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EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON THE ESTROUS CYCLE AND HORMONE LEVELS IN THE GILT

by

LAURIE CONNOR

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ABSTRACT

Two experiments were conducted using cycling crossbred gilts 7 to 9 months of age. The first experiment was divided into two treatments. In treatment 1, 3 gilts were injected intramuscularly (im) with 20 mg $PGF_{2\alpha}$ on day 9 of the cycle. Three gilts in treatment 2 were given two injections of 20 mg $PGF_{2\alpha}$ im, one on day 9 and one on day 10. Blood samples were taken daily at 10 a.m. from day 0 to 9; at 1 hr, 2 hr and subsequently every 6 hr for 96 hr after each injection; then daily until the next estrus. The $PGF_{2\alpha}$ injections were given immediately following the 10 a.m. blood collection on day 9 or 10. Blood samples were also taken from 3 gilts (control) for one cycle prior to treatment. Serum progesterone (P), estrogens and luteinizing hormone (LH) were measured by radioimmunoassay. Serum P dropped significantly ($P<.01$) on day 9 in treatment 1 from a preinjection mean ($\pm$SE) of 8.9 $\pm$ 0.7 ng/ml to 4.8 $\pm$ 0.8 ng/ml 8 hr after injection. In treatment 2 gilts, P decreased significantly ($P<.05$) on day 9 from a mean prior to injection of 11.2 $\pm$ 2.3 ng/ml to 7.1 $\pm$ 1.8 ng/ml by 8 hr post-injection. Similarly, following $PGF_{2\alpha}$ on day 10, P levels dropped sharply ($P<.05$) from a mean of 8.3 $\pm$ 0.8 ng/ml prior to injection to 3.8 $\pm$ 0.4 ng/ml 8 hr later. These decreases were not seen in controls. In all cases, except one, P then rose above preinjection levels by 96 hr after injection. Mean estrogen levels increased slightly within the first hr post-injection but were

significantly elevated ($P<.01$) above the pre-injection mean only in treatment 2 gilts on day 9. Subsequently, estrogen patterns were similar to those of the control cycles. No consistent changes were observed in mean LH concentrations following treatment. Essentially no effect was seen on cycle length. In a second experiment three gilts, which served as their own controls, were injected im with 20 mg $PGF_{2\alpha}$ on day 12 of the cycle. Blood sampling frequency prior to and following injection was the same as in experiment I. Serum P levels declined from a pre-injection mean of 12.8 $\pm$ 0.9 ng/ml to 7.9 $\pm$ 0.7 ng/ml ($P<.05$) by 2 hr after injection and continued to fall. Mean P concentrations on day 12 during the control cycle remained relatively constant. Serum estrogens and LH levels did not change significantly within the 14 hr following treatment and subsequently followed a pattern similar to that seen during the untreated control cycle. Estrus occurred an average of 1.7 days earlier than in the control cycle ($P<.05$). Side-effects noted within 5 to 10 minutes after injection in both experiments included increased respiratory rate, defecation, shivering and general signs of discomfort.

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INTRODUCTION

In recent years there has been considerable interest in prostaglandin $F_{2\alpha}$ for controlling estrus and ovulation in farm animals, possibly because of implications that it is a natural luteolysin. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been shown to be luteolytic in several mammalian species. Of the farm animals, the ability of $PGF_{2\alpha}$ to induce regression of the corpus luteum (CL) has been studied most extensively in sheep and cattle, and to a lesser degree in horses. Many laboratories have reported on studies of the association between $PGF_{2\alpha}$, both endogenous and exogenous, and luteal regression, together with the concomitant hormonal changes in these species.

The main purpose of this study was to examine the effect of $PGF_{2\alpha}$, administered during the luteal phase, on estrous cycle length and hormone levels, ie. progesterone (P), estrogens, and luteinizing hormone (LH), in the cycling gilt. One of the principle reasons for such an undertaking was the possible implication of $PGF_{2\alpha}$ as an effective synchronization agent and the lack of published research in this area.

LITERATURE REVIEW

The literature review will encompass the major highlights in recent literature pertaining to the luteolytic effect of $PGF_{2\alpha}$, primarily in sheep, cattle, horses and swine, but some reference will be made to laboratory species where applicable. The review will trace the relationship of the local uterine effect on CL regression and the various aspects of $PGF_{2\alpha}$-induction of luteolysis, including local transport to the ovary, theories pertaining to the mode of action of $PGF_{2\alpha}$ and hormonal factors affecting luteal regression. The final section will deal with the practical application of $PGF_{2\alpha}$ for controlled ovulation in farm animals.

Role of the Uterus in CL Regression

Hysterectomy. Results of hysterectomy in many species have indicated that the uterus plays an active role in the initiation of luteolysis during the later stages of the estrous cycle. It was first demonstrated by Loeb (1923) that hysterectomy in the guinea-pig during the luteal phase of the cycle extended the life-span of the CL for a period equivalent to, or longer than, pregnancy. Similar results have been reported following hysterectomy in other spontaneous ovulators such as the sheep and cow (Wiltbank and Casida, 1956), the sow (du Mesnil du Buisson and Dazier, 1959) and the mare (Ginther, 1971; Stabenfeldt et al., 1974). Hysterectomy also results in luteal maintenance in the pseudopregnant rabbit (Chu, Lee and You, 1946),

rat (Melampy, Anderson and Kragt, 1964) and hamster (Melampy and Anderson, 1968). In contrast, the CL life-span is unaffected by hysterectomy in women (Doyle et al., 1971) and monkeys (Neill, Johansson and Knobil, 1969). Therefore, results of hysterectomy indicate that, with the exception of primates, the uterus of many cyclic mammalian species produces a luteolytic substance responsible for the regression of the CL and hence initiation of a new cycle. In cattle, sheep and swine the luteolytic effect of the uterus involves, at least in part, a direct or unilateral pathway between a uterine horn and the adjacent ovary. In cattle and sheep, the removal of one uterine horn resulted in luteal regression only when the CL was on the uterine-intact side (Ginther, 1967). However, unilateral hysterectomy in swine differed in that corpora lutea usually regressed in both ovaries provided there was retention of all or more than one-quarter of the uterine tissue on the side opposite to the side of unilateral hysterectomy. If, however, less than one-quarter of the cranial tip of the uterine horn was retained, the corpora lutea usually regressed on the side of the retained fragment but were maintained on the side of complete hysterectomy (Ginther, 1967).

Uterine extracts. Studies using uterine extracts also indicate the presence of a uterine luteolysin in the late luteal phase in pigs and sheep. Pig uterine flushings from days 14 to 18 of the cycle were markedly luteolytic in their effect on cultured granulosa cells, while flushings from day 1 to 10 or day 20 had no detrimental effect (Schomberg, 1967). Similarily, Christenson and Day (1972) reported that intrauterine infusions of day 13 to 17 porcine endometrial

extracts resulted in regression of induced corpora lutea in unilaterally pregnant gilts. These results support the role of the uterus as a source of a luteolysin in the pig during the late luteal phase. Similar results have been reported in sheep (Caldwell and Moor, 1971). Day 14 freeze-dried uterine plasma of ewes was infused into the ovarian artery of intact ewes on day 8 of a 16 day cycle. The ovarian venous plasma P levels decreased rapidly over the 6 to 8 hr infusion interval and ewes returned to estrus 48 to 72 hr following the cessation of infusion. Controls, receiving day 8 freeze dried plasma, exhibited no signs of CL regression.

Local Transport of a Uterine Luteolysin:

Vascular Associations

In cattle, sheep, pigs, hamsters, rats and guinea-pigs it has been postulated that the luteolytic effect of the uterus is normally mediated via the local utero-ovarian vasculature (Del Campo and Ginther, 1972a, 1972b, 1973; Ginther, 1974). Various techniques have been employed to support this concept including; unilateral hysterectomy, embryo transplants, and ligation of the utero-ovarian vascular connections (reviewed by Melampy and Anderson, 1968; McCracken, Baird and Goding, 1971b;McCracken et al., 1973). Detailed studies by Del Campo and Ginther (1972a, 1972b, 1973 ; Ginther and Del Campo, 1974) indicated that whether the luteolytic effect of the uterus was local or systemic differed according to the vascular patterns in various species. They contended that sheep, cattle and swine had a vascular anatomy more conducive to the possible passage of a uterine luteolysin from the uterine venous blood to the ovarian

artery than did horses. In the first three species the ovarian artery is in close apposition to the utero-ovarian vein (Del Campo and Ginther, 1972a, 1973; Ginther and Del Campo, 1974). However, in the mare, the ovarian artery is caudal to the utero-ovarian vein and only contacts the uterine branch in a limited area as it passes obliquely across the vein (Del Campo and Ginther, 1973). Therefore, in the mare, a uterine luteolytic effect would seem to be exerted through systemic channels.

Barrett et al. (1971) observed luteal maintenance in the ewe by separating the ovarian artery and vein, supposedly preventing the transfer of a luteolytic substance to the artery from the utero-ovarian venous system. Auto-transplants of ovaries also resulted in CL maintenance (Harrison et al., 1972). Surgical interruption of the continuity between the uterus and ovaries resulted in maintenance of corpora lutea in swine (Anderson et al., 1969). However, a recent report by Martin and Dziuk (1974) suggested that, in swine, the usual utero-ovarian vascular relationship need not exist for normal estrous cycles. When ovaries were auto-transplanted to the uterus or body wall of gilts, both the incidence and the length of the estrous cycle was essentially unaffected. It may be possible, though, that uterine secretions could still gain access to the ovary with resultant luteolysis. Also, Torres and First (1975) observed that partial or total surgical separation of the utero-ovarian vascular relationship in gilts on day 9 did not affect the length of the cycle. While this may appear quite conclusive in support of a systemic pathway for a uterine luteolysin in pigs, it might be interpreted with reservation. Ginther (1974) has pointed out that collateral vessels, reported to

become functional after ligation of the main vessels between the uterus and ovary in guinea-pigs and sheep, may serve as an alternative pathway which would obscure the role of the ligated vessels in normal animals.

Effects of $PGF_{2\alpha}$ on Progesterone Secretion by the CL

Pharriss and Wyngarden (1969) first reported that intrauterine infusion of $PGF_{2\alpha}$ (1 mg/kg/day) resulted in a sharp decrease in plasma P and subsequent shortening of pseudopregnancy in the rat. This luteolytic effect, following $PGF_{2\alpha}$, has been duplicated by others in the rat (Behrman, Yoshinga and Greep, 1971) and in other laboratory species including hamsters (Labhsetwar, 1971, 1972a), guinea-pigs (Blatchley and Donovan, 1969), mice (Labhsetwar, 1972b) and rabbits (Keyes and Bullock, 1974). In most of the large domestic animals, $PGF_{2\alpha}$ has been reported to be luteolytic as indicated by declining P levels. More will be mentioned of this later when discussing controlled ovulation and only a few examples are cited here. Infusion of $PGF_{2\alpha}$ into the utero-ovarian vein of sheep (40 to 50 μg/hr for 4 to 6 hr) resulted in a marked depression of plasma P within 24 hr after cessation of infusion, and an early return to estrus (Thorburn and Nicol, 1971; McCracken, 1971). Convincing evidence of the luteolytic action of $PGF_{2\alpha}$ has also been provided from studies using sheep with the ovary autotransplanted to the neck. Infusion of $PGF_{2\alpha}$ into the ovarian artery was followed by a sharp fall in secretion of P and eventual degeneration of corpora lutea (McCracken, Glew and Scaramuzzi, 1970; Barrett et al., 1971; Chamley et al., 1972a, 1972b). Recently, Umo (1975) reported that the functional luteolysis

(decreasing P secretion), following administration of $PGF_{2\alpha}$ on day 10 of the ovine estrous cycle, was accompanied by limited ultrastructural changes in the CL, similar to those seen during the early stages of natural luteolysis.

Intrauterine administration of $PGF_{2\alpha}$ to cows, after day 4, resulted in a decrease in luteal diameter (Louis, Hafs and Morrow, 1972, 1974), a significant decline in blood P to near non-detectable levels by 24 to 48 hr post-treatment and a shortening of the estrous cycle (Liehr, Marion and Olson, 1972; Lamond et al., 1973; Louis, Hafs and Morrow, 1974; Oxender, 1975). Similarly, pony mares also show a luteolytic response to $PGF_{2\alpha}$ during diestrus, as measured by shortening of the estrous cycle (Douglas and Ginther, 1972, 1973a).

Results following $PGF_{2\alpha}$ administration to cycling sows during the luteal phase have indicated little or no effect (Diehl and Day, 1973, 1974). Gleeson (1974) observed that infusion of $PGF_{2\alpha}$ into the uterine veins of 4 gilts on day 11 to 12 resulted in a drop in utero-ovarian P to follicular-phase levels which remained low for a period of 8 hr to 3 days. Similarly, injections of 20 mg, intramuscularly (im) or subcutaneously (sc) on day 12, resulted in P decline but subsequent recovery. No change in P levels was observed after infusion of $PGF_{2\alpha}$ into the uterine vein of one sow on days 8 or 9. There was no detectable effect on estrous cycle length in any of these animals. It may be that swine corpora lutea are resistant to luteolytic stimuli until near the time of normal luteal regression. This aspect will be developed further in a later section.

Endogenous $PGF_{2\alpha}$ During the Estrous Cycle

Most of the evidence for the possible role of endogenous prostaglandins in luteal regression has been accumulated from studies with sheep. $PGF_{2\alpha}$ was not detectable, or was at low levels, in the uterine venous blood of ewes during the early part of the estrous cycle (Bland, Horton and Poyser, 1971). Frequent blood sampling of ewes by Thorburn and co-workers (1972, 1973) demonstrated a complex series of PGF peaks in utero-ovarian venous plasma during the time of luteal regression. Early PGF peaks seen about day 13 coincided with early histological signs of luteal regression. The PGF peaks, usually of short duration (<6 hr), increased in frequency and concentration to a maximum of 5 to 22 ng/ml on days 15 to 16. The concentration of P in the utero-ovarian vein varied considerably but increasing peaks of PGF were closely associated with declining P concentration and luteal regression. Transient, but marked, decreases in P concentration followed each PGF peak. The recovery in P levels became progressively less after each PGF peak until low levels were reached on day 15. Peaks of PGF on days 15 and 16 coincided with an overall drop in P to very low levels and marked cytological changes indicative of luteal regression. The temporary depression of P following the early PGF peaks are consistent with the observations of Thorburn and Nicol (1971) who observed that a short infusion of $PGF_{2\alpha}$ into the uterine vein resulted in only partial luteal regression and subsequent recovery of function as judged by peripheral levels of P. More specifically, a cyclic release of $PGF_{2\alpha}$ has been identified in sheep uterine venous blood towards the end of the estrous cycle (Bland, Horton and Poyser,

1971; McCracken et al., 1972; Barcikowski et al., 1974). Barcikowski et al. (1974) reported that $PGF_{2\alpha}$ peaks (3 to 8.5 ng/ml), lasting 2 hr or less, occurred on day 15 and they were associated with completion of luteal regression as evidenced by a drop in P to very low levels. In 4 sheep, with ovarian autotransplants, CL persisted and large amounts of $PGF_{2\alpha}$ accumulated in the uterine fluid, but no prostaglandins of the E series were found (Harrison et al., 1972). As well, Carlson and co-workers (1972) reported that, in contrast to $PGF_{2\alpha}$, PGE_1 or PGE_2 infused into the ovary at levels of up to 50 μg/hr for 6 hr did not induce estrus. Likewise, $PGF_{2\alpha}$ did not induce luteolysis when infused systemically at a dose (25 μg/hr) which would cause CL regression when infused locally (Thorburn et al., 1972). These, with other studies reported, indicate that $PGF_{2\alpha}$ is naturally luteolytic in the sheep and acts locally.

In the case of the cow, publications concerning the levels of prostaglandins in the endometrium or uterine venous blood are scanty. Nancarrow and co-authors (1973) reported that surges of PGF in the utero-ovarian vein of one cow, before day 0, coincided with declining P levels and active estradiol-17β secretion. However, since the results indicated that sampling began only 2 days prior to estrus, the association between endogenous levels of PGF, estradiol-17β and P in the late luteal phase are still not completely documented for the cow. Hansel et al. (1973) pointed out that the active luteolytic fraction they purified from extracts of bovine uteri was not a prostaglandin, but rather appeared to be an unsaturated fatty acid. They have demonstrated that arachidonic acid, locally applied, was luteolytic in the psudopregnant, hysterectomized hamster. Since the