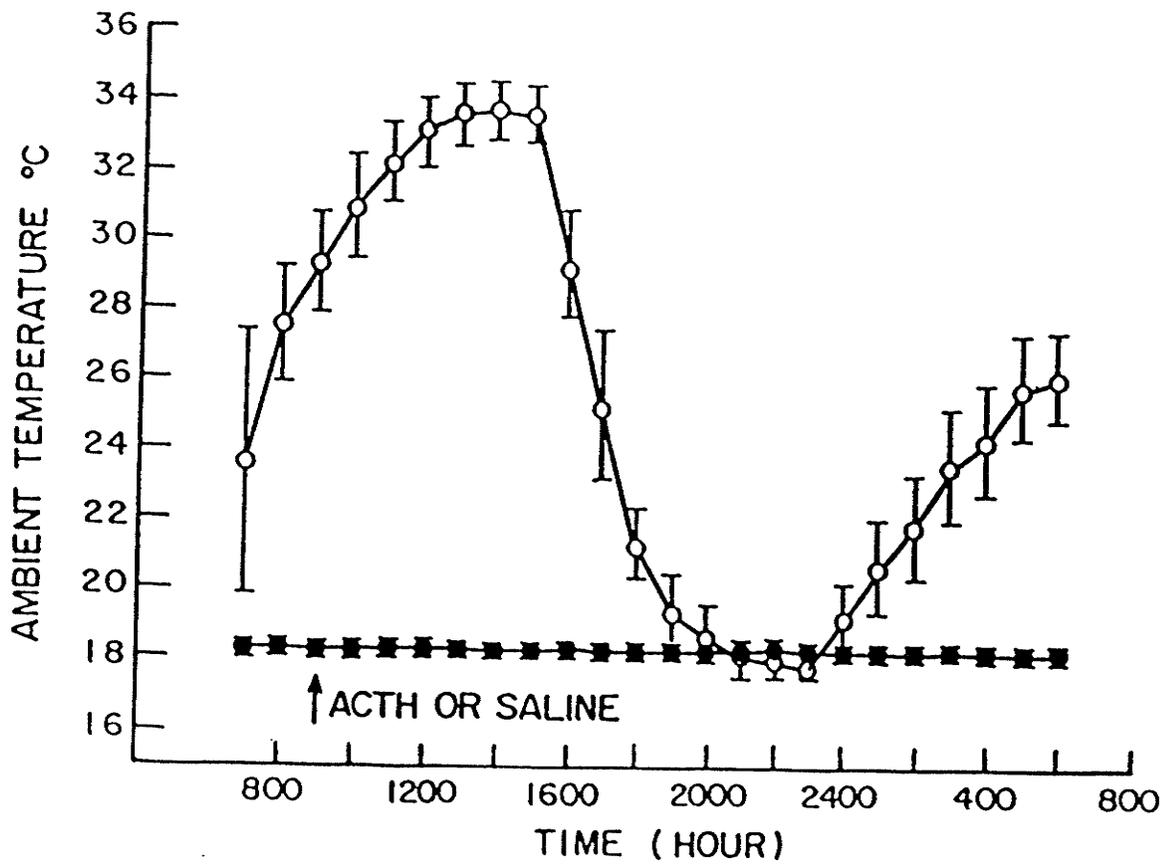


Figure 29: Mean (\pm SEM) daily fluctuation of ambient temperature at thermoneutral (\bullet) and cyclic heat stress (\circ) at the ACTH STUDY.

progressively from $23.5 \pm 3.7^\circ\text{C}$ at 700h, to $29.3 \pm 1.4^\circ\text{C}$ at 900h, to $32.1 \pm 1.2^\circ\text{C}$ at 1100h and reached a peak of $33.6 \pm 0.90^\circ\text{C}$ at 1400h. Following a short plateau between 1300-1500h T_{amb} fell steeply to $<22^\circ\text{C}$ within 2 h and remained at this temperature until the early morning hours. Due to technical problems with the chamber control system during the hyperthermic exposures the maximum T_{amb} recorded between 1400-1500h was lower than the programmed temperature of $34-35^\circ\text{C}$.

7.4.2 PROGESTERONE (P_4):

7.4.2.1 PROFILES OF P_4 FOLLOWING THE INJECTION OF SALINE:

The P_4 profiles before and after saline injection were shown in Fig. 30. Within the same temperature P_4 secretion patterns in the intervals before and after the injection were comparable. Mean P_4 concentration showed elevations but the peaks were more discrete during the normothermic exposure and less so during CHS. The magnitude of the means were greatly reduced during CHS. Despite the tendency of P_4 concentration to be lower during CHS.

7.4.2.2 CONCENTRATION OF P_4 FOLLOWING THE INJECTION OF ACTH:

Serum P_4 profiles before and after ACTH are depicted in Fig. 31. Levels prior to the injection were comparable to those observed in the same interval prior to saline injection. Subsequent to the injection of ACTH. During NT P_4 rose acutely to values significantly higher than the pre-injection concentration within 15 min ($0.48 \pm 0.05 \text{ ng mL}^{-1}$) and progressed to a peak of $0.62 \pm 0.05 \text{ ng mL}^{-1}$ at 30 min after the injection. After the peak, P_4 concentration remained elevated for 70 min and then started to decline gradually over time.

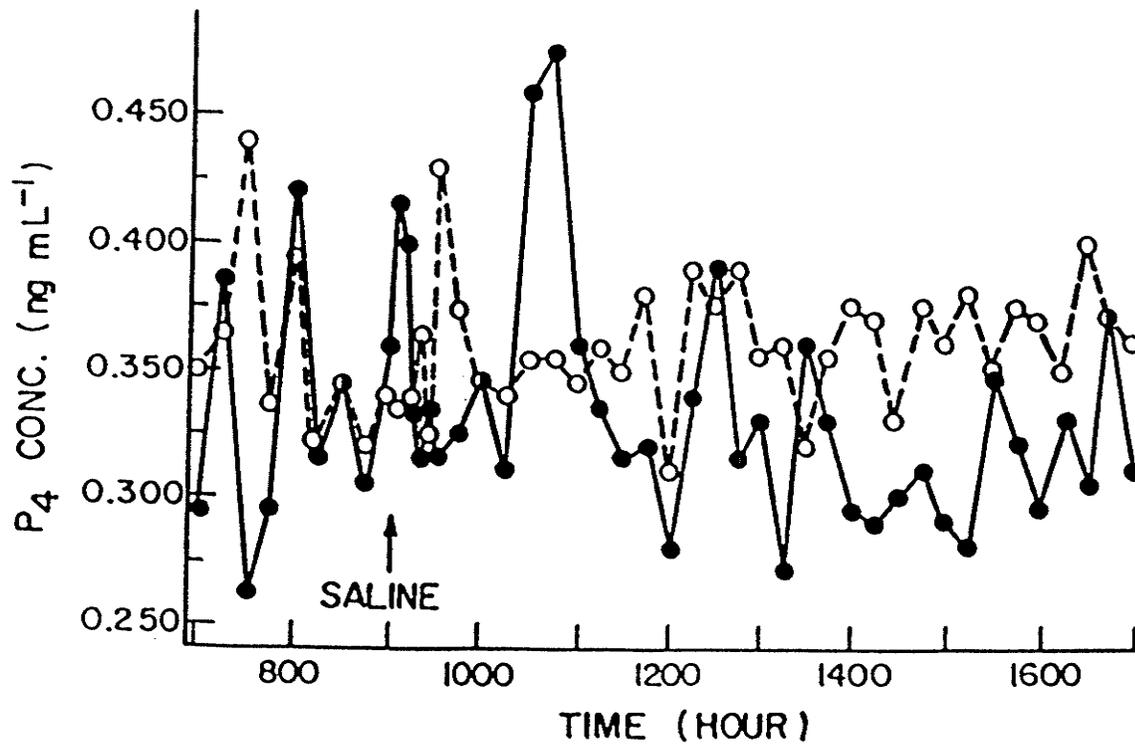


Figure 30: Mean concentration of serum progesterone (P₄) in ovx ewes in response to the infusion of saline (time=900h) at thermoneutral (●) and cyclic heat stress (○).

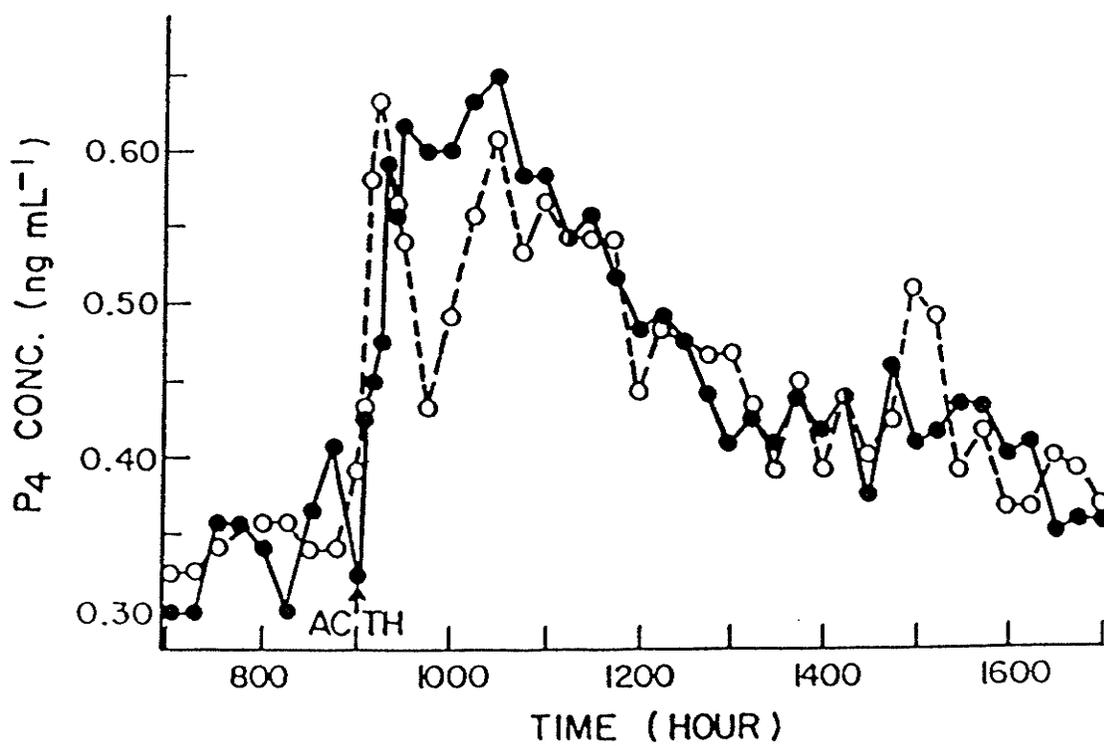


Figure 31: Mean concentration of serum progesterone (P₄) in ovx ewes in response to the infusion of an acute single dose of ACTH (time=900h) at thermoneutral (●) and cyclic heat stress (○).

In contrast to NT, P_4 secretion after ACTH during CHS revealed a biphasic response. Heat stress enhanced adrenal P_4 secretion so that at 10 min after ACTH its concentration was higher than pre-injection values. This early rise culminated in a peak at 15 min after ACTH, and then instead of the plateau in P_4 values seen during NT, concentration declined during the succeeding 30 min. A nadir value was observed at 45 min after ACTH. Subsequent to these low concentrations P_4 rose (but less steeply as compared to its initial response) to a second peak which was comparable in magnitude to the first. Like the first, the second peak was not succeeded by a plateau. Mean P_4 at 45 min after ACTH was lower than values of either peaks.

Analysis of variance of P_4 concentration at hourly periods ($n=9$) after the injection of saline or ACTH revealed significant differences between treatments ($p<0.05$) and periods ($p<0.01$) (Table 77). There were significant treat*period ($p<0.01$) and temp*period ($p<0.05$) interactions. The interaction of treat*period was due to a greater rise in P_4 following the injection of ACTH but not saline. The significant temp*period interaction was due to the biphasic response of P_4 secretion after ACTH during CHS as compared to NT. Means (\pm SEM) of the P_4 response are shown in Table 78.

Analysis of variance of the Pulsar data for the profile characteristics of P_4 in response to saline or ACTH showed no differences between treatments or temperatures and no interactions in all the parameters measured, except basal P_4 (Table 79). Basal P_4 was lower during NT with saline injection ($p<0.05$), as compared to the other treatment and temperature combinations (Table 80).

7.4.3 CORTISOL:

Data for cortisol was analyzed in a fashion similar to that of P₄.

7.4.3.1 CORTISOL CONCENTRATION FOLLOWING THE INJECTION OF SALINE:

Although cortisol profiles before and after saline injection were similar, between temperatures the secretion patterns were different. Irrespective of the temperature, an abrupt rise in cortisol followed the injection of saline (Fig. 32). However, values returned to pre-injection concentration within 60 min. Also, mean concentrations tended to be consistently lower during CHS.

7.4.3.2 CORTISOL CONCENTRATION FOLLOWING THE INFUSION OF ACTH:

Cortisol response to ACTH injection is shown in Fig. 33. Levels prior to injection were not significantly different between temperatures. Following the injection an acute increase in cortisol secretion was detectable within 10 min, irrespective of the temperature. During NT there was an acute steady rise reaching a peak at 75 min after injection. Levels subsequent to the peak reflected dramatic fluctuations in amplitudes with a tendency to fall gradually over time in a fashion similar to that of P₄ during the same temperature ($r=0.75$, $p<0.001$) (Table 81).

Alternatively, during CHS cortisol response to ACTH was enhanced between 10-25 min of the injection. However this earlier enhancement was followed by a transient decline, similar to the one observed for P₄, between 30-60 min after ACTH. Thereafter, cortisol concentration rose acutely to values seen during NT. Thus, cortisol response to ACTH

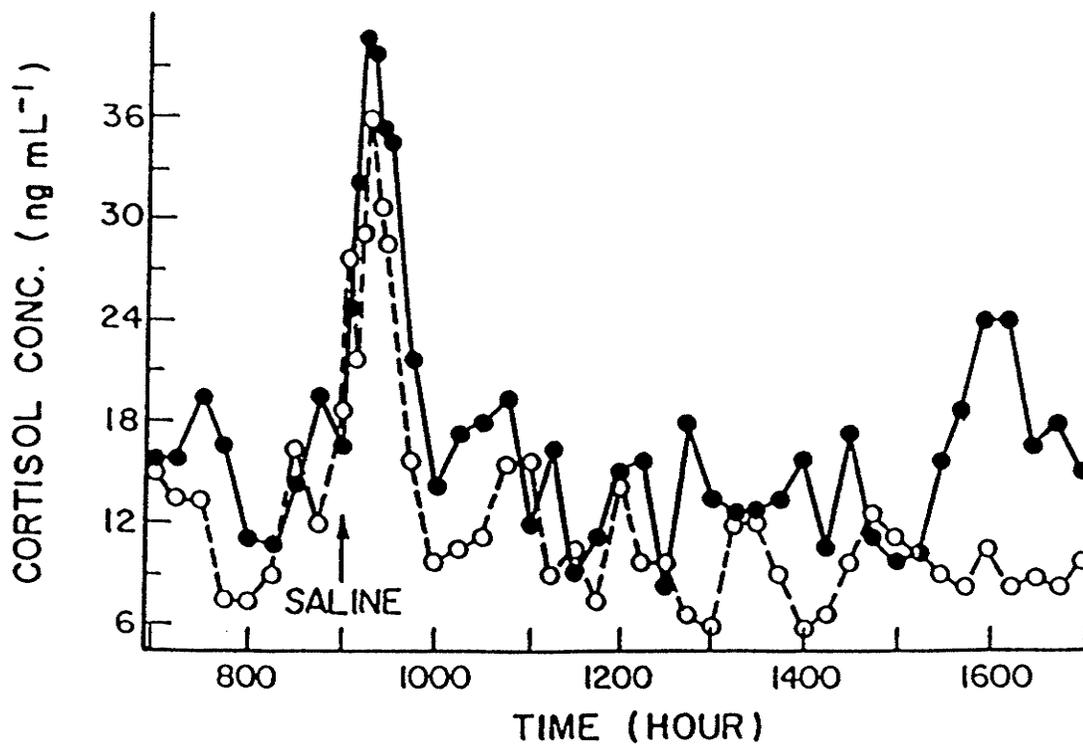


Figure 32: Mean concentration of serum cortisol in ovx ewes in response to the infusion of saline (time=900h) at thermoneutral (●) and cyclic heat stress (○).

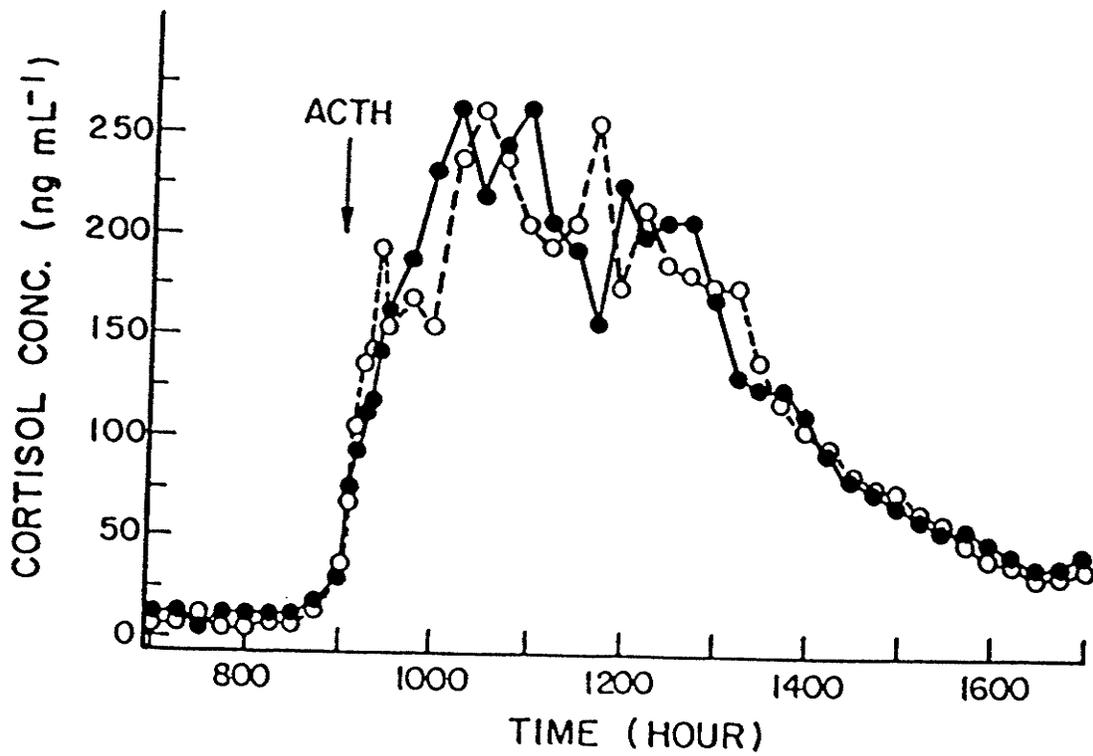


Figure 33: Mean concentration of serum cortisol in ovx ewes in response to the infusion of an acute single dose of ACTH (time=900h) at thermoneutral (●) and cyclic heat stress (○).

during CHS also showed a biphasic trend which was comparatively smaller in magnitude than that of P₄ during CHS ($r=0.50$, $p<0.001$) (Table 81). The first peak which occurred at 25 min after injection was significantly lower than the second which occurred 55 min later. Also, during the transient decline cortisol values at 60 min after ACTH tended to be lower than the corresponding concentration during NT.

The analysis of variance of the concentration of cortisol in response to the injection of saline or ACTH at hourly periods ($n=9$) showed significant differences between treatments ($p<0.001$) and periods ($p<0.01$), but no differences between temperatures (Table 82). Also, there was no interaction between treat*temp, suggesting a similar trend in the response of cortisol following saline or ACTH in either temperatures. The absence of interaction for temp*period indicated that there were no significant variation in the secretion patterns between between temperatures. The significant treat*period interaction ($p<0.01$) was due to the greater response of cortisol following the injection of ACTH. Mean (\pm SEM) concentration of cortisol response at hourly periods are given in Table 83.

The analysis of variance of the Pulsar characteristics of cortisol profiles in response to the injection of saline or ACTH revealed significant differences in basal cortisol ($p<0.01$) and pulse amplitudes ($p<0.05$) between treatments, but no differences between temperatures (Table 84). Mean (\pm SEM) of the cortisol profile characteristics are given in Table 85.

7.5 DISCUSSION:

In spite of the problems with the temperature control system during CHS, ewes in this investigation were assumed to have experienced a degree of heat stress comparable in magnitude to that reported earlier for cycling ewes (Manuscript 1). In that study, we showed the occurrence of significant physiological changes accompanied by major shifts in hormone release patterns when ambient temperature rose above 31-33°C. Although the physiological responses were not recorded during this study, the similarities between the trends of P_4 and cortisol release patterns during CHS in this and the earlier study indicated that these ewes were also heat-stressed.

By adopting a circadian pattern of T_{amb} , the aim of these studies was to reduce the severity of the homeostatic disturbance which occur when animals are exposed to constant high temperatures. Indeed, our results indicated only a moderate increase in basal P_4 (15%) and a decrease in basal cortisol (12%), as compared to values during NT. Hence, data suggested that in hyperthermic ovx ewes the adrenal tended to secrete more P_4 and less cortisol. Nevertheless, based upon previous observations on basal P_4 during the preovulatory period in cycling ewes (Manuscripts 2, 3), the estimates of P_4 values during CHS in this investigation were comparable in values to those in the earlier reports. Even after the injection of ACTH, P_4 response was comparable to the basal concentration observed during the onset of the preovulatory gonadotropin surges. Therefore, based on these findings, the importance of adrenal P_4 secretion, alone, during CHS in inhibiting the release of gonadotropins is doubtful.

Quantitatively, the peripheral concentrations of a hormone are

dependent upon its rate of secretion characteristics, its metabolic degradation and its clearance rates. Although the clearance rate plays an important role in varying hormone concentration in blood, this study was focussed on the secretion patterns and their characteristics. In previous reports (Manuscripts 1, 2, 3) we have observed that changes in the mean concentration of hormones could be brought about by variations in the pulse amplitudes without parallel significant changes in pulse frequency or the duration of the pulse peaks. In contrast, the current study revealed a different type of hormone secretion characteristics. For example, raised basal concentration of P_4 during CHS was brought about by the presence of relatively fewer pulses of longer durations; whereas, following ACTH injection basal concentration was raised as a result of the tendency to reduce the pulse frequency and extend the duration of the pulse peak, without significant changes in the pulse amplitudes.

Alternatively, lower basal cortisol concentration during the exposure to CHS resulted from the relatively low pulse amplitudes and shorter duration of pulses. Higher basal values in response to ACTH, on the other hand, was brought about by raising the magnitude of the pulse amplitudes, by the tendency to increase the frequency of the pulses but relatively, there was no reduction in the duration of the pulse peaks. Such variations in the release characteristics of P_4 and cortisol in response to either exposure to CHS or ACTH injection, strongly suggested the importance of a hormone release characteristics on its peripheral concentration.

Since adrenal P_4 functions as a precursor to cortisol biosynthesis we

did not anticipate a 5 min lag to detect significant changes in P_4 concentration as compared to those of cortisol following the injection of ACTH. Perhaps, this was due to the fact that the substrates for adrenal steroidogenesis are dually drawn from P_4 as well as from 17α -hydroxypregnenolone. The 5 min lag could also mean that, initially the rate of cortisol biosynthesis from the available pool of P_4 was faster than the rate of P_4 biosynthesis from its precursor, cholesterol. This interpretation was supported by the fact that the biphasic response of cortisol after ACTH during CHS paralleled that of P_4 at the same temperature, but its magnitude was comparatively smaller.

Moreover, adrenal steroidogenesis is under the direct control of ACTH which facilitates the transfer of cholesterol into the mitochondria and stimulates the enzymes that cleaves its side chain. ACTH also controls the activity of the enzyme 17α -hydroxylase which is vital to the biosynthesis of cortisol in sheep. Thus, the occurrence of the transient decline of P_4 and cortisol following the injection of ACTH during CHS and the absence of this feature during NT suggested that CHS might have reduced ACTH secretion and as a consequence diminished the adrenal cholesterol reserve. If this assumption was correct then why was there an inconsistency in the effect of CHS, alone, on the basal concentration of P_4 and cortisol? It was possible that low concentration of ACTH was capable of inducing a modest rise in P_4 secretion but were insufficient to stimulate the normal activity of the enzyme 17α -hydroxylase which synthesized cortisol.

In summary, the exposure of ovx ewes to chronic heat stress elevated basal P_4 concentration and depressed basal cortisol secretion.

Heat stress also modified the responses of P_4 and cortisol to exogenous ACTH into biphasic release patterns. Based on the findings of the present investigation, the response of adrenal P_4 to either CHS, alone, or to the exogenous ACTH was within the range of what is assumed to be basal concentration during the preovulatory period in cycling animals. Therefore, we conclude that, it is not likely for the adrenal P_4 , alone, to have a significant role in depressing the pituitary function in animals exposed to long-term heat-stress.

MANUSCRIPT #5

THE EFFECT OF TEMPERATURE AND BROMOCRYPTINE TREATMENT ON THE
RELEASE CHARACTERISTICS OF GnRH, LH AND FSH IN THE
OVARECTOMIZED EWE

8.1 ABSTRACT:

The effect of exposure to a thermoneutral temperature (NT) of $18 \pm 1^\circ\text{C}$ and to a cyclic heat stress (CHS) temperature of 18°C - 35°C - 18°C and bromocryptine (CB-154) treatment on the peripheral concentration and the release characteristics of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were investigated in progesterone-primed ovx ewes, prior to and following an acute single dose of GnRH.

The peripheral concentration of GnRH was very low. GnRH circulates in association with plasma proteins which interfere with the radioimmunoassay (RIA) if the procedure does not include an extraction step. In the ovx ewe, the basal concentration of endogenous GnRH, prior to the injection of exogenous GnRH was 4.8 pg mL^{-1} . During NT, GnRH was released at a pulse frequency of $0.66 \text{ pulses h}^{-1}$. The pulse frequency increased during CHS ($0.9 \text{ pulses h}^{-1}$, $p < 0.05$) and was decreased during NT in the CB-154 treated ewes ($0.48 \text{ pulses h}^{-1}$, $p < 0.05$). In addition, CHS reduced the disappearance rate of exogenous GnRH in the peripheral circulation ($p < 0.05$).

Neither the depressed pulse frequency of the GnRH following

CB-154 treatment during NT, nor its elevated concentration during CHS were paralleled by similar changes in LH and FSH. Although the CB-154 treatment tended to enhance the GnRH-induced secretion of LH and FSH during CHS, the responses were comparable irrespective of the temperatures or the treatment.

The results suggest that the pituitary response to exogenous GnRH was not significantly influenced by temperature or CB-154 treatment.

8.2 INTRODUCTION:

In sheep, the exposure to heat stress has been reported to be associated with an elevated progesterone (P_4) level (Sawyer et al. 1979), an exaggerated prolactin (PRL) release (Fraser and McNeilly 1980) and a depressed secretion of luteinizing hormone (LH) (Schillo et al. 1978; Hill and Alliston 1981). However, in cycling ewes, the LH response to exogenous GnRH was comparable during exposure to a normothermic and hyperthermic temperatures (Hooley et al. 1979). This suggests that the depressed LH concentration during CHS could have resulted from insufficient amounts of gonadotropin-releasing hormone (GnRH) (Jackson et al. 1978; Narayana and Dobson 1979; McNeilly et al. 1984), a change in the release patterns of GnRH (Levine et al. 1982; Clarke et al. 1984; Clarke and Cummins 1985a, 1985b), or interference with the pituitary release of the gonadotropins (Moss et al. 1981; Crowder et al. 1982; Wheaton and Mullett 1982). As a consequence, the estimation of the circulating GnRH concentration would be invaluable in elucidating the nature of the above mentioned changes.

Therefore, two trials were conducted to investigate:

1) the pituitary response to an acute single dose of an exogenous GnRH injection in progesterone-primed ovx ewes during exposure to NT and CHS; and 2) to evaluate the pituitary response to exogenous GnRH in the presence or the absence of elevated PRL in hyperthermic cycling ewes.

8.3 MATERIALS AND METHODS:

8.3.1 ANIMALS:

Adult, long-term ovariectomized (>5 mo), Finnish Landrace-cross ewes (n=4) were used in two trials to investigate the effect of ambient temperature (T_{amb}) on the pituitary response to exogenous gonadotropin-releasing hormone (GnRH). Ewes were shorn (fleece < 0.5 cm), individually housed in wooden crates and maintained inside a temperature-controlled environmental chamber. The daily ration was similar to the one described in Manuscript 1 for the cycling ewes. In each trial the ewes were exposed to two weeks of thermoneutral temperature (NT) of $18 \pm 1^\circ\text{C}$ and two weeks of a 12 h cyclic heat stress (CHS) temperature of 18°C - 35°C - 18°C . The relative humidity ($55 \pm 2\%$) and photoperiod (14D:10L) were kept constant.

From D6 to D13 of exposure each ewe received daily (i.m.) injections of 30 mg progesterone (P_4) prepared in 1.0 mL peanut oil. During the second trial, the ewes received twice daily (from D2 to D15 of exposure) injections (s.c., at 700h and 1900h) of 1.0 mg bromocryptine, CB-154 (2-bromo- α -ergocryptine methane sulfonate, Sigma Co. (St. Louis, MO, USA). CB-154 was prepared as described previously (Manuscript #3). Also the ewes were given 3 weeks of rest between trials. The description of the chamber, the control of temperature and photoperiod,

management and feeding of the ewes were as described in the General Materials and Methods.

8.3.2 INJECTIONS AND SAMPLING:

The GnRH used (Cystorelin, GnRH-diacetate tetrahydrate ester, P.V.U., Ltd., Guelph, Ontario, Canada) had a potency of 86.0% as compared to the native GnRH. A single injection of 20 μg GnRH ewe⁻¹ was prepared in 2.0 mL of acidified saline (adjusted to pH=6.0 with 0.2 N HCl). The injection of GnRH and the blood sampling was carried out through jugular catheters which were inserted on D13. The intensive sampling sessions extended between 800-1740h on D14 of exposure. Initially, ewes were sampled at a frequency of 20 min for 2 h prior to GnRH injection. At 1000h GnRH was injected and samples were taken at 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, and 60 min after the injections. Subsequently, sampling was continued at 20 min intervals to the end of the session.

Samples (7 mL) were drawn using 10 mL plastic syringes and were immediately transferred to pre-chilled 7 mL culture tubes containing 0.07 mL of 15% EDTA solution. The tubes were quickly (3-4 min) taken to the lab and centrifugated at 4°C (2000 rpm for 10 min). Plasma was harvested and stored at -20°C until hormone estimations.

In addition to the frequent sampling sessions, a daily plasma sample was collected at 1130h. P₄, LH, follicle-stimulating hormone (FSH), PRL and GnRH were estimated using radioimmunoassay (RIA) procedures.

8.3.3 HORMONE ASSAYS:

RIA procedures for the estimations of P₄, LH and FSH were as

described in Manuscript #2. PRL was estimated according to the method described previously (Manuscript #1).

8.3.3.1 RIA OF GnRH:

Chemicals and reagents used in this assay were all RIA grade and were maintained as described in the General Materials and Methods. The methyl alcohol for extraction (chromatography grade) was obtained from Fisher Scientific Co. (St. Louis, MO, USA). Absolute ethyl alcohol (reagent grade) was purchased from Stanchem, a Div. of PPG Industries Canada Ltd. (Winnipeg, MB, Canada).

Earlier reports by Foster et al. (1976) showed poor correlation between the concentration of GnRH in the blood and the peripheral changes in gonadotropins. The literature suggests that the GnRH molecule not only has a short half-life in peripheral blood (Nett et al. 1973; Foster et al. 1976; Wheaton 1982) but also can be degraded in a short time by aminopeptidases in samples kept at physiological pH; particularly at temperatures $>4^{\circ}\text{C}$ (Nett et al. 1974; McDermott et al. 1981). Moreover, circulating GnRH is associated with plasma proteins which renders the molecule unaccessible to the anti-GnRH serum used in the RIA procedures (Jeffcoate et al. 1974; Jonas et al. 1975; Nett and Adams 1977). To overcome these problems necessary precautions were taken to ensure the validity of GnRH estimates in our samples.

The GnRH was estimated according to an assay previously described by Jeffcoate et al. (1974). All the steps of the assay, with the exception of the recovery of the residue following methyl alcohol extraction, were carried out at 4°C . Frozen plasma samples were thawed overnight at 4°C .

All samples from an individual ewe were estimated in duplicate in the same run.

The GnRH standard (GnRH Lot #386013, Hoechst Canada Inc., Behring Diagnostics, Montreal, Que., Canada) was received in lyophilized form and was reconstituted in ewe serum which had been extracted twice with charcoal (Abraham 1974). This serum also contained 0.03 M sodium azide and 0.05 M disodium EDTA (pH adjusted to 6.0). The standard curve was run from 0-1600 pg tube⁻¹, in triplicate. The same reference material was tagged (¹²⁵I-GnRH) according to Greenwood et al. (1963), as described by Nett and Adams (1977) and was prepared in the assay buffer (pH=7.4) containing 1.0% and 0.25% of egg white and normal rabbit serum, respectively. The working solution of labelled GnRH contained 7500-9000 cpm 100 μ L⁻¹. The anti-GnRH serum was developed in rabbits (Rabbit #1729m Anti-LHRH, Lot #C0111105, Arnel Products Co., Inc., New York, N.Y., USA). According to the supplier, the anti-serum did not cross-react with leucine-enkephalin, methionine-enkephalin, oxytocin, somatostatin, thyrotropin releasing hormone (TRH) as well as three GnRH fragments. The anti-serum was also prepared in the assay buffer described above and was used at a titre of 1:10,000. Absolute alcohol was used to precipitate the hormone-bound anti-serum (Jeffcoate et al. 1974).

The assay tubes were counted for 2 min in an automatic gamma counter (Model #1185, Automatic Gamma System, Searle Analytic, Inc., Chicago, IL, USA). The calculation of the standard curve was done according to the equation

$$Y = \frac{B_i - NSB}{B_0 - NSB} \times 100$$

(Rodbard and Lewald 1970); where

B_i = counts in the standard or the unknown tubes

B_0 = counts in the reference (0) tubes

NSB = counts in the nonspecific binding tubes

The efficiency of the extraction step of a known amount of labelled GnRH was 77.5% (n=10). An attempt to estimate plasma GnRH directly without the extraction step was unsuccessful as depicted in the Table 86. In contrast to the extracted standard, increasing concentration of GnRH in the unextracted plasma failed to displace the trace, indicating that plasma factors interfered with the reaction. This finding is further confirmed in Table 87, where the concentration of GnRH in the same plasma samples (2 ewes) was compared after estimation by either direct assay or following extraction with methyl alcohol. The interference of plasma proteins with the competitiveness of the labelled hormone led to an inflated value of the (NSB). The plasma proteins also led to overestimation of GnRH concentration when concentrations were low, and to the underestimation of these values when GnRH concentrations were high.

The total binding of the anti-serum was 33.6% (n=10) and the (NSB) was 5.7%. The sensitivity of the assay at 95% binding was 1.0 pg tube⁻¹. The intra-assay C.V. of charcoal-stripped ewe serum pools containing 428.8 and 741.3 pg mL⁻¹ were 13.9% and 7.5%, respectively.

The corresponding inter-assay C.V. for the same serum pools were 14.0% and 8.5%, respectively. In addition, when 'Cystorelin' (the synthetic GnRH used as an exogenous source) was prepared in charcoal-stripped ewe serum and assayed at identical concentrations to that of the GnRH standard, it showed an identical curve.

8.3.4 STATISTICAL ANALYSIS:

The interpretation of RIA data for P_4 , LH, FSH and PRL was as described in the General Materials and Methods. The calculation of the area under LH and FSH response curves as well as the preliminary analysis of the release characteristics of GnRH, LH and FSH using the Pulsar program was also described in the General Materials and Methods section. Each of the entire data set for GnRH, LH, FSH and PRL were compared using means in which hormone values in the 2-hour intervals prior to the injection were examined against means during the first 20 min, the next 30 min and subsequently at hourly intervals following GnRH.

Hormone estimates, area under the curves and the Pulsar program-generated summaries of hormone release characteristics were analysed according to the ANOVA designs as provided in the software packages by Statistical Analysis System (SAS Institute, Inc. 1982). General comparisons between temperatures (18°C vs 35°C) and treatments (CB-154 vs No CB-154) were made using ewes as blocks (Snedecor and Cochran 1967).

8.4 RESULTS:

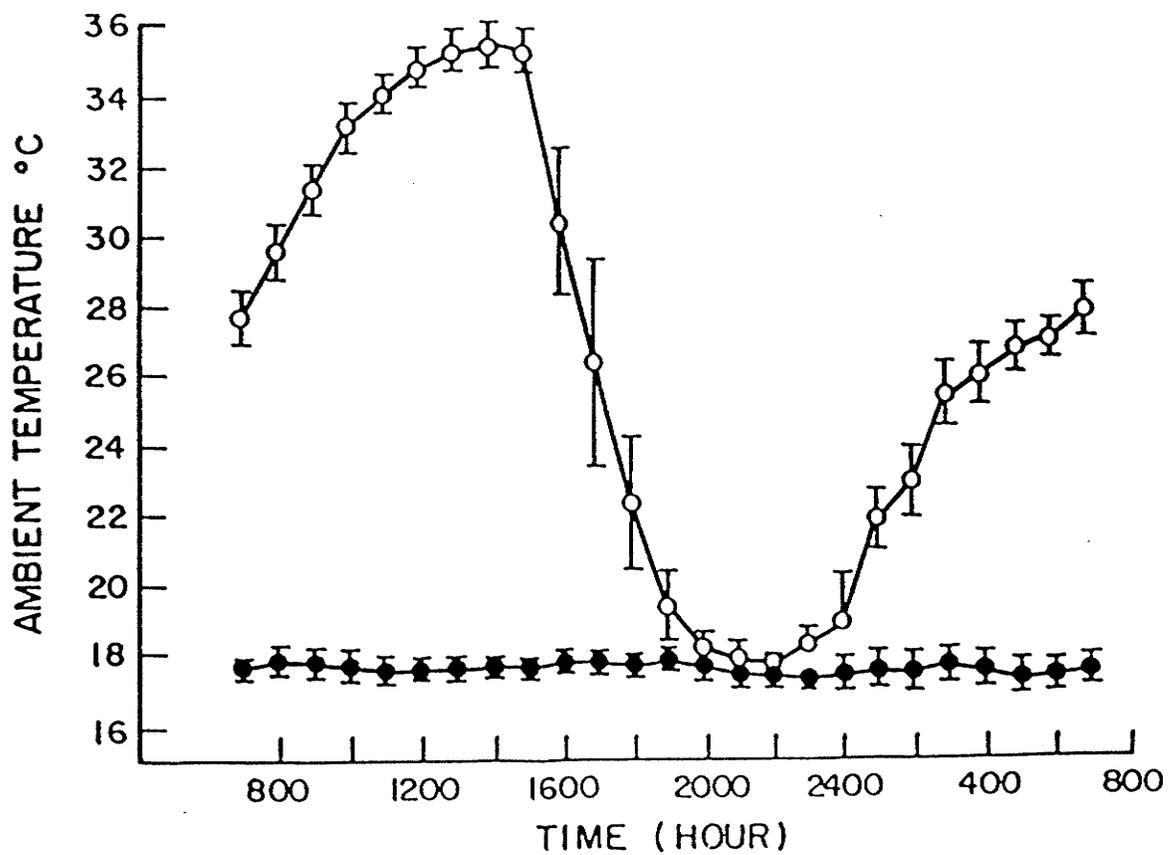


Figure 34: Mean (\pm SEM) daily fluctuation of ambient temperature at thermoneutral (\bullet) and cyclic heat stress (\circ) at the GnRH STUDY.

8.4.1 AMBIENT TEMPERATURE (T_{amb}):

The 24-h fluctuations in T_{amb} are shown in Fig. 34. During CHS the chamber temperature was $>27^{\circ}\text{C}$ for 11h, of which 6 h were spent at $T_{amb} >32^{\circ}\text{C}$ (1000-1500h). In contrast, the cooler period during the night, where T_{amb} stayed $<22^{\circ}\text{C}$ was short (7 h). According to our previous report (Manuscript #1), $T_{amb} >25^{\circ}\text{C}$ was associated with behavioral responses indicating mild discomfort; while $T_{amb} >28^{\circ}\text{C}$ was accompanied by significant physiological and endocrine (PRL) changes in shorn sheep. During similar changes in T_{amb} , in this investigation, the ewes showed behavioral responses which paralleled those observations described earlier (Manuscript #1).

8.4.2 PLASMA PROLACTIN (PRL) CONCENTRATION:

Since plasma PRL concentration in the second trial (with CB-154) was below the assay detection limit ($1.06 \pm 0.05 \text{ ng mL}^{-1}$), estimates reported here represent values from the first trial (no CB-154). Mean PRL concentration is shown in Fig. 35. Cyclic heat stress enhanced PRL release. In addition, a transient rise in PRL followed the injection of GnRH and the magnitude of this rise was greater during CHS. The analysis of variance for the comparisons of mean PRL concentration showed no differences between temperatures and periods and there was no interaction for temperature*period because PRL values tended to rise following the injection of GnRH, irrespective of the ambient temperature (Table 88).

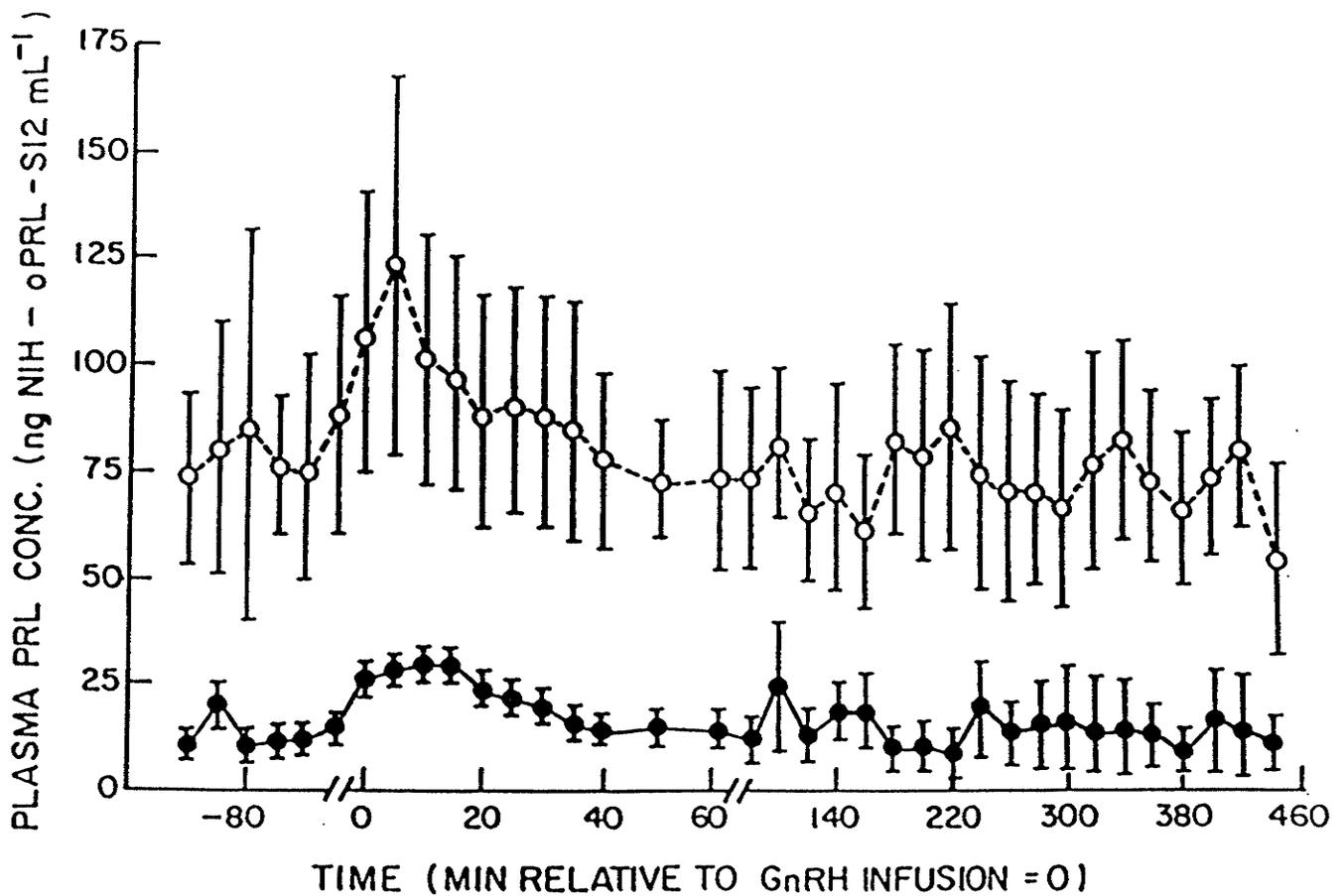


Figure 35: Mean (\pm SEM) concentration of plasma PRL following an acute single dose of an exogenous GnRH (time=0) at thermoneutral (\bullet) and cyclic heat stress (\circ) in P₄-primed ovx ewes.

8.4.3 PLASMA PROGESTERONE (P₄) CONCENTRATION:

Mean daily P₄ concentration is shown in Table 89. The estimates were made from samples collected at 4 h after the daily injection of P₄ from Day (-8) to Day (-1). Before the P₄ injections, peripheral concentrations were comparable to values observed in ovx ewes (Manuscript #4). Following the injections, P₄ rose to the mid-luteal phase concentration seen in cycling ewes (Manuscript 2, 3). However, P₄ concentration failed to fall to preovulatory values in the 24 h period following the injection. Progesterone concentration was elevated during CHS as compared to NT. Mean (\pm SEM) daily P₄ values are given in Table 68). The observation of the elevated P₄ during CHS was unexpected, since the same batch of P₄ preparation was used for the daily injections. Also, the ewes were sampled at the same time and P₄ was estimated in all the samples in one assay using the same analytical materials. P₄ concentration on the day of the intensive sampling was 1.33 ± 0.19 , 1.40 ± 0.33 , 2.25 ± 0.41 and 2.28 ± 0.50 ng mL⁻¹ during NT without CB-154, during NT with CB-154, during CHS without CB-154 and during CHS with CB-154, respectively.

8.4.4 GONADOTROPIN-RELEASING HORMONE (GnRH):

8.4.4.1 PLASMA GnRH LEVELS:

Plasma concentration of GnRH before and after the injection of GnRH is shown in Fig. 36. The immediate response following the injection was not influenced by the temperature or the CB-154 treatment. Analysis of variance of plasma GnRH in the 2-h interval before and in the first 20 min, the next 30 min and subsequently at an hourly periods (n=9)

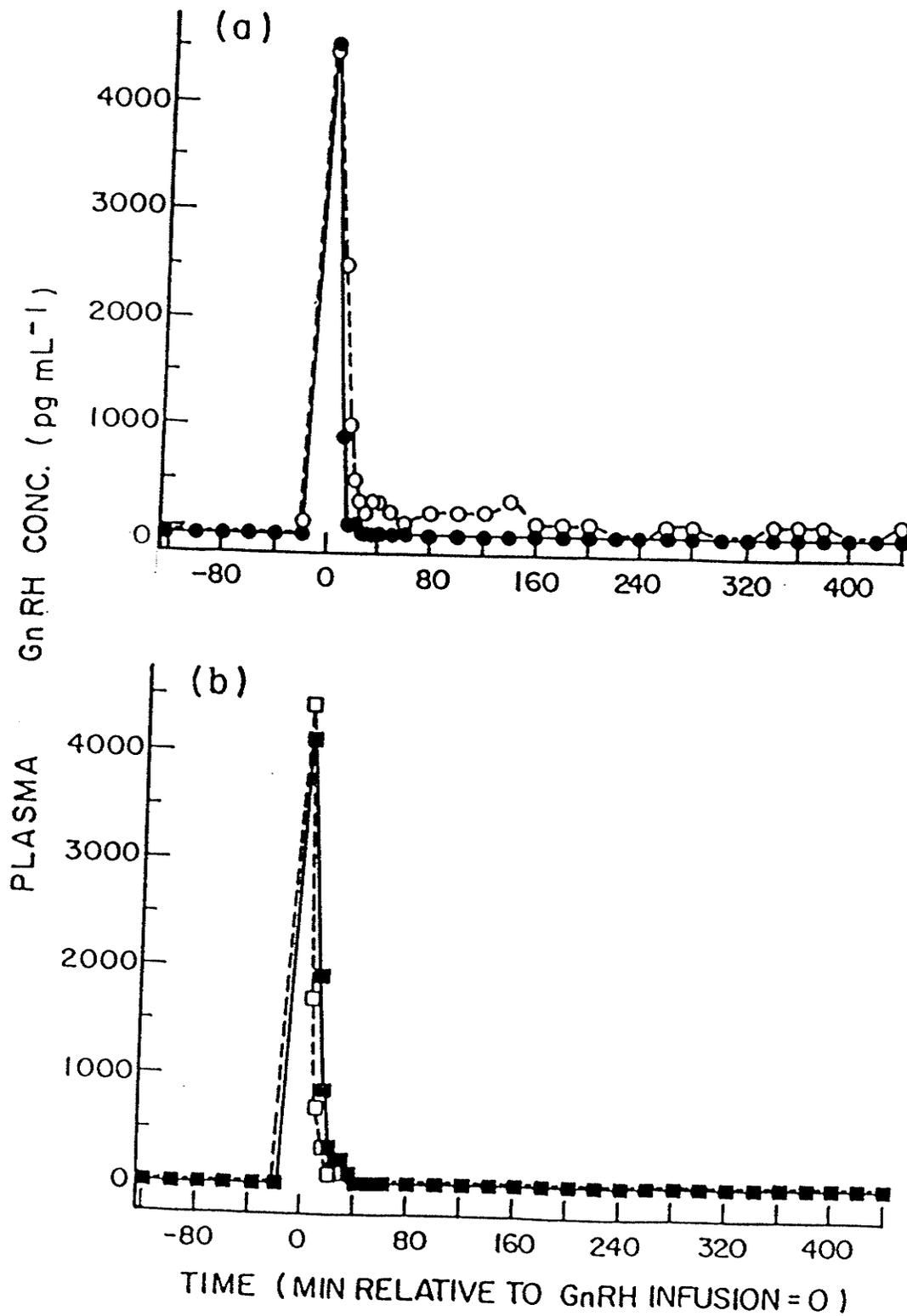


Figure 36: Mean concentration of plasma GnRH following an acute single dose of an exogenous GnRH (time=0) at thermoneutral (●, ■) and cyclic heat stress (○, □) in P₄-primed ovx ewes treated without (a) or with (b) CB-154.

following the injection of GnRH showed no differences between treatments and temperatures (Table 90). However, the interaction of treatment*temperature*period was significant ($p < 0.05$) due to the tendency of GnRH values to be higher and also to remain elevated for a relatively extended period during CHS in Trial 1 than in the other treatment and temperature combinations. Means (\pm SEM) of plasma GnRH by periods are shown in Table 91.

8.4.4.2 THE RELEASE CHARACTERISTICS OF GnRH:

The release characteristics of plasma concentration of GnRH are summarized in Table 92. During NT, mean concentration was low (4.8 pg mL^{-1}). Cyclic heat stress, significantly raised the mean values of GnRH (29.30 pg mL^{-1}) ($p < 0.05$). The peak following the injection exceeded 4000 pg mL^{-1} . The decline from peak concentration to pre-injection values occurred over a relatively longer interval during CHS without CB-154 ($137.5 \pm 47.7 \text{ min}$) as compared to NT without CB-154 ($37.5 \pm 4.3 \text{ min}$), to NT with CB-154 ($45.0 \pm 2.9 \text{ min}$) and to CHS with CB-154 ($83.8 \pm 27.0 \text{ min}$). As a result, the overall mean concentration of GnRH tended to be higher in the first trial during CHS as compared to the other treatment combinations ($p < 0.05$). Analysis of variance for the comparisons of the intervals from peak concentration of GnRH to basal values showed no differences between temperatures (Table 93).

Analysis of variance of the release characteristics of GnRH revealed significant differences between treatments ($p < .001$) in the interpeak interval ($p < 0.001$) (Table 93). Also there was a significant temperature effect on the pulse frequency ($p < 0.01$). The significant treatment*temperature interaction for pulse frequency

($p < 0.05$) reflected the fewer pulses during NT in Trial 2 ($p < 0.05$) and also due to more pulses during CHS ($p < 0.05$), irrespective of the CB-154 treatment (Table 92). The significant treatment*temperature interaction ($p < 0.05$) for the interpeak interval was due to an extended interval during NT in Trial 2 (Table 92). Further, although the analysis of variance for the duration of the GnRH pulse peaks showed no differences between treatments or temperatures and also no significant interactions (Table 93).

8.4.5 LUTEINIZING HORMONE (LH):

8.4.5.1 PLASMA LH CONCENTRATION:

The response curve of LH following exogenous GnRH is shown in Fig. 37. The LH concentration before and after the injection was comparable during NT and CHS (Fig. 37a). CB-154 treatment during CHS, on the other hand, enhanced the LH response to GnRH (Fig. 37b). The analysis of variance for the comparisons of LH concentration by periods indicated no differences between treatments (Table 94), even though mean plasma LH tended to be lower in Trial 2 than in Trial 1 (Table 95).

8.4.5.2 THE RELEASE CHARACTERISTICS OF LH:

The means (\pm SEM) for the release characteristics of LH before and after the injection of GnRH are shown in Table 96. The analysis of variance of the release characteristics of the LH response to GnRH

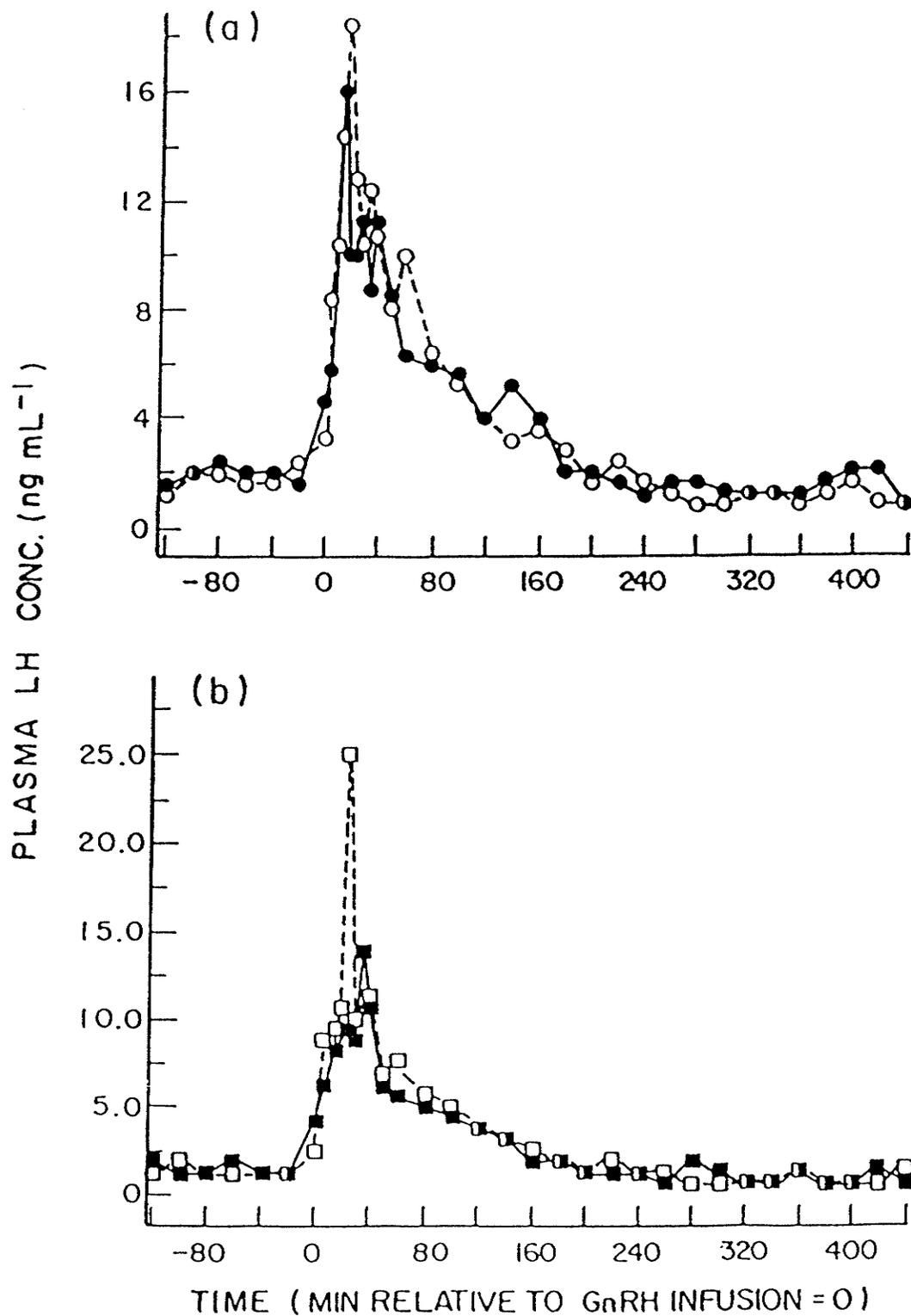


Figure 37: Mean concentration of plasma LH in response to an acute single dose of an exogenous GnRH (time=0) at thermoneutral (●, ■) and cyclic heat stress (○, □) in P₄-primed ovx ewes treated without (a) or with (b) CB-154.

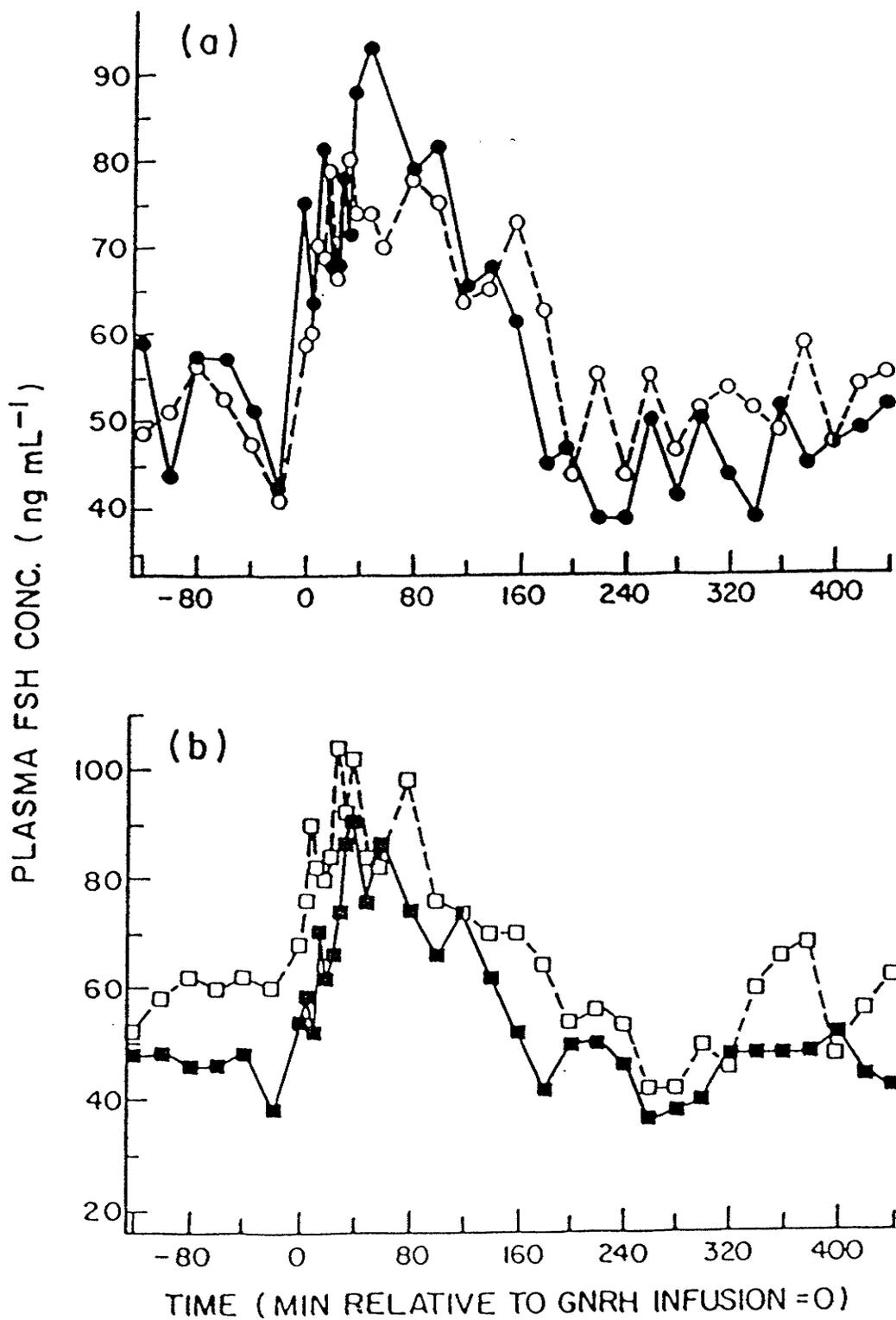


Figure 38: Mean concentration of plasma FSH in response to an acute single dose of an exogenous GnRH (time=0) at thermoneutral (●, ■) and cyclic heat stress (○, □) in P₄-primed ovs ewes treated without (a) or with (b) CB-154.

revealed no differences between treatments or temperatures and also no interactions for any of the parameters measured (Table 97).

8.4.6 FOLLICLE-STIMULATING HORMONE (FSH):

8.4.6.1 PLASMA FSH CONCENTRATION:

The mean FSH concentration before and after the injection of exogenous GnRH is shown in Fig. 38. Although the FSH response curve was comparable between temperatures, the peak was somewhat higher during NT (Fig. 38a). When the ewes were under CB-154 treatment, both basal FSH and the FSH response to GnRH tended to be higher during CHS as compared to NT (Fig. 38b). The FSH response to exogenous GnRH was analyzed in a similar fashion to that of LH.

Analysis of variance of FSH response by periods (similar to those used for LH) showed no differences between temperatures or treatments (Table 98). There was a significant treatment*temperature interaction ($p < 0.001$), which resulted from a different pattern of the FSH response between trials. In Trial 1 the FSH response tended to be lower during CHS, whereas the opposite conclusion can be said in Trial 2. Nevertheless, these changes were not reflected in the statistical differences between temperatures or treatments. Means (\pm SEM) of the FSH response to GnRH by periods are shown in Table 99.

8.4.6.2 THE RELEASE CHARACTERISTICS OF FSH:

Analysis of variance of the release characteristics of the FSH response to exogenous GnRH showed no differences between treatments of

any of the parameters measured (Table 100). However, there was a significant temperature effect ($p < 0.05$) as well as treatment*temperature interaction ($p < 0.05$) for basal FSH. These differences reflected the dramatic changes that occurred in Trial 2 as compared to Trial 1 (Table 101). During NT in Trial 2, basal FSH was lower than the values seen during CHS of either Trials ($p < 0.05$). During CHS in Trial 2, on the other hand, basal FSH was higher than the values during NT of either Trials ($p < 0.05$). The means (\pm SEM) of the FSH response to GnRH are shown in Table 101.

8.5 DISCUSSION:

Previously, we have shown that the exposure of shorn ewes to an ambient temperature above 31°C , caused significant changes in the hormone release characteristics; particularly that of PRL (Manuscripts #1, 2). Likewise, PRL concentration in the first trial of this investigation was significantly elevated during CHS, in a magnitude that was comparable to those observed in the earlier reports (manuscripts #1, 2). In addition, a transient rise in PRL followed the injection of GnRH, but the response was greater during CHS. This latter observation contradicts data from Wright et al. (1981) in which no change in PRL occurred in cycling ewes given $200 \mu\text{g}$ of GnRH. Although the physiological role of the rise in PRL following the injection of GnRH remains unclear, it did not seem to have influenced the gonadotropic response.

Unlike the cycling ewe, in which the pituitary secretion of the gonadotropins is dually controlled by the hypothalamus as well as the ovarian inhibin-like peptides, hypothalamic GnRH is the sole factor

which stimulates the release of LH and FSH in the ovx ewe. Therefore, the ovx ewe was chosen in this study as the endocrine model to test the effect of temperature on the pituitary response to GnRH, to exclude the possible interference from the ovarian factors. However, due to the inconsistency of the reports on the effects of P₄, alone, on the response of LH and FSH to exogenous GnRH in the ovx ewe (Foster et al. 1976; Clarke and Cummins 1984), we used P₄ injections to depress the endogenous release of GnRH.

The daily injections of 30 mg of P₄ caused the peripheral concentration of this hormone to rise to values similar to those seen during the mid-luteal phase of the estrous cycle in the ewe. However, at 24 h following the cessation of the injections, P₄ concentration failed to drop to the preovulatory values reported in the cycling ewe (Manuscripts 1, 2). Hence, it is possible that the elevated concentration of P₄ on the day of the intensive sampling might have depressed basal LH or even the LH response to GnRH. Indeed, basal LH concentration (prior to the injection of GnRH) was lower than the values reported in ovx ewes. Although the concentration of P₄ during CHS was almost 2-fold higher than the concentrations during NT, irrespective of the CB-154 treatment, the gonadotropic response to GnRH was not significantly influenced.

We have no explanation as to why P₄ concentration was elevated during CHS on the day of intensive sampling in either Trial. All ewes had received their daily injections from the same P₄ preparation and the

blood sample for hormone estimation was taken at the same time. In addition, P_4 was estimated for both Trials in one assay using the same analytical reagents. The question which arises is does CHS change the peripheral metabolism of P_4 to the extent that values remain elevated for a longer period as compared to concentration during NT? Therefore, this question needs to be addressed in future studies since elevated P_4 seem to be positively related to the magnitude of the preovulatory gonadotropin surges (Manuscripts 2, 3).

Our results also agree with earlier observations that peripheral concentration of GnRH is low (Wheaton 1982). In addition, the direct estimation of this hormone in plasma or serum samples, without the extraction step, would not give a true concentration because of the interference of the plasma proteins with the assay as reported earlier by Jeffcoate et al. (1974). The circulating concentration of GnRH in the ovx ewe prior to the injection of GnRH was 4.8 ± 0.71 pg mL⁻¹. These values are comparable to the concentration reported in the ovx ewe by Wheaton (1982). However, they are significantly lower than the 128-324 pg mL⁻¹ range, which was reported by Nett et al. (1974), in serum from ovx ewes.

Peripheral hormone concentration depends upon the amount released by the endocrine gland, the size of the existing hormone pool in the blood and the metabolic clearance rate. Therefore, assuming that the pool size and the metabolic clearance rate to be constant, the injection of exogenous GnRH should have resulted in an acute rise to a peak and

fairly rapid decline to the pre-injection values. This description of the plasma GnRH response curve to the exogenous injection of GnRH was observed only during NT in Trial 1 and during either temperatures in Trial 2. During CHS in Trial 1, on the other hand, the GnRH profiles after the injection of the exogenous GnRH fluctuated at elevated values for a longer duration than was expected, thus leading to a significant treatment*temperature interaction. Since the Pulsar program which we have used to analyze the hormone release patterns tended to be accurate in identifying peaks when the data series are not showing rapid changes (as was the case in the GnRH profiles in the interval subsequent to the injection), and since our assay was fairly accurate, we believe that elevated mean GnRH during CHS in Trial 1 reflected genuine endogenous release. The source of this endogenous GnRH release in response to the exogenous injection, why it occurs and what function it has require further investigation. We have shown the important role played by the plasma proteins in binding GnRH in the circulation. Since plasma proteins tend to decrease during CHS as a consequence of possible expansion in plasma volume, it was less likely that the elevated GnRH during CHS could result from a rise in plasma proteins. The role of plasma proteins was also discredited by the fact that GnRH values returned to basal concentration more rapidly during CHS in Trial 2 (with CB-154). In addition, changes in the metabolic clearance rate could explain the differences noted in plasma GnRH values during CHS with CB-154.

In spite of the elevated GnRH values during CHS in Trial 1, which was also associated with an increase in the pulse frequency, there was no

parallel changes in the LH and FSH responses, suggesting that the peripheral estimates of this hormone might be of no meaning if they have to be used to assess the release pattern of the gonadotropins. Whether the discrepancies in the literature arise because the GnRH molecule in the circulation is associated with plasma proteins which are extractable during the estimation of the hormone by RIA, yet while in the circulation these proteins render the molecule biologically unavailable to the pituitary, also needs to be evaluated. So far, promising correlations between the circulating concentration of GnRH and peripheral LH and FSH have been claimed only when GnRH was estimated in the pituitary portal blood or in the perfusates of MBH (Levine et al. 1982; Clarke and Cummins 1982; Clarke and Cummins 1985a). Even these above studies contain many inconsistencies which necessitate the re-examination of the current concept on the relationship between the GnRH release pattern and the pituitary response.

The present investigation confirmed our earlier observation (Manuscript 3) that CB-154 treatment tended to depress mean LH. Although basal LH in the current investigation was comparable between treatments, basal FSH was not only low during NT in the CB-154 ewes, but also was associated with a simultaneous reduction in the pulse frequency of GnRH. Since CB-154 failed to depress basal FSH and also did not reduce the pulse frequency of GnRH during CHS, our data implied that CB-154 produced its effect by acting on the hypothalamus. Based on our previous report (Manuscript 2), CHS depressed the preovulatory gonadotropin surges. The fact that the FSH response to exogenous GnRH tended to be greater during CHS in the CB-154-treated ovx ewes, seem to suggest the importance of the ovary in modulating the pituitary response.

The results lead to the conclusion that the depression of LH and FSH in response to heat stress might be related to changes in the release pattern of GnRH.

Our early reports showed that the preovulatory LH surge was greater during NT in the cycling ewe (Manuscript #2) and was also greater during CHS when the ewes were treated with CB-154 (Manuscript #3). The results of this investigation, on the other hand, indicated that the LH response to exogenous GnRH was comparable, irrespective of temperature or CB-154 treatment. Therefore, this latter finding suggests that the reduced pituitary function during CHS, could be due to insufficient amounts of endogenous hypothalamic GnRH. This argument is in disagreement with some of our results where mean GnRH prior to the injection of the exogenous GnRH was higher during CHS in Trial 1 than in Trial 2 where CB-154 was given. This inconsistency between the mean values of GnRH in the circulation and the argument that CHS depresses GnRH release, may be related to what we have already mentioned earlier that the concentration of this hormone in the circulation tend to be meaningless since the values do not reflect the pituitary function.

Further, we have examined the possibility that the pituitary response to exogenous GnRH might have been influenced by the apparent differences between temperatures in P_4 concentration in the day of the intensive sampling. Despite the differences in P_4 the comparable LH response to GnRH between temperatures suggested that P_4 concentration had no effect.

In contrast to the basal concentration of LH which tended to be lower

during CHS as well as after the CB-154 treatment, basal FSH concentrations were depressed by CB-154 treatment during NT, but were raised by CB-154 treatment during CHS. As with LH, the elevated concentration of GnRH during the exposure to CHS, alone, was not associated with changes in the FSH release characteristics. Instead it was accompanied by reduced pulse amplitudes of FSH, and as a consequence a lower FSH response peak.

Similarly, the significant reduction in the pulse frequency of GnRH during NT, as a result of the treatment with CB-154, was not associated with any similar changes in the FSH release characteristics, except a reduction in basal concentration.

In summary, both temperature and the CB-154 treatment induced significant changes in the release characteristics of GnRH. During NT, GnRH release occurred at a pulse frequency of 0.66 pulses h^{-1} . Cyclic heat stress increased the pulse frequency to 0.9 pulses h^{-1} ($p < 0.05$). Treatment with CB-154 caused a reduction in the pulse frequency of release during NT to 0.48 pulses h^{-1} ($p < 0.05$), but failed to modify the effect of CHS (0.9 pulses h^{-1}).

Cyclic heat stress delayed the disappearance rate of exogenous GnRH from the circulation ($p < 0.001$). Irrespective of the temperature, CB-154 treatment had the tendency of depressing basal concentration of LH before the injection of GnRH. CB-154 treatment during CHS raised the

basal FSH concentration, before the injection of GnRH ($p < 0.001$) and also tended to enhance the FSH response to GnRH.

The changes in the concentration and the release characteristics of the circulating GnRH were not paralleled by significant changes in the gonadotropins. The pituitary responded to the exogenous GnRH in a comparable fashion in all temperature and/or treatment combinations. Thus, the results of this investigation suggested that in the ovx ewe the main effects of either CHS, P_4 or CB-154 on depressing the pituitary function might have been by affecting the release of GnRH, and hence none of the three factor affected the pituitary response to exogenous GnRH. The results also indicated that CHS offsetted the tendency of CB-154 to depress the release characteristics of GnRH. Because CB-154 seemed to have influenced the hypothalamo-pituitary axis, in a manner that could be unrelated to the inhibition of PRL release, the present results agreed with our previous data (Manuscript 3) and also with the literature, in questioning the use of this chemical to investigate the effect of PRL on gonadotropin release.

GENERAL DISCUSSION

In a series of studies an attempt has been made to define the effect of hyperthermic stress as judged by the degree of displacement of physiological and hormonal homeostasis, and to investigate the effect of CHS on the relationships between the hormones of the hypothalamo-pituitary-gonadal and the pituitary-adrenal axes.

According to the literature, there is an inverse relationship between the rise in Trec in response to heat stress and the peripheral concentration of cortisol (Ingraham et al. 1979). In contrast, prolactin (PRL) concentration shows a positive correlation with Trec (Schillo et al. 1978). Our results agreed with these findings when only the the daily concentrations were considered. They did not reflect consistent relationships between short-term (24 h) changes in hormone secretion pattern and the physiological responses. This may be attributed to the fluctuating heat stress imposed, which allowed the physiological responses to recover to normothermic values, but was insufficient in time to allow the full recovery of the endocrine system.

Furthermore, the present investigation revealed that, the response of different hormones to CHS varied. For instance, even though CHS led to a general decline in basal concentration of cortisol, there was no effect on either the 24-h fluctuations or the decline of this hormone over time which may possibly be due to the animals becoming acclimatized to the stress of sampling. In contrast to the response in cortisol, the response of PRL to CHS was more dramatic. Its acute rise which accompanied the daily rise in Tamb, remained comparatively unchanged, despite the repetitive nature of the exposure. Also CHS did not

attenuate the nightly rise in PRL during the cool period. Taking these two responses of PRL together, it seems that following the exposure to CHS, the recovery of the lactotrophs occurs in a shorter time as compared to that of the adrenal zona fasciculata cells. Alvarez and Johnson (1973) have shown that in cattle subjected to chronic CHS, normal adrenal activity required 48 h to return to normothermic values. The failure of the cool period to completely restore normal endocrine function argue against management claims that the provision of cool nights to livestock during the hot summer months would be sufficient for optimum productivity.

Another difference between the PRL and the cortisol response was that, the progressive decline of PRL concentration during the period of intensive sampling was observed only during NT. Although the acute elevation in PRL which accompanied the daily rise in Tamb seemed to be the major factor, mean concentration tended to be elevated in the cool period during CHS as compared to the corresponding period during NT. These findings implied that, unlike cortisol secretion, the CHS obliterated the response of PRL to the stress of sampling. The difference between PRL and cortisol in the response to sampling during CHS could be due to variation in the susceptibility of the factors which trigger the acclimatization response for either hormone.

The presence of a 24-h rhythm for the secretion of cortisol in frequently-bled and ad libitum-fed sheep has been previously described, but there are some variations in the timing of the occurrence of the high and low concentration. Fulkerson and Tang (1979) have reported the highest concentration of cortisol occurred around midnight and the

lowest in the afternoon. These authors also have reported no change in the pulsatile release of cortisol during the day. In addition, data from Mesbah and Brudieux (1982) showed the highest cortisol concentration to be between 600-1200h and the lowest in the late afternoon hours.

During NT, in the current investigation, cortisol concentrations were high between 1100-1500h and were low during the night and the early morning hours. During CHS, elevated concentrations were seen slightly later (1300-1700h). The similarity of the 24-h rhythm of cortisol secretion between the two temperatures, and the lower basal concentration during CHS, suggests that CHS reduced the pituitary releasable pool of ACTH without significantly influencing its release patterns.

Indirect evidence for reduced circulating concentrations of ACTH during CHS is drawn from results in Manuscript #4 where the adrenal response to exogenous ACTH was quantitatively comparable between temperatures. However, the decline in cortisol over time during the period of intensive sampling could have resulted from either a reduced adrenal response to ACTH or to a reduced availability of substrate for steroidogenesis. Data from Gwazdauskas et al. (1980) showed that there was a progressive decline of the adrenal response to the same dose of ACTH when given to cows over a 3-day period, which supports our observation. In addition, McNatty and Thurley (1973) have reported that in sheep given a weekly dose of 20 μ g ACTH, the adrenal response was lowest on the third week as compared to the first and second weeks. Also by using exogenous injections of adrenaline in ewes, these latter

workers have shown that the decline in cortisol concentration over time could be due to the reduced sensitivity of the adrenal cortex to the circulating catecholamines.

Although the literature suggests that elevated catecholamines persist during exposure to CHS (Alvarez and Johnson 1973; Rosak et al. 1980) the similarity of the adrenal response to exogenous ACTH, irrespective of the temperatures (Manuscript #4), suggests that the effect of adrenaline on cortisol might be indirect; or there is no effect.

The present results do not support the report by Gwasdauskas et al. (1980) that in heifers given a single dose of 200 μg ACTH the adrenal response was lower at 32°C than at 21.3°C. The discrepancy between the two studies could be due to the differences in the dosage used. Since assuming the average liveweight of a heifer to be 350 kg ($\text{liveweight}^{0.75}=80.9$ kg), the dose of ACTH injected ($2.5 \text{ I.U. kg}^{-1} \text{ liveweight}^{0.75}$), was about 50% less than dose given to sheep (on comparable weight basis) in the present investigation. Because of a possible reduction in substrate availability and enzyme activity during CHS, a higher dose of ACTH might have been required in such circumstances to induce an adrenal response equivalent to the one which was induced during NT in those heifers.

The present results reveal that CB-154 treatment had no significant influence on the tendency of basal cortisol to be depressed during CHS. In contrast, the disappearance of the 24-h rhythm of cortisol secretion during both NT and CHS, suggests that CB-154 treatment interfered somewhat with hypothalamo-pituitary function. The persistency of the basal patterns of cortisol secretion indicates that, the hypothalamic

neurons which control ACTH release were not equally suppressible by CB-154. It is possible that, the hypothalamic neurons whose secretions control the 24-h rhythm of cortisol secretion are different from those which regulate basal secretion.

The current studies also examined the concentration and the secretory patterns of PRL and their influence on the hypothalamo-pituitary axis in ovx and cycling ewes. An acute rise in PRL concentration following the exposure to CHS has been reported by several workers (Hooley et al. 1979; Hill et al. 1980; Fraser and McNeilly 1980). Our results also confirmed these findings. Because elevated catecholamine concentrations are reported to dominate the physiological and endocrine events during CHS (Alvarez and Johnson 1973; Rosak et al. 1980), it is conceivable that PRL concentration should be depressed rather than elevated. Our results showed that CHS enhanced PRL release by the the tendency to elevate basal secretion.

Moreover, several reports have showed that hyperprolactinemia interfered with the E₂-induced LH release (Kann et al. 1976; McNeilly and Baird 1977; Miyake et al. 1985). In the present investigation, though CB-154 treatment was effective in suppressing PRL concentration to values below the detection limits of our assay ($<1.06 \pm 0.05$ ng mL⁻¹), there was no evidence that the reduced PRL concentration had any effect on pituitary function. Another important factor which seemed to have influenced the hypothalamo-pituitary axis in these studies was CB-154. Accordingly, the present data have been examined taking into consideration the events that were attributed either to fluctuations of PRL concentration, or which arose as a possible direct effect of the

treatment with CB-154.

There is little information in the literature that deals with the role of PRL on the estrous cycle of the domestic ruminant. Dopamine, the endogenous PRL release-inhibiting factor, is also the precursor of norepinephrine and epinephrine synthesis. In this vein of thought, data by Stolk et al. (1980) revealed that the activity of the enzyme, dopamine β -hydroxylase, which converts dopamine to norepinephrine and epinephrine, increases during depressed thyroid activity. Indeed, thyroid activity has been reported to be depressed during CHS (Collier et al. 1982; Ross et al. 1985; Pratt and Wettemann 1986; Manuscript #1). Because under certain conditions, a depression in the thyroid activity is counteracted by an increase in the sympatho-adrenal-medullary release of epinephrine and norepinephrine, it is believed that under such conditions the activity of the enzyme dopamine β -hydroxylase, which converts dopamine to norepinephrine, increases. Therefore, if an increase in the activity of this enzyme occurs during CHS, it could directly reduce the dopamine concentration and consequently would lead to elevated concentrations of PRL. One possible explanation could be that, during CHS the body's heat dissipation mechanism imposes a high demand for norepinephrine and epinephrine and in doing so it dissociates the lactotrophs from the inhibitory control of dopamine. Because the response of the lactotrophs to exogenous TRH has been reported to be greater during CHS (Fraser and McNeilly 1980), it would be worthwhile to investigate the factors which not only affect dopamine and its receptors on the PRL-producing cells during heat stress, but also the extent to which the CHS-induced changes in dopamine metabolism influence the hypothalamo-pituitary control of gonadotropin release and the other

endocrine rhythms as well.

Our first study indicated that Tamb affected PRL concentration more than the other hormones. However, the inhibition of its release had no significant influence on the patterns of P_4 response to CHS, in early- and mid-luteal phases of the estrous cycle. Conversely, such inhibition was associated with significant changes in basal concentration of P_4 in the preovulatory period. And perhaps as a consequence, increased the magnitude of the gonadotropic surges.

Considering its molecular mass, it is unlikely that PRL could cross the blood-brain barrier. The fact that elevated concentration of PRL had no significant effect on the pituitary response to GnRH in the ovx ewe suggests that PRL had little influence on the function of the gonadotrophs. This interpretation agrees with several reports that elevated PRL produces no significant changes in the concentration or the release characteristics of LH in response to GnRH (Kann et al. 1976; Fraser and McNeilly 1980; Miyake et al. 1985). Moreover, elevated PRL has been shown to depress the pulsatile secretion of E_2 , which always follows the LH pulses (McNeilly and Baird 1977). Therefore, since E_2 seems to potentiate the effect of GnRH on the gonadotrophs, elevated PRL during CHS might reduce the magnitude of the gonadotropic response. We have followed CL function at a 6-h interval during the peri-ovulatory period in ewes treated with or without CB-154. Our results revealed that, irrespective of of the temperature, the magnitude of the gonadotropin surge was positively related to the duration of the interval from basal P_4 to the onset of the surge. Since the LH surge curve tended to be smaller whenever the P_4 concentration failed to drop

to basal values prior to 48 h before the surge peak, and this was associated with elevated PRL during CHS, our results imply that the elevated P_4 values in the 48-h interval before the LH surge peak, might be caused by the interference of the elevated PRL with the aromatization of androgens and P_4 to E_2 . To qualify this interpretation, it would be necessary to evaluate the complete spectrum of all the major steroids secreted by the ovary during the peri-ovulatory period.

On the other hand, the investigation of the direct effect of PRL on the hypothalamo-gonadotropic function was complicated by the apparent influence of CB-154 on basal LH (Manuscript #3, #5) as well as on the release characteristics of GnRH (Manuscript #5), especially during NT. The literature shows that, CB-154 depresses LH concentration in the sheep (Hill et al. 1980; Rodway et al. 1983). Our results revealed that while CB-154 consistently tended to depress basal LH, irrespective of the temperature, its effects on basal FSH were inconsistent. In addition, CB-154 reduced the pulse frequency of GnRH with no parallel changes in the release characteristics of LH or FSH. It is worth noting that its effect on GnRH release was evident only during NT. Because of its direct interference with hypothalamic function, these findings suggest that the use of CB-154 to study the influence of PRL on the reproductive hormones is subject to many shortcomings.

In contrast to its effect on the basal level of LH and the pulsatile release of GnRH (during NT), in the cycling ewe, CB-154 enhanced the magnitude of the preovulatory LH surge during CHS, with no significant effect on the FSH surge (Manuscript #3). Additionally, pituitary response to exogenous GnRH in the CB-154-treated ovx ewes tended to

depend upon the temperature of exposure. For instance, during NT, both the pulse frequency of GnRH and basal FSH were depressed. The opposite was true during CHS. These findings suggest that changes in the release patterns of the hypothalamic neuro-hormones and as a consequence the release of the pituitary hormones are dependent upon the brain temperature. Cyclic heat stress raises the brain temperature and hence, the neuro-endocrine responses are in a state of incoordination.

How CB-154 produces its effects on the hypothalamo-pituitary function was studied by Owens et al. (1980) using ovx rats. Their study showed that prior treatment with pimozide (a dopamine receptor antagonist), blocked the inhibition of PRL and the reduction of LH concentration by CB-154 treatment. Because such treatment did not interfere with the pituitary response to exogenous GnRH, they postulated that CB-154 acts on the hypothalamus to depress LH concentration. Indeed, the present series of investigations tend to support this conclusion.

Recent data from the rat suggests that CB-154 enhances the number of E₂-induced GnRH-receptors in the pituitary (Piper et al. 1984; Duncan et al. 1986). Although, such an effect might be due to a reduced level of PRL synthesis (it has been reported by Baxter and Gorski (1981) that PRL constitutes about 13-17% of the total proteins synthesized by the pituitary), there are two reservations to this theory according to our data. Firstly, a comparison of the magnitude of the preovulatory gonadotropin surges in Manuscripts #2 and #3 reveals that the surges tended to be lower in the CB-154-treated ewes, irrespective of the temperature. This could be an overstatement, since P₄ concentration was also higher in the CB-154-treated group. Secondly, as it has been

previously noted (see the literature review section), that receptor occupancy, which follows treatment with GnRH, does not always reflect post-receptor events.

The present studies also examined the sources and the role played by P_4 during CHS. The elevated P_4 concentration during CHS (Manuscript #2, #3) confirmed earlier reports in the literature (Miller and Alliston 1974a; Abilay et al. 1975a; Sawyer et al. 1979; Adeyemo and Heath 1980). The results also agree with the observations that the inhibition of PRL release by CB-154 had no significant influence on the length of the estrous cycle or subsequent CL function (Niswender 1972; Louw et al. 1974). They further reveal that in the cycling ewe, the inhibition of PRL with CB-154 during cyclic CHS had no significant influence on P_4 concentration in early- and mid-luteal phases of the cycle.

The general hypothesis that adrenal P_4 might be involved in reproduction is based on two earlier reports. Buflour et al. (1957) have reported that in cattle, sheep and swine the P_4 content of the adrenal vein was 10-100 times higher than the contents of the adrenal artery. Later, Stott et al. (1967) have shown that the P_4 content of the CL in cows exposed to NT and CHS was comparable, but the adrenal P_4 content was 2-3 times higher during NT. Further support for the idea of the involvement of adrenal P_4 in depressing fertility came from Gwazdauskas et al. (1973) who reported that exposure of cows to CHS on the day of estrus led to the elevation of P_4 , and a reduction in the conception rate. However, the majority of data which argue for the involvement of adrenal P_4 in reproduction was based on the response to an acute exposure to CHS or to exogenous ACTH in ovx animals (Wagner et

al. 1972; Stoebel and Moberg 1979; De Silva et al. 1983; Benhaj and Cooke 1985).

As a result of the above speculation in the literature, the secretion patterns of P_4 were followed during the preovulatory period. It is evident that in the cycling ewe, the CL is the main source of fluctuations in peripheral P_4 . The absence of any significant changes in P_4 concentration following the complete regression of the CL, irrespective of the temperature or the CB-154 treatment, suggests that in the cycling ewe, the adrenal P_4 has no significant influence on the hypothalamo-pituitary function during CHS. Additional support for this conclusion is also drawn from the fact that the level of P_4 following exogenous ACTH during CHS in the ovx ewe (Manuscript #4), was comparable to values observed in the preovulatory period in the cycling ewe (Manuscript #2, #3). Therefore, the present results suggest that the source of the elevated concentration of P_4 during cyclic CHS is the ovary and not the adrenal cortex. By such conclusion, our data tend to agree with a recent report by Wheeler and Blackshaw (1986), in which P_4 secretion was studied in ewes with intact or denervated ovaries, during exposure to cold or hot temperatures.

We have examined the relationship between cyclic P_4 and the concentration of the gonadotropins. During the cycle, and irrespective of the temperature or the CB-154 treatment, cyclic P_4 concentration was inversely related to basal concentration of LH and FSH. The relationship was more pronounced during CB-154 treatment (Manuscript #3). Although, the low basal LH and FSH in Manuscript #3 seems to be related to the elevated P_4 concentration, the literature suggests that

P₄ alone is relatively ineffective in suppressing the gonadotropins (Scaramuzzi et al. 1971; Howland et al. 1978a; Clarke and Cummins 1984; Rawlings et al. 1984). Moreover, in spite of the low concentration of E₂ during the luteal phase of the cycle (Bjersing et al. 1972; Baird et al. 1976), many workers believe that the progressive decline in basal LH and FSH in early and mid luteal phases is brought about by the combined effect of E₂ and P₄.

Other experimental data also suggest that while the ovarian steroids are more effective in suppressing basal LH as well as its pulse amplitudes, their effect on FSH secretion are inconsistent (Karsch et al. 1977; Goodman and Karsch 1980; Goodman et al. 1981a; Martin et al. 1983). More recent data shows that steroid-free follicular fluid is more effective in lowering basal FSH without a significant effect on LH concentration (Ireland et al. 1983; McNeilly 1985; Findlay et al. 1985).

It follows that, since the CL of sheep does not interfere with either the progression of follicular growth nor with the secretion of E₂, in the ipsilateral ovary (Baird et al. 1975), the cyclic decline in basal LH in the current studies is likely to be caused by the increasing concentration of both E₂ and P₄. Likewise, since the growing follicles will also secrete increasing amounts of inhibitory proteins, it is conceivable that the progressive decline in basal FSH would be more related to the concentration of these macromolecules. The present results showed that daily P₄ was inversely related to basal LH and FSH, but the relationship was more consistent with FSH. We think that due to the discrete nature of the LH pulses daily samples are unlikely to reflect the true trend in the relationship between P₄ and basal LH. The

consistent relationship between daily P_4 and basal FSH emphasized the stable release pattern of FSH and the less discrete nature of its pulses.

Taking into account the time of the onset of the CL regression (P_4 withdrawal), it appears that the elevated concentration of P_4 in early- and mid-luteal phases of the estrous cycle had no significant influence on the termination of the CL function. Previous reports by Poston et al. (1962), Sawyer et al. (1979) and Yenikoye et al. (1982) have shown that the extended length of the estrous cycle in the ewe during CHS was due to the failure of ovulation. In the present studies, the preovulatory gonadotropic surge occurred in all the ewes during CHS, emphasizing the beneficial effect of the cool period to the hypothalamo-pituitary function.

The literature suggests that, in the ovx ewe treated with P_4 or E_2 , the onset of the gonadotropin surge is more related to the rate of P_4 decline, subsequent to the onset of its withdrawal, rather than to the basal concentration of P_4 (Jeffcoate et al. 1984). Our results do not necessarily contradict these findings. However, the 6-h P_4 profiles reveal that the interval from basal P_4 to the onset of the preovulatory gonadotropic surges was positively correlated to the magnitude of the gonadotropin surges. For instance, in Manuscript #2, the interval from basal P_4 to the onset of the preovulatory surge was greater during NT as compared to CHS. The reverse was true for the pattern of response observed during CHS, in the CB-154-treated ewes (Manuscript #3). Therefore, it appears that the reduction in the magnitude of the preovulatory LH and FSH surges which was associated with shorter

intervals from basal P_4 to the onset of the surges, might be due to the incomplete recovery of the hypothalamo-pituitary axis from the negative feedback of the ovarian factors. Moreover, though it has been reported that surges in gonadotropins were achievable while P_4 concentration was in excess of 1.0 ng mL^{-1} (Jeffcoate et al. 1984), elevated P_4 concentration depresses the sustained rise in E_2 which is believed to be essential for the onset and the magnitude of the preovulatory gonadotropin surges (Goodman et al. 1981b; Baird et al. 1981). The current results suggest that the interval from the initial onset of P_4 withdrawal is important since it is indirectly related to the time-dependent recovery of the hypothalamo-pituitary axis. Thence, the faster the decline rate of P_4 during its withdrawal to basal concentration, the longer would be the interval from basal P_4 to the onset of the gonadotropin surge, and consequently the greater will be the magnitude of the surges. Because one of the major problems encountered during heat stress in cycling ewes is the failure of ovulation (Yenikoye et al. 1982), the magnitude of the LH surge is of vital importance. Therefore, even though the occurrence of the preovulatory gonadotropin surge is essential, ovulation depends on whether the released concentration is optimum. However, there is no reference in the literature to indicate how much of the observed values of LH and FSH concentrations during the preovulatory surge would be optimum for the occurrence of ovulation in livestock.

Based on results in Manuscript #2 and #3, we investigated the hypothesis that CHS, either directly, or through elevated P_4 and PRL, interferes with the hypothalamic release of GnRH or its subsequent stimulation of the gonadotrophs. We have no explanation for the elevated

P₄ values during CHS in ewes receiving exogenous injections of P₄ (Manuscript 5), and as a consequence the current results indicate the need to evaluate the peripheral metabolism of this hormone in heat-stressed animals.

An important finding of the present results in Manuscript #5 is that the response of the pituitary to exogenous GnRH in the ovx ewe was not significantly influenced by the concentration of P₄, the temperature or the CB-154 treatment. This finding implies that CHS reduces the amount of the releasable GnRH. These observations also imply that the interference by P₄, CB-154 or CHS on the pituitary function is primarily achieved through the hypothalamus.

Indeed, our results have shown that CHS reduced the disappearance rate of the infused GnRH (Manuscript #5), which might be due to low activity of the aminopeptidases (McDermott et al. 1981), or perhaps, as a result a qualitative increase in the plasma proteins which seem to protect the GnRH molecule from degradation. Furthermore, the results showed that CB-154 treatment during NT reduced the pulse frequency of GnRH. However, neither the reduced rate of degradation during CHS nor the CB-154-induced reduction in its release characteristics were paralleled by similar changes in LH or FSH. Since our protein hormone assays were specific, sensitive and fairly accurate, the above findings suggest that frequent sampling intervals of <20 min (10 min or less) would have been more appropriate to study such correlations.

CONCLUSIONS

CONCLUSIONS

1. Exposure of shorn sheep to heat stress caused dramatic changes in behavior, a rise in Trec, a 3-5-fold increase in RR and concomitant changes in the peripheral concentrations of hormones (e.g. PRL).
2. During the current investigation, hyperthermia did not abolish the 24-h rhythm of cortisol, nor the decline of its concentration over time due to the stress of sampling.
3. CB-154 treatment abolished the 24-h rhythm of cortisol secretion without influencing its basal concentration or the sampling response suggesting the existence of several mechanisms that control the secretion of this hormone.
4. In the cycling ewe, the CL is the main source of peripheral P_4 , and it is unlikely for adrenal P_4 (per se) to play a significant negative feedback role on the hypothalamo-pituitary axis during hyperthermia.
5. The inhibition of PRL release has no influence on the response of the ovine CL to hyperthermia, and hence if this hormone is an anti-gonadotropin in sheep, it would possibly be through the hypothalamo-pituitary axis.
6. During hyperthermia, elevated P_4 concentration in early- and mid-luteal phases are not necessarily associated with extended estrous cycles, provided the CL starts to regress at the usual time. However, the magnitude of the preovulatory surge of gonadotropins

is positively related to the duration of the interval from basal P_4 to the onset of the surge. There is also a tendency of the magnitude of the second FSH surge to be greater when this interval was longer which suggests the involvement of other ovarian factors.

7. The poor correlation between the peripheral concentrations of GnRH and the gonadotropins necessitates the re-evaluation of the concept of correlated release patterns of hormones of the hypothalamo-pituitary axis.
8. The ability of the pituitary to respond to an acute single dose of exogenous GnRH, irrespective of the temperature, P_4 concentration or CB-154 treatment, support the hypothesis that hyperthermia reduces the circulating concentration of endogenous GnRH.
9. Irrespective of whether the basal concentration of a hormone is raised or depressed in response to hyperthermia, it is always accompanied by quantitative changes in the pulse amplitudes, suggesting that the factors which influence the releasable hormone pools are the major determinants of the peripheral concentrations of steroid and protein hormones.
10. The failure of the endocrine homeostasis to recover to normothermic values in spite of the nightly cool period, suggest that a favorable management program should be designed to protect the animal from the excessive heat load during the hot hours, rather than relying on the cooling effect during the night.

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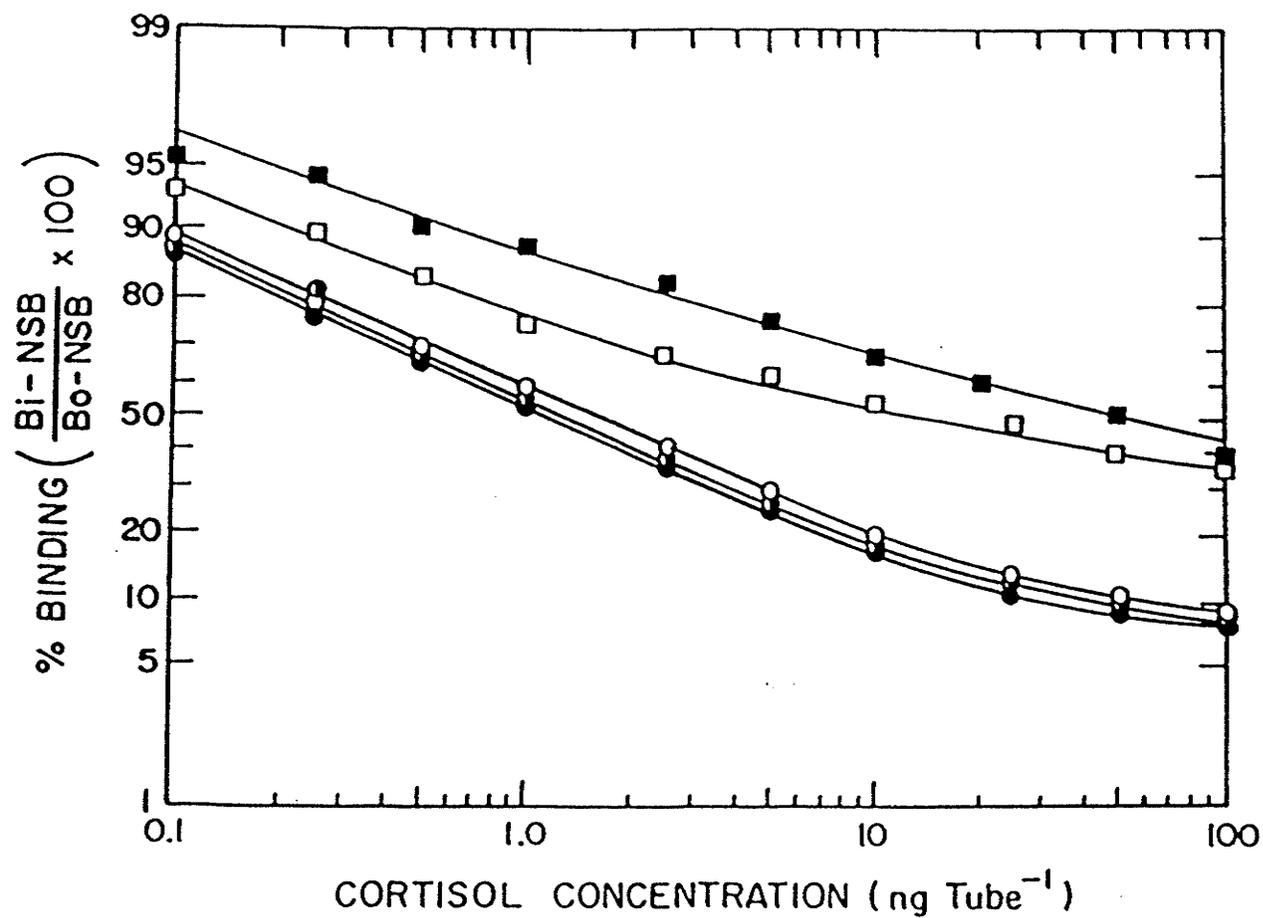
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Appendix Figure 1: The validation of cortisol RIA:
 (●) Cortisol STD, alone, pet ether extract discarded.
 (◐) Cortisol + P4 STDs, pet ether extract discarded.
 (○) Cortisol + P4 STDs, no extraction with pet ether.
 (□) P4 STD, alone, alcohol extract discarded.
 (■) P4 STD, alone, pet ether extract discarded.

APPENDIX TABLES (Statistics):

Analysis of Variance Tables

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1	1 - 28	279-306
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Table 1: Validation of cortisol RIA: the amount of progesterone (P_4) in the petroleum ether extract as estimated using anti-cortisol serum and cortisol as a reference material.

Amount of Added P_4 (ng tube ⁻¹)	Recovered Estimates (mean \pm SEM ng tube ⁻¹)	% Recovery
1.0	0.02 \pm 0.02	2.0
2.5	0.27 \pm 0.03	10.0
5.0	0.43 \pm 0.01	8.6
10.0	0.65 \pm 0.02	6.5
25.0	0.81 \pm 0.05	3.2
Mean \pm SEM		6.1 \pm 1.5

Table 2: ANOVA table for comparisons of daily ambient during Expt 1 and Expt 2.

Source	DF	MS	F
Total	447		
Expt	1	5.23	4.15 ^{NS}
Ewe(Expt)	12	1.26	
Cycle	1	11562.38	8923.06 ^{***}
Expt*Cycle	1	10.14	7.82 [*]
Cycle*Ewe(Expt)	12	1.30	
Phase	3	58.64	83.37 ^{***}
Expt*Phase	3	18.55	26.37 ^{***}
Phase*Ewe(Expt)	36	0.70	
Phase*Cycle	3	43.22	240.21 ^{***}
Phase*Cycle*Expt	3	18.80	104.51 ^{***}
Phase*Cycle*Ewe(Expt)	36	0.18	
Error	336	0.47	

*
 asterisks denote the level of significance: (* p<0.05,
 *** p<0.001, NS = not significant)

Table 3: Mean (\pm SEM) values of the ambient temperature (T_{amb}) during the estrous cycle in Expt #1 (No CB-154) and Expt #2 (CB-154).

Phase of Cycle	Thermoneutral Cycle		Heat Stress Cycle	
	NO CB-154	CB-154	NO CB-154	CB-154
	(°C)		(°C)	
Follicular D1-D4	17.78 \pm 0.09	18.00 \pm 0.00	27.19 \pm 0.35	24.54 \pm 0.46
Early-luteal D5-D8	18.00 \pm 0.06	18.00 \pm 0.00	28.58 \pm 0.32	28.00 \pm 0.51
Mid-luteal D9-D12	18.13 \pm 0.10	18.04 \pm 0.02	29.53 \pm 0.12	29.09 \pm 0.13
Late-luteal D13-D16	17.96 \pm 0.05	18.18 \pm 0.11	28.11 \pm 0.11	29.62 \pm 0.24

Table 4: ANOVA table for comparison of the response of daily rectal temperature in cycling ewes treated without (Expt 1) or with (Expt 2) bromocryptine, CB-154.

Source	DF	MS	F
Total	447		
Expt	1	0.00	0.00 NS
Ewe(Expt)	12	1.03	
Cycle	1	24.82	60.43 ***
Expt*Cycle	1	0.96	2.35 NS
Cycle*Ewe(Expt)	12	0.41	
Phase	3	1.29	35.47 ***
Phase*Expt	3	0.292	7.89 ***
Phase*Ewe(Expt)	36	0.036	NS
Phase*Cycle	3	0.44	3.38 *
Expt*Cycle*Day	3	0.86	6.58 ***
Phase*Cycle*Ewe(Expt)	36	0.13	
Error	336	0.05	

*

asterisks denote the level of significance: (* $p < 0.05$,
*** $p < 0.001$), NS = not significant)

Table 5: Mean (\pm SEM) values of rectal temperature (T_{REC}) during the estrous cycle in ewes treated without (Expt #1, n=9) and with (Expt #2, n=5) bromocryptine (CB154).

Phase of Cycle	Thermoneutral Cycle		Heat Stress Cycle	
	NO CB-154	CB-154 (°C)	NO CB-154	CB-154 (°C)
Follicular D1-D4	39.03 \pm 0.04	38.13 \pm 0.05	39.71 \pm 0.07	39.35 \pm 0.06
Early-luteal D5-D8	39.06 \pm 0.04	39.15 \pm 0.03	39.66 \pm 0.05	39.51 \pm 0.05
Mid-luteal D9-D12	39.01 \pm 0.04	39.24 \pm 0.04	39.72 \pm 0.06	39.55 \pm 0.08
Late-luteal D13D16	39.01 \pm 0.05	38.97 \pm 0.03	39.18 \pm 0.06	39.47 \pm 0.10

Table 6: Correlation coefficients (r) (Pearson's) between daily ambient temperature (Tamb), rectal temperature (Trec), respiration rate (RR), serum prolactin (PRL), serum triiodothyronine (T₃) and serum cortisol in cycling ewes at a thermoneutral temperature.

	Tamb	Trec	RR	PRL	T ₃	Cortisol
Tamb		0.05	0.03	0.11	0.18	-0.01
Trec	0.05		0.64 ^{***}	0.09	0.01	0.15
RR	-0.03	0.64 ^{***}		0.11	0.03	0.11
PRL	-0.11	0.09	0.11		0.17	0.14
T ₃	0.18	0.01	-0.03	0.17		-0.14
Cortisol	-0.01	0.15	0.11	0.04	-0.04	

*

asterisks denote the level of significance: (***) $p < 0.001$, DF=16)

Table 7: Correlation coefficients (r) (Pearson's) between daily ambient temperature (Tamb), rectal temperature (Trec), respiration rate (RR) serum prolactin (PRL), serum triiodothyronine (T₃) and serum cortisol in cycling ewes during heat stress.

	Tamb	Trec	RR	PRL	T ₃	Cortisol
Tamb		0.25 [*]	0.41 ^{***}	0.11	-0.07	-0.21
Trec	0.25 [*]		0.39 ^{***}	-0.17	-0.21	0.30 ^{**}
RR	-0.41 ^{***}	0.39 ^{***}		0.08	-0.39 ^{***}	0.04
PRL	0.11	-0.17	0.08		-0.02	-0.23
T ₃	-0.07	-0.21	-0.39 ^{***}	-0.02		-0.11
Cortisol	-0.21	0.30 ^{**}	0.04	-0.23	-0.11	

* asterisks denote the level of significance: (* p<0.05, ** p<0.01, *** p<0.001)

Table 8: ANOVA table for comparisons of the effect of temperature on the daytime hourly values of rectal temperature in cycling ewes treated without (Expt 1) or with (Expt 2) bromocryptine (CB-154).

Source	DF	MS	F
Total	69		
Expt	1	0.007	0.05 ^{NS}
Ewe(Expt)	12	0.136	
Cycle	1	3.775	89.88 ^{***}
Cycle*Expt	1	0.151	3.60 ^{NS}
Cycle*Ewe(Rep)	12	0.042	
Hour	3	1.616	202.00 ^{***}
Hour*Expt	3	0.069	8.63 ^{***}
Hour*Ewe(Expt)	36	0.008	

*

asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 9: ANOVA table for comparisons of the effect of temperature on the daytime hourly values of respiration rate (RR) in cycling ewes treated without (Expt 1, n=9) or with (Expt 2, n=5) bromocryptine (CB-154).

Source	DF	MS	F
Total	69		
Expt	1	2269.99	3.59 ^{NS}
Ewe(Expt)	12	632.09	
Cycle	1	184486.99	771.72 ^{***}
Cycle*Expt	1	489.36	2.05 ^{NS}
Cycle*Ewe(Expt)	12	239.06	
Hour	3	11846.92	108.12 ^{***}
Hour*Expt	3	622.47	5.68 ^{***}
Hour*Ewe(Expt)	36	109.57	NS

*

asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 10: ANOVA table for comparisons of the response of daily respiration rate in cycling ewes treated without (Expt 1) or with (Expt 2) bromocryptine (CB-154).

Source	DF	MS	F
Total	447		
Expt	1	15557.74	3.84
Ewe(Expt)	12	4055.52	
Cycle	1	1145018.17	1219.91
Expt*Cycle	1	13130.60	13.99
Cycle*Ewe(Expt)	12	938.61	
Phase	3	13159.41	22.32
Phase*Expt	3	7329.89	12.43
Phase*Ewe(Expt)	36	589.67	
Phase*Cycle	3	13245.04	9.21
Expt*Cycle*Phase	3	12883.83	8.96
Phase*Cycle*Ewe(Expt)	36	1438.63	
Error	336	364.15	

* asterisks denote the level of significance: (* p<0.05, ** p<0.01, *** p<0.001, NS = not significant)

Table 11: Mean (\pm SEM) of the respiration rate (RR) during the estrous cycle in ewes treated without (Expt #1, n=9) and with (Expt #2, n=5) bromocryptine (CB-154).

Phase of Cycle	Thermoneutral Cycle		Heat Stress Cycle	
	NO CB-154	CB-154 (RR min ⁻¹)	NO CB-154	CB-154 (RR min ⁻¹)
Follicular D1-D4	27.56 \pm 1.88	35.03 \pm 1.44	124.28 \pm 4.78	99.42 \pm 10.19
Early-luteal D5-D8	30.39 \pm 2.32	35.05 \pm 1.53	140.32 \pm 4.24	164.78 \pm 4.31
Mid-luteal D9-D12	37.38 \pm 3.27	35.35 \pm 1.79	150.68 \pm 3.02	174.40 \pm 3.27
Late-luteal D13-D16	44.26 \pm 3.64	38.15 \pm 2.47	96.47 \pm 8.73	167.53 \pm 4.53

Table 12: ANOVA table for comparisons of the 24-h profiles of cortisol during the preovulatory periods (D14-D18) in cycling ewes of Rep 1 (Year 1) and Rep 2 (Year 2).

Source	DF	MS	F
Total	959		
Rep	1	1.50	0.00
Ewe(Rep)	2	474.59	
Cycle	1	1314.14	2.95
Rep*Cycle	1	423.74	0.95
Cycle*Ewe(Rep)	2	445.38	
Day	4	367.36	8.41
Rep*Day	4	149.95	3.43
Rep*Day	8	104.43	
Day*Cycle	4	48.66	0.67
Day*Cycle*Rep	4	51.20	0.70
Day*Cycle*Ewe(Rep)	8	106.54	
Error	920	42.74	

*
 asterisks denote the level of significance:
 NS = not significant

Table 13: ANOVA table for comparisons of the 24-h profiles serum cortisol in cycling ewes treated without (Expt 1) or with (Expt 2) bromocryptine (CB-154).

Source	DF	MS	F
Total	1439		
Expt	1	93578.04	9.68 *
Ewe(Expt)	4	9671.80	
Cycle	1	5030.30	12.25 *
Expt*Cycle	1	1407.28	3.43 NS
Cycle*Ewe(Expt)	4	410.80	
Day	4	4443.99	7.51 ***
Expt*Day	4	6674.40	11.27 ***
Day*Ewe(Expt)	16	592.12	
Cycle*Day	4	584.10	3.65 *
Expt*Cycle*Day	4	1763.61	11.02 ***
Day*Cycle*Ewe(Expt)	16	160.02	
Error	1380	166.76	

* asterisks denote the level of significance: (* p<0.05, *** p<0.001, NS = not significant)

Table 14: Mean (\pm SEM) response of serum cortisol to ambient temperature (T_{amb}) and sampling during the preovulatory period in cycling ewes treated without (Expt 1) or with or with (Expt 2) bromocryptine (CB-154).

Expt	Day of Cycle	Thermoneutral Cycle (ng mL ⁻¹)	Cycle Mean (\pm SEM) (ng mL ⁻¹)	Heat Stress Cycle (ng mL ⁻¹)	Cycle Mean (\pm SEM) (ng mL ⁻¹)
NO CB-154	D14	12.51 \pm 0.71		10.37 \pm 0.72	
	D15	11.37 \pm 0.77		7.52 \pm 0.56	
	D16	10.70 \pm 0.68		9.31 \pm 0.67	
	D17	12.24 \pm 0.83		9.46 \pm 0.76	
	D18	8.63 \pm 0.59		7.09 \pm 0.62	
			11.09 \pm 0.69		8.75 \pm 0.62
With CB-154	D14	35.27 \pm 4.07		31.06 \pm 3.23	
	D15	35.66 \pm 4.15		39.77 \pm 4.27	
	D16	44.11 \pm 5.01		21.85 \pm 2.56	
	D17	20.01 \pm 2.17		12.29 \pm 1.50	
	D18	16.38 \pm 1.53		13.80 \pm 1.55	
			30.29 \pm 5.21		23.75 \pm 5.21

Table 15: ANOVA table for the characteristics of cortisol profiles during the preovulatory period in cycling ewes treated without (Expt #1, n=4) or with (Expt #2, n=2) bromocryptine (CB-154).

Source	DF	MS				
		Mean conc.	Pulse Amplitude	Pulse Frequency (x10 ⁻⁵)	Duration of Pulse Peak	Interpeak Interval
Total	11					
Expt	1	854.18 *	94.55	1.67	0.11	0.00
Ewe(Expt)	4	48.53	11.23	26.25	0.79	1.01
Cycle	1	87.61	2.57	15.00	0.68	0.45
Cycle*Expt	1	9.24	0.61	15.00	1.08	0.23
Cycle*Ewe(Expt)	4	14.00	2.88	26.25	0.59	0.79

* asterisks denote the level of significance: (* p<0.05)

Table 16: Means (\pm SEM) of the secretion characteristics of serum cortisol during the preovulatory period (D14-D18) in cycling ewes treated with or without bromocryptine (CB-154).

Expt	Cycle	Character				
		Basal Conc. (ng mL ⁻¹)	Pulse Amplitude (ng mL ⁻¹)	Pulse Frequency (pk h ⁻¹)	Duration of Pulse Peak (h)	Interpeak Interval (h)
NO CB-154	Thermoneutral Cycle	5.58 \pm 0.48	11.18 \pm 1.37	0.33 \pm 0.00	1.98 \pm 0.01	3.05 \pm 0.03
	Heat Stress Cycle	3.85 \pm 0.18	9.73 \pm 0.44	0.35 \pm 0.01	1.82 \pm 0.09	2.94 \pm 0.10
With CB-154	Thermoneutral Cycle	14.15 \pm 3.55	31.00 \pm 10.80	0.34 \pm 0.01	1.85 \pm 0.04	2.97 \pm 0.08
	Heat Stress Cycle	11.30 \pm 3.70	26.70 \pm 9.70	0.34 \pm 0.00	1.87 \pm 0.04	2.96 \pm 0.01

Table 17: Stepwise regression of serum prolactin, triiodothyronine and cortisol with ambient temperature, rectal temperature and respiration rate in cycling ewes.

Hormone	Step	Variables Entered/Removed	Number In	Partial R ²	Model R ²	F
Prolactin	1	Ambient Temperature	1	0.30	0.30	56.17 ***
	2	Rectal Temperature	2	0.02	0.32	3.84 *
	3	Respiration Rate	3	0.02	0.33	3.32 NS
Triiodothyronine	1	Respiration Rate	1	0.04	0.04	5.19 *
Cortisol	1	Ambient Temperature	1	0.06	0.06	8.07 **
	2	Rectal Temperature	2	0.06	0.12	9.10 **

* asterisks denote the level of significance: (* p<0.05, ** p<0.01, *** p<0.001, NS = not significant)

Table 18: Stepwise regression of daily serum cortisol with ambient temperature in cycling ewes treated with bromocryptine, CB-154.

Hormone	Step	Variables Entered/Removed	Number In	Partial R ²	Model R ²	F
Cortisol	1	Ambient Temperature	1	0.02	0.02	4.05*

* asterisks denote the level of significance: (* p<0.05)

Table 19: ANOVA table for comparisons of the 24-h profiles of triiodothyronine (T_3) in cycling ewes of Rep 1 (Expt 1) (n=4).

Source	DF	MS	F
Total	159		
Ewe	3	0.302	0.76
Cycle	1	0.017	0.04
Ewe*Cycle	3	0.399	
Day	4	0.013	0.33
Ewe*Day	12	0.039	
Cycle*Day	4	0.039	0.72
Cycle*Day*ewe	12	0.054	
Error	120	0.020	

*

asterisks denote the level of significance:
NS = not significant

Table 20: Mean (\pm SEM) comparisons of the 24-h profiles of serum triiodothyronine, T_3 (ng mL^{-1}) concentration during the preovulatory period (D14-D18) of cycling ewes during normothermia and hyperthermia (Expt 1, Rep 1; $n=4$).

Time of Day (h)	Thermoneutral Cycle (ng mL^{-1})	Heat Stress Cycle (ng mL^{-1})
700	0.44 ± 0.05	0.48 ± 0.05
1200	0.44 ± 0.05	0.39 ± 0.05
1800	0.41 ± 0.04	0.39 ± 0.04
2400	0.39 ± 0.03	0.35 ± 0.03

Table 21: Mean (\pm SEM) comparisons of the response of serum triiodothyronine, T_3 (ng mL^{-1}) to temperature and sampling during the preovulatory period (D14-D18) of cycling ewes (Expt 1, Rep 1).

Day of Cycle	Thermoneutral Cycle (ng mL^{-1})	Heat Stress Cycle (ng mL^{-1})
D14	0.36 \pm 0.04	0.40 \pm 0.03
D15	0.45 \pm 0.05	0.40 \pm 0.05
D16	0.42 \pm 0.02	0.43 \pm 0.04
D17	0.39 \pm 0.05	0.42 \pm 0.06
D18	0.49 \pm 0.07	0.36 \pm 0.06

Table 22: ANOVA table for comparisons of daily concentration of serum triiodothyronine (T_3) in cycling ewes (Rep 1, Expt #1; n=4).

Source	DF	MS	F
Total	127		
Ewe	3	0.187	3.98 ^{NS}
Cycle	1	0.033	0.71 ^{NS}
Ewe*Cycle	3	0.047	
Phase	3	0.023	1.20 ^{NS}
Phase*ewe	9	0.023	
Phase*Cycle	3	0.045	1.60 ^{NS}
Phase*Cycle*Ewe	9	0.028	
Error	96	0.019	

*

asterisks denote the level of significance:
NS = not significant

Table 23: ANOVA table for comparisons of the 24-h profiles of serum prolactin (PRL) in cycling ewes (Rep 1, Expt 1).

Source	DF	MS	F
Total	959		
Ewe	3	15151.32	0.26 NS
Cycle	1	95390.97	1.65 NS
Ewe*Cycle	3	57928.21	
Day	4	8288.26	0.34 NS
Ewe*Day	12	24420.23	
Cycle*Day	4	17453.89	1.66 NS
Day*Cycle*Ewe	12	2660.73	
Hour	23	6786.35	2.61 ***
Hour*Ewe	69	2598.48	
Cycle*Hour	23	6984.21	2.62 ***
Hour*Cycle*Ewe	69	2660.73	
Day*Hour	92	1996.70	1.09 NS
Day*Hour*Ewe	276	1833.72	
Cycle*Day*Hour	92	2633.26	1.37 *
Cycle*Day*Hour*Ewe	276	1926.13	

*

asterisks denote the level of significance:
 (* p<0.05, *** p<0.001, NS = not significant)

Table 24: Mean (\pm SEM) comparisons of daily concentration of serum prolactin, PRL (ng mL^{-1}) in response to ambient temperature and sampling during the preovulatory period (D14-D18) of cycling ewes.

Day of Cycle	Thermoneutral Cycle (ng mL^{-1})	Heat Stress Cycle (ng mL^{-1})
D14	59.55 \pm 4.51	80.85 \pm 7.87
D15	76.83 \pm 6.49	65.60 \pm 6.15
D16	50.41 \pm 4.54	73.98 \pm 7.29
D17	44.73 \pm 3.65	85.68 \pm 6.08
D18	42.48 \pm 2.53	67.58 \pm 3.51
Mean \pm SEM	54.80 \pm 2.37	74.74 \pm 2.37

Table 25: ANOVA table for the release characteristics of prolactin (PRL) during the 5 day sampling period in cycling ewes (Rep #1, Expt #1; n=4).

Source	DF	MS				
		Mean conc.	pulse Amplitude	Pulse Frequency (x10 ⁻¹)	Duration of Pulse peak	Interpeak Interval
Total	7					
Ewe	3	12.69	1.05	0.41	3.33	0.06
Cycle	1	133.61	0.53	0.84	5.00	1.29
Cycle*Ewe	3	37.76	8.03	0.82	68.33	1.42

NO differences in any of the characters

Table 26: Mean (\pm SEM) comparisons of the release characteristics of serum prolactin (PRL) during the preovulatory period (D14-D18) of cycling ewes (Expt 1, Rep 1; n=4).

Character	Thermoneutral	Cyclic Heat stress
Basal PRL (ng mL ⁻¹)	32.05 \pm 2.96	46.13 \pm 4.53
Pulse Amplitude (ng mL ⁻¹)	45.78 \pm 5.28	56.63 \pm 20.57
Pulse Frequency (pk h ⁻¹)	0.31 \pm 0.01	0.32 \pm 0.01
Duration of pulse Peak (h)	2.13 \pm 0.09	2.02 \pm 0.06
Interpeak Interval (h)	3.28 \pm 0.08	3.18 \pm 0.09

Table 27: ANOVA table for comparisons of daily concentration of serum prolactin (PRL) in cycling ewes (Rep 1, Expt 1) by phase.

Source	DF	MS	F
Total	127		
Ewe	3	6078.97	0.50 ^{NS}
Cycle	1	143012.21	11.82 [*]
Ewe*Cycle	3	12099.88	
Phase	3	7281.45	3.61 ^{NS}
Phase*ewe	9	2018.62	0.72
Phase*Cycle	3	7254.73	2.22 ^{NS}
Phase*Cycle*Ewe	9	3274.73	
Error	96	1902.59	

*

asterisks denote the level of significance:
 (* p<0.05, NS = not significant)

Table 28: Mean (\pm SEM) comparisons of rectal temperature, T_{rec} ($^{\circ}$ C) and respiration rate (min^{-1}) in cycling ewes (Expt 1, Rep 1).

Cycle	Hour of Day	Rectal Temperature ($^{\circ}$ C)	Respiration Rate (min^{-1})
Thermoneutral Cycle	700	39.01 \pm 0.12	36.65 \pm 11.44
	1200	39.22 \pm 0.09	36.33 \pm 7.86
Heat Stress Cycle	700	39.09 \pm 0.10	62.82 \pm 14.17
	1200	39.61 \pm 0.15	145.01 \pm 6.46
	1500	39.95 \pm 0.19	167.92 \pm 7.76

Table 29: ANOVA table for comparisons of the effect of temperature during Year #1 (Rep 1) and Year #2 (Rep 2) on serum progesterone (P_4) concentration of follicular (D1-D4), early-luteal (D5-D8), mid-luteal (D9-D12) and late-luteal (D13-D16) phases of cycling ewes (n=9).

Source	DF	MS	F
Total	287		
Rep	1	40.42	18.21 **
Ewe(Rep)	7	2.22	
Cycle	1	2.65	1.74 NS
Rep*Cycle	1	0.02	0.01 NS
Cycle*Ewe(Rep)	7	1.52	
Phase	3	75.11	50.07 ***
Rep*Phase	3	4.05	2.07 NS
Phase*Ewe(Rep)	21	1.50	
Cycle*Phase	3	0.94	1.54 NS
Rep*Cycle*Phase	3	0.90	1.48
Phase*Cycle*Ewe(Rep)	21	0.61	
Error	216	0.55	

* asterisks denote the level of significance: (** $p < 0.01$, *** $p < 0.001$, NS = not significant)

Table 30: Mean (\pm SEM) concentrations of serum progesterone, P_4 (ng mL^{-1}) during follicular (D1-D4), early-luteal (D5-D8), mid-luteal (D9-D12) and late-luteal (D13-D16) phases in cycling ewes ($n=9$).

Cycle Phase	Thermoneutral Cycle (ng mL^{-1})	Heat Stress Cycle (ng mL^{-1})
Follicular	0.66 ± 0.10	0.82 ± 0.12
Early-luteal	2.17 ± 0.13	2.54 ± 0.17
Mid-luteal	2.78 ± 0.16	3.14 ± 0.146
Late-luteal	1.27 ± 0.20	1.15 ± 0.18
Overall Mean	1.72 ± 0.47	1.91 ± 0.55

Table 31: Mean (\pm SEM) comparisons of the effect of temperature during Year #1 (Rep 1) and Year #2 (Rep 2) on serum progesterone (P_4) concentration (ng mL^{-1}) of follicular (D1-D4), early-luteal (D5-D8), mid-luteal (D9-D12) and late-luteal (D13-D16) phases of cycling ewes ($n=9$).

Replicate	Phase of Cycle	Thermoneutral Cycle (ng mL^{-1})	Heat Stress Cycle (ng mL^{-1})
Rep 1	Follicular	0.34 \pm 0.10	0.43 \pm 0.11
	Early-luteal	1.74 \pm 0.16	1.78 \pm 0.21
	Mid-luteal	2.10 \pm 0.22	2.52 \pm 0.23
	Late-luteal	1.07 \pm 0.25	1.21 \pm 0.23
Rep 2	Follicular	0.92 \pm 0.13	1.31 \pm 0.17
	Early-luteal	2.52 \pm 0.17	3.15 \pm 0.15
	Mid-luteal	3.33 \pm 0.14	3.64 \pm 0.16
	Late-luteal	1.43 \pm 0.29	1.10 \pm 0.27

Table 32: ANOVA table for comparisons of the effect of temperature on the area under daily progesterone curve (units) in cycling ewes (n=9).

Source	DF	MS	F
Total	17		
Rep	1	165.51	10.28*
Ewe(Rep)	7	16.10	
Cycle	1	101.77	3.05 ^{NS}
Rep*Cycle	1	13.42	0.40 ^{NS}
Error	7	33.38	

* asterisks denote the level of significance: (* $p < 0.05$,
NS = not significant)

Table 33: ANOVA table for comparisons of the effect of temperature on the regression of the corpus luteum as followed by serum progesterone (P_4) concentration during the preovulatory period in cycling ewes ($n=9$). Means were examined at 6-h periods relative to LH surge peak.

Source	DF	MS	F
Total	449		
Rep	1	16.62	14.71 ***
Ewe(Rep)	7	1.13	
Cycle	1	1.11	5.19 *
Rep*Cycle	1	0.00	0.00
Cycle*Ewe(Rep)	7	0.21	
Period	7	11.70	31.62 ***
Rep*Period	7	0.13	0.35 NS
Period*Ewe(Rep)	49	0.37	
Cycle*Period	7	0.62	3.44 **
Rep*Cycle*Period	7	0.01	0.07
Period*Cycle*Ewe(Rep)	49	0.18	
Error	306	0.09	

* asterisks denote the level of significance: (* $p < 0.05$,
 *** $p < 0.001$, NS = not significant)

Table 34: Mean (\pm SEM) comparisons for the effect of temperature on the regression of the corpus luteum between 72 h before to 72 h after the preovulatory LH surge peak (time=0) in cycling ewes (n=9).

Period #	Hours relative to LH surge peak	Thermoneutral Cycle (ng mL ⁻¹)	Heat Stress Cycle (ng mL ⁻¹)
1	-72 to -54	1.20 \pm 0.11	1.66 \pm 0.19
2	-54 to -36	0.43 \pm 0.05	0.70 \pm 0.11
3	-36 to -18	0.35 \pm 0.05	0.27 \pm 0.03
4	-18 to 00	0.33 \pm 0.04	0.26 \pm 0.03
5	00 to 18	0.31 \pm 0.04	0.30 \pm 0.04
6	18 to 36	0.28 \pm 0.04	0.34 \pm 0.05
7	36 to 54	0.47 \pm 0.05	0.44 \pm 0.06
8	54 to 72	0.70 \pm 0.08	0.77 \pm 0.08

Table 35: ANOVA table for comparisons of the effect of temperature on the length of the estrous cycle (days) in ewes (n=9).

Source	DF	MS	F
Total	17		
Rep	1	7.55	8.99 [*]
Ewe(Rep)	7	0.84	
Cycle	1	0.17	1.93 ^{NS}
Rep*Cycle	1	0.18	2.00 ^{NS}
Error	7	0.09	

* asterisks denote the level of significance: (* p<0.05, NS = not significant)

Table 36: Mean (\pm SEM) comparisons for the length of the estrous and characteristics of daily progesterone (P_4) concentration in cycling ewes of Rep 1 (n=4) and Rep 2 (n=5) .

Parameter	Replicate #	Thermoneutral Cycle	Heat Stress Cycle
Length of Estrous Cycle (days)	1	17.23 \pm 0.36	17.65 \pm 0.35
	2	16.13 \pm 0.31	16.14 \pm 0.23
Area under Progesterone Curve (arbitrary units)	1	19.08 \pm 3.14	21.90 \pm 2.76
	2	23.44 \pm 1.82	29.74 \pm 1.88

Table 37: ANOVA table for comparisons of the effect of temperature on daily serum luteinizing hormone (LH) concentration during estrus in cycling ewes (n=9).

Source	DF	MS	F
Total	305		
Rep	1	1.70	0.58
Ewe(Rep)	7	2.92	
Cycle	1	2.85	2.44
Rep*Cycle	1	2.85	2.44
Cycle*Ewe(Rep)	7	1.17	
Phase	3	3.25	5.28
Rep*Phase	3	1.85	3.00
Phase*Ewe(Rep)	21	0.62	
Cycle*Phase	3	1.72	2.64
Rep*Cycle*Phase	3	1.20	1.83
Phase*Cycle*Ewe(Rep)	21	0.65	
Error	216	0.86	

* asterisks denote the level of significance: (* p<0.05, ** p<0.01, NS = not significant)

Table 38: The Mean (\pm SEM) reponse to temperature of cyclic LH (ng mL^{-1}) in ewes of Rep 1 (n=4) and Rep 2 (n=5).

Phase of Cycle	Thermoneutral Cycle		Heat Stress Cycle	
	Rep 1	Rep 2	Rep 1	Rep 2
	(ng mL^{-1})		(ng mL^{-1})	
Follicular	1.07 \pm 0.11	1.14 \pm 0.10	1.08 \pm 0.10	1.14 \pm 0.08
Early-luteal	0.96 \pm 0.14	0.99 \pm 0.06	0.85 \pm 0.08	0.99 \pm 0.05
Mid-luteal	1.19 \pm 0.28	0.87 \pm 0.05	0.88 \pm 0.10	0.92 \pm 0.08
Late-luteal	1.10 \pm 0.13	1.14 \pm 0.11	1.03 \pm 0.09	2.21 \pm 0.70

Table 39: Effect of temperature on the correlation coefficients (r) between daily concentration of progesterone (P₄), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in cycling ewes (n=9).

Hormone	Thermoneutral Cycle			Heat Stress Cycle		
	P ₄	LH	FSH	P ₄	LH	FSH
Progesterone (P ₄)	.	NS -0.47	** -0.72	.	** -0.65	*** -0.83
Luteinizing Hormone (LH)	NS -0.47	.	*** 0.82	** -0.65	.	** 0.64
Follicle-Stimulating Hormone (FSH)	** -0.72	*** 0.82	.	*** -0.83	** 0.64	.

*

asterisks denote level of significance: (** p<0.01, *** p<0.001, NS = not significant; DF=16)

Table 40: ANOVA table for comparisons of the effect of temperature on the area under the preovulatory LH surge curve in cycling ewes (n=9).

Source	DF	MS	F
Total	17		
Rep	1	71433.49	26.02 ^{***}
Ewe(Rep)	7	2745.81	
Cycle	1	2959.37	4.95 ^{NS}
Rep*Cycle	1	98.49	0.16 ^{NS}
Error	7	598.03	

*

asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 41: ANOVA table for comparisons of the effect of temperature on the duration of the preovulatory LH surge curve in cycling ewes (n=9).

Source	DF	MS	F
Total	17		
Rep	1	12.10	5.33 [*]
Ewe(Rep)	7	2.27	
Cycle	1	0.50	1.00 ^{NS}
Rep*Cycle	1	2.50	5.00 ^{NS}
Error	7	0.50	

*
asterisks denote the level of significance:
(NS = not significant)

Table 42: ANOVA table for comparisons of the effect of temperature on preovulatory LH surge peak (ng mL^{-1}) in cycling ewes ($n=9$).

Source	DF	MS	F
Total	17		
Rep	1	113.23	7.55*
Ewe(Rep)	7	15.00	
Cycle	1	102.72	8.91*
Rep*Cycle	1	18.18	1.58 ^{NS}
Error	7	11.53	

* asterisks denote the level of significance: (* $p < 0.05$, NS = not significant)

Table 43: Means (\pm SEM) of the preovulatory LH release characteristics in cycling ewes of Rep 1 (n=4) and Rep 2 (n=5).

Character	Replicate #	Thermoneutral cycle	Heat Stress cycle
Duration of LH Surge (h)	1	9.50 \pm 0.50	9.00 \pm 0.58
	2	10.40 \pm 0.60	11.40 \pm 0.51
Area under LH Surge Curve (arbitrary units)	1	116.65 \pm 9.01	85.78 \pm 17.50
	2	238.72 \pm 23.78	217.26 \pm 19.30
LH Surge Peak (ng mL ⁻¹)	1	25.75 \pm 2.10	18.73 \pm 1.56
	2	18.68 \pm 1.85	15.70 \pm 1.33
Basal LH (ng mL ⁻¹)	1	1.24 \pm 0.21	1.52 \pm 0.21
	2	1.54 \pm 0.21	1.67 \pm 0.19
Pulse Amplitude (ng mL ⁻¹)	1	1.16 \pm 0.10	0.79 \pm 0.06
	2	0.72 \pm 0.08	0.73 \pm 0.04
Pulse Frequency (h ⁻¹)	1	0.28 \pm 0.01	0.32 \pm 0.01
	2	0.33 \pm 0.01	0.30 \pm 0.02
Duration of Pulse Peak (h)	1	2.21 \pm 0.04	1.95 \pm 0.13
	2	1.81 \pm 0.08	1.99 \pm 0.16
Interpeak Interval (h)	1	3.52 \pm 0.02	3.15 \pm 0.10
	2	3.09 \pm 0.14	3.34 \pm 0.17

Table 44: ANOVA table for the release characteristics of LH during the preovulatory period in cycling ewes of Rep #1 (n=4) and Rep #2 (n=5).

Source	DF	MS				
		Mean Conc.	Pulse Amplitude	Pulse Frequency ($\times 10^{-5}$)	Duration of Pulse Peak	Interpeak Interval
Total	17					
Rep	1	8.99	1.84*	4.33	132.25	1.01
Ewe(Rep)	7	8.71	0.33	1.94	105.39	1.44
Cycle	1	11.63	0.67	0.64	0.56	0.56
Cycle*Rep	1	0.75	0.98*	3.82*	354.69*	8.08**
Cycle*Ewe(Rep)	7	3.42	0.09	0.67	48.54	0.65

* asterisks denote the level of significance: (* $p < 0.05$, ** $p < 0.01$)

Table 45: Mean (\pm SEM) of the preovulatory release characteristics of LH in cycling ewes (n=9) in response to temperature.

Character	Thermoneutral Cycle	Heat Stress Cycle
Duration of LH surge (h)	10.00 \pm 0.41	10.33 \pm 0.55
Area under LH surge (arbitrary units)	184.47 \pm 25.11	158.82 \pm 26.23
LH surge peak (ng mL ⁻¹)	21.82 \pm 1.80	17.04 \pm 1.09
Basal LH (ng mL ⁻¹)	1.43 \pm 0.15	1.60 \pm 0.14
Pulse amplitude (ng mL ⁻¹)	0.91 \pm 0.10	0.76 \pm 0.04
Pulse Frequency (h ⁻¹)	0.31 \pm 0.01	0.31 \pm 0.01
Duration of Pulse (h)	1.99 \pm 0.08	1.97 \pm 0.10
Interpeak Interval (h)	3.28 \pm 0.11	3.26 \pm 0.11

Table 46: ANOVA table for comparisons of the effect of temperature on serum LH (ng mL^{-1}) between 48 h before to 12 h after the preovulatory LH surge peak (time=0) in cycling ewes (n=5).

Source	DF	MS	F
Total	609		
Rep	1	0.12	0.01 NS
Ewe(Rep)	3	13.82	
Cycle	1	1.59	0.32 NS
Rep*Cycle	1	16.51	3.29 NS
Cycle*Ewe(Rep)	3	5.02	
Period	9	617.63	269.71 ***
Rep*Period	9	1.22	0.53 NS
Period*Ewe(Rep)	27	2.29	
Cycle*Period	9	9.45	1.05 NS
Rep*Cycle*Period	9	11.36	1.26 NS
Period*Cycle*Ewe(Rep)	27	9.03	
Error	510	5.66	

*
 asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 47: ANOVA table for comparisons of the effect of temperature on serum LH (ng mL^{-1}) between 12 h before to 48 h after the preovulatory surge peak (time=0) in cycling ewes (n=6).

Source	DF	MS	F
Total	731		
Rep	1	25.12	1.33
Ewe(Rep)	4	18.94	
Cycle	1	0.32	0.05
Rep*Cycle	1	0.00	0.00
Cycle*Ewe(Rep)	4	6.38	
Period	9	956.64	110.88
Rep*Period	9	15.88	1.84
Period*Ewe(Rep)	36	8.63	
Cycle*Period	9	2.09	0.22
Rep*Cycle*Period	9	1.02	0.11
Period*Cycle*Ewe(Rep)	36	9.63	
Error	612	7.63	

*

asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 48: ANOVA table for comparisons of the effect of temperature on daily serum follicle-stimulating hormone (FSH) concentration in cycling ewes (n=9).

Source	DF	MS	F
Total	287		
Rep	1	3252.16	6.15 *
Ewe(Rep)	7	528.77	
Cycle	1	25.62	0.34 NS
Rep *Cycle	1	33.38	0.45 NS
Cycle*Ewe(Rep)	7	74.69	
Phase	3	354.62	6.89 **
Rep *Day	3	206.62	4.01 *
Phase*Ewe(Rep)	21	51.47	
Cycle*Phase	3	48.76	1.37 NS
Rep *Cycle*Day	3	35.97	1.01
Phase*Cycle*Ewe(Rep)	21	35.52	
Error	216	33.44	

* asterisks denote the level of significance: (* p<0.05, *** p<0.001, NS = not significant)

Table 49: Mean (\pm SEM) concentration of the cyclic FSH in ewes of Rep 1 (n=4) and Rep 2 (n=5).

Phase of Cycle	Thermoneutral Cycle		Heat Stress Cycle	
	Rep 1 (ng mL ⁻¹)	Rep 2 (ng mL ⁻¹)	Rep 1 (ng mL ⁻¹)	Rep 2 (ng mL ⁻¹)
Follicular	11.94 \pm 1.89	16.07 \pm 1.19	14.13 \pm 2.75	15.90 \pm 1.02
Early-luteal	7.96 \pm 1.51	16.18 \pm 1.07	9.25 \pm 1.54	13.01 \pm 0.84
Mid-luteal	5.76 \pm 0.94	13.16 \pm 0.97	6.28 \pm 0.80	12.71 \pm 1.08
Late-luteal	7.26 \pm 1.42	17.31 \pm 1.98	8.69 \pm 2.59	21.05 \pm 2.86

Table 50: ANOVA table for comparisons of the effect of temperature on the area under the preovulatory FSH surge curve in cycling ewes (n=9).

Source	DF	MS	F
Total	17		
Rep	1	2371365.34	8.96 [*]
Ewe(Rep)	7	264578.37	
Cycle	1	847602.00	5.18 ^{NS}
Rep*Cycle	1	170302.50	1.04 ^{NS}
Error	7	163492.86	

* asterisks denote the level of significance: (* p<0.05, NS = not significant)

Table 51: ANOVA table for comparisons of the effect of temperature on the preovulatory FSH surge peak in cycling ewes (n=9).

Source	DF	MS	F
Total	17		
Rep	1	5309.95	2.98 ^{NS}
Ewe(Rep)	7	1781.64	
Cycle	1	3078.51	3.70 ^{NS}
Rep*Cycle	1	916.49	1.10 ^{NS}
Error	7	832.51	

*
asterisks denote the level of significance:
(NS = not significant)

Table 52: ANOVA table for the release characteristics of FSH during the preovulatory period in cycling ewes of Rep #1 (n=4) and Rep #2 (n=5).

Source	DF	MS				
		Mean Conc.	Pulse Amplitude	Pulse Frequency (x10 ⁻⁵)	Duration of Pulse Peak	Interpeak Interval
Total	17					
REP	1	1670.03 ^{**}	12.51	4.00	0.07	0.03
Ewe(Rep)	7	100.18	7.46	43.43	1.27	1.22
Cycle	1	0.13	1.68	5.00	0.53	1.08
Cycle*Rep	1	25.42	1.54	25.00	0.41	0.60
Cycle*Ewe(Rep)	7	13.04	0.41	35.00	0.72	0.68

* asterisks denotes the level of significance: (** p<0.01)

Table 53: Mean (\pm SEM) of the preovulatory release characteristics of FSH in cycling ewes (n=9) in response to temperature.

Character	Thermoneutral Cycle	Heat Stress Cycle
Area under FSH surge (arbitrary units)	1813.44 \pm 240.53	1379.44 \pm 138.07
FSH Surge Peak (ng mL ⁻¹)	104.06 \pm 16.50	77.90 \pm 8.27
Basal FSH (ng mL ⁻¹)	14.50 \pm 2.05	14.92 \pm 2.61
Pulse Amplitude (ng mL ⁻¹)	8.12 \pm 0.78	7.31 \pm 0.90
Pulse Frequency (h ⁻¹)	0.33 \pm 0.01	0.33 \pm 0.01
Duration of Pulse (h)	1.99 \pm 0.05	1.91 \pm 0.05
Interpeak Interval (h)	3.08 \pm 0.05	3.01 \pm 0.07

Table 54: Means (\pm SEM) of the preovulatory FSH release characteristics in cycling ewes of Rep 1 (n=4) and Rep 2 (n=5).

Character	Replicate #	Thermoneutral cycle	Heat Stress cycle
Area under FSH Surge Curve (arbitrary units)	1	2328.00 \pm 69.22	1676.50 \pm 205.28
	2	1401.80 \pm 331.80	1141.80 \pm 107.59
FSH Surge Peak (ng mL ⁻¹)	1	76.88 \pm 15.86	66.68 \pm 15.03
	2	125.80 \pm 23.73	86.88 \pm 8.09
Basal FSH (ng mL ⁻¹)	1	11.08 \pm 2.94	9.33 \pm 2.49
	2	17.24 \pm 2.37	19.39 \pm 3.11
Pulse Amplitude (ng mL ⁻¹)	1	7.69 \pm 1.43	6.43 \pm 1.68
	2	8.46 \pm 0.94	8.02 \pm 0.98
Pulse Frequency (h ⁻¹)	1	0.33 \pm 0.03	0.33 \pm 0.01
	2	0.33 \pm 0.01	0.34 \pm 0.01
Duration of Pulse Peak (h)	1	1.93 \pm 0.04	1.94 \pm 0.12
	2	2.03 \pm 0.09	1.89 \pm 0.03
Interpeak Interval (h)	1	3.04 \pm 0.04	3.07 \pm 0.15
	2	3.12 \pm 0.09	2.96 \pm 0.04

Table 55: ANOVA table for comparisons of the effect of temperature on 6-h serum FSH (ng mL^{-1}) between 48 h before to 12 h after the preovulatory LH surge peak (time=0) in cycling ewes (n=5).

Source	DF	MS	F
Total	609		
Rep	1	8938.52	3.44
Ewe(Rep)	3	2595.67	
Cycle	1	304.95	0.54
Rep*Cycle	1	412.76	0.72
Cycle*Ewe(Rep)	3	569.67	
Period	9	11329.35	79.45
Rep*Period	9	131.15	0.92
Period*Ewe(Rep)	27	142.60	
Cycle*Period	9	313.92	1.42
Rep*Cycle*Period	9	136.20	0.62
Period*Cycle*Ewe(Rep)	27	221.19	
Error	510	141.13	

*

asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 56: ANOVA table for comparisons of the effect of temperature on 6-h concentration of serum FSH (ng mL^{-1}) between 12 h before to 48 h after the preovulatory LH surge peak (time=0) in cycling ewes (n=6).

Source	DF	MS	F
Total	731		
Rep	1	15830.10	1.18
Ewe(Rep)	4	13411.08	
Cycle	1	1298.40	3.78
Rep*Cycle	1	899.63	2.62
Cycle*Ewe(Rep)	4	343.12	
Period	9	9565.02	17.82
Rep*Period	9	167.15	0.32
Period*Ewe(Rep)	36	536.62	
Cycle*Period	9	169.96	0.70
Rep*Cycle*Period	9	309.41	1.27
Period*Cycle*Ewe(Rep)	36	244.44	
Error	612	143.16	

*

asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 57: ANOVA table for comparisons of the effect of temperature on hourly concentration of serum prolactin that were associated with the preovulatory gonadotropin surge in cycling ewes (n=4).

Source	DF	MS	F
Total	199		
Ewe	3	11144.09	0.16 ^{NS}
Cycle	1	32007.03	0.49 ^{NS}
Ewe*Cycle	3	68768.53	
Hour	24	4813.45	1.01 ^{NS}
Ewe*Hour	72	4777.54	
Cycle*Hour	24	4846.36	1.45 ^{NS}
Error	72	3351.27	

* level of significance: NS = not significant

Table 58: ANOVA for comparisons of the effect of temperature on the length of the estrous cycle and the area under daily cyclic progesterone (P₄) in cycling ewes treated with bromocryptine (CB-154).

Source	DF	MS	
		Length of the Estrous Cycle	Area under Cyclic P ₄ Curve
Total	9		
Ewe	4	3.25 ^{NS}	93.65 [*]
Cycle	1	2.84 ^{NS}	103.04 [*]
Error	5	3.04	10.59

* asterisks denote level of significance: (* p<0.05, NS = not significant)

Table 59: ANOVA table for comparisons of the effect of temperature on serum progesterone (P_4) concentration during follicular (D1-D4), early-luteal (D5-D8), mid-luteal (D9-D12) and late-luteal (D13-D16) phases of the estrous cycle in cycling ewes treated with bromocryptine (CB-154).

Source	DF	MS	F
Total	159		
Ewe	4	6.82	26.23 **
Cycle	1	5.57	21.42 **
Ewe*Cycle	4	0.26	
Phase	3	82.97	23.50 ***
Ewe*Phase	12	3.53	
Cycle*Phase	3	1.50	3.49 *
Phase*Cycle*Ewe	12	0.43	
Error	120	1.10	

* asterisks denote the level of significance: (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS = not significant)

Table 60: Mean (\pm SEM) of serum progesterone (P_4) concentration (ng mL^{-1}) during follicular (D1-D4), early-luteal (D5-D8), Mid-luteal (D9-D12) and late-luteal (D13-D16) phases in CB-154-treated cycling ewes ($n=5$) in response to temperature.

Cycle Phase	Thermoneutral Cycle (ng mL^{-1})	Heat Stress Cycle (ng mL^{-1})
Follicular	0.94 \pm 0.14	1.49 \pm 0.24
Early-luteal	3.19 \pm 0.23	4.01 \pm 0.24
Mid-luteal	3.94 \pm 0.18	4.18 \pm 0.27
Late-luteal	1.58 \pm 0.37	1.47 \pm 0.35
Overall Mean	2.41 \pm 0.70	2.79 \pm 0.75

Table 61: ANOVA table for comparisons of the effect of temperature on the regression of the corpus luteum as followed by serum progesterone (P_4) concentration during the preovulatory period in cycling ewes ($n=5$) treated with bromocryptine (CB-154). Means examined at 18-h periods relative to the LH surge peak (time=0).

Source	DF	MS	F
Total	249		
Ewe	4	2.56	5.02 ^{NS}
Cycle	1	0.45	0.80 ^{NS}
Ewe*Cycle	4	0.51	
Period	7	16.19	27.44 ^{***}
Ewe*Period	28	0.59	
Cycle*Period	7	0.62	2.14 ^{NS}
Ewe*Cycle*Period	28	0.29	
Error	170	0.08	

* asterisks denote the level of significance: (***) $p < 0.05$, NS = not significant

Table 62: Mean (\pm SEM) comparisons for the effect of temperature on the regression of the corpus luteum between 72 h before to 72 h after the preovulatory LH surge peak (time=0) in cycling ewes (n=5) treated with bromocryptine (CB-154).

Period #	Time (hours relative to LH surge peak=0)	Thermoneutral Cycle (ng mL ⁻¹)	Heat Stress Cycle (ng mL ⁻¹)
1	-72 to -54	2.03 \pm 0.28	2.60 \pm 0.21
2	-54 to -36	1.03 \pm 0.19	0.60 \pm 0.03
3	-36 to -18	0.56 \pm 0.07	0.55 \pm 0.03
4	-18 to 00	0.48 \pm 0.04	0.51 \pm 0.03
5	00 to 18	0.45 \pm 0.03	0.44 \pm 0.03
6	18 to 36	0.47 \pm 0.04	0.53 \pm 0.05
7	36 to 54	0.56 \pm 0.04	0.73 \pm 0.08
8	54 to 72	0.94 \pm 0.11	1.11 \pm 0.08

Table 63: Effect of temperature on the correlation coefficients (r) between daily concentration of serum progesterone (P₄), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in cycling ewes (n=5) treated with bromocryptine (CB-154).

Hormone	Thermoneutral Cycle			Heat Stress Cycle		
	P ₄	LH	FSH	P ₄	LH	FSH
Progesterone (P ₄)	.	-0.78 ***	-0.67 **	.	-0.47 NS	-0.75 ***
Luteinizing Hormone (LH)	-0.78 ***	.	0.76 ***	-0.47 NS	.	0.61 **
Follicle-Stimulating Hormone (FSH)	-0.67 **	0.76 ***	.	-0.75 ***	0.61 **	.

*

asterisks denote level of significance: (** p<0.01, *** p<0.001; DF=16)

Table 64: ANOVA table for comparisons of the effect of temperature on daily concentration of serum luteinizing hormone (LH) in cycling ewes treated with bromocryptine (CB-154).during

Source	DF	MS	F
Total	169		
Ewe	4	0.56	0.93 ^{NS}
Cycle	1	1.09	1.82 ^{NS}
Ewe*Cycle	4	0.60	
Phase	3	0.90	1.74 ^{NS}
Phase*Ewe	12	0.69	
Phase*Cycle	3	0.97	1.57 ^{NS}
Phase*Cycle*Ewe	12	0.61	
Error	120	0.60	

* asterisks denote the level of significance: (NS = not significant)

Table 65: Mean (\pm SEM) cyclic concentration of serum LH (ng mL^{-1}) in ewes treated with bromocryptine (CB-154).

Phase of	Thermoneutral Cycle (ng mL^{-1})	Heat Stress Cycle (ng mL^{-1})
Follicular) D1-D4	0.66 \pm 0.05	0.70 \pm 0.15
Early-luteal D5-D8	0.51 \pm 0.03	0.50 \pm 0.03
Mid-luteal) D9-D12	0.50 \pm 0.03	0.50 \pm 0.04
Late-luteal D13-D16	0.55 \pm 0.03	1.18 \pm 0.46

Table 66: ANOVA for comparisons of effect of temperature on the preovulatory LH and FSH surges in cycling ewes treated with bromocryptine (CB-154) (n=5).

Source	DF	MS				
		Area under LH Surge Curve	Duration of LH Surge	LH Surge Peak	Area under FSH Surge Curve	FSH Surge Peak
Total	9					
Ewe	4	425.95 ^{**}	1.25 ^{NS}	41.26 ^{NS}	690494.15 ^{***}	316.30 ^{NS}
Cycle	1	341.06 ^{**}	0.40 ^{NS}	34.23 ^{NS}	1932.10 ^{NS}	54.76 ^{NS}
Error	5	19.00	0.65	25.91	9131.98	151.80

* asterisks denote level of significance: (** p<0.01, *** p<0.001, NS = not significant)

Table 67: Mean (\pm SEM) of the preovulatory release characteristics of LH in cycling ewes treated with bromocryptine (CB-154) in response to temperature.

Character	Thermoneutral Cycle	Heat Stress Cycle
Duration of LH surge (h)	10.20 \pm 0.58	9.80 \pm 0.20
Area under LH surge curve (arbitrary units)	101.22 \pm 6.09	112.90 \pm 7.20
LH surge peak (ng mL ⁻¹)	16.84 \pm 0.88	20.54 \pm 3.56
Basal LH (ng mL ⁻¹)	1.25 \pm 0.07	1.39 \pm 0.05
Pulse Amplitude (ng mL ⁻¹)	0.74 \pm 0.12	0.74 \pm 0.15
Pulse Frequency (h ⁻¹)	0.28 \pm 0.01	0.28 \pm 0.01
Duration of Pulse Peak (h)	2.02 \pm 0.07	2.09 \pm 0.11
Interpeak Interval (h)	3.61 \pm 0.09	3.57 \pm 0.16

Table 68: ANOVA table for the release characteristics of LH during the preovulatory period in cycling ewes treated with bromocryptine (CB-154).

Source	DF	MS				
		Mean Conc.	Pulse Amplitude	Pulse Frequency ($\times 10^{-5}$)	Duration of Pulse Peak	Interpeak Interval
Total	9					
Ewe	4	0.44	1.71	40.00	0.45	0.79
Cycle	1	0.64	0.03	0.00	0.02	0.16
Cycle*Ewe	4	0.35	0.31	100.00	1.34	0.93

NO differences between any of the characters examined

Table 69: ANOVA table for comparisons of the effect of temperature on 6-h concentration of serum LH (ng mL^{-1}) between 48 h before to 12 h after the preovulatory surge peak (time=0) in cycling ewes (n=3) treated with bromocryptine (CB-154).

Source	DF	MS	F
Total	365		
Ewe	2	17.01	13.08 ^{NS}
Cycle	1	9.51	7.52 ^{NS}
Ewe*Cycle	2	1.30	
Period	9	507.35	55.81 ^{***}
Ewe*Period	18	9.09	
Cycle*Period	9	12.04	4.14 ^{**}
Ewe*Cycle*Period	18	2.91	
Error	306	8.05	

*
 asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 70: ANOVA table for comparisons of the effect of heat stress on luteinizing hormone (LH) concentration between 12 h before to 48 h after the preovulatory LH surge peak (time=0) in cycling ewes treated with bromocryptine (CB-154).

Source	DF	MS	F
Total	365		
Ewe	2	3.93	1.28 ^{NS}
Cycle	1	7.22	2.34 ^{NS}
Ewe*Cycle	2	3.08	
Period	9	558.26	73.26 ^{***}
Ewe*Period	18	7.62	
Cycle*Period	9	14.99	8.24 ^{***}
Period*Cycle*Ewe	18	1.82	
Error	306	7.61	

* asterisks denote the level of significance: (***) $p < 0.05$, NS = not significant)

Table 71: ANOVA table for comparisons of the effect of heat stress on daily serum follicle-stimulating hormone (FSH) concentration in cycling ewes treated with bromocryptine (CB-154).

Source	DF	MS	F
Total	159		
Ewe	4	406.39	11.38 [*]
Cycle	1	20.45	0.57 ^{NS}
Ewe*Cycle	4	35.71	
Phase	3	224.52	4.13 [*]
Phase*Ewe	12	54.30	
Phase*Cycle	3	63.97	1.72 ^{NS}
Phase*cycle*Ewe	12	37.24	
Error	120	47.23	

*
 asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 72: Mean (\pm SEM) cyclic concentration of FSH in ewes treated with bromocryptine (CB-154).

Phase of	Thermoneutral Cycle	Heat Stress Cycle
Follicular	9.12 \pm 0.98	8.88 \pm 1.31
Early-luteal	6.05 \pm 0.84	5.36 \pm 0.83
Mid-luteal	9.75 \pm 0.82	4.04 \pm 0.56
Late-luteal	6.85 \pm 1.77	11.34 \pm 3.78

Table 73: ANOVA table for the release characteristics of FSH during the preovulatory period in cycling ewes treated with bromocryptine (CB-154).

Source	DF	MS				
		Mean Conc.	Pulse Amplitude	Pulse Frequency ($\times 10^{-5}$)	Duration of Pulse Peak	Interpeak Interval
Total	9					
Ewe	4	117.50 ^{**}	2.24	106.00	0.55	2.03
Cycle	1	0.52	0313	25.00	0.34	0.24
Cycle*Ewe	4	6.47	1.91	140.00	0.65	0.71

* asterisks denote the level of significance: (** $p < 0.01$)

Table 74: Mean (\pm SEM) of the preovulatory release characteristics of FSH in cycling ewes treated with bromocryptine (CB-154) in response to temperature.

Character	Thermoneutral Cycle	Heat Stress Cycle
Area under FSH surge peak (arbitrary units)	1764.60 \pm 289.39	1736.80 \pm 237.50
FSH surge peak (ng mL ⁻¹)	68.80 \pm 9.02	64.12 \pm 3.51
Basal FSH (ng mL ⁻¹)	8.81 \pm 1.99	9.57 \pm 1.53
Pulse Amplitude (ng mL ⁻¹)	4.61 \pm 0.80	5.05 \pm 0.72
Pulse Frequency (h ⁻¹)	0.31 \pm 0.02	0.32 \pm 0.01
Duration of Pulse Peak (h)	2.02 \pm 0.10	1.91 \pm 0.02
Interpeak Interval (h)	3.23 \pm 0.15	3.10 \pm 0.08

Table 75: ANOVA table for comparisons of the effect of temperature on 6-h concentration of serum FSH (ng mL^{-1}) between 48 h before to 12 h after the preovulatory LH surge peak (time=0) in cycling ewes (n=3) treated with bromocryptine (CB-154).

Source	DF	MS	F
Total	365		
Ewe	2	891.31	94.51 ^{***}
Cycle	1	146.17	15.52 ^{NS}
Ewe*Cycle	2	9.42	
Period	9	4452.09	54.21 ^{***}
Ewe*Period	18	82.13	
Cycle*Period	9	205.86	3.06 [*]
Ewe*Cycle*Period	18	67.29	NS
Error	306	52.12	

* asterisks denote the level of significance:
 (***) $p < 0.001$, NS = not significant)

Table 76: ANOVA table for comparisons of the effect of heat stress on follicle-stimulating hormone concentration between 12 h before to 48 h after the preovulatory LH surge peak (time=0) in cycling ewes (n=3) treated with bromocryptine (CB-154).

Source	DF	MS	F
Total	365		
Ewe	2	519.27	10.91 ^{NS}
Cycle	1	556.97	11.70 ^{NS}
Ewe*Cycle	2	47.59	
Period	9	3452.45	14.68 ^{***}
Ewe*Period	18	235.24	
Cycle*Period	9	219.74	2.83 [*]
Period*Cycle*Ewe	18	77.54	
Error	306	53.56	

*

asterisks denote the level of significance:
 (* p<0.01, *** p<0.001, NS = not significant)

Table 77: ANOVA for the comparison of mean hourly (n=9) concentration of serum progesterone in response to an acute dose of ACTH or saline in ovx ewes.

Source	DF	MS	F
Total	719		
Ewe	3	0.089	0.58
Treat	1	1.970	12.88
Ewe*Treat	3	0.153	
Temp	1	0.033	0.30
Ewe*Temp	3	0.110	
Treat*Temp	1	0.034	0.30
Ewe*Treat*Temp	3	0.113	
Period	8	0.167	8.84
Ewe*Period	24	0.019	
Treat*Period	8	0.111	4.27
Ewe*Treat*Period	24	0.026	
Temp*Period	8	0.013	2.60
Ewe*Temp*Period	24	0.005	
Treat*Temp*Period	8	0.003	0.23
Ewe*Treat*Temp*Period	24	0.013	
Erroe	576	0.0057	

*

asterisks denote the level of significance: (* p<0.05, ** p<0.01, NS = not significant)

Table 78: Mean (\pm SEM) of the hourly (n=9) concentration of progesterone (ng mL^{-1}) in response to an acute dose of ACTH (5 I.U. kg^{-1} liveweight^{0.75}) or saline in ovx ewes as influenced by temperature.

Treatment	Period #	Time (min Relative to Inj.=0)	Thermoneutral Temperature (ng mL^{-1})	Heat Stress Temperature (ng mL^{-1})
Saline	1	(-120- 0)	0.33 \pm 0.02	0.36 \pm 0.02
	2	(0- 60)	0.35 \pm 0.02	0.36 \pm 0.01
	3	(60-120)	0.40 \pm 0.02	0.35 \pm 0.02
	4	(120-180)	0.31 \pm 0.01	0.35 \pm 0.01
	5	(180-240)	0.34 \pm 0.01	0.38 \pm 0.01
	6	(240-300)	0.31 \pm 0.02	0.35 \pm 0.01
	7	(300-360)	0.30 \pm 0.01	0.36 \pm 0.01
	8	(360-420)	0.31 \pm 0.02	0.37 \pm 0.02
	9	(420-480)	0.33 \pm 0.02	0.37 \pm 0.01
ACTH	1	(-120- 0)	0.34 \pm 0.02	0.34 \pm 0.01
	2	(0- 60)	0.51 \pm 0.03	0.52 \pm 0.01
	3	(60- 120)	0.61 \pm 0.04	0.57 \pm 0.03
	4	(120-180)	0.53 \pm 0.02	0.52 \pm 0.02
	5	(240-240)	0.46 \pm 0.02	0.47 \pm 0.01
	6	(240-300)	0.42 \pm 0.02	0.42 \pm 0.01
	7	(300-360)	0.42 \pm 0.02	0.44 \pm 0.03
	8	(360-420)	0.42 \pm 0.01	0.42 \pm 0.02
	9	(420-480)	0.37 \pm 0.02	0.38 \pm 0.02

Table 79: ANOVA for comparisons of the secretion characteristics of progesterone in response to ACTH or saline infusion in ovx ewes (n=4).

Source	DF	MS				
		Basal Conc.	Pulse Amplitudes	Pulse Frequency ($\times 10^{-5}$)	Duration Pulse Peak	Interpeak Intervals
Total	15					
Ewe	3	93.82 ^{NS}	0.71 ^{NS}	1.1 ^{NS}	1.11 ^{NS}	2.34 ^{NS}
Treat	1	1279.04 [*]	3.14 ^{NS}	2.8 ^{NS}	4.31 ^{NS}	2.57 ^{NS}
Ewe*Treat	3	92.28 ^{NS}	1.60 ^{NS}	0.27 ^{NS}	0.55 ^{NS}	0.74 ^{NS}
Temp	1	200.37 ^{NS}	1.10 ^{NS}	1.1 ^{NS}	0.29 ^{NS}	0.50 ^{NS}
Ewe*Temp	3	261.43 ^{NS}	0.49 ^{NS}	0.94 ^{NS}	1.29 ^{NS}	0.93 ^{NS}
Treat*Temp	1	20.20 ^{NS}	0.06 ^{NS}	5.1 ^{NS}	0.53 ^{NS}	0.20 ^{NS}
Error	3	103.34	1.58	0.47	1.02	0.43

* asterisks denote level of significance: (* $p < 0.05$, NS = not significant)

Table 80: Means (\pm SEM) of the secretion characteristics of P_4 in response to an acute dose of ACTH (5.0 I.U kg^{-1} liveweight $^{0.75}$) or saline (5 mL) in ovx ewes.

Treatment	Saline		ACTH	
	18°C	35°C	18°C	35°C
Mean Conc. (ng mL^{-1})	0.27 \pm 0.02	0.31 \pm 0.01	0.37 \pm 0.02	0.38 \pm 0.03
Pulse Amplitudes (ng mL^{-1})	0.12 \pm 0.01	0.11 \pm 0.01	0.14 \pm 0.02	0.13 \pm 0.01
Pulse Frequency (min^{-1})	0.025 \pm 0.001	0.022 \pm 0.002	0.021 \pm 0.001	0.021 \pm 0.001
Duration of Pulse Peak (min)	25.93 \pm 2.02	31.52 \pm 4.72	33.09 \pm 2.90	32.67 \pm 2.74
Interpeak Interval (min)	41.77 \pm 2.81	47.35 \pm 4.12	48.00 \pm 2.64	49.81 \pm 3.04

Table 81: The correlation coefficients (r) between progesterone (P₄) and cortisol secretion patterns in ovx ewes following the infusion of an acute dose of ACTH or saline.

Treatment	Hormone	Thermoneutral Temperature	Heat Stress Temperature
		Cortisol	Cortisol
Saline	P ₄	0.15 *	0.14 NS
ACTH	P ₄	0.75 ***	0.50 ***

* asterisks denote the level of significance: (* p<0.05, *** p<0.001, NS = not significant, DF=35)

Table 82: ANOVA for the comparison of mean hourly (n=9) concentration of serum cortisol in response to an acute dose of ACTH or saline in ovx ewes.

Source	DF	MS	F
Total	719		
Ewe	3	26481.49	0.83
Treat	1	1665905.02	52.15
Ewe*Treat	3	31943.34	
Temp	1	778.54	0.12
Ewe*Temp	3	6375.41	
Treat*Temp	1	1149.38	0.18
Ewe*Treat*Temp	3	6534.36	
Period	8	123830.40	26.59
Ewe*Period	24	4676.13	
Treat*Period	8	12383.40	22.26
Ewe*Treat*Period	24	5561.12	
Temp*Period	8	790.52	0.27
Ewe*Temp*Period	24	2914.49	
Treat*Temp*Period	8	640.00	0.22
Ewe*Treat*Temp*Period	24	2879.11	
Error	576	858.17	

*

asterisks denote the level of significance:
 (** p<0.01, *** p<0.001, NS = not significant)

Table 83: Mean (\pm SEM) of the hourly (n=9) concentration of serum cortisol (ng mL^{-1}) after an acute dose of ACTH (5 I.U. kg^{-1} liveweight^{0.75}) or saline in ovx ewes .

Treatment	Hour #	Time (min Relative to Inj.=0)	Thermoneutral Temperature (ng mL^{-1})	Heat Stress Temperature (ng mL^{-1})
Saline	1	(-120- 0)	15.32 \pm 2.01	11.89 \pm 1.10
	2	(0- 60)	28.74 \pm 3.03	24.36 \pm 2.31
	3	(60-120)	16.62 \pm 2.23	13.13 \pm 1.87
	4	(120-180)	12.90 \pm 2.40	10.34 \pm 1.56
	5	(180-240)	13.78 \pm 1.86	8.02 \pm 0.84
	6	(240-300)	13.66 \pm 1.86	9.72 \pm 1.23
	7	(300-360)	12.15 \pm 1.73	10.03 \pm 1.20
	8	(360-420)	17.12 \pm 2.42	9.43 \pm 0.86
	9	(420-480)	18.48 \pm 1.92	8.91 \pm 0.85
ACTH	1	(-120- 0)	12.41 \pm 1.50	12.49 \pm 1.60
	2	(0- 60)	128.27 \pm 14.40	130.89 \pm 11.00
	3	(60-120)	248.28 \pm 25.29	235.59 \pm 22.74
	4	(120-180)	195.79 \pm 22.47	207.14 \pm 15.81
	5	(180-240)	195.76 \pm 19.01	187.97 \pm 10.87
	6	(240-300)	122.56 \pm 6.14	132.89 \pm 15.66
	7	(300-360)	79.63 \pm 5.04	81.67 \pm 4.19
	8	(360-420)	56.62 \pm 2.98	53.98 \pm 3.83
	9	(420-480)	41.78 \pm 2.86	40.14 \pm 2.71

Table 84: ANOVA for comparisons of the secretion characteristics of cortisol in response to ACTH or saline infusion in ovx ewes (n=4).

Source	DF	MS				
		Basal Conc.	Pulse Amplitudes	Pulse Frequency ($\times 10^{-5}$)	Duration of Pulse peak	Interpeak Intervals
Total	15					
Ewe	3	17.46 ^{NS}	1.54 ^{NS}	1.9 ^{NS}	3.19 ^{NS}	2.39 ^{NS}
Treat	1	1656.60 ^{**}	38.56 [*]	3.9 ^{NS}	5.07 ^{NS}	4.35 ^{NS}
Ewe*Treat	3	19.04 ^{NS}	1.39 ^{NS}	0.61 ^{NS}	0.71 ^{NS}	0.69 ^{NS}
Temp	1	5.35 ^{NS}	0.08 ^{NS}	0.76 ^{NS}	0.86 ^{NS}	1.01 ^{NS}
Ewe*Temp	3	63.07 ^{NS}	0.46 ^{NS}	0.17 ^{NS}	0.39 ^{NS}	0.12 ^{NS}
Treat*Temp	1	8.61 ^{NS}	2.35 ^{NS}	1.4 ^{NS}	2.22 ^{NS}	1.20 ^{NS}
Error	3	3.77	1.48	6.2	0.84	0.71

* asterisks denote level of significance: (* p<0.05, ** p<0.01, NS = not significant)

Table 85: Means (\pm SEM) of the secretion characteristics of cortisol in response to an acute dose of ACTH (5.0 I.U. kg^{-1} liveweight $^{0.75}$) or saline (5 mL) in ovx ewes.

Treatment	Saline		ACTH	
	18°C	35°C	18°C	35°C
Meanl Conc. (ng mL^{-1})	8.78 \pm 0.31	7.68 \pm 1.44	77.20 \pm 7.84	86.15 \pm 10.05
Pulse Amplitudes (ng mL^{-1})	19.53 \pm 2.24	10.40 \pm 1.42	57.98 \pm 15.15	59.98 \pm 11.41
Pulse Frequency (min^{-1})	0.017 \pm 0.002	0.020 \pm 0.001	0.022 \pm 0.001	0.022 \pm 0.002
Duration of Pulse Peak (min)	41.20 \pm 4.72	32.25 \pm 1.47	29.18 \pm 1.63	30.60 \pm 2.35
Interpeak Interval (min)	62.35 \pm 5.90	51.83 \pm 3.40	48.00 \pm 1.95	48.73 \pm 4.01

Table 86: Comparisons of relative tracer displacement and the binding kinetics of GnRH standard curves prepared in charcoal-stripped serum, after the extraction or non-extraction with methyl alcohol prior to the incubation with anti-GnRH serum.

Conc (pg tube ⁻¹)	Unextracted GnRH Standard [*]		Extracted GnRH Standard	
	cpm tube ⁻¹	%binding	cpm tube ⁻¹	%binding
		$\frac{\text{Bi}}{\text{Tcpm}} \times 100$		$\frac{\text{Bi}}{\text{Tcpm}} \times 100$
Total	18500		15332	
NSB	5720	30.90	798	5.20
Reference(0)	6666	36.03	5780	37.70
0.78	6562	35.47	5486	35.78
1.56	6621	35.79	5421	35.36
3.125	6642	35.90	5202	33.93
6.25	6575	35.54	4854	31.66
12.5	5825	31.49	4680	30.52
25	5811	31.41	4187	27.31
50	6071	32.82	5876	22.74
100	5745	31.05	2997	19.55
200	5784	31.26	2395	15.62
400	5861	31.68	2120	13.83
800	5899	31.89	1635	10.66
1600	6063	32.77	1364	8.90

*

(Bi = count tube⁻¹, Tcpm = total counts)

Table 87: Comparisons of the effect of plasma proteins on the estimation of GnRH concentration (pg mL^{-1}) in the same plasma samples (2 ewes) as assayed directly without extraction with methyl alcohol or following the extraction. Ewes were challenged with an acute single dose of exogenous GnRH (20 ug) at time=0.

Time Relative to GnRH Injection=0 (min)	Unextracted Samples (pg mL^{-1})	Extracted Samples (pg mL^{-1})
-120	274.7±69.9	5.3±1.5
-100	163.8±38.4	8.9±5.1
-80	198.1±48.7	5.1±1.3
-60	352.1±196.6	4.0±0.2
-40	226.3±52.9	6.2±2.4
-20	321.3±174.2	7.4±3.5
0	1596.3±1080.1	5078.3±249.8
5	1150.6±671.3	2601.5±961.6
10	637.5±125.3	935.1±495.7
15	616.3±113.3	216.7±108.2
20	536.3±106.8	50.3±25.1
25	488.0±63.3	32.4±10.4
30	471.3±54.0	36.3±4.1
35	476.3±41.9	23.8±3.5
40	478.8±132.2	18.6±3.4
50	295.6±69.0	15.0±4.2
60	215.6±17.7	8.7±2.9
80	196.9±29.9	18.3±12.3
100	171.3±26.2	20.3±10.3
120	178.1±25.5	9.3±4.6
140	128.8±20.4	13.4±3.5

Table 88: ANOVA table for comparisons of the periodic concentration of plasma prolactin following an acute dose of exogenous GnRH (20 ug) in P₄-primed ovx ewes.

Source	DF	MS	F
Total	143		
Ewe	3	17957.13	0.68 ^{NS} *
Temp	1	78274.05	2.98 ^{NS}
Ewe*Temp	3	26265.48	
Period	8	573.62	1.52 ^{NS}
Ewe*Period	24	376.68	
Temp*Period	8	319.49	1.72 ^{NS}
Ewe*Temp*Period	24	185.97	
Error	72	202.26	

*

asterisks denote the level of significance: (NS = not significant)

Table 89: Means (\pm SEM) of plasma progesterone (P_4) (ng mL^{-1}) in response to daily injections of 30 mg P_4 (Days -8 to Days -1) in ovx ewes treated with or without CB-154 (n=4).

Day	Thermoneutral Temperature		Heat Stress Temperature	
	No CB-154	With CB-154	No CB-154	With CB-154
-13	0.67 \pm 0.06	0.64 \pm 0.06	0.54 \pm 0.10	0.55 \pm 0.11
-12	0.67 \pm 0.02	0.68 \pm 0.08	0.73 \pm 0.07	0.58 \pm 0.13
-11	0.42 \pm 0.08	0.54 \pm 0.10	0.58 \pm 0.14	0.39 \pm 0.07
-10	0.55 \pm 0.11	0.35 \pm 0.08	0.49 \pm 0.10	0.57 \pm 0.08
-9	0.45 \pm 0.07	0.37 \pm 0.05	0.86 \pm 0.05	0.49 \pm 0.08
-8	2.59 \pm 0.30	2.22 \pm 0.29	3.44 \pm 0.34	3.05 \pm 0.79
-7	3.33 \pm 0.67	3.28 \pm 1.01	3.86 \pm 0.24	3.35 \pm 0.77
-6	3.79 \pm 0.69	4.65 \pm 0.55	4.08 \pm 0.70	3.41 \pm 0.55
-5	4.67 \pm 0.65	4.69 \pm 0.75	3.75 \pm 0.61	3.60 \pm 0.74
-4	4.89 \pm 0.27	5.34 \pm 0.38	3.78 \pm 0.81	4.68 \pm 0.59
-3	3.57 \pm 0.69	3.77 \pm 0.43	5.57 \pm 1.09	5.30 \pm 0.27
-2	4.36 \pm 1.17	4.46 \pm 0.42	5.49 \pm 0.82	5.99 \pm 0.89
-1	4.43 \pm 0.22	6.12 \pm 0.89	7.01 \pm 1.97	3.73 \pm 1.01
* 0	1.33 \pm 0.19	1.40 \pm 0.33	2.25 \pm 0.41	2.28 \pm 0.50

(*) = day of intensive sampling

Table 90: ANOVA for comparisons of plasma concentration of GnRH (pg mL⁻¹) before and after an acute dose of exogenous GnRH (20 ug) in P₄-primed ovx ewes treated with or without CB-154.

Source	DF	MS	F
Total	575		
Ewe	3	1796745.57	2.15 NS
Treat	1	651247.39	0789 NS
Ewe*Treat	3	836979.17	
Temp	1	239733.78	0.34 NS
Ewe*Temp	3	701269.14	
Treat*Temp	1	1795861.34	6.48 NS
Ewe*Treat*Temp	3	124216.44	
Period	8	3615506.30	2.90 *
Ewe*Period	24	1248139.75	
Treat*Period	8	135085.59	0.22 NS
Ewe*Treat*Period	24	621627.87	
Temp*Period	8	50285.04	0.13 NS
Ewe*Temp*Period	24	391294.23	
Treat*Temp*Period	8	667506.73	6.36 *
Ewe*Treat*Temp*Period	24	104896.72	
Error	432	409004.72	

*

asterisks denote the level of significance:
NS = not significant)

Table 91: Mean (\pm SEM) concentration of plasma GnRH (pg mL^{-1}) before and after an acute dose of exogenous GnRH (20 μg) in P_4 -primed ovx ewes treated with or without CB-154 . normothermia (18°C) and hyperthermia (18°C - 35°C - 18°C).

Treatment	Period #	Time (min Relat. to GnRH=0)	Thermoneutral Temperature (pg mL^{-1})	Heat Stress Temperature (pg mL^{-1})
NO CB-154	1	-120- -20	4.79 \pm 6.5	29.30 \pm 14.64
	2	0- 20	2148.68 \pm 532.93	2758.51 \pm 524.62
	3	20- 50	28.99 \pm 6.57	346.94 \pm 100.34
	4	50-100	8.83 \pm 2.73	180.43 \pm 55.33
	5	100-160	4.79 \pm 1.18	220.97 \pm 79.92
	6	160-220	11.53 \pm 5.43	101.03 \pm 31.62
	7	220-280	6.12 \pm 1.47	33.72 \pm 11.00
	8	280-340	5.32 \pm 1.15	63.94 \pm 34.15
	9	340-440	7.75 \pm 1.25	92.36 \pm 33.97
With CB-154	1	-120- -20	8.65 \pm 1.90	5.97 \pm 1.20
	2	0- 20	2498.06 \pm 448.55	1800.23 \pm 486.00
	3	20- 50	149.34 \pm 32.48	98.01 \pm 25.37
	4	50-100	11.88 \pm 4.43	20.49 \pm 3.44
	5	100-160	7.40 \pm 1.80	26.34 \pm 13.34
	6	160-220	7.02 \pm 2.65	12.95 \pm 2.40
	7	220-280	9.46 \pm 2.88	24.04 \pm 9.19
	8	280-340	6.03 \pm 1.77	18.14 \pm 1.25
	9	340-440	4.36 \pm 0.55	9.28 \pm 1.21

Table 92: Means (\pm SEM) of the release characteristics of GnRH before and after an acute dose of exogenous GnRH (20 ug) in P₄-primed ovx ewes treated with or without CB-154 (n=4).

Character	Thermoneutral Temperature		Heat Stress Temperature	
	NO CB-154	With CB-154	NO CB-154	With CB-154
Peak Conc. at Time=0 After GnRH (pg mL ⁻¹)	4431.38 \pm 866.89	4416.43 \pm 911.58	4044.25 \pm 861.90	4437.25 \pm 605.42
Basal Conc. Prior to GnRH (pg mL ⁻¹)	4.80 \pm 0.71	8.70 \pm 2.30	29.30 \pm 14.40	6.00 \pm 1.50
Period from GnRH to Basal Conc. (min)	37.50 \pm 4.33	45.00 \pm 2.89	137.50 \pm 47.68	83.75 \pm 27.03
Pulse Amplitudes (pg mL ⁻¹)	652.48 \pm 99.51	299.86 \pm 144.93	626.85 \pm 105.94	595.85 \pm 111.45
Pulse Frequency (min ⁻¹)	0.011 \pm 0.002	0.008 \pm 0.001	0.015 \pm 0.002	0.015 \pm 0.001
Duration of Pulse Peak (min)	39.35 \pm 3.06	47.70 \pm 5.33	38.58 \pm 2.38	36.10 \pm 5.42
Interpeak Interval (min)	65.43 \pm 13.77	154.68 \pm 43.48	59.53 \pm 4.08	63.31 \pm 8.67

Table 93: ANOVA for comparisons of plasma GnRH release characteristics following an acute dose of exogenous GnRH (20 ug) in P₄-primed ovx ewes treated with or without CB-154 (n=4).

Source	DF	Time from GnRH to Basal Conc.	GnRH Pulse Amplitudes	Pulse Frequency (x10 ⁻⁵)	Duration of Pulse Peak	Interpeak Interval
Total	15					
Ewe	3	4793.23 ^{NS}	0.53 [*]	0.51 ^{NS}	0.62 ^{NS}	411.54 ^{NS}
Treat	1	2139.06 ^{NS}	0.05 ^{NS}	1.06	0.11 ^{NS}	4168.70 ^{***}
Ewe*Treat	3	851.56	0.04	3.86	0.16	116.87
Temp	1	19251.56	0.12 ^{NS}	11.56 ^{**}	0.68 ^{NS}	75.83 ^{NS}
Ewe*Temp	3	5264.06	0.18	0.09	0.67	31.96
Treat*Temp	1	3751.56 ^{NS}	0.13 ^{NS}	1.06 [*]	0.54 ^{NS}	92.12 [*]
Error	3	1214.06	0.04	0.09	0.31	9.67

* asterisks denote level of significance: (* p<0.05, ** p<0.01, *** p<0.001, NS = not significant)

Table 94: ANOVA for the comparison of plasma concentration of LH (ng mL⁻¹) before and after an acute dose of exogenous GnRH (20 ug) in P₄-primed ovx ewes treated with or without CB-154.

Source	DF	MS	F
Total	575		
Ewe	3	91.93	1.34 NS
Treat	1	50.31	0.73 NS
Ewe*Treat	3	68.67	
Temp	1	3.53	0.32 NS
Ewe*Temp	3	10.98	
Treat*Temp	1	4.77	0.33 NS
Ewe*Treat*Temp	3	14.29	
Period	8	963.00	52.85 ***
Ewe*Period	24	18.22	
Treat*Period	8	5.67	0.51 NS
Ewe*Treat*Period	24	11.04	
Temp*Period	8	18.75	2.34 NS
Ewe*Temp*Period	24	8.02	
Treat*Temp*Period	8	0.69	0.14 NS
Ewe*Treat*Temp*Period	24	4.78	
Error	432	11.95	

*

asterisks denote the level of significance:
 *** p<0.001, NS = not significant)

Table 95: Mean (\pm SEM) concentration of LH (ng mL^{-1}) in response to an acute dose of exogenous GnRH (20 μg) in P_4 -primed ovx ewes treated with or without CB-154.

Treatment	Period #	Time (min Relat. to GnRH Inj.=0)	Thermoneutral Temperature (ng mL^{-1})	Heat Stress Temperature (ng mL^{-1})
NO CB-154	1	-120- -20	1.93 \pm 0.24	1.76 \pm 0.16
	2	0- 20	9.29 \pm 2.33	9.04 \pm 1.28
	3	20- 50	10.16 \pm 0.80	13.07 \pm 1.58
	4	50-100	7.00 \pm 0.84	8.04 \pm 1.01
	5	100-160	5.01 \pm 0.82	4.06 \pm 0.29
	6	160-220	2.73 \pm 0.55	2.64 \pm 0.49
	7	220-280	1.33 \pm 0.15	1.59 \pm 0.29
	8	280-340	1.39 \pm 0.28	1.01 \pm 1.13
	9	340-440	1.53 \pm 0.29	1.05 \pm 1.14
With CB-154	1	-120- -20	1.56 \pm 0.17	1.29 \pm 0.14
	2	0- 20	6.69 \pm 0.70	7.40 \pm 1.00
	3	20- 50	10.17 \pm 1.29	13.41 \pm 2.05
	4	50-100	5.79 \pm 0.64	6.73 \pm 0.53
	5	100-160	3.75 \pm 0.32	3.93 \pm 0.43
	6	160-220	1.79 \pm 0.16	1.89 \pm 0.18
	7	220-280	1.06 \pm 0.14	1.56 \pm 0.22
	8	280-340	1.18 \pm 0.16	0.83 \pm 0.16
	9	340-440	0.88 \pm 0.10	0.88 \pm 0.12

Table 96: Means (\pm SEM) of the release characteristics of LH in response to an acute dose of an exogenous GnRH (20 ug in P₄-primed ovx ewes treated with or without CB-154 (n=4).

Character	Thermoneutral Temperature		Heat Stress Temperature	
	NO CB-154	With CB-154	NO CB-154	With CB-154
Area Under Surge Curve (arbitrary units)	107.78 \pm 28.81	119.48 \pm 6.83	118.63 \pm 27.63	98.80 \pm 16.24
LH Surge Peak (ng mL ⁻¹)	18.00 \pm 7.86	17.98 \pm 2.53	20.23 \pm 7.59	25.15 \pm 7.63
Time from GnRH to LH Surge Peak (min)	25.00 \pm 3.54	27.50 \pm 4.79	25.00 \pm 5.40	20.00 \pm 5.00
Basal LH (ng mL ⁻¹)	2.45 \pm 0.49	2.35 \pm 0.43	2.26 \pm 0.30	2.23 \pm 0.48
Pulse Amplitudes (ng mL ⁻¹)	3.60 \pm 1.77	3.05 \pm 0.63	4.53 \pm 0.68	3.96 \pm 1.02
Pulse Frequency (min ⁻¹)	0.018 \pm 0.001	0.018 \pm 0.002	0.017 \pm 0.001	0.018 \pm 0.001
Duration of Pulse Peak (min)	38.83 \pm 2.44	37.73 \pm 2.96	41.13 \pm 5.98	37.69 \pm 1.02
Interpeak Interval (min)	57.85 \pm 2.44	61.45 \pm 5.63	60.17 \pm 4.64	58.57 \pm 2.31

Table 97: ANOVA for comparisons of the release characteristics of LH in response to an acute dose of exogenous GnRH (20 ug) in P₄-primed ovx ewes treated with or without CB-154 (n=4).

Source	DF	MS							
		Area Under LH Surge Curve	LH Surge Peak	Time from GnRH to Surge Peak	Basal Level	Pulse Amplitude	Pulse Frequency (x10 ⁻⁵)	Duration of Pulse Peak	Interpeak Interval
Total	15								
Ewe	3	3772.50	476.99	18.75	112.77*	2.35	0.52	0.52	0.58
Treat	1	66.02	24.01	6.25	16.61	0.002	0.06	0.01	0.15
Ewe*Treat	3	3156.39	163.59	135.42	9.85	1.20	0.62	0.22	0.16
Temp	1	96.53	88.36	56.25	1.37	3.09*	0.06	0.03	0.004
Ewe*Temp	3	427.57	69.79	110.42	4.92	0.31	0.96	1.34	0.58
Treat*Temp	1	493.83	24.50	56.25	1.37	0.96	0.06	0.00	0.18
Error	3	259.54	25.78	93.75	11.38	0.28	0.05	0.21	0.08

* asterisks denote level of significance: (* p<0.05)

Table 98: ANOVA for comparisons of plasma concentration of FSH (ng mL⁻¹) before and after an acute dose of exogenous GnRH (20 ug) in P₄-primed ovx ewes treated with or without CB-154.

Source	DF	MS	F
Total	575		
Ewe	3	1700.94	0.07
Treat	1	312.79	0.01
Ewe*Treat	3	22742.26	
Temp	1	5635.32	1.89
Ewe*Temp	3	2988.25	
Treat*Temp	1	3451.05	28.83
Ewe*Treat*Temp	3	119.69	
Period	8	11603.20	20.31
Ewe*Period	24	571.04	
Treat*Period	8	228.18	0.66
Ewe*Treat*Period	24	344.46	
Temp*Period	8	157.51	0.38
Ewe*Temp*Period	24	418.61	
Treat*Temp*Period	8	394.79	1.05
Ewe*Treat*Temp*Period	24	374.65	
Error	432	190.83	

* asterisks denote the level of significance: (***) p<0.01, NS = not significant)

Table 99: Means (\pm SEM) of plasma concentration of FSH (ng mL^{-1}) in response to an acute dose of exogenous GnRH (20 μg) in P_4 -primed ovx ewes treated with or without CB-154.

Treatment	Period #	Time (min Relat. to GnRH Inj.=0)	Thermoneutral Temperature (ng mL^{-1})	Heat Stress Temperature (ng mL^{-1})
NO CB-154	1	-120- -20	51.66 \pm 3.76	49.54 \pm 1.75
	2	0- 20	73.53 \pm 7.86	64.51 \pm 3.77
	3	20- 50	79.29 \pm 5.42	73.83 \pm 5.41
	4	50-100	86.88 \pm 9.00	73.52 \pm 4.83
	5	100-160	71.26 \pm 5.85	67.77 \pm 3.76
	6	160-220	51.26 \pm 4.41	59.84 \pm 6.44
	7	220-280	42.35 \pm 4.97	50.84 \pm 4.80
	8	280-340	45.15 \pm 4.97	50.48 \pm 3.97
	9	340-440	47.08 \pm 3.25	52.43 \pm 3.70
With CB-154	1	-120- -20	45.60 \pm 3.04	58.99 \pm 2.72
	2	0- 20	58.26 \pm 5.72	79.69 \pm 4.84
	3	20- 50	75.34 \pm 6.16	92.78 \pm 4.86
	4	50-100	78.44 \pm 9.00	87.23 \pm 5.19
	5	100-160	67.33 \pm 5.33	73.03 \pm 7.28
	6	160-220	47.58 \pm 4.06	62.52 \pm 7.54
	7	220-280	43.13 \pm 4.74	50.97 \pm 5.31
	8	280-340	42.28 \pm 3.61	46.37 \pm 5.52
	9	340-440	47.28 \pm 3.61	60.11 \pm 4.76

Table 100: ANOVA for comparisons of the release characteristics of FSH in response to an acute dose of exogenous GnRH (20 ug) in P₄-primed ovx ewes treated with or without CB-154 (n=4).

Source	DF	MS							
		Area Under FSH Surge Curve	FSH Surge Peak	Time from GnRH to Surge Peak	Basal Level	Pulse Amplitude	Pulse Frequency (x10 ⁻⁵)	Duration of Pulse Peak	Interpeak Interval
Total	15								
Ewe	3	125892.92	809.96	2026.56	46.69	0.96	0.36	1.12	0.18
Treat	1	195806.25	121.55	564.06	6.14	0.20	0.16	0.19	0.02
Ewe*Treat	3	28060.92	2372.56	1505.73	312.64	5.68	0.47	1.26	0.26
Temp	1	100.00	0.11	76.56	148.82	0.09	1.06	2.47	1.18
Ewe*Temp	3	59011.00	490.19	393.23	141.71	1.16	0.97	0.70	0.39
Treat*Temp	1	27225.00	403.01	689.06	94.27*	1.80	0.31	1.06	0.36
Error	3	52013.33	194.27	1855.73	9.27	0.26	0.96	1.45	0.45

* asterisks denote level of significance: (* p<0.05).

Table 101: Means (\pm SEM) of the release characteristics of FSH in response to an acute dose of exogenous GnRH (20 ug) in P₄-primed ovx ewes treated with or without CB-154 (n=4).

Character	Thermoneutral Temperature		Heat Stress Temperature	
	No CB-154	With CB-154	No CB-154	With CB-154
Area Under Surge Curve (arbitrary units)	1000.25 \pm 46.18	696.50 \pm 160.54	922.75 \pm 105.37	784.00 \pm 165.04
FSH Surge Peak (ng mL ⁻¹)	109.00 \pm 16.54	104.48 \pm 19.79	99.13 \pm 12.11	114.68 \pm 12.44
Time from GnRH to FSH Surge Peak (min)	57.50 \pm 8.54	58.75 \pm 22.40	75.00 \pm 27.54	50.00 \pm 10.61
Basal FSH (ng mL ⁻¹)	45.20 \pm 7.06	42.33 \pm 4.40	47.70 \pm 5.06	53.53 \pm 5.71
Pulse Amplitudes (ng mL ⁻¹)	26.15 \pm 2.86	24.30 \pm 3.29	21.40 \pm 3.04	27.63 \pm 4.41
Pulse Frequency (min ⁻¹)	0.019 \pm 0.001	0.017 \pm 0.001	0.019 \pm 0.001	0.020 \pm 0.002
Duration of Pulse Peak (min)	35.58 \pm 2.86	41.25 \pm 3.66	34.95 \pm 2.00	34.10 \pm 4.20
Interpeak Interval (min)	55.40 \pm 1.70	61.18 \pm 2.71	53.08 \pm 1.57	51.80 \pm 3.98