REPRODUCTIVE PHYSIOLOGY OF CAPTIVE FEMALE AND MALE FISHERS (*Martes pennanti*)

By

Richard Blaine Cherepak

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree of

MASTER OF SCIENCE

Department of Animal Science

University of Manitoba

Winnipeg, Manitoba

Canada. R3T 2N2

October 1993

ISBN 0-315-85962-8

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REPRODUCTIVE PHYSIOLOGY OF CAPTIVE FEMALE AND MALE FISHERS (*Martes pennanti*)

BY

RICHARD BLAINE CHEREPAK

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

ABSTRACT

Cherepak, Richard Blaine. M.Sc., The University of Manitoba, June, 1993. Various Aspects of the Reproductive Physiology of Captive Female and Male Fishers (*Martes pennanti*). Major Professor; M. Laurene Connor.

In order to obtain a more complete understanding of the reproductive biology of the fisher, 694 blood samples, 235 vaginal swabs and 120 testes diameter measurements were collected from a captive population of fishers over a 3 year period. In the 1st year, blood samples and vaginal swabs were collected from female fishers (n=14) monthly from September 1989 until January 1990, bi-weekly until March 1990, weekly until the end of May and once in August 1990. Second year blood sampling of females (n=13) was conducted once in October 1990, then weekly from mid-December 1990 until mid-June 1991. Three male fishers were blood sampled in the 2nd year of the study, and in the 3rd year blood samples and testes diameter measurements were taken from 5 animals. Sampling of males was conducted monthly starting in September 1990, then weekly from mid-December 1990 to mid-June 1991, and in the final year, sampling was conducted weekly from late-November 1991 to early-June 1992.

Blood samples collected from females were analyzed for 17ß-estradiol and progesterone by radioimmunoassay. Smears were made from the vaginal swabs and observations were made for estrous behavior. Monthly mean 17ß-estradiol ranged from 19.74 to 40.77 pg ml^{-1} over the entire research period, but monthly means within research years were not found to be significantly different. Individual profiles indicate episodic elevations of 17ß-estradiol throughout the year with the frequency of peaks increasing as the breeding season approached. Examination of vaginal smears revealed that the percentage of cornified epithelial cells and the number of leukocytes were negatively correlated, but that 17ß-estradiol was not correlated to any aspects of vaginal cytology.

Progesterone was monitored as an indicator of luteal activity and to provide information regarding ovulation, the onset of implantation, duration of the post-implantation period, and which environmental cues trigger reproductive events in the fisher. A temporary increase in progesterone levels indicated that ovulation had occurred during April or May, the normal breeding season, in 8 animals. Levels returned to near baseline until the following January or February when progesterone was again elevated. The profiles suggest the lengthening days following the winter solstice stimulate reactivation of dormant corpora lutea capable of

producing peripheral progesterone levels of over 40 ng ml^{-1}. Observations strongly suggest that pseudopregnancy may occur in female fisher and the post-implantation period is approximately 40 to 45 days. Ovulation may have occurred spontaneously in several animals. Progesterone levels remained low throughout the year in 5 animals.

In the male fishers, initial increases in testosterone, from a baseline of <1 ng ml^{-1}, occurred in November/December with the most dramatic increases occurring in February/March. Peak levels of testosterone, ranging from 11 to 18 ng ml^{-1}, were reached in late-March/early-April and were followed by a rapid return to baseline by late-April/May. Testes diameter ranging from 10.6 ± 0.5 mm in late-November to 16.8 ± 0.3 mm in early-May, was positively correlated with testosterone concentration. Testosterone levels declined 3 to 4 weeks before the decrease in testes diameter. Semen was collected by electroejaculation and sperm dimensions and morphology were recorded. Sperm heads measured 5 μm wide and 7 μm long. The total length of the sperm was 71 to 75 μm.

This research provides the first descriptions of dynamic reproductive characteristics of live fishers, and in doing so may increase our ability to manage wild populations of fishers. It also adds to the database of information regarding reproduction in mustelids and may prove useful in

discussions regarding delayed implantation and various aspects of comparative endocrinology.

DEDICATION

This thesis is dedicated to my loving parents, who through their hard work and belief in me, gave me the opportunity and confidence to pursue a goal I've had since my childhood.

This is also dedicated to the fishers that I worked with over the last 6 years, especially the few that are no longer around.

ACKNOWLEDGMENTS

I would like to offer my most sincere gratitude to my M.Sc. supervisor, Dr. Laurie Connor. She appeared on the scene from the east coast just in time to accept me as a graduate student in a "new" department to me, Animal Science. Her sincere interest in my research ideas, the care for the fishers, and the confidence and encouragement she provided me with, made my time as a graduate student truly enjoyable. Most importantly, her friendship and understanding were always a welcome sight in what were once in a while difficult times. I cannot imagine having done my research with anyone else. Thank you!

Thanks to the other members of my graduate committee, Drs. Martyn Palmer, Borden Howland and Robert MacArthur. A committee meeting was never missed, nobody asked when I would be finished, and advice was cheap.

Thank you to Dr. Norman Stanger, who performed delicate work with the fishers, helped heal the sick, and was ever a source of knowledge and friendship. Drs. Gary Crowe and Loreen Onischuk provided undivided attention when help was needed with statistics.

Thank you to all the staff at Glenlea Research Station.

Almost everyone working there had some sort of role with my research, be it assisting me with the animals, holding pens, enclosure construction,... and the list goes on. Special thanks to Rosaire Menard, and to Lorne Dawydiuk, Bob Lavallee and Gilbert Perron for caring for my animals during the study.

Norman and Lawrence Stansell provided a safe, secure place for my fishers for the 2 years I had them before my research started. I hope I can repay the debt.

Thanks to Lori Iverson and Ricky Araneda for technical assistance in the laboratory. Without them I still wouldn't know what a standard curve is. Thanks to Janice Haines for a diversity of tasks, from ordering materials to assisting Dr. Stanger with surgery on the fishers.

At one time or another, all the staff of the Department of Animal Science provided me with needed input, and/or assistance. Special thanks to Margaret-Anne Baker, who never failed to have a smile and an answer to my plethora of word processing questions, and to Terri Garner for "just being there".

It is not possible to express the gratitude and friendship I have felt towards my fellow students. They were study partners, sounding stones and inspirations. Special thanks to two special women, Dr. Tracy Gilson, and soon to be Dr., Kim Ominski. We shared happiness and

sorrows together, thank you both. Also, to Carolyn Tucker, forever a friend.

Finally, thank you to Dyck Stardom of the Manitoba Department of Natural Resources for my start in work with furbearers, Dr. Ann McRae of the University of Saskatchewan for the use of an electroejaculator, Darryl Wright of the Department of Entomology for use of microscope/camera equipment, and Midge Strickland, Bill Berg and Dr. Rodney Mead for sending me some hard to get references.

Funding for my research was provided in part by a University of Manitoba operating grant, a University of Manitoba Graduate Fellowship, and a Manitoba Department of Natural Resources, Wildlife Branch Research Award.

FOREWORD

The preparation of this thesis followed a manuscript format. Portions of Manuscript II have been published as: Cherepak, R.B. and Connor, M.L., 1992. Constantly pregnant... well almost. Reproductive hormone levels of the fisher (*Martes pennanti*), a delayed implanter. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 150-154. Manuscripts I and III will be submitted to "Biology of Reproduction", and the authors of both will be R.B. Cherepak and M.L. Connor, Department of Animal Science, Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

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LIST OF ABBREVIATIONS

SYMBOL	NAME
E2	17ß-Estradiol
P4	Progesterone
T	Testosterone
PBS	Phosphate-Buffered Saline
sem	Standard Error of the Mean
LHRH	Luteinizing Hormone Releasing Hormone
HCl	Hydrochloride
RIA	Radioimmunoassay
cpm	Counts Per Minute

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INTRODUCTION

The fisher (*Martes pennanti*) is a large member of the family Mustelidae, order Carnivora, that has long been cherished for its' luxurious pelt. High fur prices early in the 20th century placed a great deal of pressure on wild populations of fisher, so attempts to raise them commercially were initiated. All early attempts to obtain litters in captivity failed, for reasons that were later found to be related to a lack of accurate knowledge regarding reproduction in the species.

It was discovered on fur farms that fishers breed for the first time at 1 year of age, are pregnant for approximately 51 weeks, and breed within 2 weeks of producing a litter in April or May (Hodgson 1937, Hall 1942, Douglas 1943). Carcass analyses revealed that the exceptionally long gestation period was due to an extended period of embryonic diapause prior to implantation (Enders and Pearson 1943a). Also documented were: age of sexual maturity; ovulation rates; blastocyst counts; placental scar counts; population-based pregnancy rates; estimates of litter size; and dimensions of reproductive organs (Hamilton and Cook 1955, Eadie and Hamilton 1958, Wright and Coulter 1967, Shea et al. 1985, Leonard 1986, Douglas and Strickland

1987).

However, very little is known about basic reproductive physiology in the live animal. Some of the unknowns include: what are the environmental cues that trigger reproductive events such as implantation and estrus in this species; what is the duration of the post-implantation period; is ovulation an induced or spontaneous event; how late in the season can successful matings occur; what are typical fisher semen characteristics; how do externally visible characteristics such as vulval swelling and testes diameter relate to reproductive status; and can vaginal smears be used to predict estrus?

Seasonally delayed implantation is a trait the fisher shares with many members of the family Mustelidae, as well as representatives from 9 other taxonomic families (Sandell 1990). However, the mechanisms that control the phenomenon are poorly understood. Research conducted on this trait in the fisher may provide information applicable to other species and increase our knowledge of this mode of reproduction.

With these unknowns in mind, the objectives of this research were to achieve a more complete understanding of the reproductive biology of the fisher. One method of confronting these issues was to determine circulating levels of 17ß-estradiol (E2) and progesterone (P4) in female

fishers and testosterone (T) in males. The determination of E2 levels in conjunction with the collection of vaginal smears and visual observations could provide information regarding when estrus occurs and its' duration in the fisher. Progesterone has been used as an indicator of ovulation, luteal function, pregnancy and implantation, and has been used to monitor gestation length in many species of mustelids. We, therefore, intend to use it for similar purposes in the fisher. Testosterone profiles provide a picture of the timing of reproductive events in males of many species. To obtain a better understanding of reproduction in the male fisher, T profiles were determined along with seasonal changes in testes size.

The collection of this information would greatly increase our knowledge of the biology of this furbearer. In a time when human activities are seriously threatening much of our wild spaces, this fundamental knowledge is becoming increasingly more difficult to obtain, and at the same time more important to know.

LITERATURE REVIEW

This review will address the information currently available in the literature regarding reproduction in the fisher, and for comparison, other mustelids. Species from other taxonomic groups will be cited when appropriate. Topics to be discussed include general aspects of reproduction, delayed implantation, reproductive hormone levels, ovulation, gestation length, pseudopregnancy, vaginal cytology and vulval swelling, and sperm characteristics.

Reproduction in Female Fisher

Early Information

The fisher is a secretive animal, seldom observed in the wild even where abundant, so early reports regarding its reproductive history were largely anecdotal. Hodgson (1937) cited several authors from the period of 1905 to 1930 and essentially all that was "known" about fisher reproduction was that breeding presumably took place about the 1st of March and that litters of 1 to 5 young were born about the

1st of May. Seton (1953) further stated that most trappers were of the opinion that the species pairs for breeding.

Age of Sexual Maturity

It was discovered on fur farms that captive female fishers breed for the first time at 12 months of age and thereafter one litter is produced per year (Hodgson 1937, Hall 1942). This finding has since been supported by analysis of trapper-collected specimens in which researchers found corpora lutea and/or blastocysts in the reproductive tracts only of animals believed to be older than 1 year of age (Hamilton and Cook 1955, Eadie and Hamilton 1958, Wright and Coulter 1967, Shea et al. 1985, Leonard 1986).

Mating Dates

Hodgson (1937) lists the widest range of mating dates recorded for captive fishers, extending from March 23rd to May 16th. Similarly, mating dates of captive fishers reported by other authors (Laberee 1941, Hall 1942, Douglas 1943) are within this range. There is no literature regarding observed matings in wild fisher, although there is speculation that increased movement of male fishers in March and April is associated with the breeding season (Wright and Coulter 1967, Leonard 1986, Johnson 1984, Arthur et al. 1989).

Parous females exhibit a post-partum estrus within 3 to 11 days of parturition (Hodgson 1937, Laberee 1941, Hall 1942, Douglas 1943). Douglas (1943) also reported a single mating 18 days post-partum that may be explained by an observation of Hodgson (1937), who stated that if the female does not conceive at the first mating period, she may come into heat again after about 14 days. It has been reported that female fishers remain in heat for a period of 2 to 3 days (Laberee 1941, Hall 1942).

Gestation

The belief that fishers had a gestation period of about 2 months resulted in a complete failure of all early attempts to obtain offspring in captive breeding operations. Finally Hodgson (1937), documenting his work and that of others, reported that the gestation period of fishers was actually much longer, and sited a range of 336 to 360 days. Subsequently, Hall (1942) and Douglas (1943) reported successful matings of fishers in captivity with gestation lengths ranging from 327 to 358 days.

The long gestation period was determined by Enders and Pearson (1943a) to be attributable to delayed implantation of the blastocysts. Specimens collected by these authors in January and February, each contained 3 unimplanted blastocysts. Since copulation is known to occur in the

spring months, and there was no evidence of delayed ovulation or fertilization, these authors concluded that blastocysts remain unimplanted for a period of 9 months or more.

Post-implantation duration in the fisher is unknown but has been estimated by several authors. Wright and Coulter (1967) assumed that the interval between implantation and parturition was about 30 days, while Hamilton and Cook (1955) suggested that the post-implantation period may be as long as 60 days.

As the duration of the post-implantation period is unknown, implantation dates can only be interpolated from known parturition dates and estimates of the post-implantation length. Powell (1982), using known parturition dates from several authors and a gestative phase duration estimate of 30 days (from Wright and Coulter 1967), indicated that implantation could occur as early as January and as late as early-April. Indeed, Wright and Coulter (1967) found implanted embryos in January, February and March. In contrast, Douglas and Strickland (1987) cited a report of 3 fishers that had fetuses so well developed by mid- to late-December that implantation would have had to occur in early to mid-December, although they question the validity of the dates reported to them by the trappers who collected the specimens.

Parturition Dates

Like mating, the date of parturition is highly variable. Documented parturition dates have been reported to occur between March 12th and April 20th (Hodgson 1937, Hall 1942, Douglas 1943, Coulter 1966, Powell 1982, Leonard 1980). Wright and Coulter (1967) estimated a late-February parturition date by the crown-rump measurement of embryos taken from a female collected on February 7th. Powell (1982) suggested that parturition and breeding dates may correspond with the length of winter in different parts of the fishers' range and speculated that they should show a north-south correlation, both occurring later at northern latitudes.

Litter Size

Litter sizes of captive and wild fishers have been documented by only a few authors, who found an average litter size of 2 to 3, with a range of 1 to 6 (Hodgson 1937, 1949, Seton 1953, Douglas 1943, Leonard 1986, Douglas and Strickland 1987).

Counts of corpora lutea and blastocysts have been used to estimate litter size of fisher, although they are usually considered to be high. Several authors reported on the relationship between corpora lutea numbers and late term embryos (Douglas and Strickland 1987, Wright and Coulter 1967, Leonard 1980), and on blastocyst counts (Hamilton and

Cook 1955, Wright and Coulter 1967, Strickland 1981 in Douglas and Strickland 1987).

Placental scars do not usually persist from pregnancy until the following trapping season, when specimens would be collected. Coulter (1966 in Douglas and Strickland 1987) did however find sufficiently distinct placental scars in 27 females to report a mean scar count of 2.93. This value was lower than the mean number of corpora lutea, blastocysts or embryos derived from the same specimens. These results suggest that fecundity rate may increase in older females as has been speculated by Shea et al. (1985) and Douglas and Strickland (1987).

Reproduction in Male Fisher

There is little information available regarding reproduction in male fisher. It is known from mating records on fur farms that breeding occurs from March to May (Hodgson 1937, Laberee 1941, Hall 1942, Douglas 1943). Carcass analyses is the source of all other information regarding reproduction in male fishers.

Sperm Production

Douglas and Strickland (1987), found that males were spermatic by 12 months of age. In fact, they found sperm in

males of all ages during February and March, but in only 8% of males examined from November to January. Sperm was also found in both juveniles and adults during February and March by Wright and Coulter (1967), and a single January juvenile specimen was aspermatic. They also reported sperm in an April specimen and in 1 of 2 specimens collected in May. Leonard (1980) found sperm in males only in the month of March.

Reproductive Organs

Testes size increases progressively starting in early winter in both adults and juveniles, with those of the juveniles reaching adult size by March (Douglas and Strickland 1987, Wright and Coulter 1967). However, the mean weight of bacula of juvenile males was found to be significantly lighter than those of adult males during March and April. This finding led Douglas and Strickland (1987) to hypothesize that juvenile males may be ineffective breeders because the smaller baculum may be of insufficient size to induce ovulation in the female. It is unknown how long the testes remain enlarged because specimens are generally lacking from the months of April to October (Douglas and Strickland 1987).

Reproduction in Mustelids

Delayed Implantation

Delayed implantation is the phenomenon whereby the zygote develops to the blastocyst stage, but instead of implanting in the uterine wall and continuing development, it remains unimplanted and relatively quiescent for the duration of the delay period. During the diapause there is a very slow rate of cell division which is primarily restricted to cells within the trophoblast (Aitken 1977, Mead and Wright 1983).

Types of Delayed Implantation. Delayed implantation has been described in about 100 mammalian species, representing 8 mammalian orders and 17 of the 139 families (Sandell 1990). Two forms of delayed implantation have been distinguished: lactational (or facultative) and seasonal (or obligate). Lactational delay is found in species bearing multiple litters per year and in which ovulation and fertilization occur before the previous litter is weaned. Embryonic development resumes after heavy lactation ceases. If the newborn litter dies the embryos from the post-partum mating do not enter diapause but implant without delay. This mode of delay has been found in marsupials, insectivores and rodents. Seasonal delay is found in

species which invariably produce only a single litter per year and has been reported in carnivores, pinnipeds, edentates, bats, and one species each of both deer and moles. The diapausing embryos are activated in all females of the population at a certain time of year in response to what are assumed to be appropriate environmental cues (Aitken 1977, Sandell 1990). The following will be directed towards discussion of seasonal delays with an emphasis on information available for mustelids.

Evolution of Delayed Implantation. Delayed implantation is believed to have evolved independently many times within the family Mustelidae (Mead 1989, Ridley 1983 in Sandell 1990). Powell (1982) however believed that delayed implantation likely had a common origin among mustelids. Seventeen species of mustelids are currently known to exhibit delayed implantation (Sandell 1990).

Mead (1989) reviewed the literature regarding the evolution of delayed implantation and found that 5 basic hypotheses can be identified. The first, and still one of the most accepted of these, states that delayed implantation is of selective advantage in that it ensure the young are born as early as possible in the year. This provides the young with a longer period to develop physically and behaviorally, thereby increasing their chance of survival in

a harsh winter environment. A cooling environment, as encountered during periods of glaciation, provided the selection pressure to produce rapidly developing young early in the spring.

Other theories include: a reduction in core body temperature and consequently, embryonic development; the presence of selection pressures that make it advantageous to the adults (mate selection, paternal assistance with the young, avoidance of inclement weather, synchrony of reproductive processes) to breed later in the year; the facilitation of the prolonged separation of the sexes; and a population self-regulation mechanism for small voracious predators.

Sandell (1990) provided an extensive argument for the evolution of delayed implantation based on the ability of females to choose their mate and the belief that the timing of parturition is determined by ecological factors which can enhance survival of the young.

Control of Delayed Implantation. Considerable effort has been made to elucidate the environmental and physiological factors responsible for the initiation, maintenance and termination of delayed implantation in mustelids.

Photoperiod is the environmental cue which is generally believed to be responsible for the timing of reproductive

events in mustelids. Artificial manipulation of photoperiod has been found to induce premature implantation of diapausing embryos in many mustelids, most requiring lengthening days (Pearson and Enders 1944, Hansson 1947, Holcomb et al. 1962, Kirk 1962, Wright 1963, Mead 1971, Murphy and James 1974). The European badger (*Meles meles*) and river otter (*Lutra canadensis*) require shortening days (Canivenc et al. 1971, Canivenc and Bonnin 1981, Stenson 1985). While light appears to be necessary for the synchronization of nidation in a population, it is not ultimately required for implantation to occur. Blinded skunks (*Spilogale putorius*) (Mead 1971) and mink (*Mustela vison*) kept in darkness (Kirk 1962) have been found to implant their diapausing embryos, albeit out of synchrony with the rest of the population.

Neuroendocrine Role in Delayed Implantation. The effect of light on reproduction appears to be mediated through melatonin production by the pineal gland (Wurtman et al. 1968). In skunks (May et al. 1985) and other mammals, (Nishino et al. 1976, Bearden and Fuquay 1992) the neural input from the retina passes along the optic nerves to the suprachiasmatic nuclei within the hypothalamus. Multi-synaptic neural pathways pass through the superior cervical ganglia from the suprachiasmatic nuclei to the pineal gland.

During periods of darkness, increased neuronal activity within postganglionic fibres of the superior cervical ganglia results in increased enzyme activity and melatonin levels in the pineal gland. Denervation of the pineal gland eliminated diurnal variation of serum melatonin in mink (Ravault et al. 1986 in Mead 1989) indicating a similar pathway exists in other mustelids. Indeed, melatonin concentrations are higher at night and remain elevated for a longer duration during the long nights of winter in the ferret (*Mustela putorius*) (Baum et al. 1986) and mink (Ravault et al. 1986 in Mead 1989).

While the site and mechanism of action of melatonin remains unknown, it undeniably has an affect on reproductive events. Elimination of diurnal variation of melatonin secretion in mink (Ravault et al. 1986 in Mead 1989) inhibited nidation in animals exposed to artificially lengthened photoperiods (Murphy and James 1974). The treatment of skunks with exogenous melatonin during the period of embryonic delay has been found to increase the length of the delay period (May and Mead 1986 in Mead 1989). This is perhaps related to the fact that mink treated with melatonin exhibit marked reduction in plasma prolactin levels (Martinet et al. 1983, Rose et al. 1985). Murphy (1983) has shown that treatments which increase plasma prolactin levels shorten the pre-implantation period in

mink. Other experiments have demonstrated that treatments which suppress prolactin secretion result in decreased luteal function and delay or prevent blastocyst implantation (Papke et al. 1980, Martinet et al. 1981).

Delayed implantation in mustelids is associated with histologically inactive corpora lutea (Deanesly 1935, 1943, Wright 1942, Hansson 1947, Wright and Rausch 1955, Neal and Harrison 1958, Enders 1962, Canivenc and Bonnin-Laffargue 1963, Canivenc et al. 1967, Enders and Enders 1963, Wright 1963, Hamilton and Eadie 1964, Wright and Coulter 1967, Mead and Eik-Nes 1969b, Mφller 1973b) and correspondingly low P4 levels (Mead and Eik-Nes 1969b, Mφller 1973a, Sinha and Mead 1975). Of the ovarian compartments, the corpus luteum is the only constituent that consistently undergoes pronounced morphological and physiological changes that temporally coincide with cessation and subsequent resumption of embryonic development and implantation in mustelids (Mead 1981, 1986). These changes in the corpora lutea parallel changes in plasma and/or luteal concentrations of P4 in skunk (Mead and Eik-Nes 1969b, Sinha and Mead 1975, Mead 1981), mink (Mφller 1973b, Mφller 1974), European badger (Bonnin et al. 1978), beech or stone marten (*Martes foina*) (Bonnin et al. 1977, Canivenc et al. 1981) and weasel (*Mustela erminea*) (Gulamhusein and Thawley 1974). These observations are consistent with the hypothesis that the

resumption of embryonic development and blastocyst implantation are dependent upon the renewed activity of luteal tissue (Mead 1986). In fact, it has been shown in mink and ferrets that the corpus luteum is the only ovarian compartment required to induce implantation in these species (Foresman and Mead 1978, Murphy et al. 1983, Mead 1986).

Progesterone is the predominant steroid produced by the corpora lutea in the skunk (Ravindra et al. 1984) and ferret (Kintner and Mead 1983), however corpora lutea of both have the capability to aromatize androgens to estrogens. While estrogens are required to initiate implantation in species whose corpora lutea are fully active during diapause, such as red deer (*Capreolus capreolus*), rats (*Rattus* spp.), mice (*Mus* spp.) and armadillo (*Dasypus* spp.) (Aitken 1977), there is very little evidence that it is required for the termination of diapause in mustelids (Mead and Eik-Nes 1969a).

It is clear however, that P4 is not the only ovarian product required for delayed implantation to be terminated in mustelids (Mead 1981). Indeed, all attempts to hasten implantation in mustelids by treatment with P4 and/or other steroids have failed (reviewed by Mead 1981, Mead et al. 1981). Interestingly, Mead et al. (1988) recently reported that extracts from ferret corpora lutea will induce implantation in ovariectomized, P4-treated ferrets.

Preliminary findings indicate that the luteal factor is a protein.

The consensus appears to be that luteal inactivity associated with diapause is due to an insufficiency of one or more pituitary hormones (Aitken 1977, Mead 1989). Hypophysectomy during the delay period in skunks and mink abolished the pre-implantation increase in P4 and caused an indefinite extension of the diapause (Mead 1975, Murphy and Moger 1977). Histological studies of the pituitary of mink (Baevskii 1964, Murphy 1972) and European badger (Herland and Canivenc 1960 in Mead 1989) have revealed a marked paucity of gonadotrophin-secreting cells during the delay period and a subsequent increase at the time of blastocyst reactivation. Several authors (Foresman and Mead 1974, Canivenc and Bonnin 1981, Martinet et al. 1981) found that LH and prolactin secretion was low during the mid-pre-implantation period and increased gradually towards the time of implantation in skunks, badger and mink. The rise in LH and prolactin was paralleled by a similar rise in P4. The role of prolactin in the European badger is unclear as it is at low levels at the time of blastocyst reactivation and implantation.

The occurrence of delayed implantation was first documented in the 18th century, but still only speculation is available regarding the environmental pressures that led to

its evolution, how many times it evolved, the hormonal mechanisms regulating the phenomenon, and its current significance to species such as fisher. This remains an area of active research and our increasing knowledge should fill some of the considerable gaps still present.

Reproductive Steroids

The determination of reproductive steroid levels generally provides an insight into the state of reproductive readiness of an animal. The steroids E2 and P4 are considered to characterize various reproductive events in the female, as is T in the male. The adrenal glands may be a source of various androgens and estrogens, however during reproductive events the gonads (testes and ovaries) are the main site of this production (Hadley 1984).

17ß-Estradiol. 17ß-estradiol is produced in the granulosa cells of maturing follicles of the ovary through FSH-stimulated aromatization of androgens which were derived from LH-stimulated thecal cells. Estrogens are responsible for ovum maturation, preparation of the reproductive tract for mating, pregnancy and lactation, and the expression of female sexual behaviour (Hadley 1984).

17ß-estradiol levels have been studied in 8 species of mustelids in various reproductive states. In sexually

immature and anestrous animals, E2 was found to be undetectable in mink (Stoufflet et al. 1989) and spotted skunk (Mead and Eik-Nes 1969a), but found to be between 12 and 63 pg ml^{-1} in sable (*Martes zibellina*) (Polynstev et al. 1975, Shul'gina et al. 1981), river otter (Stenson 1985), wolverine (*Gulo gulo*) (Mead et al. 1991) and mink (Pilbeam et al. 1979).

In 2 species of mustelids, levels of E2 are higher in the months before estrus and declines as estrus approaches. In the river otter, E2 peaked at 30 pg ml^{-1} in December and was at 15 pg ml^{-1} at estrus (Stenson 1985). In mink, levels of E2 approached 100 pg ml^{-1} before the breeding season and were at 50 to 64 pg ml^{-1} by the time breeding season arrived (Travis et al. 1978, Pilbeam et al. 1979).

During the breeding season E2 levels were found to be between 80 and 90 pg ml^{-1} in the wolverine (Mead et al. 1991) and as high as 3750 pg ml^{-1} in pooled plasma samples from spotted skunks (Mead and Eik-Nes 1969a). Individual peaks of E2 of up to 120 pg ml^{-1} have been found in river otter (Stenson 1985) and sable (Polynstev et al. 1975), and as high as 930 pg ml^{-1} in ferrets under artificial photoperiods (Donovan et al. 1983). Juvenile sable have been found to have E2 levels equal to those of adult (95.7 pg ml^{-1}) during the breeding season, but are still classified as reproductively immature (Shul'gina et al. 1981).

The common finding that estrogen is required for implantation to occur in rodents (Cochrane and Meyer 1957 in Kintner and Mead 1983, Smithberg and Runner 1960) has stimulated investigation of estrogen levels in pregnant mustelids. Total unconjugated estrogens were found to be undetectable in ferrets during pregnancy (Heap and Hammond 1974). Levels of E2 have been reported at between 8.5 and 18 pg ml^{-1} in pregnant European badger (Mondain-Monval et al. 1980), mink (Pilbeam et al. 1979) and spotted skunk (Ravindra and Mead 1984). In sable, estrogens have been found to be: lower during diapause than during estrus; elevated several days before parturition; and subsequently reduced at the time of parturition (Polynstev et al. 1975). Similar findings have been described in the spotted skunk (Mead and Eik-Nes 1969a, Ravindra and Mead 1984), along with a correlation between increasing blastocyst size and decreasing E2 levels during the late pre-implantation period (Ravindra and Mead 1984). Interestingly, there are regular cycles of increased E2 levels in the pregnant European badger, indicating ovarian activity during diapause (Mondain-Monval et al. 1980). Thus, there is no conclusive evidence that estrogens are required for implantation in mustelids (Wu and Chang 1972, Murphy and Mead 1976, Mead and McRae 1982).

Progesterone. The corpus luteum formed after ovulation is considered the main source of P4 production in the mink (Møller 1974, Mead and Swannack 1978) and presumably in most mustelids (Mead 1981). Low level P4 secretion by corpora lutea is required for the long-term survival of diapausing blastocysts. Equally important, in mustelids, is the increased secretion of P4 by corpora lutea prior to implantation (Mead 1981). The absolute requirement for P4 in several stages of reproduction has led to extensive research involving this steroid in mustelids.

In immature and non-pregnant adult mustelids, P4 levels are typically low, being below 3 ng ml^{-1} in river otter (Stenson 1985), wolverine (Mead et al. 1991), sable (Polynstev et al. 1975, Shul'gina et al. 1981, Song et al. 1988), beech marten (Bonnin et al. 1977, Canivenc et al. 1981), ferret (Carlson and Rust 1969), mink (Pilbeam et al. 1979), short-tailed weasel (Gulamhusein and Thawley 1974), striped skunk (*Mephitis mephitis*) (Wade-Smith et al. 1980) and spotted skunk (Mead and Eik-Nes 1969b). Møller (1974) reported somewhat higher P4 levels in mink unmated throughout the breeding season.

In all mustelids studied, P4 levels during pregnancy generally follow the same pattern. Progesterone may temporarily increase shortly after ovulation due to partial luteinization of the granulosa cells of the corpus luteum.

Secretion of P4 is then reduced to near baseline levels as the granulosa cells become involuted for the duration of the delay period. Reactivation of the corpus luteum in response to appropriate environmental stimuli results in elevated P4 secretion, which along with other unknown factors, initiates changes in the endometrial tissues of the uterus making it conducive to embryo implantation and development. Luteal tissue typically begins to regress, due to increased prostaglandin secretion by the uterus, a few days after implantation and P4 levels, which were maximal at the time of implantation, fall to near baseline levels during the last few days of pregnancy (Mead and Wright 1983, Stenson 1985).

Progesterone levels have been monitored in many mustelids, including mink (Mϕller 1973a, 1974, Murphy and Moger 1977, Allais and Martinet 1978, Pilbeam et al. 1979, Stoufflet et al. 1989), ferret (Carlson and Rust 1969, Heap and Hammond 1974, Daniel 1976, Foresman and Mead 1978), short-tailed weasel (Gulamhusein and Thawley 1972, 1974, Rowlands 1974), polecat (*Mustela eversmanni*) (Mead et al. 1990), beech marten (Bonnin et al. 1977), sable (Polynstev et al. 1975, Song et al. 1988), striped (Wade-Smith et al. 1980) and spotted skunks (Mead and Eik-Nes 1969b, Sinha and Mead 1975, Mead 1981), river otter (Stenson 1985) and European badger (Bonnin et al. 1978, Canivenc and Bonnin

1979, 1981). Generally, maximum P4 levels are considerably higher (> 30 ng ml^{-1}) in the smaller species such as mink and ferret, compared to the larger species such as river otter and wolverine (< 20 ng ml^{-1}).

Testosterone. Testosterone is produced in the Leydig cells of the testes in response to stimulation of FSH-induced LH receptors. Subsequently, T acts in concert with FSH to initiate and maintain spermatogenesis and is also responsible for expression of mating behaviour or libido (Hadley 1984). Given the close relationship between T and a male's reproductive state, many researchers have characterized this steroid's annual profile, and been able to correlate it to various physiological and behavioral events in mustelids.

Testosterone levels of male mustelids in non-reproductively active months of the year range from < 0.2 ng ml^{-1} in European pine marten (*Martes martes*) and stone marten (Audy 1976, 1978a, b), to 1 to 2 ng ml^{-1} in the European badger (Audy 1976, Maurel et al. 1981, Maurel and Boissin 1982), river otter (Stenson 1985) and wolverine (Mead et al. 1991). Testosterone levels in non-breeding mink have been reported to be near 1 ng ml^{-1} under natural (Nieschlag and Bieniek 1975, Boissin-Agasse et al. 1981) and artificial photoperiods (Boissin-Agasse et al. 1982). Pilbeam et al.

(1979) reported total androgen levels of 0.9 ng ml^{-1} in non-breeding mink. Testosterone in non-breeding ferrets has been found to be higher, from 2.8 to 5 ng ml^{-1} (Neal et al. 1977, Erskine and Baum 1982).

In various mustelids, maximum levels of T are attained either in the months prior to, or early in, the breeding season. This is seen in the mink (Nieschlag and Bieniek 1975, Sundqvist et al. 1984, 1986, Pilbeam et al. 1979, Boissin-Agasse et al. 1981), stone marten (Audy 1976, 1978a, b), European pine marten (Audy 1976), short-tailed weasel (Gulamhusein and Tam 1974), European badger (Audy 1976, Maurel and Boissin 1981, Audy et al. 1982, 1985), wolverine (Mead et al. 1991), ferret (Neal et al. 1977) and river otter (Stenson 1985).

Maximum circulating concentrations of T are typically higher in the smaller species of mustelids. Testosterone levels of > 10 ng ml^{-1} have been reported in most of the smaller species (Audy et al. 1982, 1985, Pilbeam et al. 1979, Boissin-Agasse et al. 1981, Sundqvist et al. 1984, 1986, Neischlag and Bieniek 1975, Neal et al. 1977, Reiger and Murphy 1977, Erskine and Baum 1982, Wildt et al. 1989, Gulamhusein and Tam 1974, Audy 1976) and in one larger species (Audy 1976). Maximum average T values of < 10 ng ml^{-1} are typical of the larger species of mustelids (Maurel et al. 1981, Maurel and Boissin 1982, Stenson 1985, Mead et

al. 1991), again with 2 smaller species being exceptions (Audy 1978a, b, Boissin-Agasse et al. 1982). Testosterone has been found to be secreted in a pulsatile fashion in European badger (Maurel et al. 1981) and ferrets (Reiger and Murphy 1977). In virtually all species of mustelids studied, T levels rapidly decrease to baseline levels at or near the end of the breeding season.

Increased T concentration prior to the breeding season has been found to coincide or be positively correlated with: increased testes weight in short-tailed weasel (Gulamhusein and Tam 1974), beech marten (Audy 1976, 1978), European badger and pine marten (Audy 1976); increased testes volume in river otter (Stenson 1985), mink (Pilbeam et al. 1979, Boissin-Agasse et al. 1982) and ferret (Wildt et al. 1989); increased testes size in ferret (Neal et al. 1977) and wolverine (Mead et al. 1991); the onset of spermatogenesis in short-tailed weasel (Gulamhusein and Tam 1974) and wolverine (Mead et al. 1991); and fertility in mink (Sundqvist et al. 1984).

Ovulation

Ovulation is generally considered to be induced in mustelids (Ewer 1973, Mead and Wright 1983) even though it has been studied in only a few species. Induced ovulation has been confirmed, or implied in mink (Hansson 1947, Møller

1974), ferret (Hammond and Marshall 1930, Carrol et al. 1985), long-tailed weasel (*Mustela frenata*) (Wright 1963), river otter (Stenson 1985), striped skunk (Wade-Smith et al. 1978), sable (Bernatskii et al. 1976), wolverine (Mead et al. 1991) and European badger (Canivenc 1966). Formation of corpora lutea as a result of spontaneous ovulations or luteinization of unruptured follicles have been documented in short-tailed weasels (Deanesly 1935), spotted (Mead 1968a, Greensides and Mead 1973) and striped skunks (Wade-Smith et al. 1980), mink (Hansson 1947, Mϕller 1974), river otter (1985) and European badger (Neal and Harrison 1958). Ovulation may also have been induced by collection of vaginal smears in river otter (Stenson 1985) and striped skunk (Wade-Smith et al. 1980).

Gestation Length

The duration of pregnancy, including the period of delayed implantation when applicable, has been documented for most mustelids (see review of Mead 1989). The duration of the post-implantation period however, has only been either estimated or determined for a few of these species. In the larger species this period is > 40 days, as in the river otter (Stenson 1985) and European badger (Canivenc 1966, Canivenc and Bonnin 1981). In the smaller weasels, the post-implantation period has been reported to be between

21 and 35 days (Wright 1942, Pearson and Enders 1944, Wright 1948, Enders 1952, Enders and Enders 1963, Jonkel and Weckworth 1963, Wright 1963, Mead and Eik-Nes 1969b, Heidt 1970, Foresman and Mead 1973, Gulamhusein and Thawley 1974, Heap and Hammond 1974, Concannon et al. 1980, Mead et al. 1990).

Pseudopregnancy

Pseudopregnancy as a result of a non-fertile mating, spontaneous ovulation or luteinization of unruptured follicles has been confirmed or is suspected in the ferret (Hammond and Marshall 1930, Carlson and Rust 1969, Heap and Hammond 1974), short-tailed weasel (Deanesly 1935), river otter (Stenson 1985), striped skunk (Wade-Smith et al. 1980), mink (Mϕller 1974) and polecat (Mead et al. 1990).

Vaginal Cytology and Vulval Swelling

The characterization of changes in vaginal cytology and vulval swelling during the year, and specifically as related to reproductive periods, has been studied in several mustelids. During anestrus, smears contain mostly non-cornified epithelial cells and varying numbers of leukocytes, while the vulva is relatively flush with the surrounding area and essentially skin-colored. At estrus, vaginal smears contain epithelial cells which are

essentially all cornified and no leukocytes are present. The vulva is pink to reddish in color and is visibly swollen in appearance. This general picture of vaginal cytology and/or vulval swelling has been observed in wolverine (Mead et al. 1991), short-tailed weasel (Deanesly 1935), least weasel (*Mustela nivalis*) (Deanesly 1944), long-tailed weasel (Wright 1948), ferret (Hammond and Marshall 1930, Bissonnette 1932, Donovan et al. 1983), marten (*Martes americana*) (Enders and Leakly 1941), river otter (Stenson 1985), mink (Hansson 1947, Enders 1952, Holcomb et al. 1962, Enders and Enders 1963, Travis et al. 1978, Pilbeam et al. 1979, Travis and Pilbeam 1980), polecat (Mead et al. 1990) and striped (Wight 1931) and spotted skunk (Mead 1968a, b, Mead and Eik-Nes 1969b, Greensides and Mead 1973).

Sperm Characteristics

Dimensions of mammalian sperm have been determined for only a very few species. In an extensive review by Cummins and Woodall (1985), the dimensions of sperm from only 2 mustelids were cited, those of the mink and badger. Further to these reports, sperm dimensions of mink (Kim et al. 1979) and river otter (Stenson 1985) are available.

Physical abnormalities of sperm have only been reported in mink (Aulerich et al. 1972, Sundqvist et al. 1986) and ferret (Curry et al. 1989).

Summary

While there is extensive literature available regarding reproduction in a few species of mustelids, this topic has been largely unexplored in most others. For example, Sundqvist et al. (1989) located over 700 scientific papers dealing with various aspects of reproduction in mink. On the other hand, our knowledge of reproduction in most other species entails little more than when breeding occurs and when litters are born (Mead 1989).

Female mustelids follow 3 general patterns of reproduction: no delay of implantation; a short and variable delay of implantation; or an extended period of delayed implantation. With the exceptions of the western spotted skunk and European badger, on which considerable research has been conducted, research on members of the family Mustelidae, which undergo an extended period of delayed implantation, is generally lacking. Recently, Stenson (1985) added to our knowledge by reporting on his extensive work with the river otter. Other species which follow this pattern of reproduction, including the fisher, have to date not been investigated intensively.

It is crucial that we increase our knowledge of reproduction of mustelids. Many species are threatened by various aspects of human encroachment (habitat loss, chemical contamination, etc.), and in order to effectively

manage these species we must know what their reproductive potential and problems are likely to be. Human involvement may extend as far as artificial breeding programs and re-population attempts, as are underway with the black-footed ferret (*Mustela nigripes*). The substantial data-base available from work conducted on reproduction in mink, skunk, badger and ferret, provides a strong foundation for future research with other species of mustelids.

MANUSCRIPT I

Detection of Estrus in Captive Fishers (*Martes pennanti*)

Abstract. Vaginal smears and blood samples were collected from 14 female fishers over 2 research years so that serum 17ß-estradiol levels could be monitored and its relationship to vaginal cytology and behavioral estrus ascertained. Monthly mean 17ß-estradiol ranged from 19.74 to 40.77 pg ml^{-1} over the entire research period, but monthly means within research years were not found to be significantly different. Individual profiles indicate transient elevations of 17ß-estradiol throughout the year with the interval between elevations decreasing as the breeding season approached. In our research population, the percentage of cornified epithelial cells and the number of leukocytes were negatively correlated. Mean 17ß-estradiol was not correlated with any aspects of vaginal cytology in the research population, but correlations were found in individual animals.

Introduction

The information currently available regarding the estrous cycle of the fisher (*Martes pennanti*) is limited to observations that describe behavioral events. For example, Hall (1942) reported "... that the female comes into heat six to eight days after the young are born (in March or April), as evidenced by her action in leaving the nest box, running nervously in the pen and scratching on the partition separating her pen from that of the male. She remains in heat two or three days." Leonard (1986) found that a female travelled most of her home range during the breeding period and another extended her movements out of her previous home range, eventually leaving the research area. There is no quantitative physiological information regarding estrus in the fisher.

In mustelids, the timing and/or duration of estrus has been determined by several methods including: the characterization of estrogen profiles (Mead and Eik-Nes 1969a, Heap and Hammond 1974, Travis et al. 1978, Pilbeam et al. 1979, Mondain-Monval et al. 1980, Stenson 1985, Mead et al. 1990, Mead et al. 1991); the monitoring of vaginal cell cytology (Deanesly 1944, Enders 1952, Mead 1968a, b, Travis et al. 1978, Stenson 1985, Mead et al. 1990, Mead et al. 1991); and vulvar swelling (Wright 1931, Bissonnette 1932, Enders and Leekley 1941, Deanesly 1944, Wright 1948, Enders

1952, Mead 1968a, b, Travis et al. 1978, Pilbeam et al. 1979, Stenson 1985, Mead et al. 1991). This investigation was conducted to determine if estrus could be detected in captive fisher, its duration monitored using estrogen profiles and vaginal cytology, and if a relationship exists between these characteristics.

Materials and Methods

Animals and Maintenance

Fourteen female fishers (*Martes pennanti*) were maintained in southern Manitoba, at the University of Manitoba Glenlea Research Station (49° 53'N, 97° 09'W). Eight of the animals were captured in south-eastern Manitoba in the fall of 1987 and one in the fall of 1988. Two litters were born in captivity, producing 4 animals (3 born in 1988 and 1 in 1989) used in this research. The animals were housed individually in wire mesh pens (1 m x 1 m x 2 m) with attached nest-boxes (0.125 m^3) and exposed to natural weather conditions and photoperiods. The animals were fed a commercial wet mink ration (see appendix 1) at a level that maintained body weight and allowed water *ad libitum*. When the study began the animals were 0.5 (n=1), 1.5 (n=3) and 2.5 to 6.5 (n=10) years of age. Pre-molars of animals of unknown age were collected and ages assigned by annuli counts performed at Matson's Laboratory (Milltown, MT). The

method was confirmed by using the technique on teeth collected from animals of known age.

Sampling

First year blood sampling (14 animals) was conducted monthly from September 1989 until January 1990, bi-weekly until March 1990, weekly until the end of May and once in August 1990. Second year sampling (13 animals) was conducted once in October, then weekly from mid-December 1990 until mid-June 1991. Fishers were immobilized for all procedures using ketamine HCl (Ayerst Lab., Montreal, PQ) with xylazine HCl (Chemagro Ltd., Etobicoke, ON) mixed 10:1, and administered intramuscularly at approximately 20 mg ketamine HCl kg^{-1} body weight. Rogar-Mycine eye ointment (Rogar/STB Inc., London, ON) was applied to the eyes of the anaesthetized fishers to prevent drying.

Blood (7 ml) was collected by jugular venipuncture using a sterile 20 gauge 2.5 cm vacutainer needle and sterile, siliconized 7 ml vacutainer (Becton Dickinson and Company, Rutherford, NJ). The samples were refrigerated for 16 to 24 h, centrifuged for 20 min at ~1000 g (Jouan Inc., Winchester, VA) and the serum removed and stored at -20°C until analyzed for 17ß-estradiol (E2). Blood was collected in the same order each sampling day, between 07:35 and 17:45 h during the 1st year and between 08:30 and 17:05 h in the 2nd

year.

17ß-Estradiol Analysis

17ß-estradiol was extracted from serum and quantified by RIA using methods based on those of Yu et al. (1974). In brief, 1000 µl serum was mixed with 6.5 ml ethyl ether (Mallinckrodt Specialty Chemicals Co., Mississauga, ON), vortexed for 4 min and equilibrated for 15 min before freezing over dry ice/ethanol (-43°C). The supernatant was decanted and dried under a stream of nitrogen gas in a shaking water bath maintained at 37°C. The dried precipitate was resuspended in 1.25 ml phosphate buffered saline (PBS). Percentage recovery of ^{3}H-E2 (New England Nuclear, Boston MA) added to pooled fisher serum averaged (± sem) 79 ± 1%.

For the RIA, the standard curve dilution ranged from 1.56 to 400 pg E2 500 μl^{-1} PBS and was prepared with E2 stock 1,3,5(10)-estratrien-3,17ß-diol (Steraloids, Inc., Wilton, NH). The E2 antibody (A11), prepared by N.C. Rawlings, Univ. of Saskatchewan, was raised in rabbits, and used (200 µl) at 1:8000 initial dilution in PBS. Radioactively labelled E2[2,4,6,7,16,17-^{3}H(N)] (New England Nuclear, Boston, MA) was diluted in PBS to ~11,000 cpm 200 μl^{-1}. Ecolume (ICN Biomedicals Inc., Irvine, CA) was the scintillation cocktail used. Maximum binding of the

antiserum was 37%, non-specific binding was 3.3% and the sensitivity of the assay was 6.25 pg ml^{-1} at 95% of maximum binding. Both years' samples were analyzed together in 7 assays with intra and inter-assay coefficients of variation of 15.9% and 21.1%, respectively.

Vaginal Smears

Vaginal smears were collected during routine sampling periods from mid-November 1989 until June 1990. Smears were collected by the insertion of a saline moistened cotton swab (3 mm in diameter) approximately 10 to 15 mm into the vagina. The swab was rotated once and withdrawn. The swab was then immediately rolled onto a clean glass microscope slide and allowed to air dry. Cells were stained (Leukostat, Fisher Scientific Company, Fairlawn, NJ) for characterization.

Cell characterization was done using a light microscope at magnifications of 100x and 400x. Epithelial cells were classified as non-stratified (parabasal, small and large intermediates) or stratified (partly and fully cornified superficials) and counted in a predetermined vertical transect at 400x. Leukocytes were quantified by estimating their abundance in a predetermined horizontal transect at 100x and given an abundance score as follows: 1, <10 leukocytes in a typical field of view; 2, between 10 and 100

in a typical field; 3, between 100 and 1000 in a typical field; and 4, >1000 in a typical field.

Statistics

Monthly average E2 for each animal was calculated and the monthly averages of all animals, over the entire research period and over each research year were subsequently analyzed by analysis of variance. All values are expressed as monthly means ± standard error of the mean (sem).

Correlation coefficients were calculated to determine whether correlations existed (within the research population and in individual animals) between: E2 levels and % cornified epithelial cells; E2 levels and leukocyte score; and % cornified epithelial cells and leukocyte score.

Results

A total of 495 serum samples were analyzed for E2 over the research period, 244 in the 1st year and 251 in the 2nd. Analysis of variance revealed that E2 changed significantly over the entire research period ($F = 3.90$, $df = 18,13$, $P < 0.0001$), but did not change within research years (year 1: $F = 1.59$, $df = 9,13$, $P < 0.1227$; year 2: $F = 1.83$, $df = 8,12$, $P < 0.0839$).

Maximum monthly average E2 levels (40.77 pg ml^{-1}) were observed in September 1989, after which E2 varied between 27.60 and 33.14 pg ml^{-1} for the remainder of the 1st research year. Second year values were lower, ranging from 19.74 to 27.54 pg ml^{-1} (Figure 1). Individual profiles are in Appendix 2.

Vaginal epithelial cells were characterized on 235 slides with 45,828 cells described. In 6 females (Appendices 2a,b,d,e,h,j), cornified epithelial cells increased from < 10% prior to March to > 30% in April and May. The percentage of cornified cells peaked in January and February in 2 females (Appendices 2m and f, respectively) and fluctuated considerably throughout the research period in the remainder of the animals (Appendices 2c,g,i,k,l,n).

The percentage of cornified epithelial cells was found to be negatively correlated to leukocyte score within the research population ($r = -0.34861$; $P = .0001$) and in 5 individual animals ($P < 0.05$; ex. Figure 2)(Appendices 2a,b,f,i,j). E2 was not found to be correlated to either vaginal cornification ($r = 0.09141$; $P = 0.1797$) or leukocyte score ($r = -0.09901$; $P = 0.1460$) in the research population. With in individual animals however, E2 was positively correlated to vaginal cornification ($P < 0.10$)(Appendix 2c), and leukocyte score ($P < 0.10$)(Appendix 2k) in one animal

each, and negatively correlated to leukocyte score in another ($P < 0.05$)(Appendix 21).

Vulvar swelling was not quantified in our fishers, however changes in appearance of the vulva (color, rigidity, shape) were noted. In general, vulvar swelling was first noticed in late-February or early-March when the color of the vulva changed from creamy-white to pink and the tissue became more relaxed in appearance. Swelling typically reached maximal levels, denoted by a reddish-pink color and loose appearance, in mid- to late-April. This condition usually lasted 1 to 2 weeks followed by regression of the swelling. Often a second increase in vulvar swelling was noted in the weeks following regression and was usually of a shorter duration. During anestrus, the vulva is typically whitish in color, with a small opening and rigid appearance.

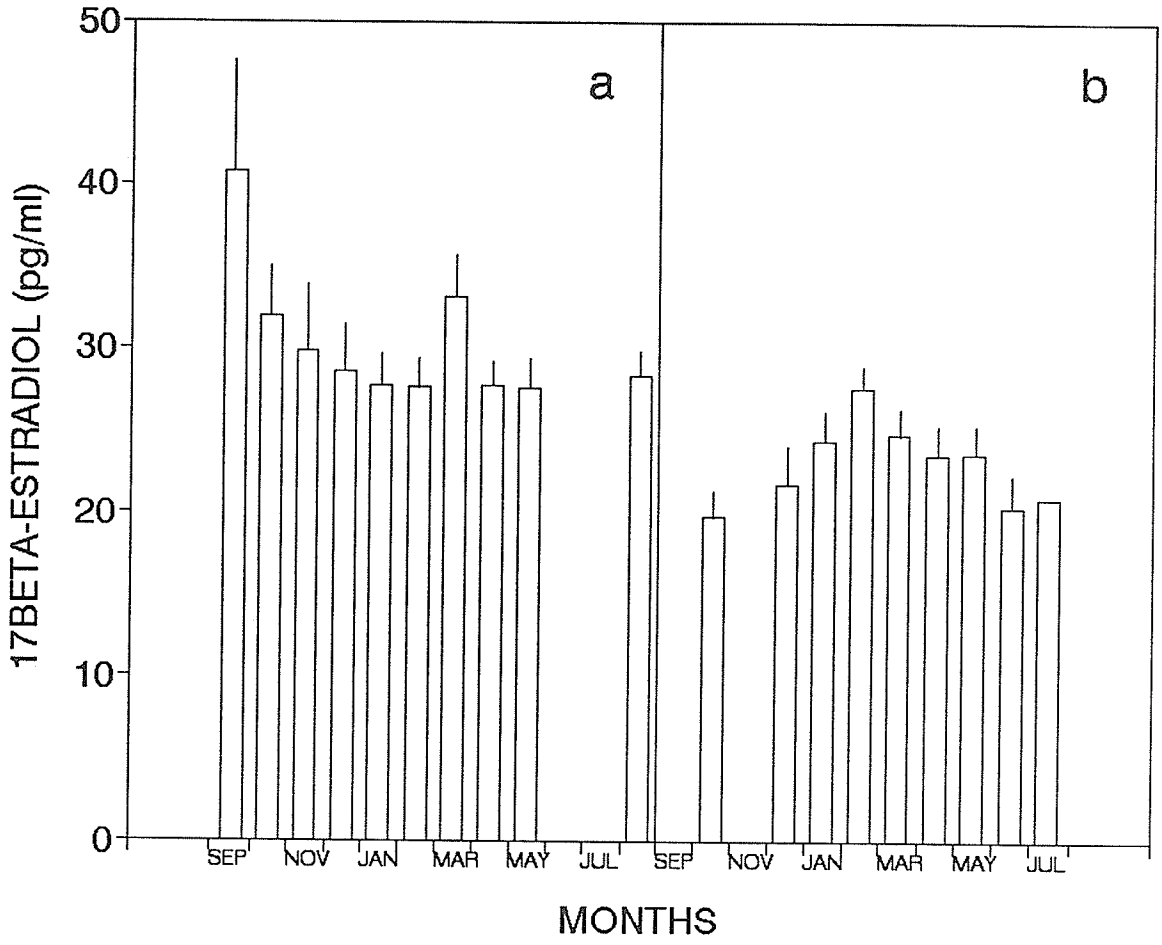


Figure 1. Monthly average (+ sem) 17ß-estradiol levels of female fishers: a) 1st research year (n=14) and b) 2nd research year (n=13). (No sem value is assigned to July of the 2nd year as only one sample was collected in that month).

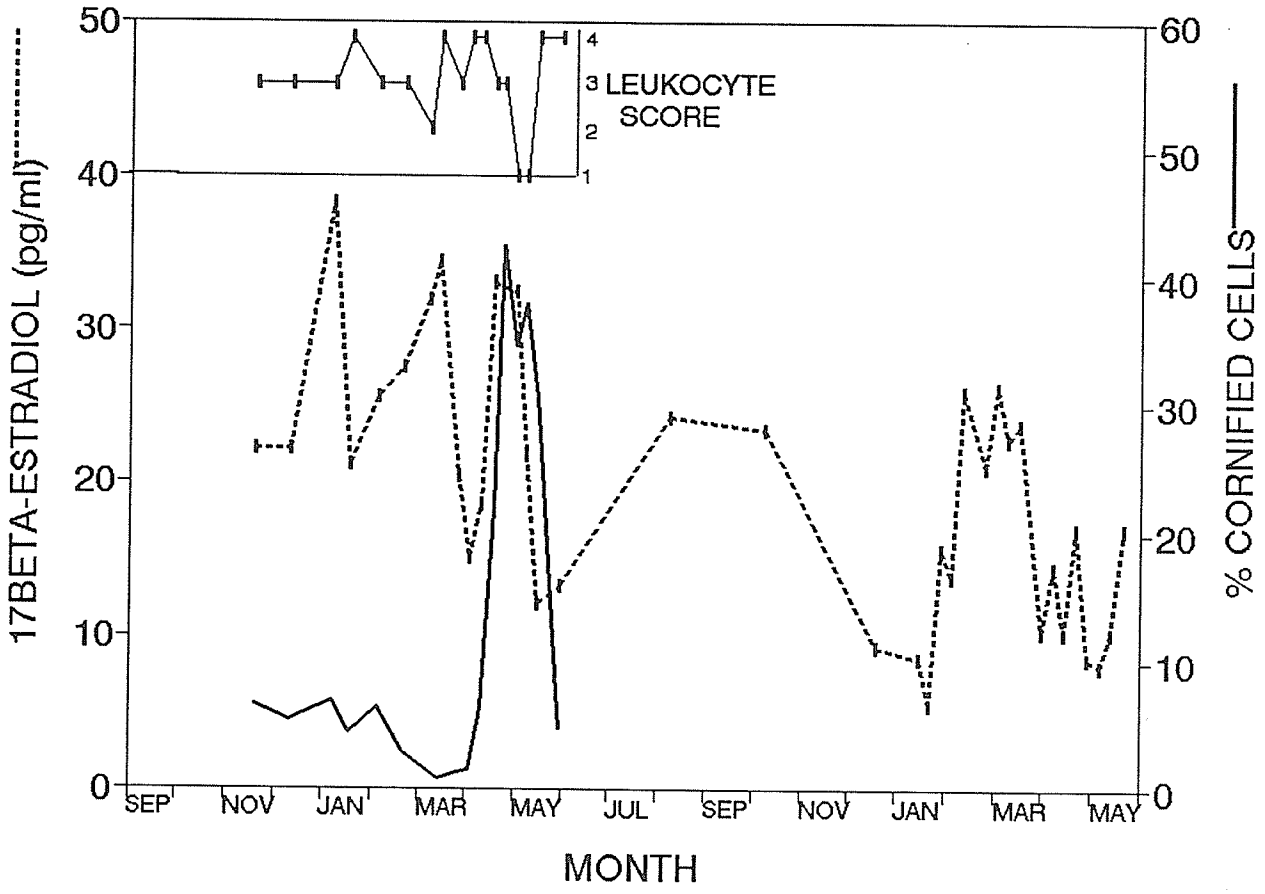


Figure 2. E2, % cornified epithelial cells and leukocyte score of a female fisher (F2) which showed a negative correlation between leukocyte score and % cornified epithelial cells.

Discussion

17ß-Estradiol

The E2 levels I observed in fishers are of similar magnitude to levels in other non-pregnant mustelids which have been studied. While the baseline levels observed were in the 20 to 30 pg ml^{-1} range, anestrus levels of E2 have been found to be between 10 and 20 pg ml^{-1} in sable (Shul'gina 1981) and river otter (Stenson 1985), and in the 40 to 60 pg ml^{-1} range in mink (Pilbeam et al. 1979) and wolverine (Mead et al. 1991). Mead and Eik-Nes (1969a) working with spotted skunks and Heap and Hammond (1974) working with ferrets found undetectable levels of E2 and unconjugated estrogens, respectively, in anestrous animals.

The E2 profile in Figure 1 suggests gradual changes or trends throughout the year, however individual profiles in Appendix 2 show the much more dynamic nature of E2 production. Second year sampling was conducted weekly from mid-December to mid-June and clearly shows E2 fluctuated by up to 20 pg ml^{-1} at 2- to 5-week intervals in several animals. First year sampling was less frequent during the winter months and while obvious peaks in E2 are evident, short-term variations may have went undetected. Hodgson (1937) noted that if a female was not bred during the 1^{st} heat period of the year, she may come into heat again after about 10 days. Short-term fluctuations in E2 were observed

in many of individual fisher profiles (Appendices 2b,c,d,e,g,h,k) which may have been indicative of recurring follicular development even though behavioral estrus and receptivity were observed in only 2 females. One female (Appendix 2j) was in heat as determined by behavioral changes, on April 27, 1990 and again on May 5, 1990. E2 peaked at 88.36 pg ml^{-1} 31 days before she was first noticed to be in heat and E2 was at only 24.83 pg ml^{-1} on April 27th. On May 5th, E2 was up to 36.04 pg ml^{-1} and 34.87 pg ml^{-1} on May 10th when she was no longer in heat. Changes in percentage of cornified epithelial cells and leukocyte score in this female are discussed below. The single female (Appendix 2f) that mated in 1991 had a maximal E2 level of 54.14 pg ml^{-1} on Apr 9th. E2 fell to 26.22 pg ml^{-1} on April 23rd. On May 3rd, 0.0125 μg synthetic LHRH (Factrel, Ayerst Lab., Montreal PQ) was administered as part of another experiment and 4 days later (May 7th) this female appeared to be in heat, as evidenced by behavioral changes, and mated. E2 was at 36.64 pg ml^{-1} eight days after mating and decreased to 27.35 pg ml^{-1} by May 24th. It is unknown whether the hormone treatment induced ovulation and if mating occurred as a result of an induced second wave of follicular development. No litter was produced from this mating.

The timing of E2 peaks in relation to other reproductive events appears to be variable in the mustelids.

While monthly mean E2 was not correlated with vaginal cornification or leukocyte counts in our study, several of the individual profiles suggest that relationships exist (Appendices 2a,b,d,e,f). Elevated E2 was found to be associated with maximal vaginal cornification in wolverine (Mead et al. 1991) and Stenson (1985) found that E2 levels of river otters peaked prior to or during estrus. However, Pilbeam (1979) found that in mink E2 decreased from proestrus levels as estrus approached and that the decrease was associated with an increase in vulvar swelling and the exhibition of behavioral estrus.

Vaginal Cytology

The cycle of vaginal cytology of the fisher appears to follow closely the cycles of other mustelids (Enders 1952, Mead 1968a, b, Stenson 1985, Mead et al. 1990, Mead et al. 1991) and dogs (Concannon and DiGregorio 1986). Smears are comprised mostly of non-cornified epithelial cells during anestrus and leukocytes are present in varying degrees of abundance. As follicular activity and associated E2 production increases, the percentage of cornified cells increases and the number of leukocytes decreases. At estrus, cornified cells may make up nearly 100% of epithelial cells collected and leukocytes are typically absent. In metestrus, leukocyte numbers increase

dramatically and the number of cornified epithelial cells decreases.

The maximum values for cornified epithelial cells we observed during the anticipated estrous period were higher than those obtained from mink (Travis et al. 1978) but considerably lower than those reported by Mead et al., in polecats (1990) and wolverines (1991), and by Stenson (1988) in river otter. The lavage technique used by others (Travis et al. 1978, Mead et al. 1990) collects only cells which are readily removed from the vaginal epithelial lining, while the swab method we used mechanically removes cells. This may have contributed to the lower percentages of cornified cells we obtained. As many parabasal cells were collected by our technique, the relative abundance of cornified cells was likely underestimated.

Leukocyte counts were variable in our fishers throughout the year. Levels of between 100 and 1000 cells in a typical field of view at 100X were typical for most of the animals, and fluctuations of up to 2 leukocyte score levels between sampling periods were common. Decreases in leukocyte score were clearly associated with increased percentages of cornified epithelial cells in 8 ($P < 0.05$ in 5) of the females (Appendices 2a,b,c,d,e,f,h,j). For the research population as a whole, a significantly negative correlation was found. These findings are in complete

agreement with those of Enders (1952) who stated that if one must designate one type of smear as the estrous smear, the smear containing a high proportion of cornified cells and lacking leukocytes should be so labelled.

While it is probable that under natural circumstances, E2 levels, vaginal cornification and leukocyte counts, and behavioral estrus are correlated in the fisher (as suggested by Figure 2), their relationship is confounded by evidence gathered from the only female (Appendix 2j) that exhibited what we perceived as obvious behavioral estrus in the 1st year of the study. This female exhibited a rise in percentage of cornified epithelial cells to > 50 % and a decrease in leukocyte score from 4 to 2 over the 2 week period surrounding peak cornification. However, this change in vaginal cytology occurred almost 2 weeks after behavioral estrus ceased and 2 months after maximum E2 values were reached, although a smaller increase in E2 occurred in the weeks preceding the changes. During the days that she was in behavioral estrus, vaginal cornification was only 4.8 and 9.3 %, and leukocyte scores were 4 and 3, respectively. Interestingly, Travis et al. (1978) stated that although vaginal smears could be used to determine when mink would not mate, they could not be used to predict when mink would mate. Perhaps the same holds true for the fisher.

Stresses

Whether stresses associated with captivity, conspecific interactions or handling for sample collection affected physiological characteristics or the behavioral expression of estrus is unknown. Stress associated with captivity may increase adrenal output of corticosterone which can affect normal gonadotropin secretion, thereby leading to reproductive irregularities (Eckstein 1977, Ramaley 1981). Throughout 5 breeding seasons, we obtained only 2 matings in captive animals (the same pair in 2 consecutive years), and in recent years similar results are common from other captive populations which are maintained under "fur-farming" conditions (D. Ross pers. comm.). These fur-farms utilize relatively small wire pens which are elevated above the ground and are fairly sterile in terms of stimuli to the fishers. Successful breeding programs have invariably incorporated large pens, situated on the ground, containing rocks, trees and other enrichments (Hodgson 1937, R. Mead pers. comm.). From our observations, male fishers would appear to be less stressed by their "captive environment" than the females as breeding behavior was observed in 2 of 5 males and they seemed to be less distracted by human activities.

Fishers are solitary animals in the wild except during the breeding season, and high densities of animals as occurs

under farm and/or research conditions are unnatural. Socially-induced stresses have been reported to interfere with reproductive events in coyotes (*Canis latrans*) (Hodges et al. 1990), marmosets (Tardiff et al. 1990) and in a variety of other mammals (Drickamer 1979, McClintock 1983), but Mead et al. (1991) questions whether social stresses interfere with reproductive success in mustelids as many species (*Mustela*, *Martes*) are raised successfully under intensive farming conditions. Indeed, ranch mink (Gilbert and Bailey 1967) and possibly skunks (Greensides and Mead 1973) experience delayed estrus and inhibited sexual development when visually isolated from conspecifics.

The effect of chemical immobilization on E2 levels and related physiological traits in fishers is unknown. It has been established in rhesus monkeys (*Macaca mulatta*) that chemical immobilization using ketamine HCl after cage restraint does not effect menstrual cycle length, blood steroid or gonadotropin levels (Channing et al. 1977, Fuller et al. 1984). While no similar research has been conducted on the effect of anaesthetics on steroid levels in mustelids, Bonnefond et al. (1988) did study its effect on prolactin and LH in mink. These authors found that ketamine HCl had no effect on prolactin levels of pregnant, or LH levels of ovariectomized, mink. They did however, discover that plasma prolactin concentrations increased within 5 min

of administration in ovariectomized mink. Even though blood samples from our fishers were generally collected within 5 min of injection of ketamine HCl and xylazine HCl, the possibility that they may have a rapid effect on E2 levels clearly exists. If steroid levels of the fisher are modified by these immobilizing agents, E2 levels and all reproductive characteristics involving its presence may have been altered by the repeated immobilization necessary for sample collection.

Vulvar Swelling

Vulvar swelling has been identified as a sign of estrus in the marten (Enders and Leekley 1944), ferret (Bissonnette 1932), polecat (Mead et al. 1990), long-tailed weasel (Wright 1948), spotted skunk (Mead 1969a, b) and mink (Pilbeam et al. 1979). In these species, maximal vulvar swelling is often associated with a willingness to mate, however in wolverine (Mead et al. 1991) and river otter (Stenson 1985) swelling of the vulva does not indicate receptivity to mating. We placed female fishers with males when vulvar swelling was at all stages, including maximal swelling, and with few exceptions the female violently opposed all advances made by the male. Since the female fisher is in heat for only 2 to 3 days (Hall 1942) it may be difficult to detect small changes in an already edemic vulva

that may indicate estrus is occurring. It is apparent however, that the vulva does not become as swollen as found in other mustelids and it is unlikely that vulva swelling alone is useful as an indicator of estrus.

Summary

The most conclusive evidence to indicate that an animal is in estrus is the actual occurrence of mating. As mentioned, seldom has mating been achieved with captive fishers and until such time, inferences made about the timing of estrus are largely speculative. The information presented here indicates that the fisher is similar to most other mustelids with regard to E2 secretion and vaginal cytology. The correlation or relationship between vaginal cornification and leukocyte counts is the easiest to determine and likely the most dependable, though unproven, method of estrus detection in the fisher given the dynamic nature of E2 secretion. With this in mind, future research with a breeding population of fishers should focus on this relationship between estrus and measurable physiological indicators.

MANUSCRIPT II

Progesterone Levels of the Fisher (*Martes pennanti*), a Delayed Implanter

Abstract. Progesterone levels of 14 female fishers were monitored over a 2-year period to provide information about reproductive events in the live animal. Progesterone levels, determined by RIA, indicated the presence of ovulation during the breeding season in several animals, followed by near baseline levels until the following January or February when progesterone was elevated. In sexually inactive animals, progesterone remained low throughout the year. The profiles suggest the lengthening days following the winter solstice stimulate reactivation of dormant corpora lutea capable of producing progesterone levels of over 40 ng ml^{-1}. Pseudopregnancy likely occurs in female fishers and active gestation is estimated at 40 to 45 days. Ovulation may have occurred spontaneously in several animals. Attempts to induce ovulation by hormone therapy were inconclusive.

Introduction

Information pertaining to reproduction in the female fisher (*Martes pennanti*) has been collected by observations made under fur farm conditions (Hall 1942, Douglas 1943) and gleaned from trapper collected carcasses (Enders and Pearson 1943a, Hamilton and Cook 1955, Eadie and Hamilton 1958, Wright and Coulter 1967, Shea et al. 1985, Leonard 1986, Douglas and Strickland 1987). These fur producers and researchers described the breeding season and total gestation length, discovered that fishers exhibit delayed implantation and described anatomical characteristics of the reproductive tract. Despite these contributions, many unanswered questions remain regarding the reproductive physiology of the fisher.

Intensive studies of many mustelids which share some reproductive characteristics with the fisher have clarified much of their reproductive histories (Møller 1973a, Bonnin et al. 1978, Canivenc et al. 1981, Stenson 1985, Mead et al. 1991). The description of progesterone (P4) profiles has been a cornerstone of much of this research. Therefore the main objective of this research was to determine P4 levels of the fisher throughout the year and to attempt to correlate the information with several aspects of reproduction. This could provide evidence to confirm that ovulation in the fisher is induced by mating (Mead and

Wright 1983); provide information regarding which environmental cues regulate delayed implantation in this species; and enable the determination of active gestation length.

While the fisher has been bred successfully in captivity (Hodgson 1937, Hall 1942, Douglas 1943), recent attempts have generally met with poor success (D. Ross pers. comm.). The use of artificial breeding programs such as artificially induced ovulation and/or artificial insemination, could be extremely beneficial to the captive propagation of this species. Therefore, to provide information as to whether ovulation could be artificially induced in the fisher, we attempted to do so with hormone therapy.

Materials and Methods

Animals and Maintenance

Fourteen female fishers (*Martes pennanti*) were maintained in southern Manitoba, at the University of Manitoba Glenlea Research Station (49° 53'N, 97° 09'W). Eight of the animals were captured in south-eastern Manitoba in the fall of 1987 and one in the fall of 1988. Two litters were born in captivity, producing 4 animals (3 born in 1988 and 1 in 1989) used in this research. The animals were housed individually in wire mesh pens (1 m x 1 m x 2 m)

with attached nest-boxes (0.125 m^3) and exposed to natural weather conditions and photoperiods. The animals were fed a commercial wet mink ration (see Appendix 1) at a level which maintained body weight and allowed water *ad libitum*.

When the study began the animals were 0.5 (n=1), 1.5 (n=3) and 2.5 to 6.5 (n=10) years of age. Pre-molars of animals of unknown age were collected and ages assigned by annuli counts performed at Matson's Laboratory (Milltown, MT). The method was confirmed by using the technique on teeth collected from animals of known age.

The 2 females which produced litters in captivity were placed with males in the days following parturition. However, it is unknown whether matings took place. Most of the remaining females were placed with males during the spring of each year in captivity. When paired, the animals were allowed to remain together for varying time periods, ranging from 30 min to overnight. Two matings were observed between the same pair, in 2 consecutive years.

Sampling

First year blood samples were collected monthly from September 1989 until January 1990, bi-weekly until March 1990, weekly until the end of May 1990 and once in early-August. Second-year samples were collected once in October and weekly from mid-December 1990 until mid-June 1991. The

samples were collected in the same order each sampling day, between 07:35 and 17:45 h during the 1st year and between 08:30 and 17:05 h in the 2nd year. Ketamine HCl (Ayerst Lab., Montreal, PQ) with xylazine HCl (Chemagro Ltd., Etobicoke, ON) mixed 10:1 were used to immobilize the animals for all procedures (approximately 20 mg ketamine HCl kg^{-1} body weight). Rogar-Mycine eye ointment (Rogar/STB Inc., London, ON) was applied to the eyes of the anaesthetized fishers to prevent drying.

Blood (7 ml) was collected by jugular venipuncture using a sterile 20 gauge 2.5 cm vacutainer needle and sterile, siliconized 7 ml vacutainer (Becton Dickinson and Company, Rutherford, NJ). The samples were refrigerated for 16 to 24 h, then centrifuged for 20 min at ~1000 g (Jouan Inc., Winchester, VA). Serum was removed and stored at -20°C until assayed for P4.

Hormone Treatment

Between May 3 and May 17, 1991, six animals were given an intramuscular injection of synthetic luteinizing hormone releasing hormone (LHRH)(Factrel, Ayerst Lab., Montreal, PQ) during blood sampling sessions. Total administration of the hormone was 12.5 μg (0.25 ml of solution at 50 mg ml^{-1}). Controls (n=7) were blood sampled as usual. After the treatments were completed, all animals, including controls,

were sampled weekly. The occurrence of ovulation was monitored by changes in P4 between samples collected on May 1 (pre-treatment) and those collected on May 24 or May 25 (post-treatment) in both treatment and control groups.

Progesterone Analysis

Progesterone was extracted from serum and quantified by RIA using methods based on those of Yuthasastrakosol et al. (1974). In brief, for extraction, 250 μl serum combined with 750 μl PBS (pH 7.2) was mixed with 5.5 ml ethyl ether (Mallinckrodt Specialty Chemicals Co., Mississauga, ON), vortexed for 4 min and equilibrated for 15 min before freezing over dry ice/ethanol (-43°C). The supernatant was decanted and dried under a stream of nitrogen gas in a shaking water bath maintained at 37°C. The dried precipitate was reconstituted in 1.250 ml phosphate buffered saline (PBS). Percentage recovery of ^{3}H-P4 (New England Nuclear, Boston, MA) added to pooled fisher serum averaged (± sem) 81 ± 1%.

For the RIA, the standard curve (prepared with P4 stock 4-pregnen-3,20 dione (Steraloid Inc., Wilton, NH)) ranged from 10 to 800 pg P4 500 μl^{-1} PBS. The P4 antibody, prepared by N.C. Rawlings, Univ. of Saskatchewan, was raised in rabbits (A18), and used at 1:2500 initial dilution in PBS. Radioactively labelled P4[1,2-^{3}H(N)] (New England Nuclear,

Boston, MA) was diluted in PBS to ~10,000 cpm 100 μl^{-1}. Ecolume (ICN Biomedicals Inc., Irvine, CA) was the scintillation cocktail used. Maximum binding to the antiserum was 50%, non-specific binding was 2.8 to 6.0% and the sensitivity of the assay was 20 pg ml^{-1} at 95% of maximum binding. First year samples were analyzed in two assays with intra- and inter-assay coefficients of variation (CV) of 10.3% and 10.7%, respectively. Second year samples were also analyzed in two assays with intra- and inter-assay CV of 14.9% and 22.9%, respectively. Inter-assay CV for all assays was 20.4%.

Statistics

Individual P4 profiles fell into 2 general categories, static and dynamic. Whether a profile was static or dynamic was determined by the following criteria. Profiles from the entire research period with 1 or no samples containing > 3 ng ml^{-1} P4 were considered to be static. A dynamic profile was one in which 2 or more P4 values were > 3 ng ml^{-1}, or a profile in which a single sample exceeded 4 ng ml^{-1}. Only the dynamic group was subjected to further statistical evaluation.

Monthly average P4 for each animal in the dynamic group was calculated. The monthly averages of all animals in this group, over each year and over the entire research period,

were subjected to analysis of variance. In the presence of a significant F value, Scheffe's test was carried out on unmatched pairs within research years to determine which months had significantly different average P4 values. All values are expressed as monthly means ± standard error of the mean (sem).

The data from the hormone treatment experiment was subjected to analysis of variance to determine if the P4 levels of treatment animals were significantly different from those of control animals before and after LHRH treatment. Results are expressed as averages ± sem for both control and treatment animals.

Results

Over the study period, 508 blood samples were collected. Five of the 14 fisher P4 profiles were considered to be static, and 9 were considered to be dynamic.

During the breeding season of the 1st research year, sampling at weekly intervals indicated that all females except F8 and Y89 had some degree of vulvar swelling. Also, a distinct increase in the percent cornification of vaginal epithelial cells was noted in all females except F9, Y88 and BJ. During the 2nd research year, vulvar swelling was observed in all females except F11 and BJ (see Manuscript

#1).

It is unknown which females in the research population had contact with males in the breeding season of 1989. In 1990, seven females (F1,3,5,8,9,10,11) were in contact with males for varying time periods, including overnight on several occasions. Consequently, it is unknown if any matings occurred during this breeding season. In 1991, F8, F11 and BJ were the only females not to be placed with males for at least a short period of time. Animals were not left together overnight during this breeding season and only one mating was observed.

P4 levels in the static group were essentially unchanged throughout the research period with an overall mean of 1.28 ± 0.04 ng ml^{-1} (Figure 3). Individual profiles of the static group are in appendices 3.g,h,j,m and n.

Statistical analyses of the dynamic group found that there were significant differences throughout the entire research period ($F = 5.13$, $df = 18,8$, $P < 0.0001$) and throughout each research year (year 1: $F = 4.30$, $df = 9,8$, $P < 0.0002$; year 2: $F = 7.00$, $df = 8,8$, $P < 0.0001$). Mean monthly serum P4 levels of the dynamic group are shown in Figure 4. Individual profiles of the dynamic group are in appendices 3.a-f,i,k and l.

Dynamic P4 levels followed similar patterns in both years of the study, being between 1.45 and 2.57 ng ml^{-1} for

all monthly means from September to January. February levels of P4 were significantly higher ($P < 0.05$) than January in both years, and declined slightly in March. Monthly means after March in year 1 ranged from 1.45 to 1.98 ng ml^{-1} and in year 2 from 2.10 to 2.83 ng ml^{-1}. Individual profiles (Appendix 3) clearly show small increases in P4 above baseline, occurring during or after the 1st breeding season in 6 females (F1,3,4,5,6,Y88), and in 9 females (F1,3,4,5,6,7,9,11,Y88) during or after the 2nd breeding season.

Treatment of fishers with LHRH did not significantly affect P4 levels. Pre-treatment levels of P4 were not significantly different between treatment and control animals (1.87 ± 0.30 and 1.18 ± 0.18 ng ml^{-1}, respectively; $F = 3.53$, $df = 1,10$, $P < 0.0896$), nor were post-treatment levels (3.67 ± 0.74 and 2.44 ± 0.59 ng ml^{-1}, in treatment and controls, respectively; $F = 4.36$, $df = 1,10$, $P < 0.0633$). One female from the treatment group was excluded because she mated during the experimental period.

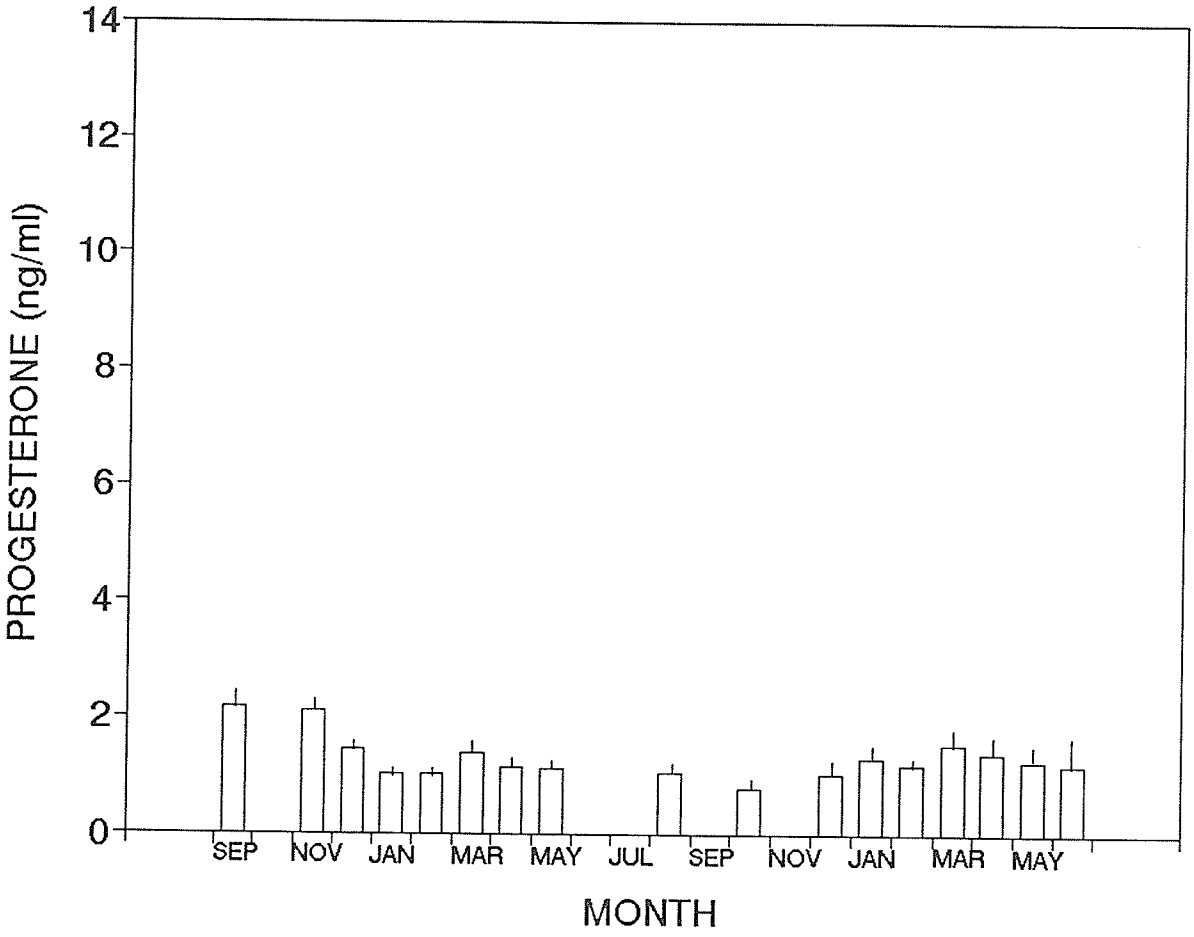


Figure 3. Monthly average (+ sem) progesterone levels of female fishers (n=5) with static profiles over entire research period.

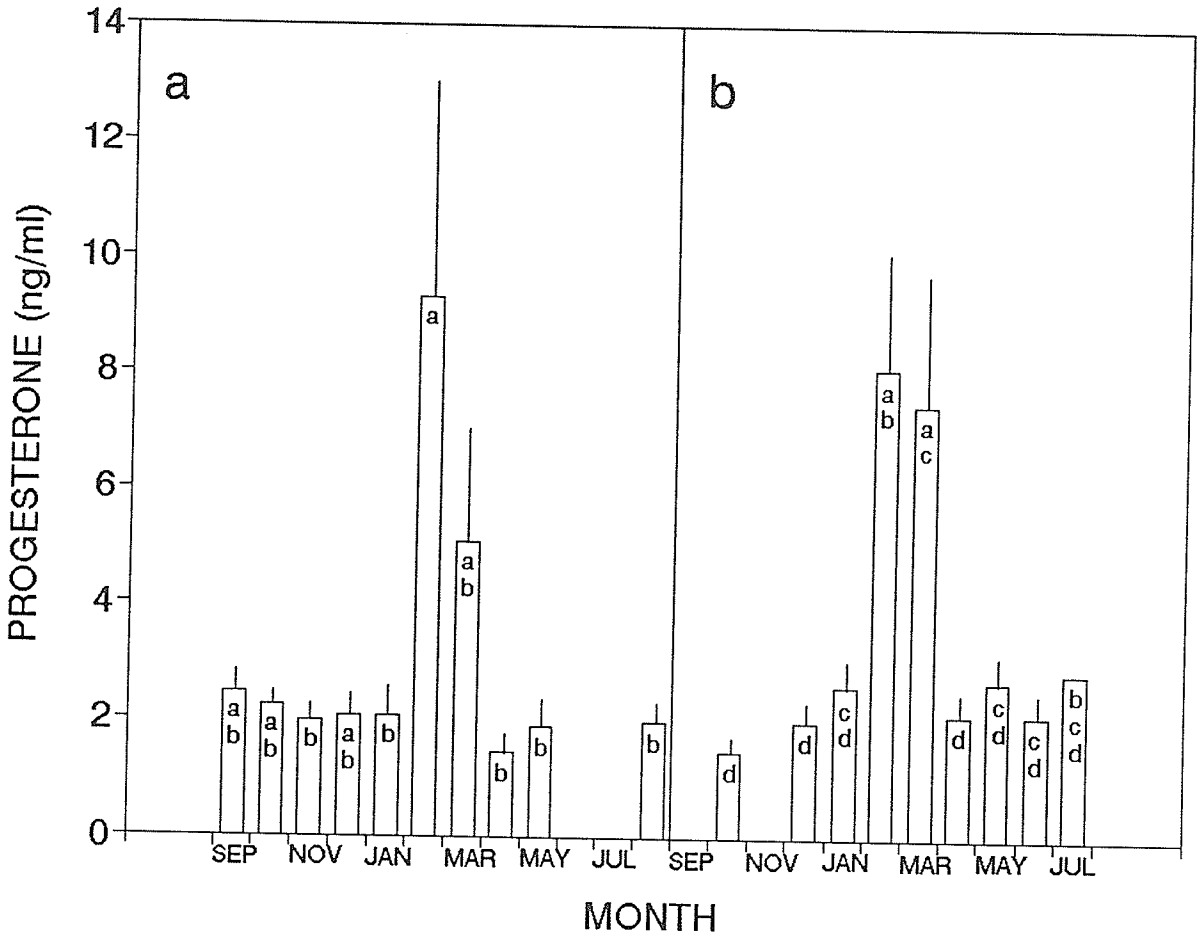


Figure 4. Monthly average (+ sem) progesterone levels of female fishers (n=9) with dynamic profiles: a) 1st research year and b) 2nd research year. Months with same letter are not significantly different ($P < 0.05$). (No sem value is assigned to July of the 2nd year as only one sample was collected in that month)

Discussion

Given that animals with static and dynamic P4 profiles represent different reproductive states, these groups will be discussed separately.

Static P4 Profiles

Presumably, P4 levels would remain static throughout the year if ovulation failed to occur. Two instances may lead to ovulatory failure: the inability to ovulate (sexual immaturity); and failure to ovulate in response to appropriate stimuli (in sexually mature animals).

It has been well-documented in both live fishers and from carcass analyses that breeding normally occurs at 1 year of age (Hall 1942, Hamilton and Cook 1955, Eadie and Hamilton 1958, Wright and Coulter 1967). Since all of our animals were at least 1 year old, it is likely that animals with chronically low P4 levels simply failed to ovulate.

As with fishers, P4 levels tend to remain low throughout the year in other mature mustelids that are unmated (Mead and Eik-Nes 1969b, Stenson 1985, Mead et al. 1991) and in which spontaneous ovulations have not occurred. Indeed, Mead et al. (1991) assumed failure of ovulation based on low P4 levels in mature wolverines. The source of baseline levels of P4 may be the ovarian interstitium (Møller 1973b), the ovarian follicle (Hadley 1984) or the

adrenal gland (Mead and Eik-Nes 1969b). However, corpora lutea (CL) appear to be the most significant source of elevated P4 in mustelids (Mφller 1974, Mead and Swannack 1978).

It is unlikely that our fishers ovulated and failed to exhibit pre-implantation increases in P4 due to degeneration of ovum or blastocysts. Mead and Swannack (1978) showed that hysterectomy during delayed implantation in the spotted skunk had no effect on normal luteal function, as P4 increased at the normal time and was elevated for a normal duration. Similar findings have been reported in the mink (Duby et al. 1972, Mφller 1973a) and ferret (Carlson and Rust 1969, Heap and Hammond 1974).

In the few sexually immature mustelids which have been studied, P4 levels remain low until sexual maturity (Canivenc et al. 1981, Shul'gina et al. 1981, Stenson 1985). Small fluctuations seen in individual P4 profiles (Appendix 3) of our fishers throughout the winter and spring months may be due to the onset of follicular development as seen in the mink (Pilbeam et al. 1979) or may be coincident with estrous, as noted in the spotted skunk (Mead and Eik-Nes 1969b).

Dynamic P4 Profiles

Two terms that will be used throughout the remainder of

this manuscript in reference to dynamic profiles are post-ovulatory and pre-implantation increases in P4. These terms refer to increased P4 secretion by luteal tissue at different periods of the reproductive cycle. A typical pattern of P4 secretion for a reproductively active mustelid exhibiting delayed implantation is as follows: a transient (post-ovulatory) increase occurs shortly after ovulation due to partial luteinization of the granulosa cells of the CL; reduced secretion as the granulosa cells become involuted for the duration of the delay period; and elevated P4 secretion (pre-implantation) due to reactivation of CL in response to appropriate environmental stimuli. This final increase in P4, along with other unknown factors, initiates changes in the endometrial tissues of the uterus which make it conducive to embryo implantation and development. Shortly after implantation, luteal tissue regresses and P4 is near baseline at parturition (Stenson 1985, Mead and Wright 1983).

While no histological evidence was obtained from our fishers, we will assume elevations in P4 during or shortly after the breeding season were the result of ovulations, and will be referred to as post-ovulatory increases. The river otter, which exhibits a reproductive cycle very similar to that of the fisher has been shown to experience a small increase in P4 immediately after mating, followed by a

return to near baseline until the pre-implantation rise is initiated (Stenson 1985). The short-tailed weasel exhibits a similar transient rise during the breeding season likely associated with ovulation (Gulamhusein and Thawley 1974).

Secondarily, while no litters were produced during the research period, increases in P4 at the anticipated time of implantation will be referred to as pre-implantation increases and could have represented either pregnancies that were not completed or pseudopregnancies.

All of our fishers that showed ovulatory increases in P4 during May 1990 exhibited pre-implantation increases in the winter of 1991, adding to the supposition that ovulation had occurred. However, 1 female (F2) that exhibited a pre-implantation increase in P4 in the winter of 1991 did not exhibit an obvious post-ovulatory increase the previous spring. While it is possible that a short-term increase in P4 may have been missed due to the frequency of sampling, it is also possible that the granulosa cells failed to undergo the partial luteinization associated with formation of the corpora lutea. P4 levels during the pre-implantation period of this animal were however, comparable to those of animals which showed obvious post-ovulatory increases.

The P4 profiles of our fishers in the dynamic group show slightly higher P4 levels during the period of luteal inactivity (July to December) than seen over the same period

in females with static P4 profiles, suggesting low but continuous production of P4 by the CL during the delay period. Indeed, the female (F6) that mated in May of the 2nd year of research had P4 levels of 3.4 and 2.8 ng ml^{-1} in June and July, respectively, compared to expected levels of < 1 ng ml^{-1} in non-mated females. As in fisher, the P4 levels of several other mustelids remains elevated above baseline levels during most or all of the period of diapause (Mead and Eik-Nes 1969b, Canivenc et al. 1981, Stenson 1985).

Pre-implantation increases in P4 were observed in 4 females in the dynamic group in the 1st year and in all 9 of these females in the 2nd year. These elevations ranged from 4.83 to 42.52 ng ml^{-1} in individual animals (Appendix 3). Two animals which exhibited maximum P4 levels > 20 ng ml^{-1}, had P4 levels elevated > 5 ng ml^{-1} for more than 55 days, while females that had maximum P4 levels between 7 and 18 ng ml^{-1} had P4 levels > 5 ng ml^{-1} for only 23 to 35 days. The 2 animals with maximum P4 levels > 20 ng ml^{-1} were both previously parous animals that had produced litters in captivity, and had been given opportunities to mate at the appropriate time. While matings were never observed with these females, at least 1 (F3) was believed to have been pregnant in the spring of 1990, as fetuses could be palpated. No litter was produced but it is likely that the P4 profile of this animal is that of pregnancy. The P4

profiles of the other females in this group may have been indicative of pseudopregnancy, the reasons for which are discussed below.

Ovulation

Since it is unknown which females were in the presence of males or whether any matings took place in the year prior to the commencement of this study, it is impossible to speculate whether the pre-implantation increases in P4 observed in several of the animals (F1,3,4,5) were the result of matings. Records of animal interactions from the breeding season of 1990 and P4 profiles from the proceeding year do however provide a basis for discussion.

The elevated P4 in February and March 1991, in 9 of the females are indicative of ovulation having occurred the previous spring. Seven of these females were in contact with males the previous spring, so the possibility exists that unseen mating or associated behavior occurred. However, for reasons related to inexperience of the males in our research population at the time, and their lack of libido, we doubt that any matings took place. Only minimal courtship behavior was observed. We therefore feel that ovulations during the breeding season of 1990 were the result of collection of vaginal smears, spontaneous ovulations or luteinization of unruptured follicles.

During the 1st research year vaginal smears were obtained weekly from all animals while they were anaesthetized for blood sampling. Enders (1952) reported one case where collection of vaginal smears induced ovulation in mink and another instance where it failed to do so. Stenson (1985) also stated that collection of vaginal smears may have induced ovulation in 1 of his otters, although it was with a male during breeding season and may actually have been pregnant.

While ovulation is believed to be induced in most mustelids including the fisher (Mead and Wright 1983), spontaneous ovulations in mink and skunks have been found (Møller 1974, Mead 1968a). Evidence collected from the reproductive tract of a fisher (Y89) in August 1991 and the P4 profile of another female (F11) from the spring of 1991 suggest that spontaneous ovulations may occur in the fisher as well.

Vaginal smears were not collected in the spring of 1991, but upon flushing the uterine horns, an unfertilized egg was recovered and CL were present on the ovary of Y89. Similar findings by Mead (1968a) led him to conclude spontaneous ovulations occur in spotted skunks. Secondarily, the P4 profile of F11 shows what clearly appears to be a post-ovulatory increase in P4 in the spring of 1991. Neither of these females were in direct contact

with a male during the breeding season of 1991.

Interestingly, the ovaries of Y89 contained luteinized tissue similar to that of a CL, but with an unexpelled ovum still present. Møller (1974) suggested that unovulated follicles with incipient luteinization may have been responsible for small but measurable amounts of P4 found in the blood of mink, producing a P4 profile similar to that of pregnancy.

Pseudopregnancy

Elevated P4 levels in late winter, resulting from reactivation of luteal tissue could be the due to either one of two situations in the fisher: pregnancy; or pseudopregnancy. Pseudopregnancy could result for several reasons: intra-uterine loss of blastocysts; matings which induced ovulation but were infertile; spontaneous ovulations; luteinization of unruptured follicles and; ovulations induced by collection of vaginal smears. While it is likely that pseudopregnancy occurred in our fishers, 2 facts render it impossible to confirm from our data. First, the breeding history and therefore occurrence of mating in our fishers from the year preceding our research and the spring of 1990 are uncertain. Secondly, no histological analysis of reproductive tracts was carried out which could have indicated relationships between P4 profiles and

reproductive status. For these reasons the occurrence of pseudopregnancy must remain speculative.

Environmental Cues

The timing of the pre-implantation increase in the P4 profiles of fishers in the dynamic group had a very high degree of synchrony. In almost all animals which exhibited a pre-implantation increase, P4 was low until mid-January, but by early-February was increasing dramatically to peak in late-February or early-March. Therefore, it would not be unreasonable to speculate that the fisher appears to be sensitive to the increasing daylength following the winter solstice. Indeed, direct correlations between changes in photoperiod and luteal reactivation, as characterized by P4 production, have been found in several species (Allais and Martinet 1978, Mead 1971, Canivenc and Bonnin 1981). Additionally, research has shown that timing of breeding, nidation and parturition in many mustelids are regulated by photoperiod (Enders and Pearson 1943b, Canivenc and Bonnin 1981, Stenson 1985). Sundqvist (1989) reviewed work by several authors which implicated photoperiod in control of reproduction in the mink, including the length of the delay period before implantation. Our data, while not conclusive, supports the contention that photoperiod is the major environmental cue regulating reproduction in the fisher.

Gestation

Active gestation length in the fisher is unknown but has been estimated to be between 30 days (Wright and Coulter 1967) and 60 days (Hamilton and Cook 1955). Two lines of circumstantial evidence point towards an active gestation period approaching 40 to 45 days in the fisher. First, the literature available on post-implantation duration in mustelids clearly shows a relationship between body size and active gestation period. Active gestation periods range from 23 to 31 days in the smaller weasels (Wright 1963, Foresman and Mead 1973, Heap and Hammond 1974) and up to 40 to 65 days in the largest (Canivenc 1966, Stenson 1985). The fisher is of intermediate size being considerably larger than the weasels and mink, but smaller than badgers and otters. By this comparison, active gestation length could be estimated to be 35 to 45 days.

Second, P4 data from our fishers provides additional evidence which would support a similar estimate of active gestation length. If the fisher is like most mustelids in that implantation occurs within the few days around peak P4 levels (Mead and Eik-Nes 1969b, Møller 1973a, Canivenc and Bonnin 1981) it can be estimated that implantation in our fishers would have occurred between February 20 and March 5. As mentioned, no litters were produced during the study period, however 2 of the females had whelped on the 8th and

9th of April in previous years. If these dates are representative, active gestation length could be estimated at between 35 and 49 days. This circumstantial evidence coupled with the relationship between body size and active gestation length mentioned above make it reasonable to estimate active gestation length in the fisher to be 40 to 45 days.

Hormone Therapy

Our research did not clarify whether hormone therapy is an effective method of inducing ovulation in the fisher. While no statistical difference in P4 levels was observed between treatment animals and controls, before or after hormone therapy, the data suggests that there was an effect. Hormone therapy increased P4 by an average of 1.8 ng ml^{-1} in the treatment group. Over the same period P4 increased an average of only 0.70 ng ml^{-1} in the control group if the levels in 1 animal were excluded. This individual animal (F11) had a dramatic increase in P4 (over 5 ng ml^{-1}) which is likely indicative of ovulation. In this case spontaneous ovulation is suspected. Further research with a larger sample size and conducted earlier in the breeding season may clarify whether ovulation can be artificially induced in the fisher.

Summary

Our data suggests that P4 profiles of fishers in both reproductively inactive and active states are similar to those of the other mustelids that have been studied in detail. Increases in P4 were observed, coincident with ovulatory and pre-implantation periods, the latter of which is likely triggered by photoperiodic cues. Pseudopregnancy may occur in fishers that ovulate either spontaneously or in response to mechanical stimulation, or that possess unruptured luteinized follicles. Active gestation length is estimated at 40 to 45 days.

MANUSCRIPT III

Dynamic Reproductive Characteristics of Captive Male Fishers (*Martes pennanti*)

Abstract. To provide information on dynamic aspects of male fisher reproductive physiology, blood samples were collected weekly from 3 captive males throughout the winter and spring of 2 consecutive years. In the 2nd year, two additional males were sampled and measurements of testes diameter were taken from all 5 animals. Serum was analyzed for testosterone by RIA. Initial increases in testosterone, from a baseline of <1 ng ml^{-1}, occurred in November/December with the most dramatic increases occurring in February/March. Peak levels of testosterone, ranging from 11 to 18 ng ml^{-1}, were reached in late-March/early-April and were followed by a rapid return to baseline by late-April/May. Testes diameter ranging from 10.6 ± 0.5 mm in late-November to 16.8 ± 0.3 mm in early-May, was positively correlated to testosterone concentration. Testosterone levels declined 3 to 4 weeks before the decrease in testes diameter. During April/May of the 1st year and April of the 2nd year, semen was collected by electroejaculation and sperm dimensions and morphology were recorded. Sperm heads

measured 5 μm wide and 7 μm long. The total length of the sperm was 71 to 75 μm.

Introduction

Information regarding the reproductive biology of the male fisher (*Martes pennanti*) is largely anecdotal from notes regarding time of breeding on fur farms (Hall 1942) and the dimensions of reproductive organs gathered from carcasses (Wright and Coulter 1967, Leonard 1986, Douglas and Strickland 1987). Annual testosterone (T) profiles, testes size and their seasonal relationship have not been reported for fisher, as they have for other mustelids (Gulamhusein and Tam 1974, Nieschlag and Bieniek 1975, Audy 1976, Neal et al. 1977, Stenson 1985, Mead et al. 1991, Audy 1978a, Pilbeam et al. 1979, Boissin-Agasse et al. 1982, Wildt et al. 1989). As well, reports of sperm dimensions (Stenson 1985, Kim et al. 1979, Cummins and Woodall 1985, Altman and Dittmer 1962) and morphology (Aulerich et al. 1972, Sundqvist et al. 1986, Curry et al. 1989) are available for a very few mustelids, again the fisher not being among them.

The objective of this research was to achieve a more complete understanding of the reproductive biology of the male fisher by characterizing seasonally dynamic reproductive events in the live animal. Blood samples and

measurements of testes diameter were taken enabling the characterization of T profiles and the elucidation of the relationship between testes diameter and T. This information could indicate the likely timing of the onset of spermatogenesis, the timing and duration of the breeding season, and which environmental cues regulate these events in this species. Monitoring of testes diameter could also provide a non-invasive technique for evaluating reproductive potential in the fisher.

Materials and Methods

Animals and Maintenance

Five male fishers (*Martes pennanti*) were maintained in southern Manitoba, at the University of Manitoba Glenlea Research Station (49° 53'N, 97° 09'W). The animals were housed individually, exposed to natural weather conditions and photoperiods, fed a commercial wet mink ration (see appendix 1) at a level that maintained body weight and allowed water *ad libitum*.

Two of the animals used in the 1st year of the study (1990/91) were captured in south-eastern Manitoba in the fall of 1987, while the third was born in captivity in the spring of 1989. The 2 wild-captured animals were assigned ages of 3.5 and 4.5 years in the first year of the study, as determined by counting annuli present in the roots of

premolars (Matson's Laboratory, Milltown, MT). The remaining male was 1.5 years of age when the study began. Second year sampling (1991/92) included 2 additional males believed to be 8 months old, as determined by tooth wear and skeletal structure.

Sampling

First year blood samples were collected monthly from September 1990, then weekly from mid-December 1990 to mid-June 1991. Second year blood sampling and testes measurements were conducted weekly from late-November 1991 to early-June 1992. Ketamine HCl (Ayerst Lab., Montreal, PQ) and xylazine HCl (Chemagro Ltd., Etobicoke, ON) were used to immobilize the animals for all procedures (10:1 ratio, 20 mg ketamine HCl kg^{-1} body weight). Whenever immobilized, Rogar-Mycine ointment (Rogar/STB Inc., London, ON) was applied to the eyes of the fishers to prevent drying.

Blood (7 ml) was collected by jugular venipuncture using a sterile 20 gauge 2.5 cm vacutainer needle and sterile, siliconized 7 ml vacutainer (Beckton Dickinson and Company, Rutherford, NJ). The samples were refrigerated for 18 to 24 h, centrifuged (Jouan Inc., Winchester, VA) for 20 min at ~1000 g and the serum removed and stored at -20°C until assayed for T. Blood samples were collected between

09:10 and 16:10 h and 09:00 and 15:23 h on sampling days during the 1st and 2nd years, respectively.

Testes diameter was measured by palpating the testes with the thumb and index finger, and measuring the gap with a millimetre ruler. The accuracy of the technique was confirmed with ultrasonography equipment (Siemens Electric Ltd., W. Germany).

Testosterone Analysis

Testosterone was extracted and quantified by RIA based on the method used by Yarney and Sanford (1983). In brief, for extraction, 250 μl serum combined with 750 μl PBS (pH 7.2) was mixed with 5.5 ml ethyl ether (Mallinckrodt Specialty Chemicals Co., Mississauga, ON), vortexed for 4 min and equilibrated for 15 min before freezing over dry ice/ethanol (-43°C). The supernatant was decanted and dried under a stream of nitrogen gas in a shaking water bath maintained at 37°C. The dried precipitate was resuspended in 1.250 ml PBS. Percentage recovery of ^{3}H-T (New England Nuclear, Boston, MA) added to pooled fisher serum averaged (± sem) 83 ± 3%.

The standard curve ranged from 12.5 to 1600 pg T 500 μl^{-1} PBS and was prepared with T stock 4-androsten-17ß-ol-3-one (Steraloids Inc., Wilton, NH). The T antibody (Sanford et al. 1976), was raised in sheep immunized with

testosterone-3-carboxy-methyloxime conjugated to bovine serum albumin and used at 1:2500 initial dilution in PBS. Radioactively labelled T[1,2,6,7-^{3}H(N)] (New England Nuclear, Boston, MA) was diluted in PBS to ~10,000 cpm 100 μl^{-1}. Ecolume (ICN Biomedicals Inc., Irvine, CA) was the scintillation cocktail used. Maximum binding of the antibody was 35%, non-specific binding was 1.5 to 2.3% and sensitivity of the assay at 85% of maximum binding was 12.5 pg T 500 μl^{-1}. First year samples were analyzed in a single assay with an intra-assay coefficient of variation (CV) of 7.1%. Second year samples were analyzed in 2 assays with intra- and inter-assay CV of 8.5% and 9.6%, respectively. Inter-assay CV for all assays from both years was 10.9%.

Semen Collection

Semen was collected from anaesthetized fishers by electroejaculation during April and May 1991 and April 1992. The electroejaculator used was constructed to a size that could be used specifically for fishers and was modelled after commercially available units. The procedure was performed in a heated trailer maintained at 15 to 25 °C, and evaluation materials and samples were kept warm (37 °C) in a heated microscope box. The risk of urine contamination was reduced by stimulating the animals to urinate during the immobilization procedure. The bi-polar rectal electrode

(Figure 5) was inserted to a depth of 6 to 10 cm and impulses were directed dorsally when possible. Each impulse was of 3 sec duration, followed by a 5 to 10 sec pause. Impulses were initially 4 to 6 mA, and were increased progressively throughout the procedure to a maximum of 30 mA. Ejaculation usually occurred after 10 to 15 impulses (range 8 to 40), when stimulation reached 20 to 24 mA. Ejaculates were collected in graduated centrifuge tubes enabling volumes to be determined immediately after collection. Samples of the semen were then placed on warmed microscope slides for motility assessment (400x light microscope) and smear preparation. Smears were later stained with rose bengal stain for sperm dimension and morphology evaluations. A 20 μl sample of the ejaculate was also fixed in acetic acid and sperm concentration was determined using the ejaculate volume and a haemocytometer (Reichert Scientific Instruments, Buffalo, NY) count of sperm cells. Sperm dimensions were measured under a 1000x oil-immersion lens with a calibrated eye-piece. Morphology and abnormalities were assessed at 400x magnification using a light microscope.

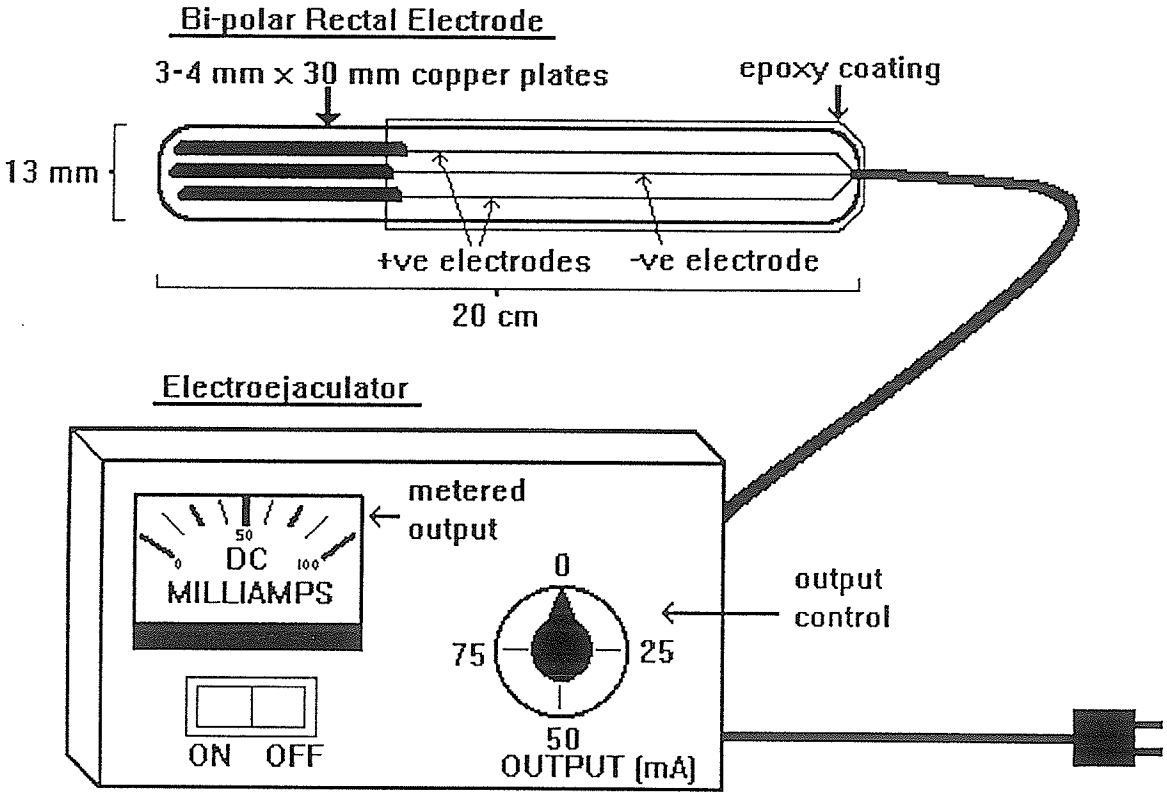


Figure 5. Diagram of electroejaculator used to collect semen samples from fishers.

Statistics

Monthly average T for each of the 3 adult animals was calculated and the monthly averages from this group over the entire research period and over each research year were analyzed by analysis of variance. Also, monthly average T for each of the 2 juvenile animals was calculated and the monthly averages from this group over the 2nd research year were analyzed by analysis of variance. In the presence of a significant F value, Scheffe's test was performed on unmatched pairs within research years of both groups to determine which months were significantly different. In the 2nd year, the data from the 2 juvenile animals were compared to that of the 3 adult animals by analysis of variance.

Correlation coefficients were calculated to determine whether 2nd year T levels and associated testes diameters were correlated. A Student's *t*-test was used to test Ho, the correlation coefficient = 0. All values are expressed as means ± sem.

Results

During the 2-year study period, 186 blood samples were collected. Monthly average serum T levels of the 3 adult males (Figure 6a, b) changed significantly throughout the study ($F = 15.55$, $df = 16,2$, $P < 0.0001$) and throughout each research year (year 1: $F = 24.39$, $df = 8,2$, $P < 0.0001$; year

2: $F = 13.05$, $df = 7,2$, $P < 0.0001$). The mean monthly T levels of the 2 juvenile males (Figure 7) used in year 2 also changed significantly throughout the year ($F = 49.89$, $df = 7,1$, $P < 0.0001$), and were significantly different from those of the adult males ($F = 11.45$, $df = 7,1$, $P < 0.0001$).

During the 1st year (Figure 6a) mean monthly T levels were 0.68 ± 0.11 and 0.66 ± 0.08 ng ml^{-1} during September and October, respectively, and increased gradually to 2.61 ± 0.41 ng ml^{-1} by February. Mean T concentrations in March (6.77 ± 0.74 ng ml^{-1}) was significantly higher ($P < 0.05$) than all months except February and April. April at 9.83 ± 0.80 ng ml^{-1} was found to be significantly higher than all months except March ($P < 0.05$). Mean T level then decreased to 2.59 ± 0.72 ng ml^{-1} in May and further to 0.53 ± 0.04 ng ml^{-1} in June. The 2nd year T profiles of the adult males (Figure 6b) and the juvenile males (Figure 7) were similar, but in the juveniles, T peaked in March ($P < 0.05$). (Individual profiles are presented in Appendix 4).

Mean weekly testes diameter was 10.6 ± 0.5 mm in late November 1991 and fluctuated between 12.1 ± 2.7 mm and 15.8 ± 0.8 mm over the next 3 months. Testes diameter increased to 16.3 ± 0.3 mm in early-March and was maintained at ≥ 15.8 mm for 2 months. Maximum mean testes diameter was 16.8 ± 0.3 mm, reached in early-May. A rapid decrease in testes diameter was seen in mid-May when it declined to 12.8 ± 1.5

mm and further to 10.2 ± 0.3 mm by early-June. Mean weekly testes diameter was significantly correlated with the mean weekly T concentration (Figure 8; $r = 0.596$; $P = 0.0021$).

Semen was collected on 17 occasions over the 2 year study period. Ejaculate volumes were < 50μl. Sperm concentrations were 1 to 1.5×10^9 ml^{-1} semen as determined from 5 samples. Dimensions of fisher sperm are given in Table 1 and normal fisher spermatozoa are shown in Figure 9. Abnormalities observed in the samples included: retained cytoplasmic droplets (5 to 20% in individual samples); bent/coiled midpieces and tails (< 5 to almost 100% in individual samples); double tails/heads (very few observed); and head-less/tail-less spermatozoa (few observed). Progressive motility varied considerable ranging from 20 to 70% and was evident for 5 min to 1 h following collection. One male showed an increase in the number of cytoplasmic droplets from late-April to mid-May, while another showed a drastic increase in the incidence of bent/coiled tails and clumping of sperm.

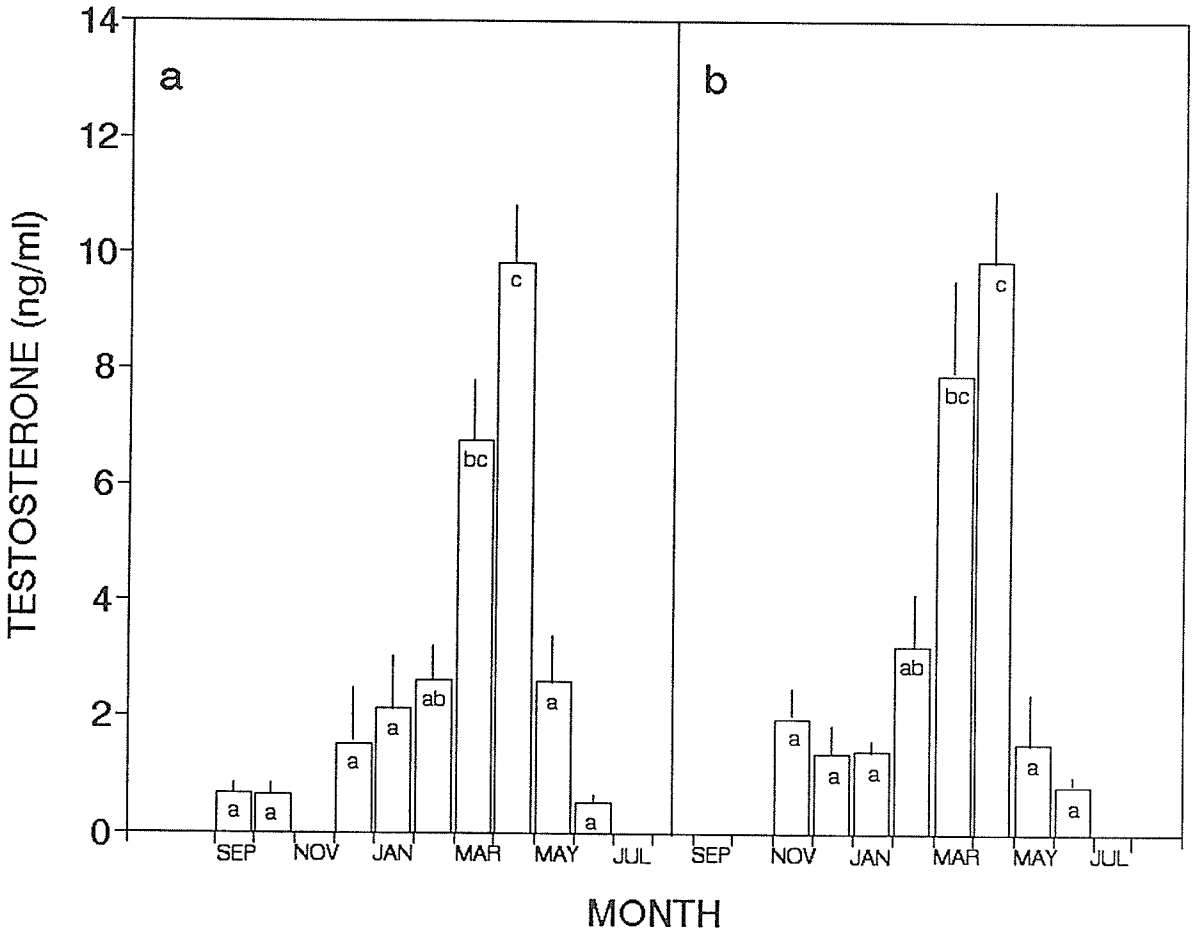


Figure 6. Monthly average (+sem) testosterone levels of 3 adult male fishers: a) 1st research year and b) 2nd research year. Months with the same letters within each year are not significantly different ($P < 0.05$)

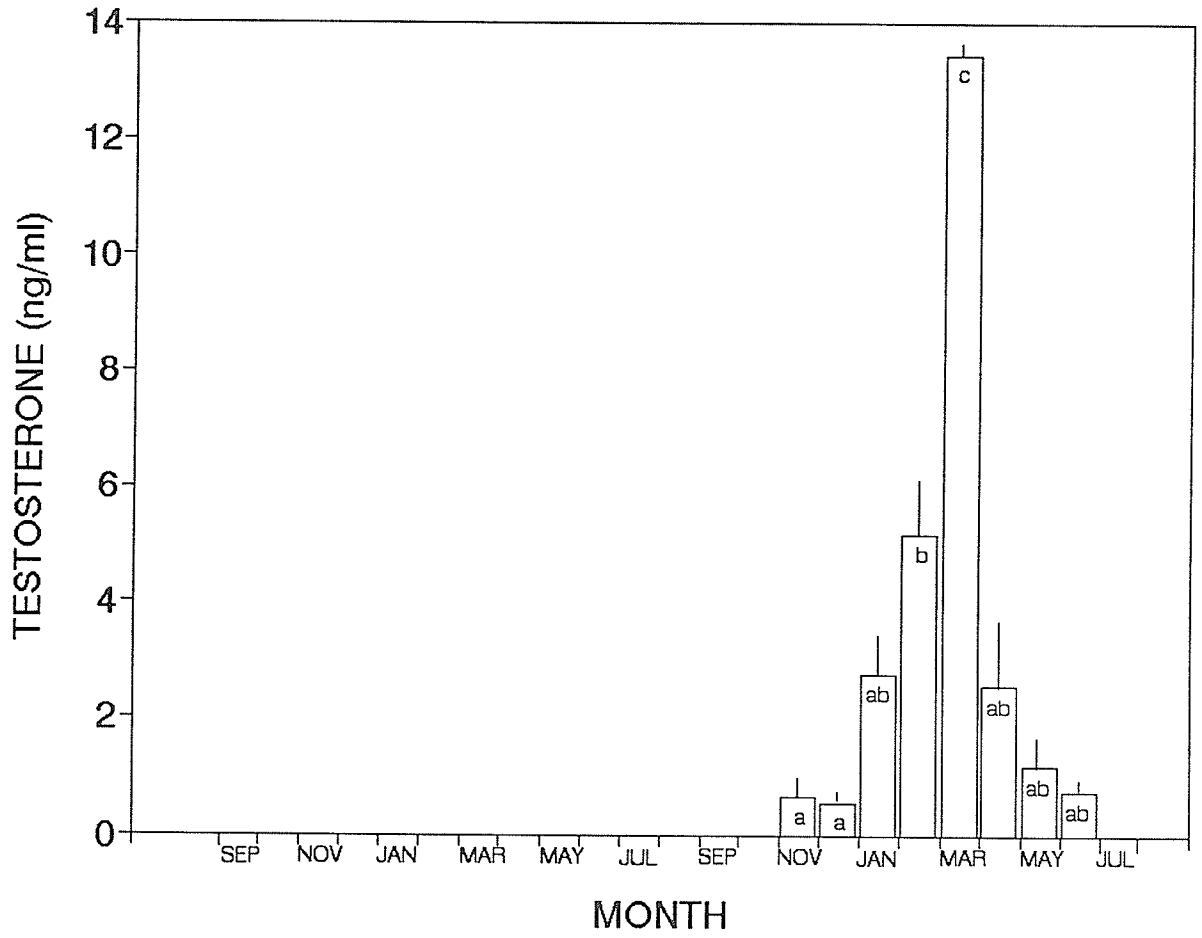


Figure 7. Monthly average (+sem) testosterone levels of 2 juvenile male fishers from 2nd research year. Months with the same letters within each year are not significantly different ($P < 0.05$)

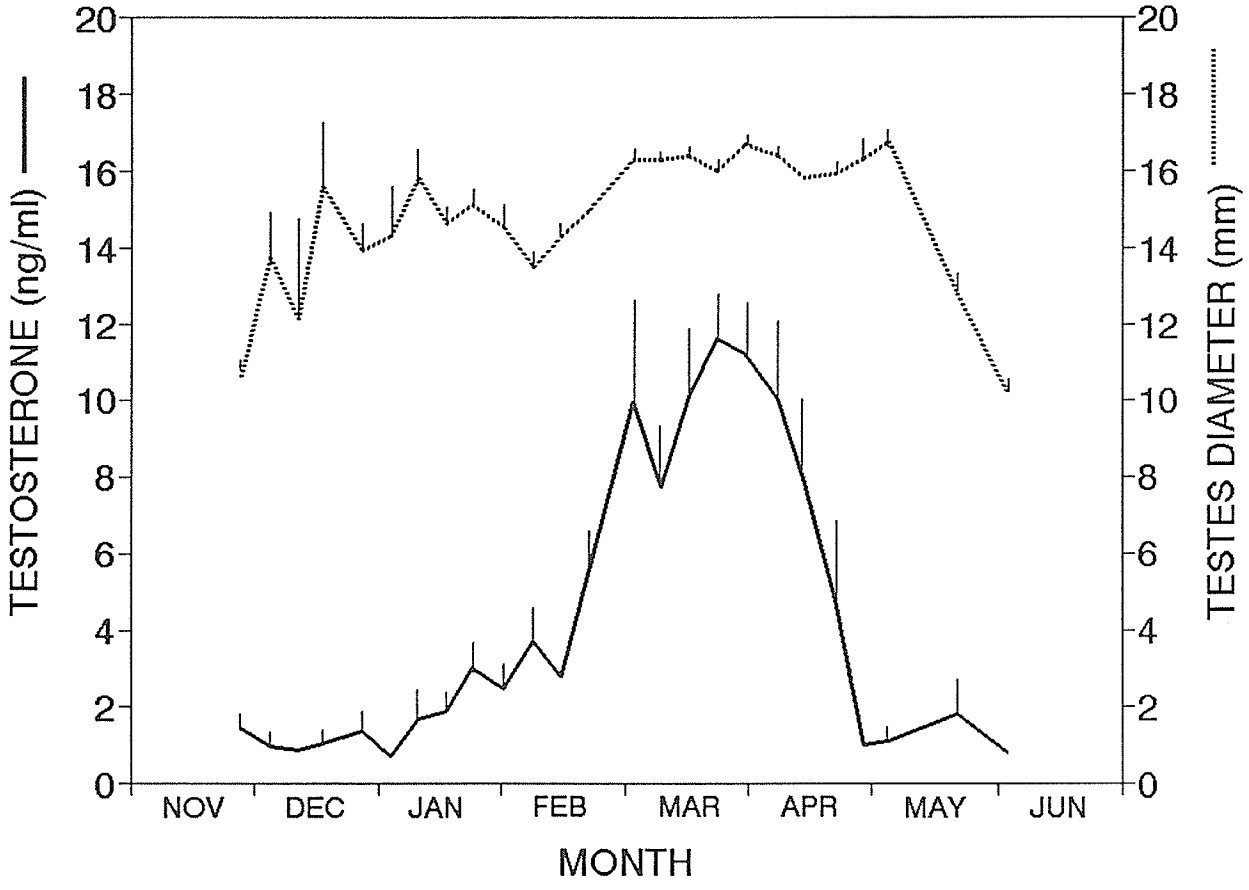


Figure 8. Second year mean (+ sem) weekly testosterone levels and associated mean (+ sem) weekly testes diameters of 5 male fishers.

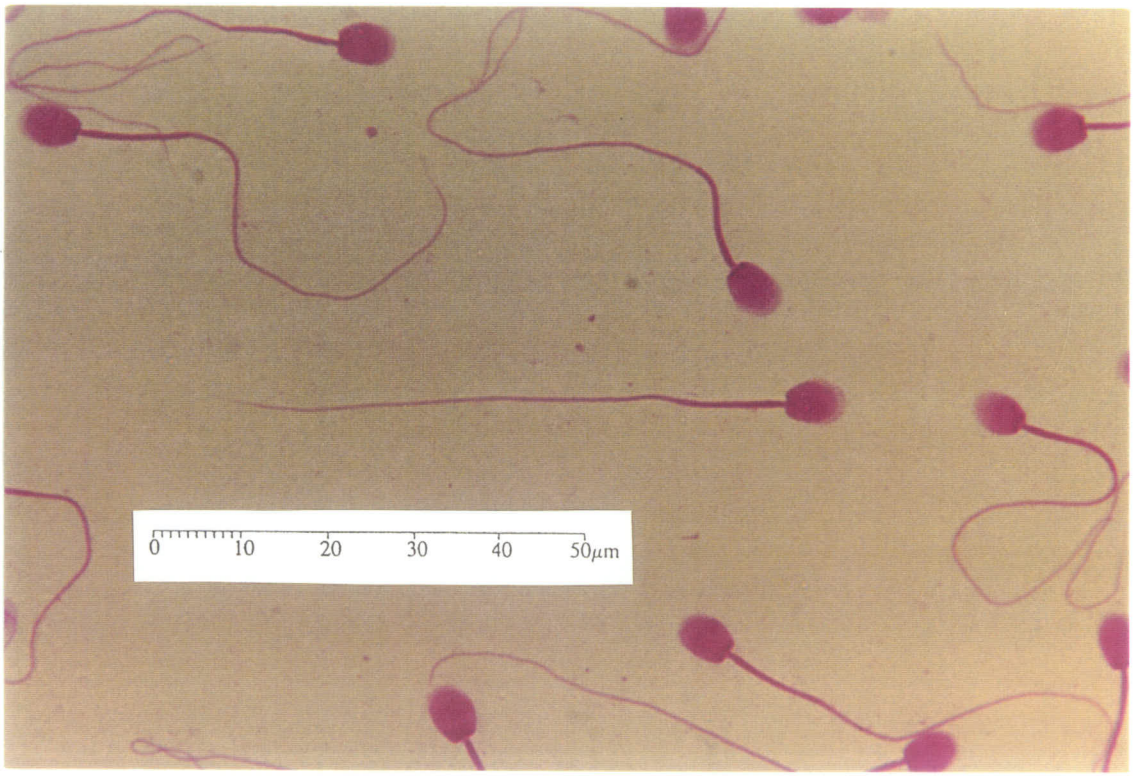


Figure 9. Normal fisher spermatozoa (magnified 1150x).

Table 1. Dimensions of fisher sperm (n=15) collected by electroejaculation in the spring of 1991.

HEAD		TAIL	MIDPIECE	TOTAL
LENGTH	WIDTH	LENGTH	LENGTH	LENGTH
7 μm	5 μm	64-68 μm	12-13 μm	71-75μm

Discussion

Until this study, seasonal changes in the male fishers' T level, live animal testes size and sperm morphology were undocumented. Results of this research confirm and substantiate predictions that could be made based on knowledge about the fisher breeding season (Hall 1942), carcass testes measurements (Wright and Coulter 1967, Leonard 1986, Douglas and Strickland 1987) and about other mustelids. Using the known breeding season of the fisher (April and May) as a reference point in time it can be predicted that T levels should begin to increase between December and February, peak in March or April and return to baseline levels sometime in April or May. As T production is dependent on testes development, testes size should follow a similar pattern but could be advanced by 2 to 8 weeks. Finally, sperm dimensions and observable abnormalities would not be expected to differ from those found in other mustelids.

Testosterone

The general appearance of the fisher T profiles are similar to those of several other mustelids. During periods of testicular quiescence T levels in the fisher were <1 ng ml^{-1}, which is similar to levels reported in the majority of mustelids studied (Nieschlag and Bieniek 1975, Audy 1976,

Neal et al. 1977, Stenson 1985, Mead et al. 1991, Pilbeam et al. 1979, Boissin-Agasse et al. 1982, Boissin-Agasse et al. 1981, Maurel et al. 1981, Maurel and Boissin 1982).

Each of the individual fisher T profiles (Appendix 4) exhibited sporadic, small-scale increases in T levels in the 2 to 4 months preceding the sustained peak associated with the breeding season. Similar findings have been reported in the T profiles of other mustelids (Gulamhusein and Tam 1974, Stenson 1985, Mead et al. 1991). These increases may be a function of the blood collection regimes used and the episodic nature of T production, as has been demonstrated in 2 species of mustelids (Maurel et al. 1981, Rieger and Murphy 1977), but may also be necessary for initiating breeding behavior and physiological changes associated with reproduction.

The maximum monthly mean T level found in our fishers is similar to that found in the badger (Audy et al. 1982) and slightly higher than the 5 to 7 ng ml^{-1} reported by other authors for larger mustelids (Stenson 1985, Mead et al. 1991, Maurel et al. 1981, Maurel and Boissin 1982) and one smaller species (Audy 1976). Most smaller species have been found to have considerably higher maximum monthly mean T levels (Gulamhusein and Tam 1974, Nieschlag and Bieniek 1975, Neal et al. 1977, Wildt et al. 1989, Erskine and Baum 1982, Sundqvist et al. 1984).

T concentrations in the fisher were found to peak prior to, or very early in the breeding season. Most breedings on fur farms have been reported to occur in April or early May (Hall 1942), and the two matings we observed occurred during these months. The breeding season occurs following attainment of peak T levels in most other mustelids (Nieschlag and Bieniek 1975, Audy 1976, Neal et al. 1977, Pilbeam et al. 1979, Sundqvist et al. 1984, Stenson 1985, Sundqvist et al. 1989, Mead et al. 1991).

Douglas and Strickland (1987), having found that juvenile (1 year old) male fishers have a smaller baculum than adults in March and April, suggested that juveniles may be unable to induce ovulation during mating. Their theory, that young males might be ineffective breeders, may be further supported by our finding from the 2nd year of our study, that the T profiles of juvenile and adult fishers were significantly different. The T levels of the juveniles (Appendices 4d and e) remained below 1 ng ml^{-1} until mid-January, and increased rapidly to peak in March. In contrast, the T levels of the adults (Appendices 4a, b and c) were over 1.5 ng ml^{-1} by late-December and increased gradually to peak in April. The mean T level of the adults was significantly higher than that of the juveniles in April ($P < 0.05$). This difference may be of some consequence. Sundqvist et al. (1984) found that mink with gradual

increases in T, followed by sharp declines were found to be fertile whereas mink with rapid increases of T to maximum levels that were maintained for a longer period were sterile. Our juvenile fishers exhibited a rapid increase in T and due to this "unfavorable" T profile may have been rendered ineffective breeders. Ultimately, spermatogenesis, baculum size, aggressiveness and mobility during the breeding season could be affected by differences in timing of T production.

Testes Size

This study revealed a positive correlation between testes diameter and T concentration in the fisher. Increases in testes diameter and T level began in December and January, respectively. Pre-breeding season increases in the T levels of many mustelids have been found to coincide or be positively correlated with increases in testes weight (Gulamhusein and Tam 1974, Audy 1976a), testes volume (Stenson 1985, Pilbeam et al. 1979, Boissin-Agasse et al. 1982, Wildt et al. 1989), testes size (Neal et al. 1977, Mead et al. 1991), the onset of spermatogenesis (Gulamhusein and Tam 1974, Stenson 1985) and fertility (Sundqvist et al. 1984).

The trend in testes diameter is in agreement with measurements of testes volumes (Leonard 1986) and weights

(Wright and Coulter 1967, Douglas and Strickland 1987) taken from fisher carcasses. Our data include measurements from later in the season than were previously reported and demonstrate that testes diameter remains elevated for approximately 2 months before rapidly declining after T returns to baseline levels. Also apparent, is that the fisher achieves maximum testes diameter early in the breeding season. While some mustelid species seem to follow a similar pattern (Audy 1976a, Pilbeam et al. 1979, Allanson 1932, Basrur and Ramos 1973, Walton 1976, Anhlund 1980), in the majority testes size (weight, volume or length) peaks prior to the breeding season (Gulamhusein and Tam 1974, Neal et al. 1977, Stenson 1985, Mead et al. 1991, Sundqvist et al. 1989, Wright 1969, Schowalter and Gunson 1982, Madsen and Rasmussen 1985, Mead et al. 1990).

Timing of Reproduction

Photoperiod has been found to be a critical factor in regulating reproduction in countless species, including many mustelids. Danilov and Tumanov (1972) stated that the sexual cycle of 4 Asian mustelids lasted about 4 months, but species specific responses to photoperiodic changes dictated when in the year breeding occurred. Male reproductive characteristics of several mustelid species have been manipulated by alterations to photoperiod (Stenson 1985,

Boissin-Agasse et al. 1982, Wildt et al. 1989, Bissonnette 1932, Hammond 1951, Duby and Travis 1972, Travis and Pilbeam 1980). If the sexual cycle of the male fisher lasts 4 months as found in some other mustelids, records of breeding dates and our findings of T profiles suggests that the fisher responds to increasing daylength. However, the issue is confounded by our somewhat erratic findings regarding changes in testes diameter in December, indicating that some activity may be occurring while daylength is still decreasing. Further research with fishers conducted from earlier in the autumn may elucidate whether they are short-day breeders like the mink (Pilbeam et al. 1979), or long-day breeders like the ferret (Neal et al. 1977).

Sperm Characteristics

There is little documentation of mustelid sperm characteristics. Dimensions reported herein for fisher sperm heads are very similar to those reported for the river otter (Stenson 1985) and the mink (Altman and Dittmer 1962), whereas the length of the midpiece is longer than that of mink (Kim et al. 1979, Altman and Dittmer 1962). The total length of fisher sperm appears to be intermediate between that of the mink (Altman and Dittmer 1962) and the European badger (Cummins and Woodall 1985).

Abnormalities observed in fisher sperm are similar to

those described in the mink (Aulerich et al. 1972, Sundqvist et al. 1986) and the ferret (Curry et al. 1989). One male which had recovered from food poisoning and severe muscular atrophy of the hind limbs had high numbers of sperm with coiled tails and midpieces in all samples collected. Curry et al. (1989) sited chemical contamination, chronic psychological stress and extreme population homozygosity as possible reasons for high incidence of abnormal sperm. These factors could affect spermatogenesis or the excurrent duct system, resulting in abnormal sperm production or maturation.

Motility values were a function of several factors including urine contamination, number of abnormal sperm and temperature of collection vessels and slides. Sperm with bent or coiled/midpieces or tails made up nearly 100% of samples collected from one male (Blacky), and motility was very low. The incidence of retained cytoplasmic droplets increased between sample collections in another male (Junior) and may have adversely affected motility. Samples were not collected prior to, nor after the breeding season, so effects of season relative to sperm motility are unknown. While glass material used for collection was kept warm in a heated microscope box, significant deviations from 37°C may have occurred, resulting in cold or heat shock of the sperm and possibly effected motility values.

Summary

Our characterization of the dynamic reproductive events in the male fisher confirm and extend information obtained from carcass analysis and is consistent with our knowledge of other related species. Testicular recrudescence is initiated 3 to 4 months prior to the April/May breeding season and follows a developmental pattern typical of mustelids. T levels increase shortly after testicular growth is initiated, peak early in the 2-month breeding season and rapidly return to baseline levels over a 2 to 4 week period. Sperm morphology, frequency and type of abnormalities are similar to those reported for other mustelids. Still unclear is whether photoperiod is the environmental cue initiating reproductive events in the fisher and, if so, whether shortening or lengthening daylength is ultimately responsible.

GENERAL DISCUSSION

This study provides the first descriptions of steroid levels for the fisher, and describes several physical characteristics that have not previously been reported for the live animal. These results confirm and extend information based on captive breeding programs (Hall 1942, Douglas 1943) and carcass analysis of fisher (Wright and Coulter 1967, Douglas and Strickland 1987), and complement the extensive literature available regarding reproduction in other mustelids. The one unexpected finding, that ovulation may have occurred without mating in some of our fishers, while unusual, is not without parallel. Striped skunks and mink have been found to ovulate due to non-mating interactions with members of the same or opposite sex (Enders 1952, Wade-Smith et al. 1980), and also to ovulate spontaneously (Møller 1974).

The reproductive pattern of the female fisher appears to be very similar to that of the river otter and the much-studied European badger, although specific events occur about 2 to 3 months earlier in the badger. In each of these species, a post-partum estrus occurs within 2 weeks of parturition in early spring, followed by a period of delayed implantation that may last up to 11 months (Hall 1942,

Canivenc and Bonnin 1981, Stenson 1985). While the badger has been found to initiate implantation in response to shortening daylength (Canivenc and Bonnin 1981), the fisher appears to be responsive to increasing photoperiod, similar to the river otter (Stenson 1985).

We have estimated the duration of the post-implantation period in the fisher to be 40 to 45 days, which is slightly longer than has been found in many of the smaller mustelids (Wright 1963), but not as long as estimates for the larger species (Canivenc 1966, Stenson 1985). Previous estimates for the duration of the post-implantation period in the fisher of 30 days (Wright and Coulter 1967) to as much as 60 days (Hamilton and Cook 1955) seem unreasonably short and long, respectively.

The characterization of progesterone (P4) profiles in the fisher appears to have been extremely informative with respect to the above mentioned reproductive events. It also provided circumstantial evidence that pseudopregnancy may occur in the fisher. This characteristic has only been confirmed in small mustelids with no, or only a short period of delayed implantation (Møller 1973a, Heap and Hammond 1974). Pseudopregnancy has been suggested to occur, but not confirmed, in only 1 species of mustelid with a long period of delayed implantation, the river otter (Stenson 1985), and has yet to be confirmed in any *Martes*.

If intensive breeding programs are to be undertaken with the fisher, artificially induced ovulation and artificial insemination will likely be cornerstones to their success. While our results regarding hormone-induced ovulation were inconclusive, we feel confident that further research in this area could provide favorable results as has been obtained with sable (Bernatskii et al. 1976). We obtained good quality semen on several occasions by electroejaculation and it is likely that cryo-preservation of fisher semen is possible given the successes realized with other mustelid species (Curry et al. 1989).

The monitoring of 17ß-estradiol (E2) secretion does not appear to be a satisfactory method of qualifying the reproductive condition of female fishers. Due to the dynamic nature of the steroid's secretion, blood levels would have to be determined much more regularly than was the case in our research. However, fishers must be chemically immobilized in order to collect any sample, making it impractical and potentially harmful to the animal. This fact, coupled with the paucity of obvious behavioral signs of estrus, and our observation that vulval swelling does not appear to be a useful indicator of reproductive status in the fisher, makes the characterization of vaginal cells the most likely and practical method of estrous detection.

A relationship appears to exist between the percentage

of cornified vaginal epithelial cells and the number of leukocytes present in vaginal smears of the fisher. If vaginal cells could be obtained by the lavage technique (Mead et al. 1990), a stronger relationship between these parameters may be found. Also, a gaseous anaesthetic has recently been used for immobilizing fishers (Breton et al. 1991) which may prove easier to administer and less stressful to the animals, making more frequent collection of all kinds of samples safer and more practical.

The characterization of testosterone (T) profiles in the fisher provided evidence that supports previous observations regarding the timing of breeding in this species (Hall 1942, Douglas 1943). It remains unclear, however, whether decreasing photoperiod before the winter solstice, or increasing daylength is the environmental cue which triggers testicular development in the fisher. Testes diameter was found to increase in December, but T concentrations did not increase until January or February. Supportive evidence for delayed sexual maturity in young male fisher however, as suggested by Douglas and Strickland (1987), was provided by our T profiles. The initial seasonal increase in T occurred earlier in the older males when compared to 1-year old males. This may confer physical or behavioral advantages to older males. We have also reported the first data regarding when testicular

recrudescence occurs in the fisher. The fisher appears to be similar to most other mustelids in that T levels return to near baseline early in the breeding season, at approximately the same time that testicular regression is initiated. Testosterone concentrations peaked at approximately the time of the vernal equinox and declined thereafter, strongly suggesting photoperiodic involvement in the timing of the fishers' reproductive cycle. Testes were apparently fully regressed by the end of the breeding season in early-June.

Our reported characteristics of fisher semen are among the few available for any mustelid. Dimensions we reported for the fisher are similar to those recorded for other species (Altman and Dittmer 1962, Stenson 1985), and abnormalities we observed are consistent with others reported in mustelids (Aulerich et al. 1972, Sundqvist et al. 1986, Curry et al. 1989).

This research has added greatly to our knowledge regarding the reproductive biology of the fisher. This information may prove useful to personnel that manage wild populations of fishers, and would undoubtedly benefit anyone attempting to propagate the fisher in captivity. Also, the information provided here may be useful to researchers investigating delayed implantation, and various aspects of the reproductive physiology of mustelids.

CONCLUSIONS

1. The fisher is similar to most mustelids with respect to reproductive hormone levels and physical characteristics.

2. Mean monthly E2 levels in female fishers ranged from 19 to 40 pg ml^{-1}, and were not found to be correlated to any aspect of vaginal cytology.

3. The percentage of cornified vaginal epithelial cells was negatively correlated with the number of leukocytes in vaginal smears, and is likely the most reliable method to detect estrus in the fisher.

4. In female fisher with corpora lutea, baseline levels of P4 of 1 to 2 ng ml^{-1} can increase to 40 ng ml^{-1} at the anticipated time of embryo implantation, likely in response to increasing daylength following the winter solstice.

5. Pseudopregnancy and spontaneous ovulations likely occur in fishers.

6. The post-implantation period is estimated to be 40 to 45 days.

7. In male fisher, baseline levels of T of 1 to 2 ng ml^{-1} increases dramatically in January or February to peak at 11 to 18 ng ml^{-1} in late-March or April. Testosterone is back to baseline by late-April or May.

8. Testes diameter, ranging from 10.6 mm in late-November to 16.8 mm in early-May, is positively correlated to T levels.

9. Semen, collected by electroejaculation in April and May, was of small volume and with sperm concentrations and dimensions similar to those of the few other mustelids thus characterized.

LITERATURE CITED

Aitken, R.J., 1977. Embryonic diapause. In: Johnson M.H., ed. Development in mammals. pp. 307-359. North-Holland Publishing Company, New York.

Allais, C. and Martinet, L., 1978. Relation between daylight ratio, plasma progesterone levels and timing of nidaion in mink (*Mustela vison*). J. Reprod. Fertil., 54:133-136.

Allanson, M., 1932. The reproductive processes of certain mammals. III.-The reproductive cycle of the male ferret. Proc. R. Soc. London, Ser. B Biol. Sci., 110:295-312.

Altman, P.L. and Dittmer, D.S., 1962. Growth, including reproduction and development. Federation of American Societies for Experimental Biology, Handbook. pp. 163.

Anhlund, H., 1980. Sexual maturity and breeding season of the badger, *Meles meles* in Sweden. J. Zool. (London), 190:77-95.

Arthur, S.M., Krohn, W.B. and Gilbert, J.R., 1989. Home range characteristics of adult fishers. J. Wildl. Manage., 53:674-679.

Audy, M.C., 1976. Le cycle sexuel saisonnier du mâle des mustélidés Europeens. Gen. Comp. Endo., 30:117-127.

Audy, M.C., 1978a. Etude ultrastructurale des cellules de leydig et de sertoli au cours du cycle sexuel saisonnier de la fouine (*Martes foina* Erx.). Gen. Comp. Endo., 36:462-476.

Audy, M.C., 1978b. Variations saisonnières de la testostérone plasmatique chez la Fouine (*Martes foina* Erx.). C.R. Acad. Sci. Paris, Ser. D, 287:721-724.

Audy, M.C., Bonnin, M., Souloumiac, J., Ribes, C., Kerdelhue, B., Mondain-Monval, M., Scholler, R. and Canivenc, R., 1985. Seasonal variations in plasma luteinizing hormone and testosterone levels in the

European badger *Meles meles* L.. Gen. Comp. Endo., 57:445-453.

Audy, M.C., Martin, B., Charron, G. and Bonnin, M., 1982. Steroid-binding proteins and testosterone level in the badger plasma during the annual cycle. Gen. Comp. Endo., 48:239-246.

Aulerich, R.J., Ringer, R.K. and Sloan, C.S., 1972. Electro-ejaculation of mink (*Mustela vison*). J. Anim. Sci., 34:230-233.

Baevskii, Y.B., 1964. Changes in the anterior lobe of the hypophysis, corpora lutea of pregnancy, and the thyroid in the mink (*Mustela vison*) associated with implantation of embryos. Acad. Sci. USSR, Proc. Biol. Sci., 157:522-525.

Basrur, P.K. and Ramos, A.S., 1973. Seasonal changes in the accessory sex glands and gonadoducts of male mink. Can. J. Zool., 51:1125-1132.

Baum, M.J., Lynch, H.J., Gallagher, C.A. and Deng, M.H., 1986. Plasma and pineal melatonin levels in female ferrets housed under long or short photoperiods. Biol. Reprod., 34:96-100.

Bearden, H.J. and Fuquay, J.W., 1992. Applied animal reproduction. Prentice Hall, Englewood Cliffs. pp. 33-52.

Bernatskii, V.G., Snytko, É.G. and Nosova, H.G., 1976. Natural and induced ovulation in the sable (*Martes zibellina* L.). Acad. Sci. USSR, Proc. Biol. Sci., 230:458-459.

Bissonnette, T.H., 1932. Modification of mammalian sexual cycles; Reactions of ferrets (*Putorius vulgaris*) of both sexes to electric light added after dark in November and December. Proc. R. Soc. London, Series B, Biol. Sci., 110:322-336.

Boissin-Agasse, L., Boissin, J. and Ortavant, R., 1982. Circadian photosensitive phase and photoperiodic control of testis activity in the mink (*Mustela vison* Peale and Beauvois), a short-day mammal. Biol. Reprod., 26:110-119.

Boissin-Agasse, L., Maurel, D. and Boissin, J., 1981. Seasonal variations in thyroxine and testosterone

levels in relation to the moult in the adult male mink (*Mustela vison* Peale and Beauvois). Can. J. Zool., 59:1062-1066.

Bonnefond, C., Fouche, L.G. and Martinet, L., 1988. A chronic jugular catheterization for remote blood sampling in freely moving mink. Physiol. Behav., 44:141-146.

Bonnin, M., Canivenc, R. and Aitken, J., 1977. Variations saisonnières du taux de la progestérone plasmatique chez la Fouine, *Martes foina*, espèce à ovo-implantation différée. C.R. Acad. Sci. Paris, Ser. D, 285:1479-1481.

Bonnin, M., Canivenc, R. and Ribes, C., 1978. Plasma progesterone levels during delayed implantation in the European badger (*Meles meles*). J. Reprod. Fertil., 52:55-58.

Breton, L., Garant, Y. and Crête, M., 1991. Field anesthesia of pine marten and fisher with isoflurane. Symposium on the Biology and Management of Martens and Fishers. Laramie, Wyoming. Abstract

Canivenc, R., 1966. A study of progestation in the European badger (*Meles meles* L.). Symp. Zool. Soc. London, 15:15-26.

Canivenc, R. and Bonnin, M., 1979. Delayed implantation is under environmental control in the badger (*Meles meles* L.). Nature (London), 278:849-850.

Canivenc, R. and Bonnin, M., 1981. Environmental control of delayed implantation in the European badger (*Meles meles*). J. Reprod. Fertil., Supple. 29:25-33.

Canivenc, R. and Bonnin-Laffargue, M., 1963. Inventory of problems raised by the delayed ova implantation in the European badger (*Meles meles* L.). In: Enders, A.C., ed. Delayed implantation. pp. 115-128. University of Chicago Press, Chicago.

Canivenc, R., Bonnin-Laffargue, M. and Lajus, M., 1967. Preuve expérimentale de la réactivation lutéale chez le blaireau européen (*Meles meles* L.). C. R. Acad. Sci. Paris, Ser. D, 264:1486-1489.

Canivenc, R., Bonnin-Laffargue, M. and Lajus-Boué, M., 1971. Réalisation expérimentale précoce de l'ovo-implantation

chez le blaireau européen (*Meles meles* L.) pendant la péiode de latence blastocytaire. C. R. Acad. Sci. Paris, Ser. D, 273:1855-1857.

Canivenc, R., Mauget, C., Bonnin, M. and Aitken, R.J., 1981. Delayed implantation in the beech marten (*Martes foina*). J. Zool. (London), 193:325-332.

Carlson, I.H. and Rust, C.C., 1969. Plasma progesterone levels in pregnant, pseudopregnant and anestrous ferrets. Endocrinol., 85:623-624.

Carrol, R.S., Weaver, C.E. and Baum, M.J., 1985. Evidence implicating aromatization of testosterone in the regulation of male ferret sexual behavior. Physiol. Behav., 42:457-460.

Channing, C.P., Fowler, S., Engel, B. and Vitek, K., 1977. Failure of daily injections of ketamine HCl to adversely alter menstrual cycle length, blood estrogen, and progesterone levels in the rhesus monkey. Proc. Soc. Exp. Biol. Med., 155:615-619.

Concannon, P., Pilbeam, T. and Travis, H., 1980. Advanced implantation in mink (*Mustela vison*) treated with medroxyprogesterone acetate during early embryonic diapause. J. Reprod. Fertil., 58:1-6.

Concannon, P.W. and DiGregorio, G.B., 1986. Canine vaginal cytology. In: Burke, T.J., ed. Small Animal Reproduction and Infertility. pp. 96-111. Lea and Febiger, Philadelphia

Coulter, M.W., 1966. Ecology and management of fishers in Maine. Ph.D. Thesis, Syracuse University, Syracuse. pp. 183.

Cummins, J.M. and Woodall, P.F., 1985. On mammalian sperm dimensions. J. Reprod. Fertil., 75:153-175.

Curry, P.T., Ziemer, T., Van der Horst, G., Burgess, W., Straley, M., Atherton, R.W. and Kitchin, R.M., 1989. A comparison of sperm morphology and silver nitrate staining characteristics in the domestic ferret and the black-footed ferret. Gam. Res., 22:27-36.

Daniel, J.C. Jr., 1976. Plasma progesterone levels before and at the time of implantation in the ferret. J. Reprod. Fertil., 48:437-438.

Danilov, P.I. and Tumanov, I.L., 1972. Reproductive cycles of males in some Mustelidae. Zool. Zh., 51:871-880.

Deanesly, R., 1935. Growth and reproduction in the stoat (*Mustela erminea*). Philo. Trans. R. Soc. London, Ser. B, 225:459-492.

Deanesly, R., 1943. Delayed implantation in the stoat (*Mustela mustela*). Nature (London), 151:365-366.

Deanesly, R., 1944. The reproductive cycle of the female weasel (*Mustela nivalis*). Proc. R. Soc. London, Ser. B Biol. Sci., 114:339-349.

Donovan, B.T., Matson, C. and Kilpatrick, M.J., 1983. Effect of exposure to long days on the secretion of oestradiol, oestrone, progesterone, testosterone, androstenedione, cortisol and follicle-stimulating hormone in intact and spayed ferrets. Endocrinol., 99:361-368.

Douglas, W.O., 1943. Fisher farming has arrived. American Fur Breeder, 16:18-20.

Douglas, C.W. and Strickland, M.A., 1987. Fisher. In: Novak, M., Baker, J.A., Obbard, M.E. and Malloch, B., eds. Wild furbearer management and conservation in North America. pp. 510-529. Ontario Trappers Association, North Bay

Drickamer, L.C., 1979. Acceleration and delay of first estrus in wild *Mus musculus*. J. Mammal., 60:215-216.

Duby, R.T., Pilbeam, T. and Travis, H.F., 1972 The influence of melatonin and hysterectomy on plasma progesterone levels of the mink (*Mustela vison*). Biol. Reprod., 7:125 (Abstract 73).

Duby, R.T. and Travis, H.F., 1972. Photoperiodic control of fur growth and reproduction in the mink (*Mustela vison*). J. Exper. Zool., 182:217-226.

Eadie, W.R. and Hamilton, W.J. Jr., 1958. Reproduction in the fisher in New York. New York Fish Game J., 5:77-83.

Eckstein, P., 1977. Endocrine activity of the ovary. In: Zuckerman, S. and Weir, B.J., eds. The Ovary. Volume II, Physiology. pp. 275-313. Academic Press, New York.

Enders, A.C., 1962. Observations on the fine structures of lutein cells. J. Cell Biol., 12:101-113.

Enders, R.K., 1952. Reproduction in the mink (*Mustela vison*). Proc. Amer. Philo. Soc., 96:691-755.

Enders, R.K. and Enders, A.C., 1963. Morphology of the female reproductive tract during delayed implantation in the mink. In: Enders, A.C., ed. Delayed implantation. pp. 129-139. University of Chicago Press, Chicago.

Enders, R.K. and Leakly, J.R., 1941. Cyclic changes in the vulva of the marten (*Martes americana*). Anat. Rec., 79:1-5.

Enders, R.K. and Pearson, O.P., 1943a. The blastocyst of the fisher. Anat. Rec., 85:285-287.

Enders, R.K. and Pearson, O.P., 1943b. Shortening gestation by inducing early implantation with increased light in the marten. Am. Fur Breed., 15:18.

Erskine, M.S. and Baum, M.J. 1982. Plasma concentrations of testosterone and dihydrotestosterone during perinatal development in male and female ferrets. Endocrinol., 111:767-772.

Ewer, R.F., 1973. The carnivores. Cornell University Press, Ithaca, New York. pp. 494.

Foresman, K.R. and Mead, R.A., 1973. Duration of post-implantation in a western subspecies of the spotted skunk (*Spilogale putorius*). J. Mammal., 54:521-523.

Foresman, K.R. and Mead, R.A., 1974. Pattern of luteinizing hormone secretion during delayed implantation in the spotted skunk (*Spilogale putorius latifrons*). Biol. Reprod., 11:475-480.

Foresman, K.R. and Mead, R.A., 1978. Luteal control of nidation in the ferret (*Mustela putorius*). Biol. Reprod., 18:490-496.

Fuller, G.B., Hobson, W.C., Reyes, F.I., Winter, J.S.D. and Faiman, C., 1984. Influence of restraint and ketamine anaesthesia on adrenal steroids, progesterone and gonadotropins in rhesus monkeys. Proc. Soc. Exp. Biol. Med., 175:487-490.

Gilbert, F.F. and Bailey, E.D., 1967. The effect of visual isolation on reproduction in the female ranch mink. J. Mammal., 48:113-118.

Greensides, R.D. and Mead, R.A., 1973. Ovulation in the spotted skunk (*Spilogale putorius latifrons*). Biol. Reprod., 8:576-584.

Gulamhusein, A.P. and Tam, W.H., 1974. Reproduction in the male stoat, *Mustela erminea*. J. Reprod. Fertil., 41:303-312.

Gulamhusein, A.P. and Thawley, A.R., 1972. Ovarian cycle and plasma progesterone levels in the female stoat, *Mustela erminea*. J. Reprod. Fertil., 31:492-493.

Gulamhusein, A.P. and Thawley, A.R., 1974. Plasma progesterone levels in the stoat. J. Reprod. Fertil., 36:405-408.

Hadley, M.E., 1984. Endocrinology. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. pp. 547.

Hall, E.R., 1942. Gestation period in the fisher with recommendations for the animals protection in California. Calif. Game Fish, 28:143-147.

Hamilton, W.J. Jr. and Cook, A.H., 1955. The biology and management of the fisher in New York. New York Fish Game J., 2:13-35.

Hamilton, W.J. Jr. and Eadie, W.R., 1964. Reproduction in the otter, *Lutra canadensis*. J. Mammal., 45:242-252.

Hammond, J. Jr. and Marshall, F.H.A., 1930. Oestrus and pseudopregnancy in the ferret. J. Exper. Biol., 11:307-319.

Hammond, J., 1951. Control by light of reproduction in ferrets and mink. Nature (London), 167:150-151.

Hansson, A., 1947. The physiology of reproduction in mink (*Mustela vison*, Schreb.) with special reference to delayed implantation. Acta Zool., 28:1-136.

Heap, R.B. and Hammond, J. Jr., 1974. Plasma progesterone levels in pregnant and pseudopregnant ferrets. J. Reprod. Fertil., 39:149-152.

Heidt, G.A., 1970. The least weasel (*Mustela nivalis* Linnaeus): developmental biology in comparison with other North American *Mustela*. Michigan State University. Mus. Publ. Biol. Ser. 4:227-282.

Hodges, C.M., Magyar, S.J. and Amoss, M.S. Jr., 1990. Antagonistic behavior induces a reduction in fecundity in subordinate female coyotes (*Canis latrans*). Biol. Reprod., Supple. 42:51 (Abstract).

Hodgson, R.G., 1937. Fisher farming. Fur Trade Journal of Canada, Toronto, Ontario. pp. 105.

Hodgson, R.G., 1949. Successful marten raising. Fur Trade Journal of Canada, Toronto, Ontario. pp. 84.

Holcomb, L.C., Schaible, P.J. and Ringer, R.K., 1962. The effects of varied lighting regimes on reproduction in mink. Mich. Quart. Bull., 44:666-678.

Johnson, S.A., 1984. Home range, movements, and habitat use of fishers in Wisconsin. M.Sc. Thesis, University of Wisconsin, Stevens Point. pp. 78.

Jonkel, C.J. and Weckworth, R.P., 1963. Sexual maturity and implantation of blastocysts in the wild pine marten. J. Wildl. Manage., 27:93-98.

Kim, J.W., Kitts, W.D., Ahmad, M.S. and Krishnamurti, C.R., 1979. The ultrastructure of mink (*Mustela vison*) spermatozoa. Can. J. Zool., 57:924-933.

Kintner, P.J. and Mead, R.A., 1983. Steroid metabolism in the corpus luteum of the ferret. Biol. Reprod., 29:1121-1127.

Kirk, R.J., 1962. The effect of darkness on the mink reproductive cycle. Fur Trade J. Can., 40: 8, 15.

Laberee, E.E., 1941. Breeding and reproduction in fur bearing animals. Fur Trade J. Can., Toronto. pp. 166.

Leonard, R.D., 1980. The winter activity and movements, winter diet, and breeding biology of the fisher (*Martes pennanti*) in southeastern Manitoba. M.Sc. Thesis, University of Manitoba, Winnipeg. pp. 181.

Leonard, R.D., 1986. Aspects of reproduction of the fisher, *Martes pennanti*, in Manitoba. Can. Field-Nat., 100:32-44.

Møller, O.M., 1973a. The progesterone concentrations in the peripheral plasma of the mink (*Mustela vison*) during pregnancy. J. Endocrinol., 56:121-132.

Møller, O.M., 1973b. The fine structure of the ovarian interstitial gland cells in the mink (*Mustela vison*). J. Reprod. Fertil., 34:171-174.

Møller, O.M., 1974. Plasma progesterone before and after ovariectomy in unmated and pregnant mink, *Mustela vison*. J. Reprod. Fertil., 37:367-372.

Madsen, A.B. and Rasmussen, A.M., 1985. Reproduction in the stone marten *Martes foina* in Denmark. Nat. Jutl., 21:145-148.

Martinet, L., Allain, D. and Meunier, M., 1983. Regulation in pregnant mink (*Mustela vison*) of plasma progesterone and prolactin concentrations and regulation of the spring moult by daylight ratio and melatonin injection. Can. J. Zool., 61:1959-1963.

Martinet, L., Allais, C. and Allain, D., 1981. The role of prolactin and LH in luteal function and blastocyst growth in mink (*Mustela vison*). J. Reprod. Fertil., Suppl. 29:119-130.

Maurel, D. and Boissin, J., 1982. Métabolisme périphérique de la testostérone en relation avec le cycle annuel de la testostérone et de la 5α-dihydrotestostérone plasmatiques chez le blaireau européen (*Meles meles* L.) et le renard roux (*Vulpes vulpes* L.). Can. J. Zool., 60:4406-416.

Maurel, D., Laurent, A.M. and Boissin, J., 1981. Short-term variations of plasma testosterone concentrations in the European badger (*Meles meles*). J. Reprod. Fertil., 61:53-58.

May, R., DeSantis, M., Mead, R.A., 1985. the suprachiasmatic nuclei and retinohypothalamic tract in the western spotted skunk. Brain Res., 339:378-381.

McClintock, M.K. 1983. Pheromonal regulation of the ovarian cycle: enhancement, suppression and synchrony. In: Vandenbergh, J.G., ed. Pheromones and Reproduction in Mammals. pp. 113-149. Academic Press, New York.

Mead, R.A., 1968a. Reproduction in eastern forms of the spotted skunk (genus *Spilogale*). J. Zool. (London),

156:119-136.

Mead, R.A., 1968b. Reproduction in western forms of the spotted skunk (genus *Spilogale*). J. Mammal., 49:373-390.

Mead, R.A., 1971. Effects of light and blinding upon delayed implantation in the spotted skunk. Biol. Reprod., 5:214-220.

Mead, R.A., 1975. Effects of hypophysectomy on blastocyst survival, progesterone secretion and nidation in the spotted skunk. Biol. Reprod., 12:526-533.

Mead, R.A., 1981. Delayed implantation in mustelids, with special emphasis on the spotted skunk. J. Reprod. Fertil., Suppl. 29:11-24.

Mead, R.A., 1986. Role of the corpus luteum in controlling implantation in mustelid carnivores. Annals New York Acad. Sci., 476:25-35.

Mead, R.A., 1989. The physiology and evolution of delayed implantation in carnivores. In: Gittleman, J.L., ed. Carnivore behavior, ecology and evolution. pp. 437-464. Cornell University Press, Ithaca, New York.

Mead, R.A., Concannon, P.W. and McRae, M., 1981. Effect of progestins on implantation in the western spotted skunk. Biol. Reprod., 25:128-133.

Mead, R.A. and Eik-Nes, K.B., 1969a. Oestrogen levels in peripheral blood plasma of the spotted skunk. J. Reprod. Fertil., 18:351-353.

Mead, R.A. and Eik-Nes, K.B., 1969b. Seasonal variation in plasma levels of progesterone in western forms of the spotted skunk. J. Reprod. Fertil., Supple. 6:397-403.

Mead, R.A., Joseph, M.M., Neirinckx, S., 1988. Partial characterization of a luteal factor that induces implantation in the ferret. Biol. Reprod., 38:798-803.

Mead, R.A. and McRae, M., 1982. Is estrogen required for implantation in the ferret? Biol. Reprod., 27:540-547.

Mead, R.A., Neirinckx, S. and Czekala, N.M., 1990. Reproductive cycle of the steppe polecat (*Mustela eversmanni*). J. Reprod. Fertil., 88:353-360.

Mead, R.A., Rector, M., Starypan, G., Neirinckx, S., Jones, M. and DonCarlos, M.N., 1991. Reproductive biology of captive wolverines. J. Mammal., 72:807-814.

Mead, R.A. and Swannack, A., 1978. Effects of hysterectomy on luteal function in the western spotted skunk (*Spilogale putorius latifrons*). Biol. Reprod., 18:379-383.

Mead, R.A. and Wright, P.L., 1983. Reproductive cycles of Mustelidae. Acta Zool. Fenn., 174:169-172.

Mondain-Monval, M., Bonnin, M., Canivenc, R. and Scholler, R., 1980. Plasma estrogen levels during delayed implantation in the European badger (*Meles meles* L.). Gen. Comp. Endo., 41:143-149.

Murphy, B.D., 1972. Population of gonadotrophic cells in the adenohypophysis of the mink during embryonic diapause and early postimplantation. Anat. Rec., 172:372 (Abstract).

Murphy, B.D., 1983. Precocious induction of luteal activation and termination of delayed implantation in mink with the dopamine antagonist pimozide. Biol. Reprod., 29:658-662.

Murphy, B.D. and James, D.A., 1974. The effects of light and sympathetic innervation to the head on nidation in mink. J. Exper. Zool., 187:267-276.

Murphy, B.D. and Mead, R.A., 1976. Effects of antibodies to oestrogens on implantation in ferrets. J. Reprod. Fertil., 46:261-263.

Murphy, B.D., Mead, R.A. and McKibbin, P.E., 1983. Luteal contribution to the termination of preimplantation delay in mink. Biol. Reprod., 28:497-503.

Murphy, B.D. and Moger, W.H., 1977. Progestins of mink gestation: The effects of hypophysectomy. Endo. Res. Comm., 4:45-60.

Neal, E.G. and Harrison, R.J., 1958. Reproduction in the European badger (*Meles meles* L.). Trans. Zool. Soc. London, 29:67-131.

Neal, J., Murphy, B.D., Moger, W.H. and Oliphant, L.W., 1977. Reproduction in the male ferret: Gonadal activity during the annual cycle; Recrudescence and

maturation. Biol. Reprod., 17:380-385.

Nieschlag, E. and Bieniek, H., 1975. Endocrine testicular function in mink during the first year of life. Acta Endocrinol., 79:375-379.

Nishino, H., Koizumi, K. and Brooks, C.M., 1976. The role of suprachiasmatic nuclei of the hypothalamus in the production of circadian rhythm. Brain Res., 112:45-59.

Papke, R.L., Concannon, P.W., Travis, H.F. and Hansel, W., 1980. Control of luteal function and implantation in the mink by prolactin. J. Anim. Sci., 50:1102-1107.

Pearson, O.P. and Enders, R.K., 1944. Duration of pregnancy in certain mustelids. J. Exper. Zool., 95:21-35.

Pilbeam, T.E., Concannon, P.W. and Travis, H.F., 1979. The annual reproductive cycle of mink (*Mustela vison*). J. Anim. Sci., 48:578-584.

Polynstev, Y.V., Volchek, A.G., Ilyinsky, V.M. and Rozen, V.B., 1975. Progesterone and estrogen content during the reproduction in sables. Probl. Endokrinol., 21:86-94.

Powell, R.A., 1982. The fisher: life history, ecology and behavior. University Minnesota Press, Minneapolis. pp. 217.

Ramaley, J.A., 1981. Stress and fertility. In: Gilmore, D., ed. Environmental factors in mammalian reproduction. pp. 125-141. University Park Press, Baltimore.

Ravindra, R., Bhatia, K. and Mead, R.A., 1984. Steroid metabolism in corpora lutea of the western spotted skunk (*Spilogale putorius latifrons*). J. Reprod. Fertil., 72:495-502.

Ravindra, R. and Mead, R.A., 1984. Plasma estrogen levels during pregnancy in the western spotted skunk. Biol. Reprod., 30:1153-1159.

Reiger, D. and Murphy, B.D., 1977. Episodic fluctuations in plasma testosterone and dihydrotestosterone in male ferrets during the breeding season. J. Reprod. Fertil., 51:511-514.

Rose, J., Stormshak, F., Oldfield, J. and Adair, J., 1985. The effects of photoperiod and melatonin on serum prolactin levels of mink during the autumn molt. J. Pineal Res., 2:13-19.

Rowlands, I.W., 1974. Reproductive studies in Mustelidae. J. Zool. (London), 173:116-118.

Sandell, M., 1990. The evolution of seasonal delayed implantation. Quart. Rev. Biol., 65:23-42.

Sanford, L.M., Faiman, C., Howland, B.E. and Palmer, W.M., 1976. The profile of follicle-stimulating hormone secretion in the ram. Can. J. Anim. Sci., 56:497-504.

Schowalter, D.B. and Gunson, J.R., 1982. Parameters of population and seasonal activity of striped skunks, *Mephitis mephitis*, in Alberta and Saskatchewan. Can. Field-Nat., 96:409-420.

Seton, E.T., 1953. Lives of game animals. Charles T. Branford, Company, Boston. pp. 451-479.

Shea, M.E., Rollins, N.L. Bowyer, R.T. and Clark, A.G., 1985. Corpora lutea number as related to fisher age and distribution in Maine. J. Wildl. Manage., 49:37-40.

Shul'gina, N.K., Polyntsev, Y.V., Rozen, V.B. and Diveeva, G.M., 1981. Contents of sex steroids in the blood plasma of female sable at the initial stages of postnatal ontogenesis. Acad. Sci. USSR, Proc. Biol. Sci., 258:255-258.

Sinha, A.A. and Mead, R.A., 1975. Ultrastructural changes in granulosa lutein cells and progesterone levels during preimplantation, implantation, and early placentation in the western spotted skunk. Cell Tissue Res., 164:179-192.

Smithberg, M. and Runner, M.N., 1960. Retention of blastocysts in nonprogestational uteri of mice. J. Exp. Zool., 143:21-31.

Song, J.H., Tong, Y. and Xiao, Y., 1988. Effects of light on reproduction and molting of *Martes zibellina*. J. Ecol. China, 7:27-29.

Stenson, G.B., 1985. The reproductive cycle of the river otter, *Lutra canadensis* in the marine environment of

southwestern British Columbia. Ph.D. Thesis, University of British Columbia, Vancouver. pp. 259.

Stenson, G.B., 1988. Oestrus and the vaginal smear cycle of the river otter, *Lutra canadensis*. J. Reprod. Fertil., 83:605-610.

Stoufflet, I., Mondain-Monval, M., Simon, P. and Martinet, L., 1989. Patterns of plasma progesterone, androgen and estrogen concentrations and in-vitro ovarian steroidogenesis during embryonic diapause and implantation in the mink (*Mustela vison*). J. Reprod. Fertil., 87:209-221.

Sundqvist, C., Amador, A.G. and Bartke, A., 1989. Reproduction and fertility in the mink (*Mustela vison*). J. Reprod. Fertil., 85:413-441.

Sundqvist, C., Lukola, A. and Valtonen, M., 1984. Relationship between serum testosterone concentrations and fertility in male mink (*Mustela vison*). J. Reprod. Fertil., 70:409-412.

Sundqvist, C., Toppari, J., Parvinen, M., Fagerström, R. and Lukola, A., 1986. Elimination of infertile male mink from breeding using sperm test, testicular palpation, testosterone test and fine-needle aspiration biopsy of the testis. Anim. Reprod. Sci., 11:295-305.

Tardiff, S., Hyde, K. and Rigby, L., 1990. Social suppression of ovulation in singly housed common marmosets. Biol. Reprod., Suppl. 42:48 (Abstract).

Travis, H.F. and Pilbeam, T.E., 1980. Use of artificial light and daylength to alter the life cycles of mink. J. Anim. Sci., 50:1108-1112.

Travis, H.F., Pilbeam, T.E., Gardner, W.J. Sr. and Cole, R.S., 1978. Relationship of vulvar swelling to estrus in mink. J. Anim. Sci., 46:219-224.

Wade-Smith, J. and Richmond, M.E., 1978. Induced ovulation, development of the corpus luteum, and tubal transport in the striped skunk (*Mephitis mephitis*). Amer. J. Anat., 153:123-142.

Wade-Smith, J., Richmond, M.E., Mead, R.A. and Taylor, H., 1980. Hormonal and gestational evidence for delayed implantation in the striped skunk, *Mephitis*. Gen. Comp. Endo., 42:509-515.

Walton, K.C., 1976. The reproductive cycle of the male polecat *Putorius* in Britain. J. Zool. (London), 180:498-503.

Wight, H.M., 1931. Reproduction in the eastern skunk (*Mephitis mephitis nigra*). J. Mammal., 12:42-47.

Wildt, D.E., Bush, M., Morton, C., Morton, F. and Howard, J.G., 1989. Semen characteristics and testosterone profiles in ferrets kept in a long-day photoperiod, and the influence of hCG timing and sperm dilution medium on pregnancy rate after laparoscopic insemination. J. Reprod. Fertil., 86:349-358.

Wright, P.L., 1942. Delayed implantation in the long-tailed weasel (*Mustela frenata*), the short-tailed weasel (*Mustela cicognani*), and the marten (*Martes americana*). Anat. Rec., 83:341-353.

Wright, P.L., 1948. Breeding habits of captive long-tailed weasel (*Mustela frenata*). Amer. Midl. Nat., 39:338-344.

Wright, P.L., 1963. Variations in reproductive cycles in North American mustelids. In: Enders, A.C., ed. Delayed implantation. pp. 77-97. University of Chicago Press, Chicago.

Wright, P.L., 1969. The reproductive cycle of the male American badger, *Taxidea taxus*. J. Reprod. Fertil., Suppl. 6:435-445.

Wright, P.L. and Coulter, M.W., 1967. Reproduction and growth in Maine fishers. J. Wildl. Manage., 31:70-87.

Wright, P.L. and Raucsh, R., 1955. Reproduction in the wolverine, *Gulo gulo*. J. Mammal., 36:346-355.

Wu, J.T. and Chang, M.C., 1972. Effects of progesterone and estrogen on the fate of blastocysts in ovariectomized pregnant ferrets: a preliminary study. Biol. Reprod., 7:231-237.

Wurtman, R.J., Larin, F., Axelrod, J., Shein, H.M. and Rosasco, K., 1968. Formation of melatonin and 5-hydroxyindole acetic acid from ^{14}C-tryptophan by rat pineal glands in organ culture. Nature (London), 217:953-954.

Yarney, T.A. and Sanford, L.M. 1983. The reproductive-

endocrine response of adult rams to sexual encounters with estrual ewes is season dependent. Horm. Behav., 17:169-182.

Yu, J.Y.L., Yuthasastrakosol, P., Marquardt, R.R. and Palmer, W.M., 1974. Estrogen levels in peripheral plasma of immature female chickens as determined by radioimmunoassay. Endocrinol., 94:1168-1171.

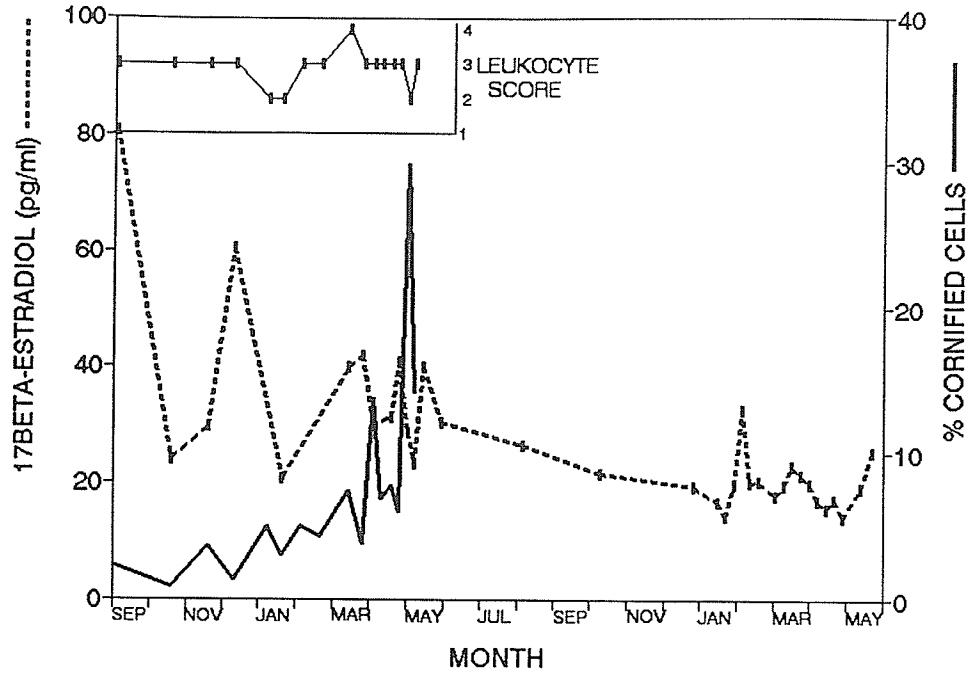
Yuthasastrakosol, P., Howland, B.E., Simaraks, S. and Palmer, W.M., 1974. Estrogen-induced LH release in progesterone-treated ovariectomized ewes. Can. J. Anim. Sci., 54:565-572.

APPENDICES

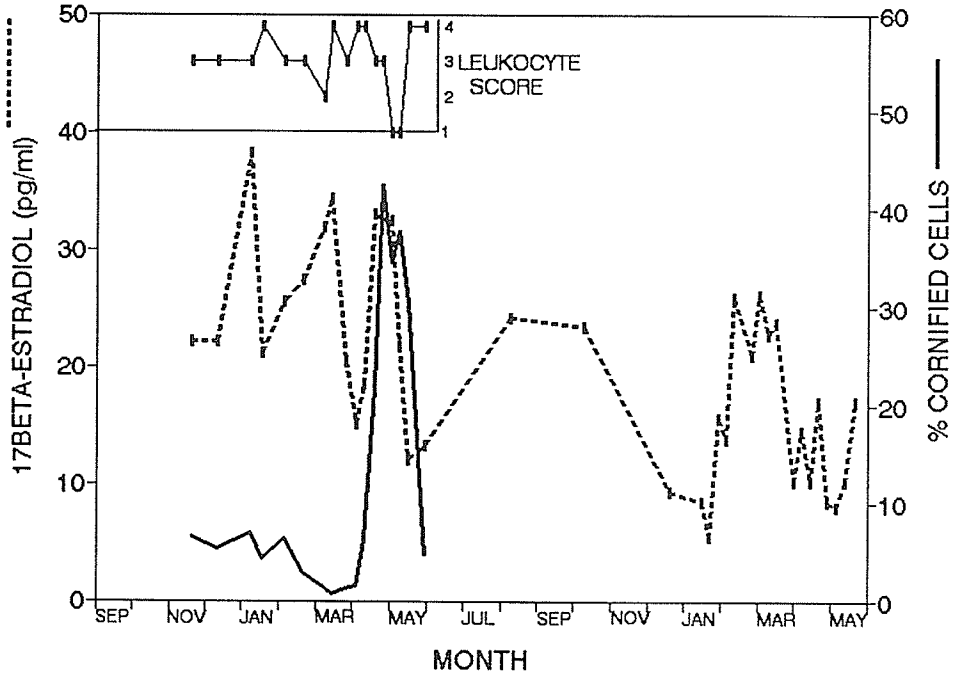
Constituent	Units	Maintenance (mature)	Gestation
Energy			
males	kcal ME kg^{-1} BW	275-330	-
females	kcal ME kg^{-1} BW	280-355	200
Amount per 100 kcal metabolizable energy[a]:			
Digestible protein	kcal ME	20-24	35
Fat-soluable vitamins			
Vitamin A	IU	b	b
Vitamin E	mg	b	b
Water-soluable vitamins			
Thiamine	mg	b	b
Riboflavin	mg	b	b
Pantothenic acid	mg	b	b
Vitamin B_6	mg	b	b
Niacin	mg	b	b
Folic acid	μg	b	b
Biotin	μg	b	b
Vitamin B_{12}	μg	b	b

[a]When original data were not presented on the basis of ME, requirements were calculated according to the following formula (ME = .77 E).
[b]Quantitative requirements not determined, but dietary need demonstrated.

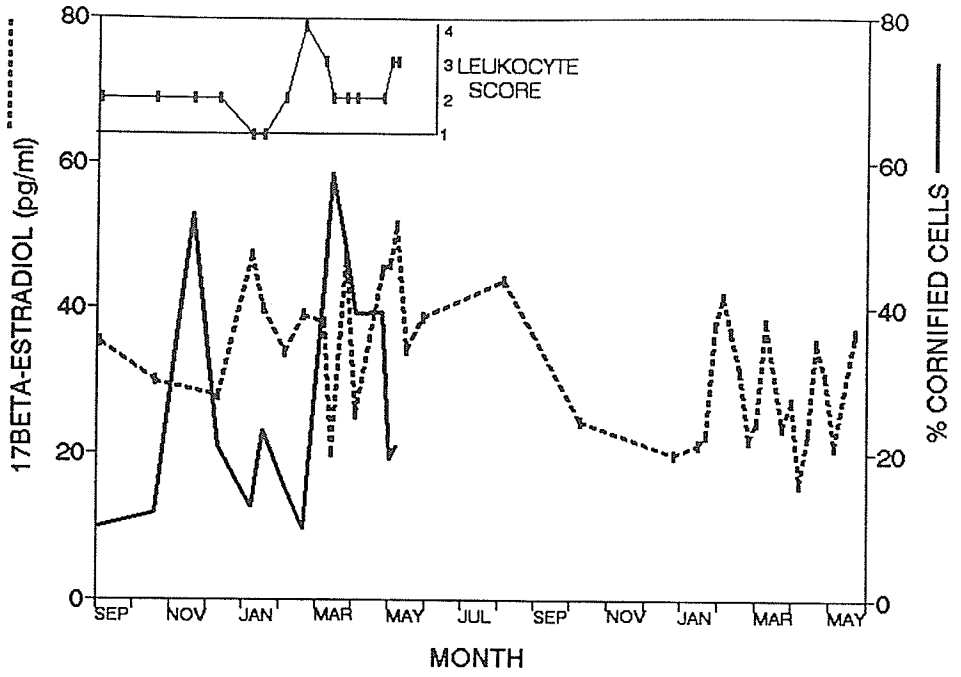
Appendix 1. Nutrient requirements of mink. From: Nutrient Requirements of Domestic Animals. Number 7. Nutrient Requirements of Mink and Foxes. Second Edition, 1982.



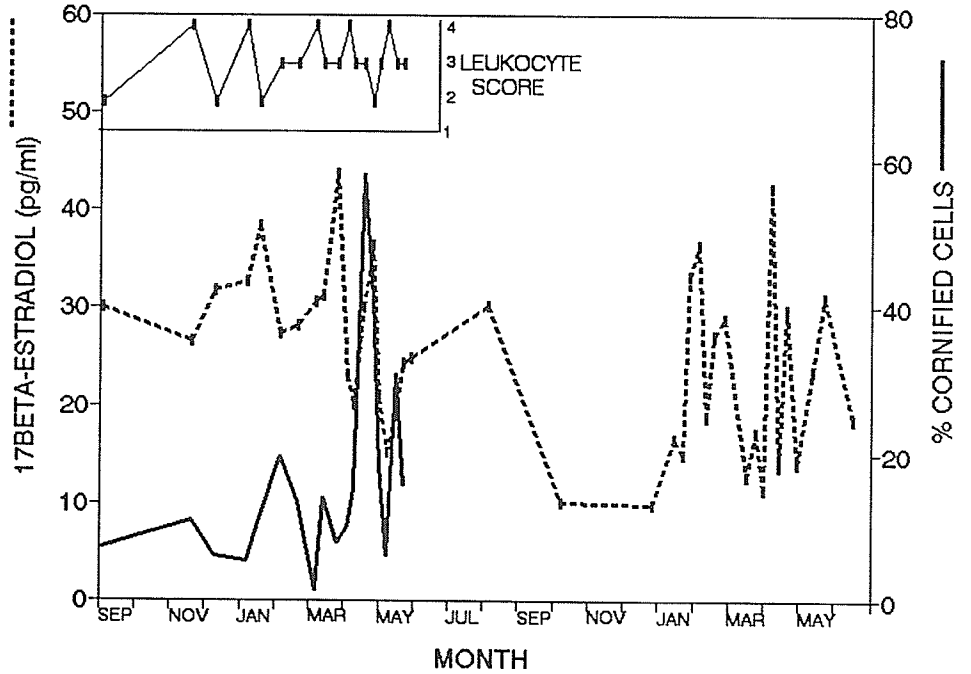
Appendix 2.a Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F1) over 2 years.



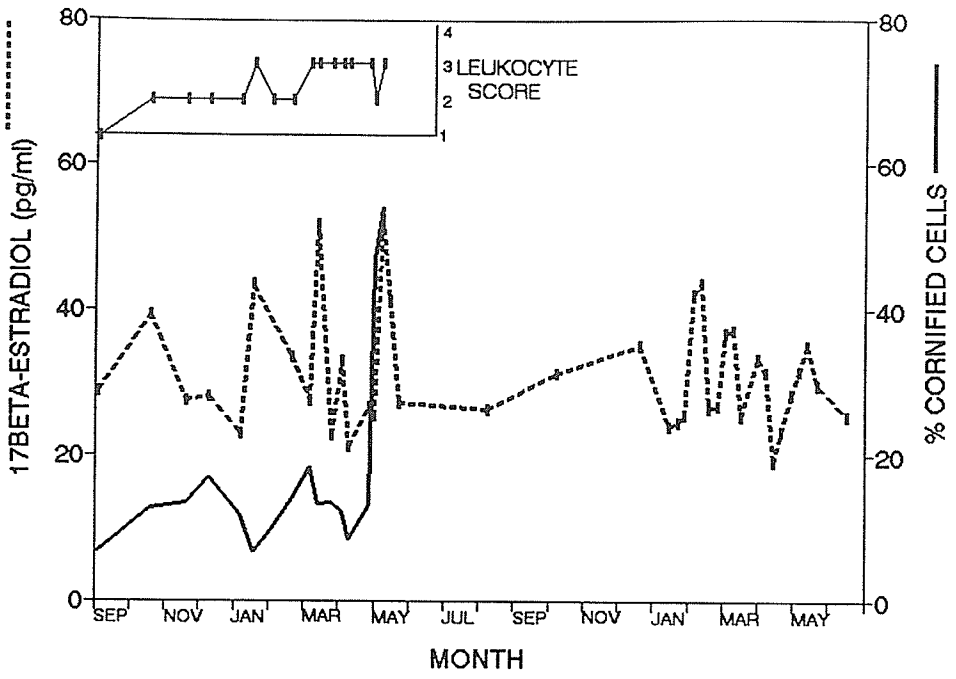
Appendix 2.b Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F2) over 2 years.



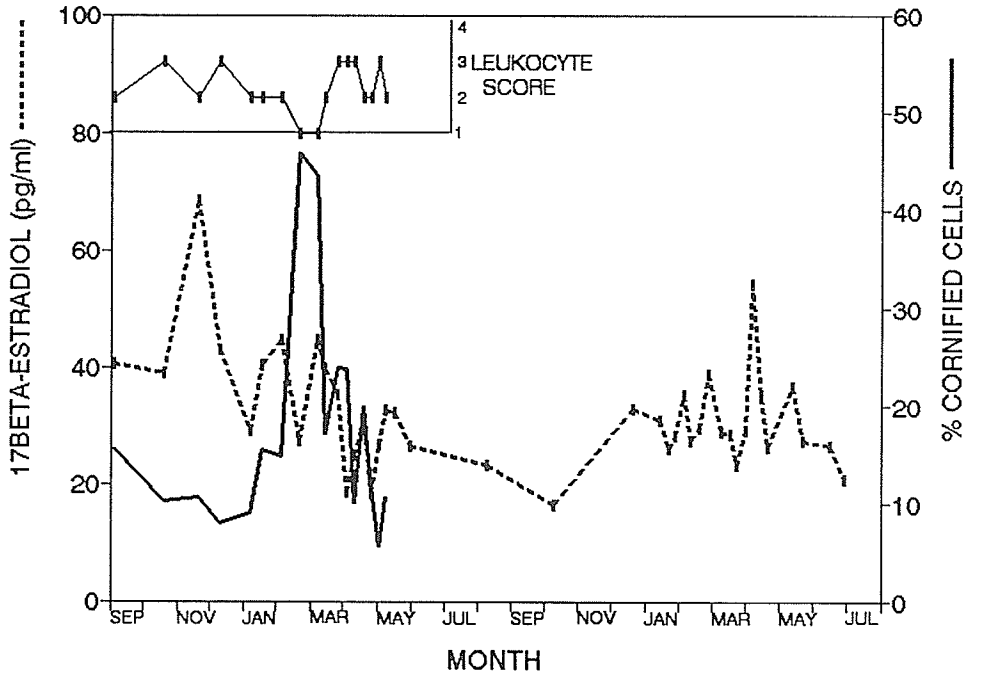
Appendix 2.c Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F3) over 2 years.



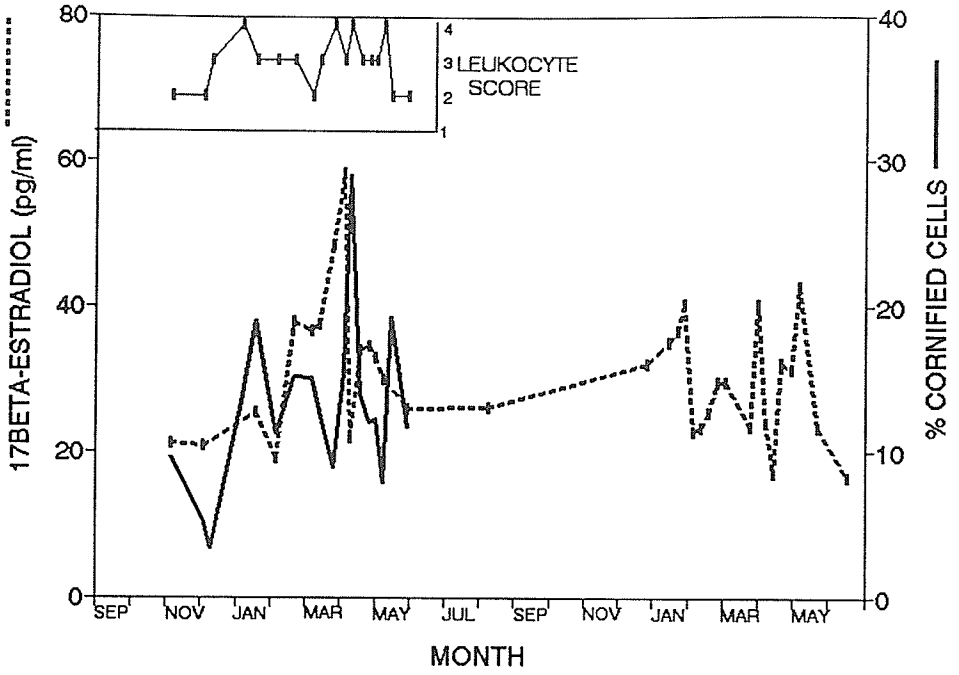
Appendix 2.d Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F4) over 2 years.



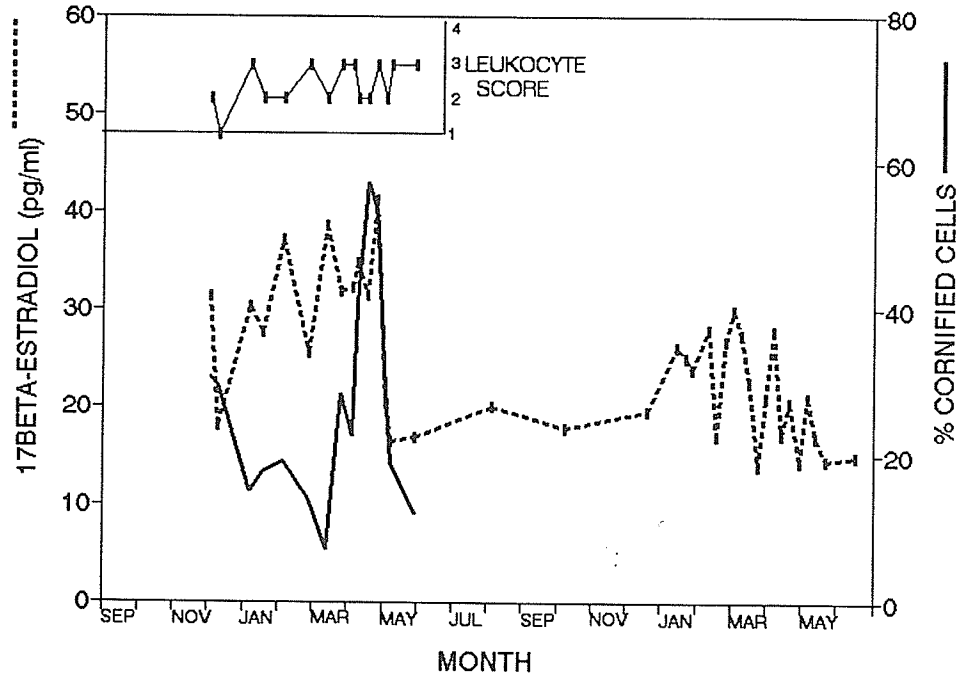
Appendix 2.e Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F5) over 2 years.



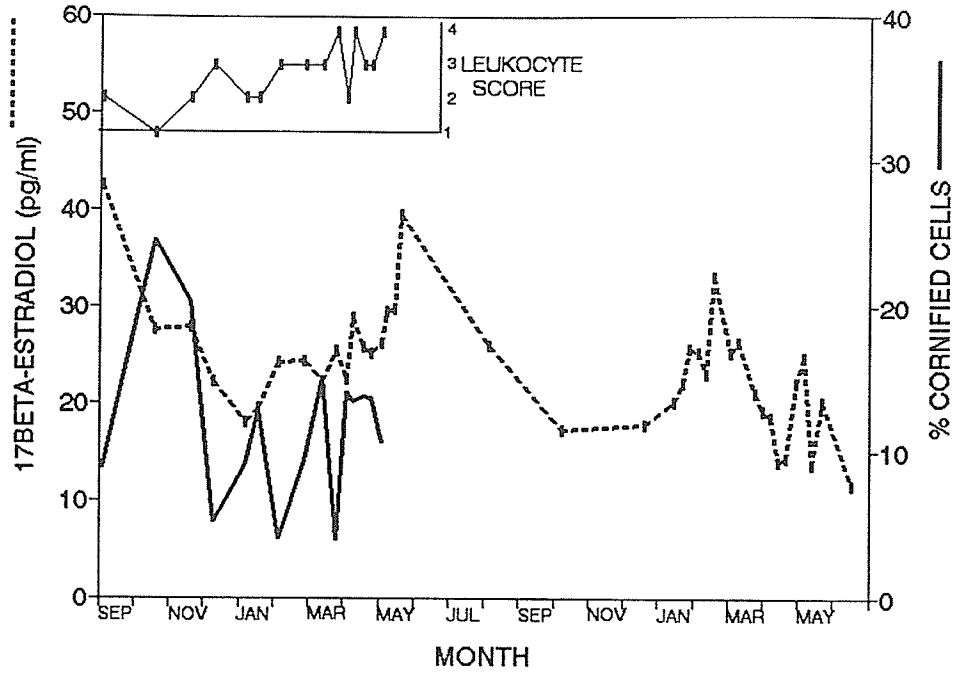
Appendix 2.f Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F6) over 2 years.



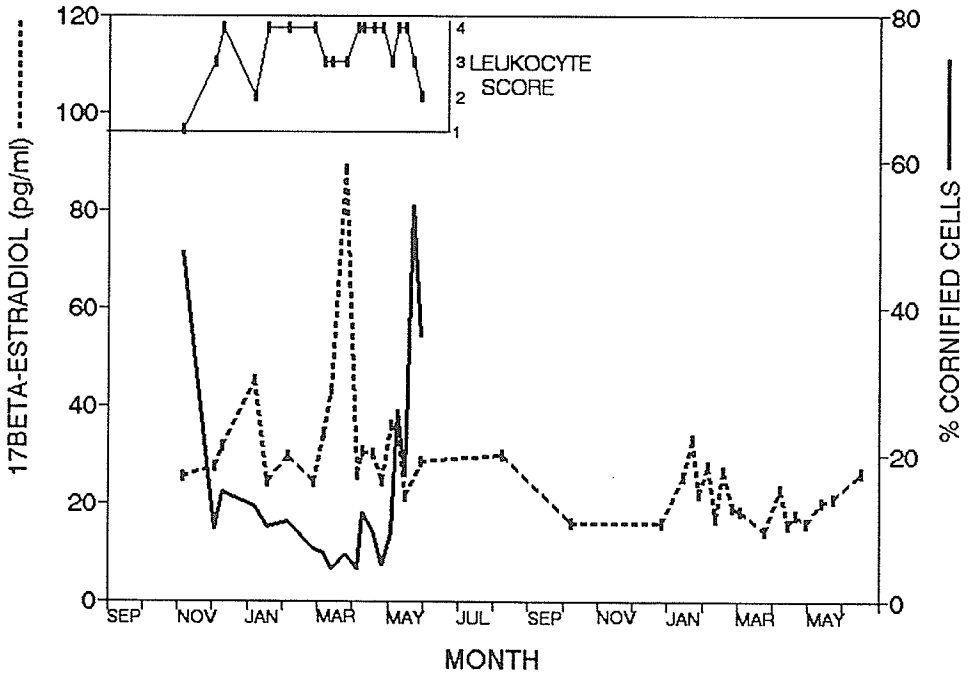
Appendix 2.g Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F7) over 2 years.



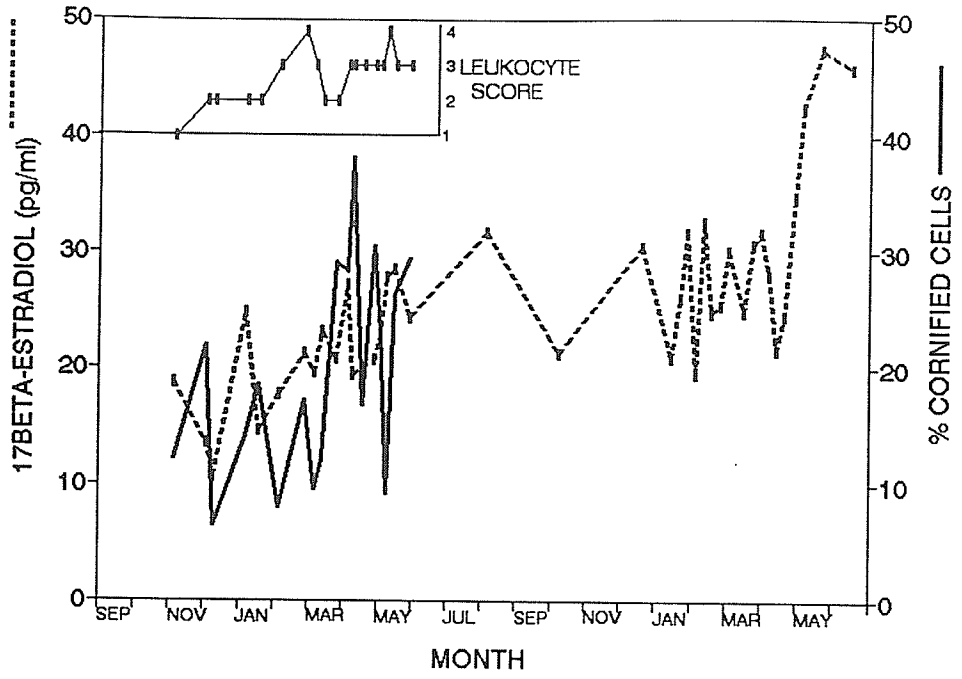
Appendix 2.h Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F8) over 2 years.



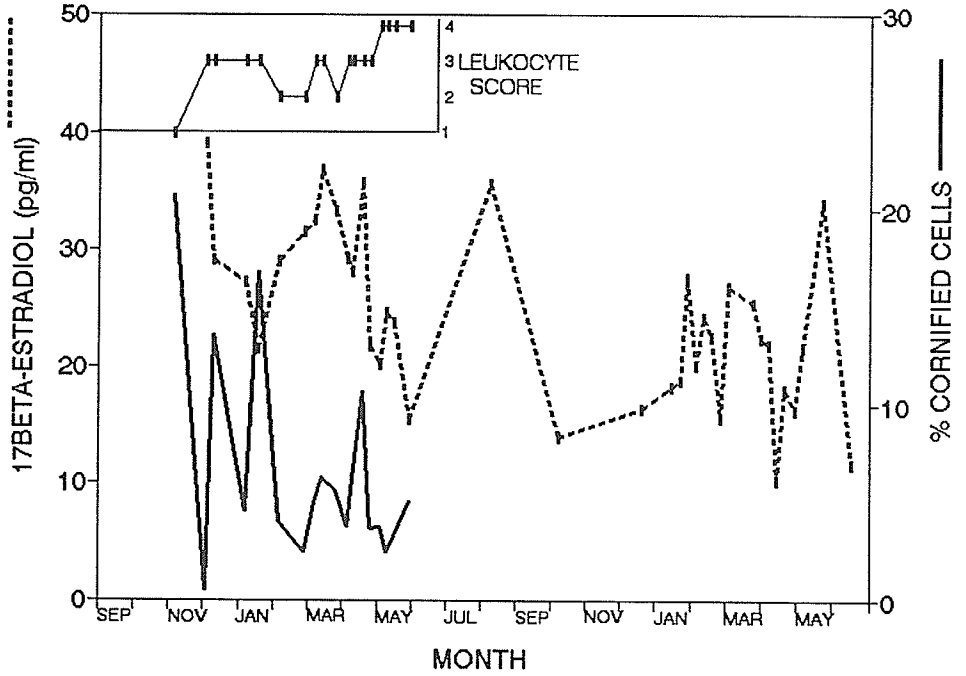
Appendix 2.i Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F9) over 2 years.



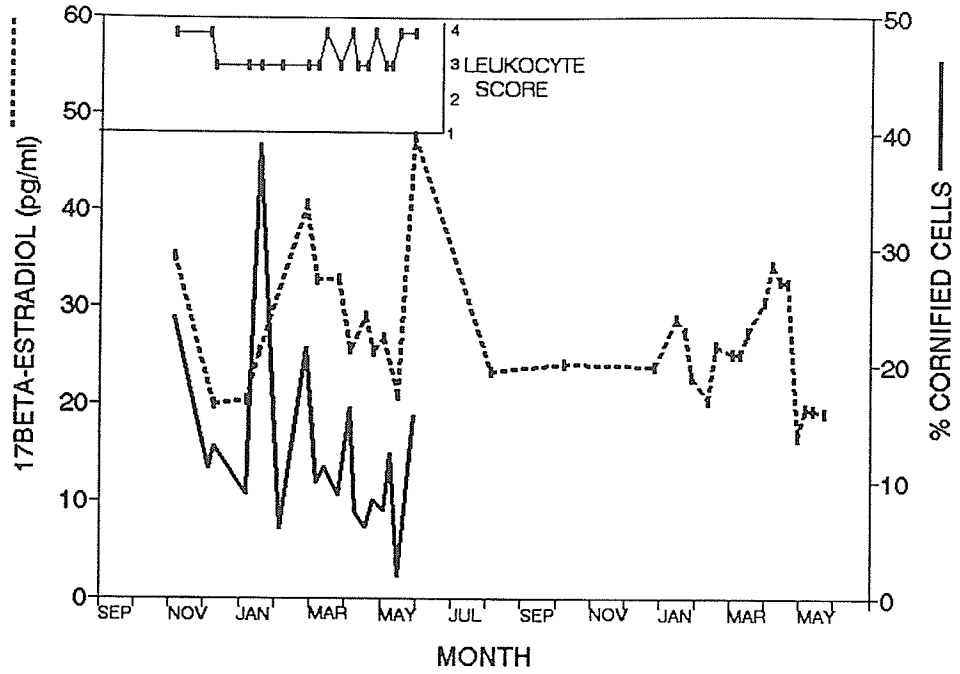
Appendix 2.j Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F10) over 2 years.



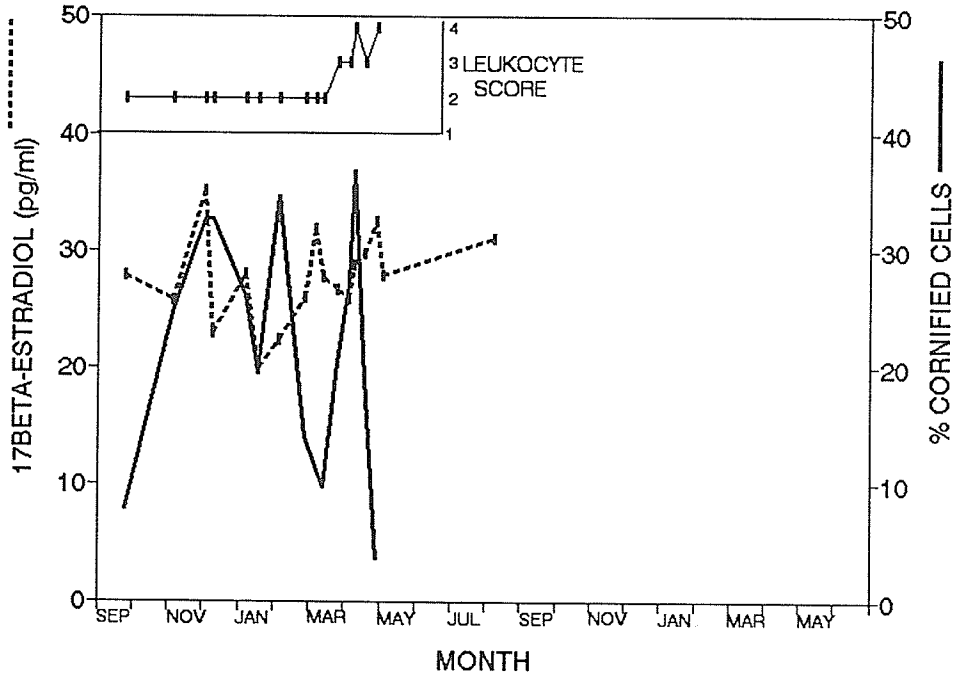
Appendix 2.k Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (F11) over 2 years.



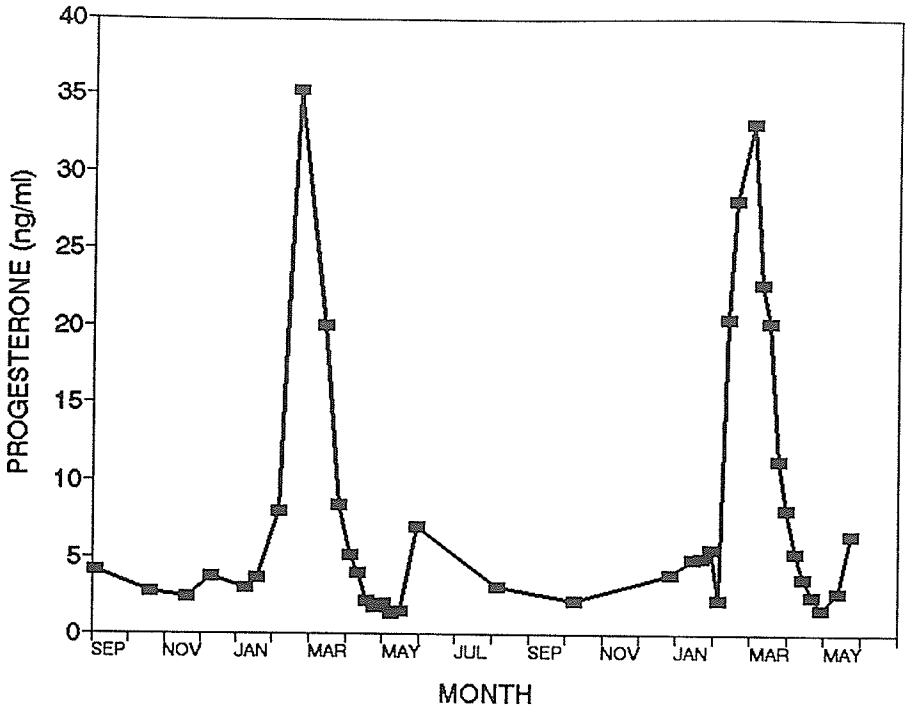
Appendix 2.l Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (Y88) over 2 years.



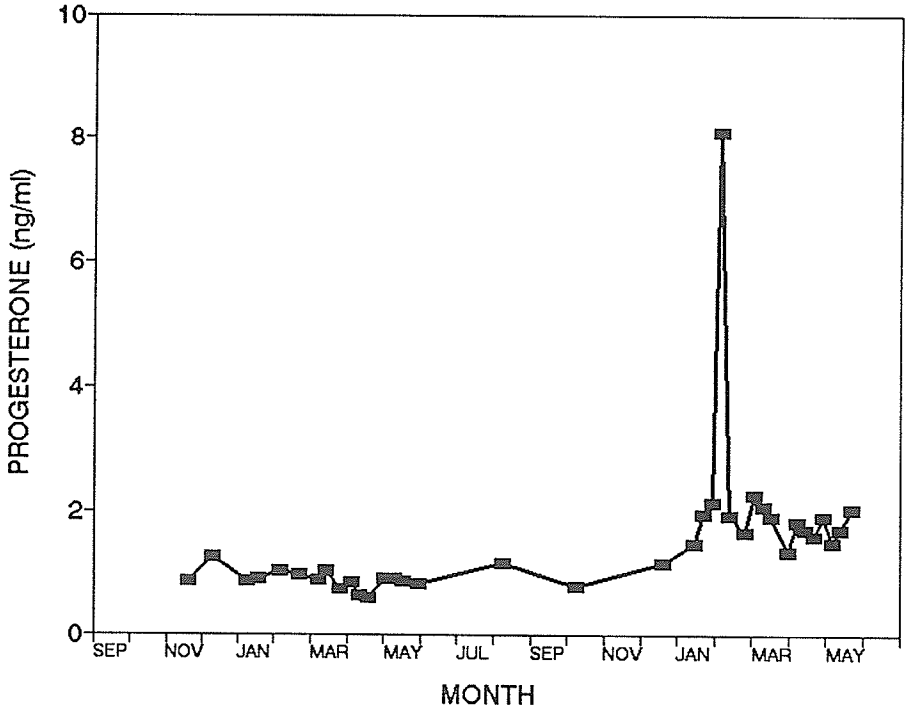
Appendix 2.m Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (BJ) over 2 years.



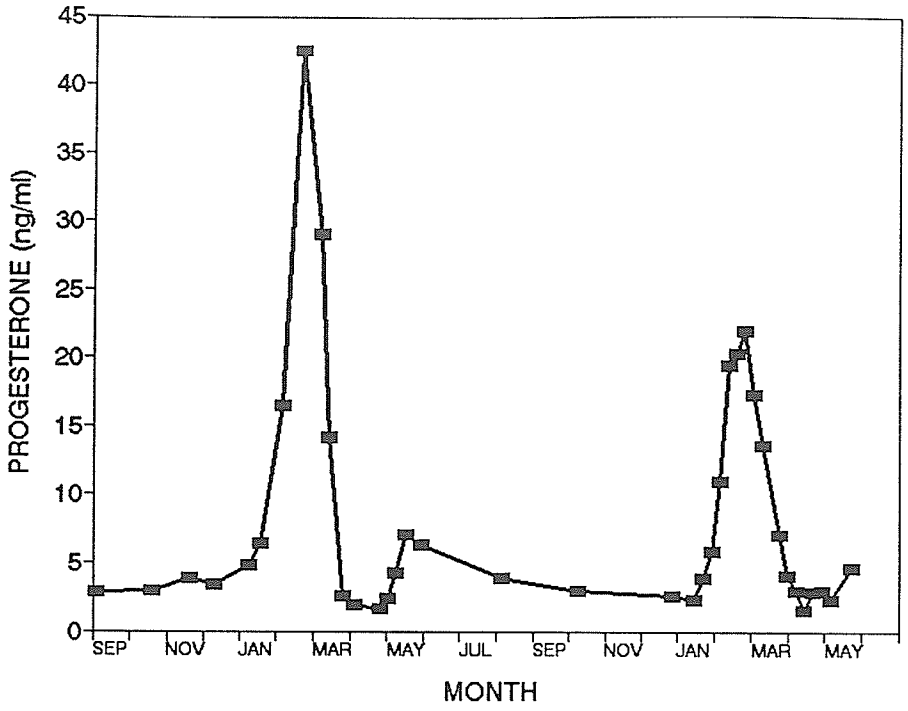
Appendix 2.n Profiles of 17ß-estradiol, % cornified epithelial cells and leukocyte scores of individual female fisher (Y89) over 1 year.



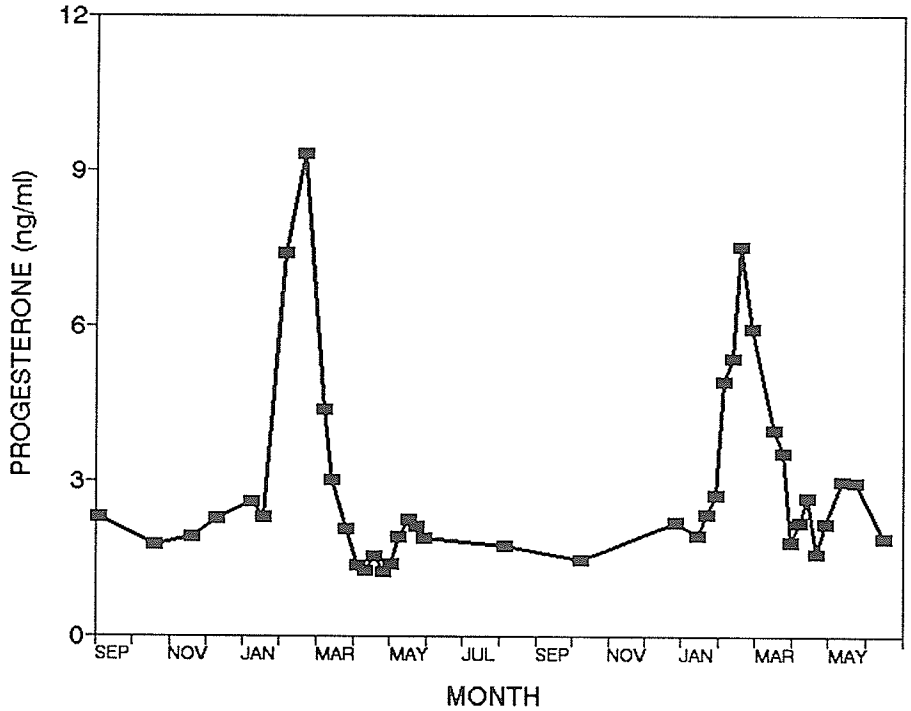
Appendix 3.a Individual progesterone profile of female fisher (F1) over 2 years.



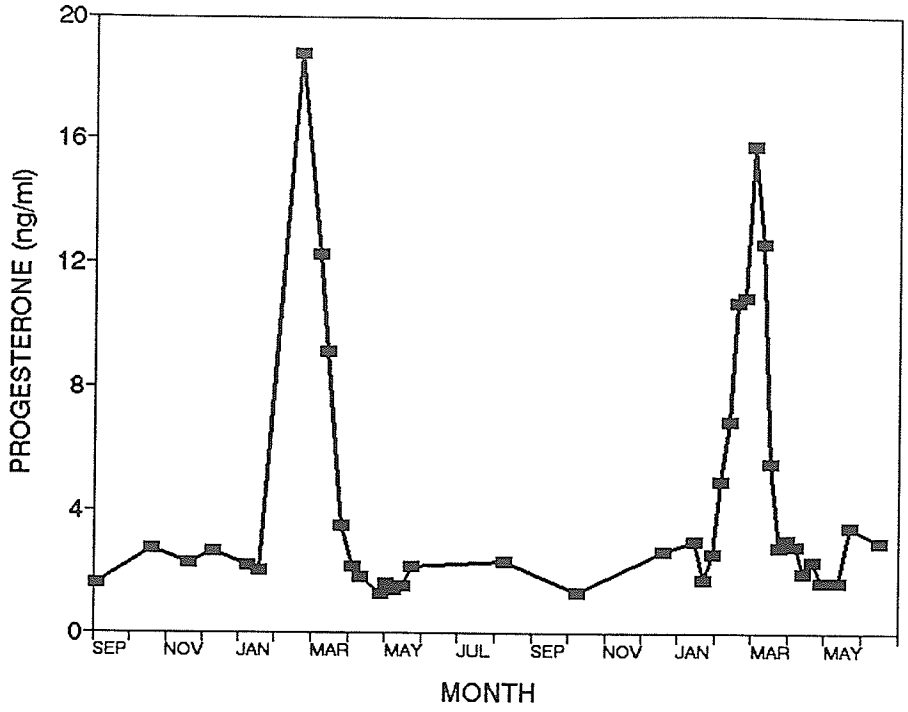
Appendix 3.b Individual progesterone profile of female fisher (F2) over 2 years.



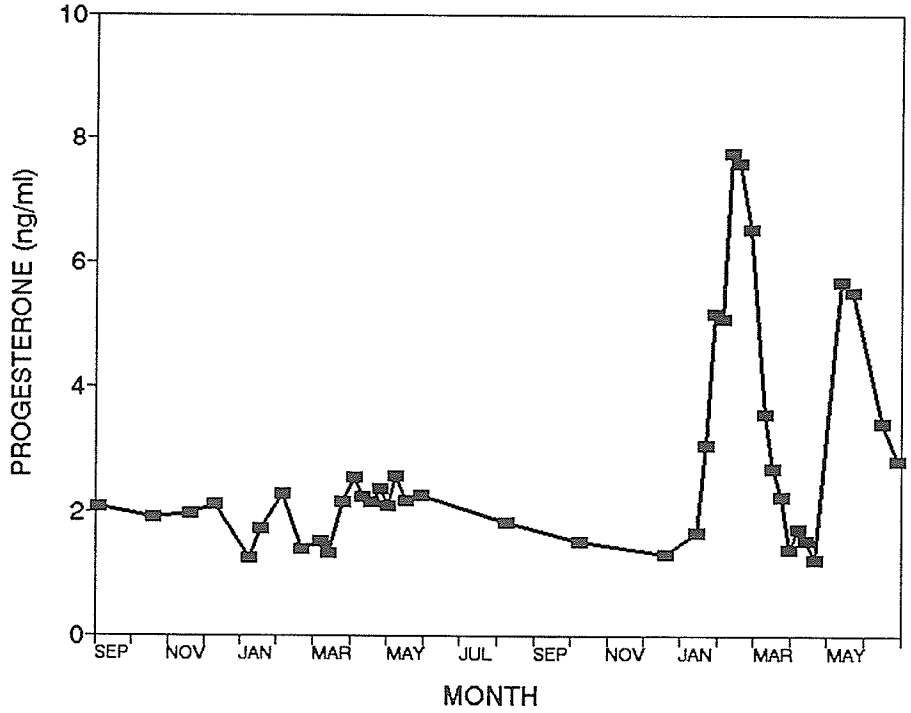
Appendix 3.c Individual progesterone profile of female fisher (F3) over 2 years.



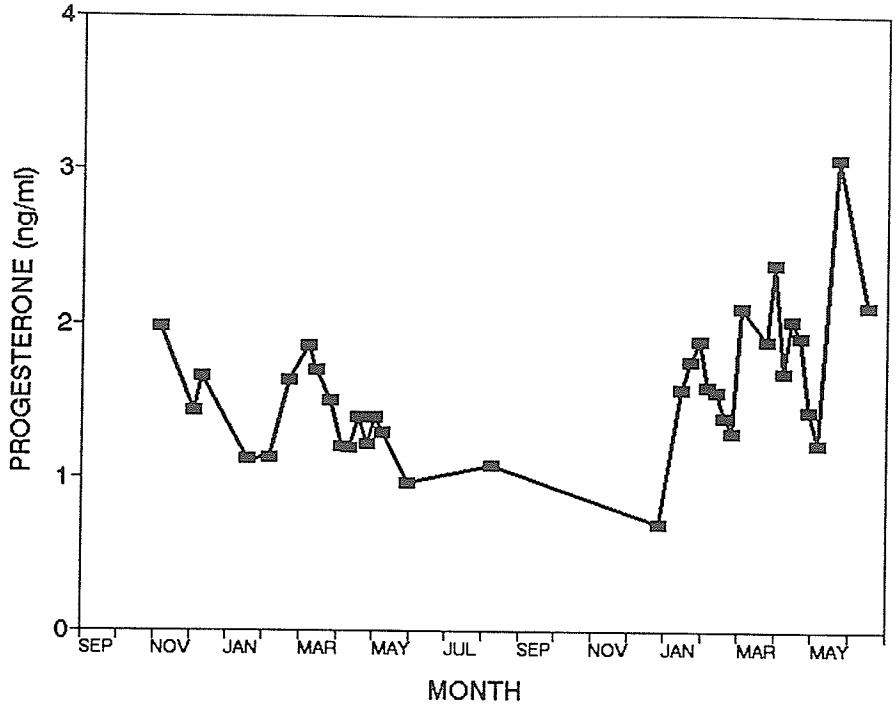
Appendix 3.d Individual progesterone profile of female fisher (F4) over 2 years.



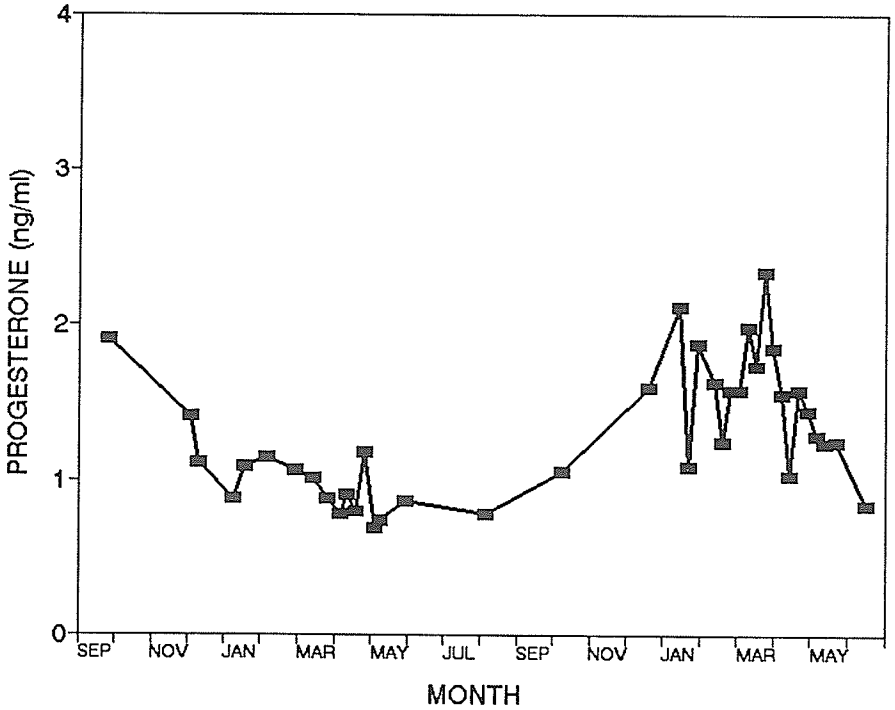
Appendix 3.e Individual progesterone profile of female fisher (F5) over 2 years.



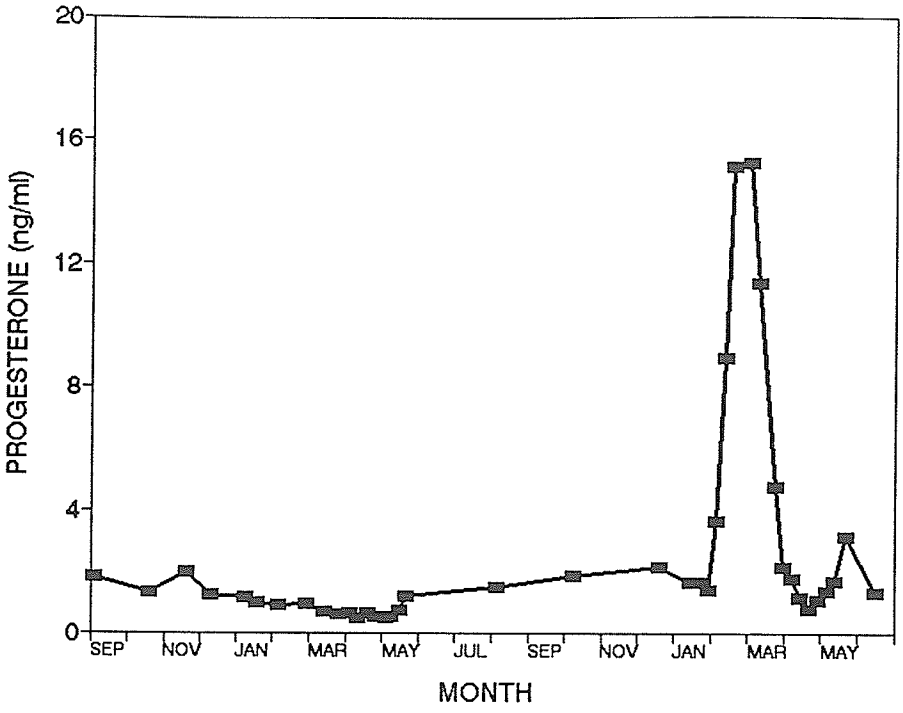
Appendix 3.f Individual progesterone profile of female fisher (F6) over 2 years.



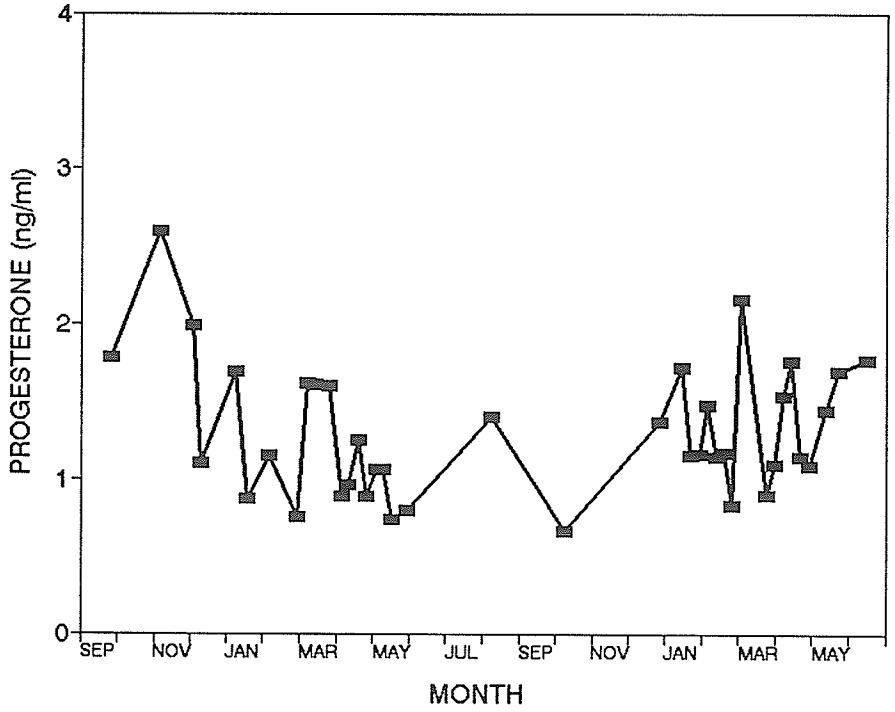
Appendix 3.g Individual progesterone profile of female fisher (F7) over 2 years.



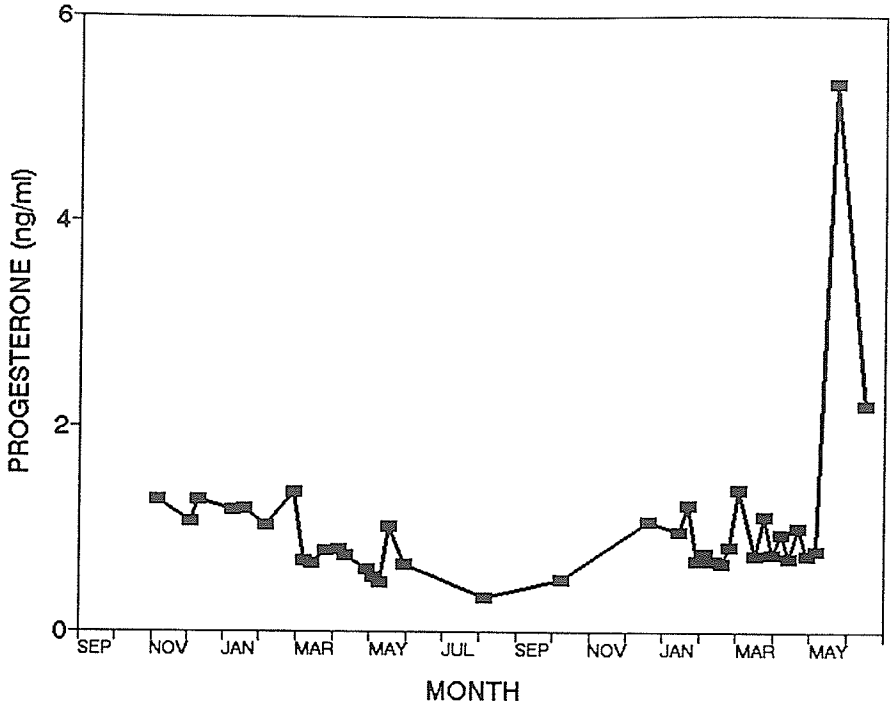
Appendix 3.h Individual progesterone profile of female fisher (F8) over 2 years.



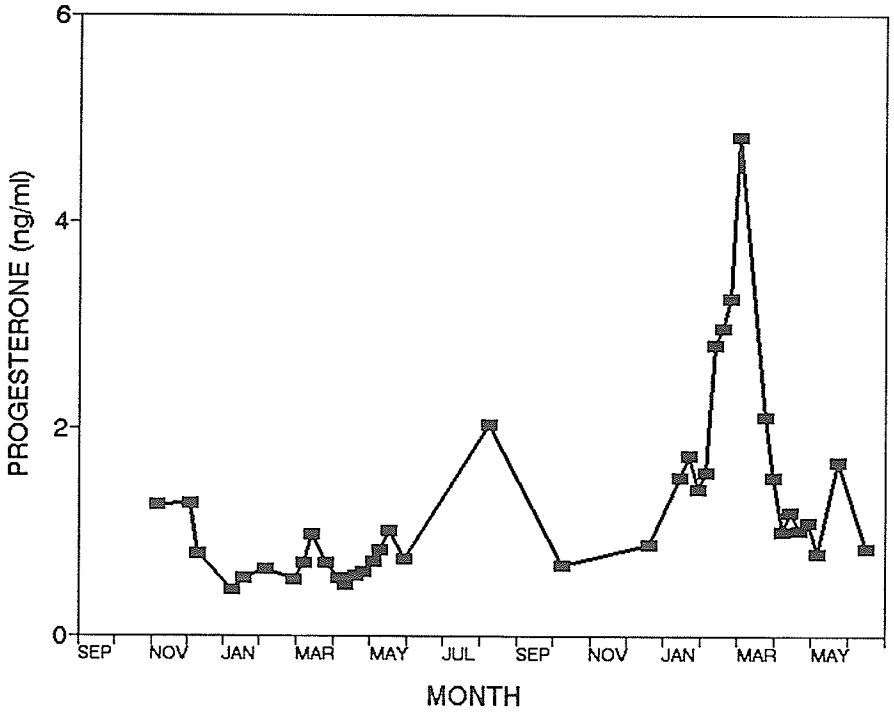
Appendix 3.i Individual progesterone profile of female fisher (F9) over 2 years.



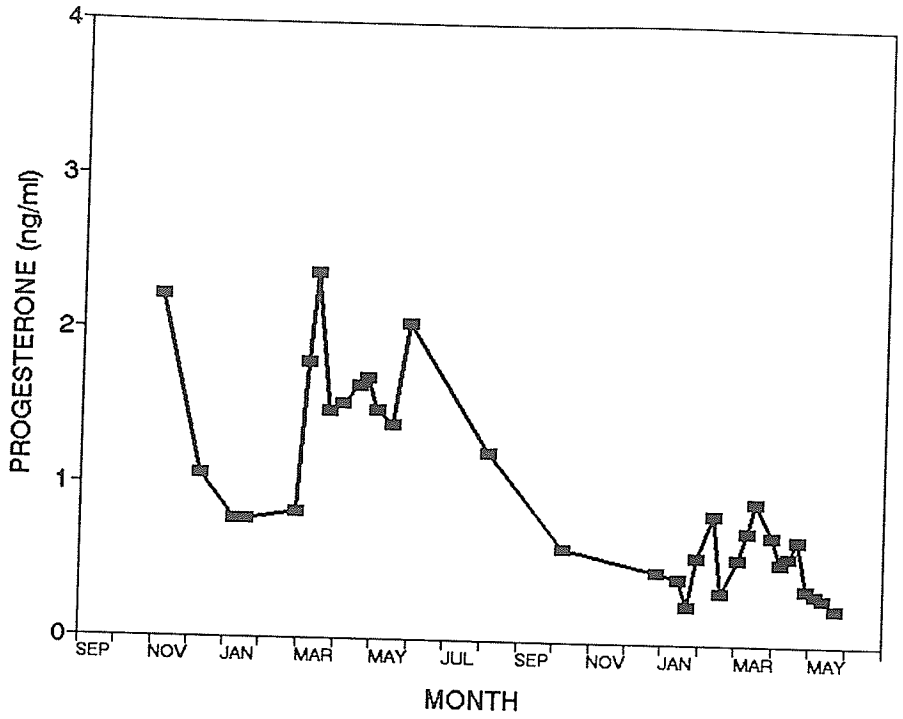
Appendix 3.j Individual progesterone profile of female fisher (F10) over 2 years.



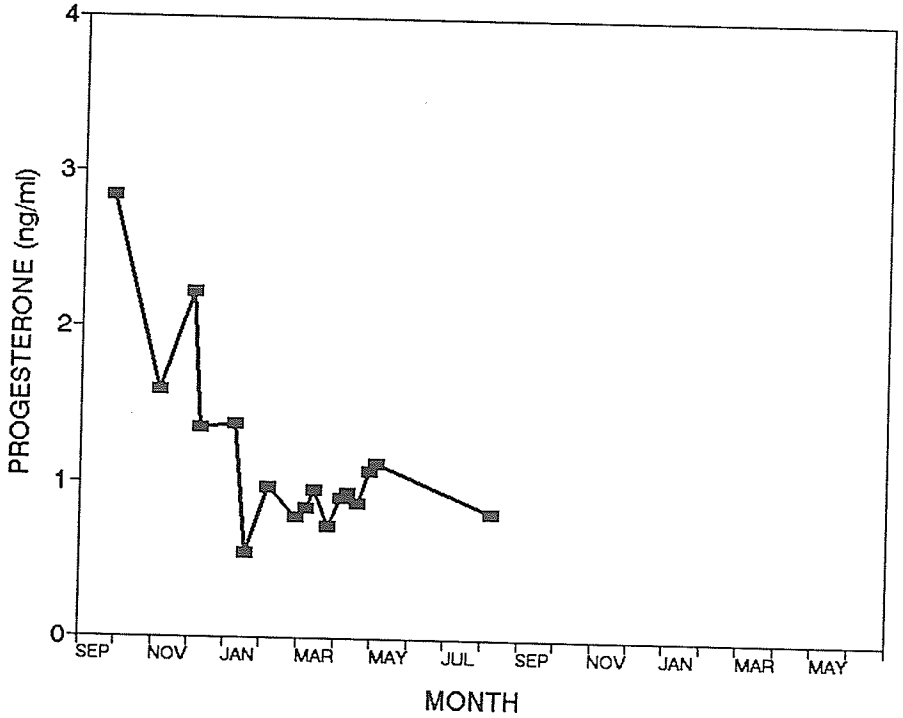
Appendix 3.k Individual progesterone profile of female fisher (F11) over 2 years.



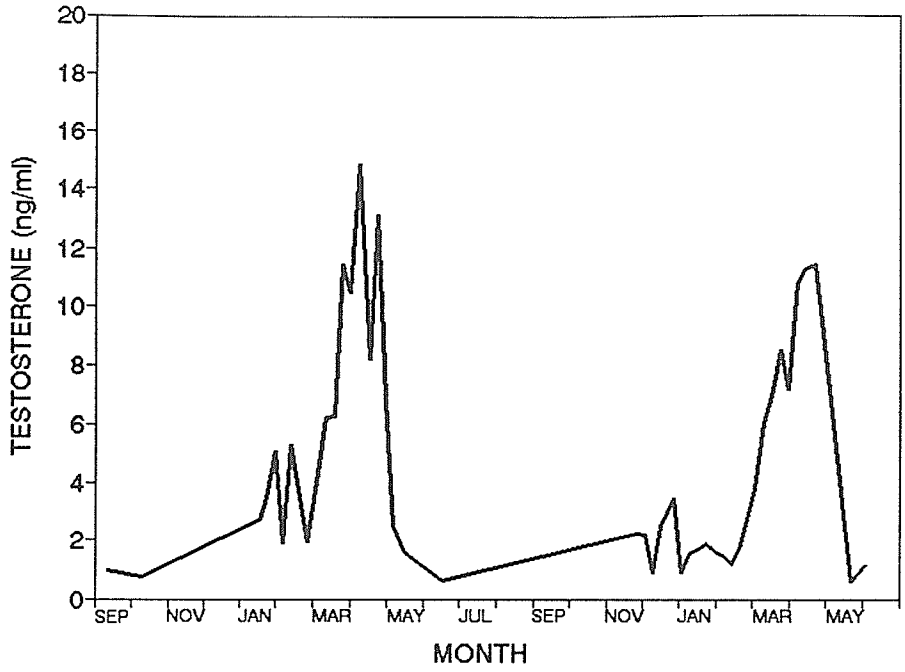
Appendix 3.l Individual progesterone profile of female fisher (Y88) over 2 years.



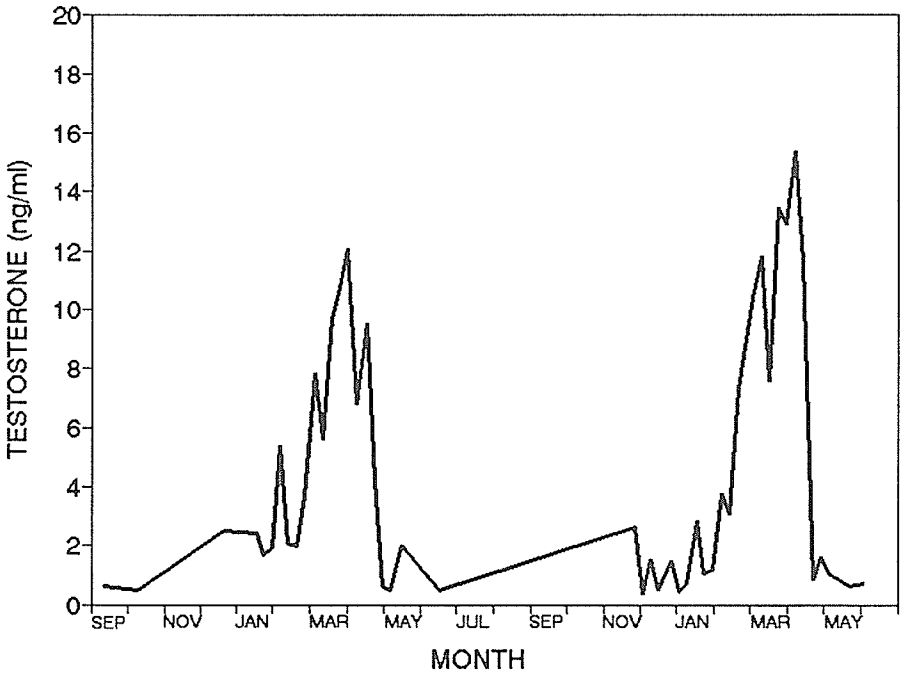
Appendix 3.m Individual progesterone profile of female fisher (BJ) over 2 years.



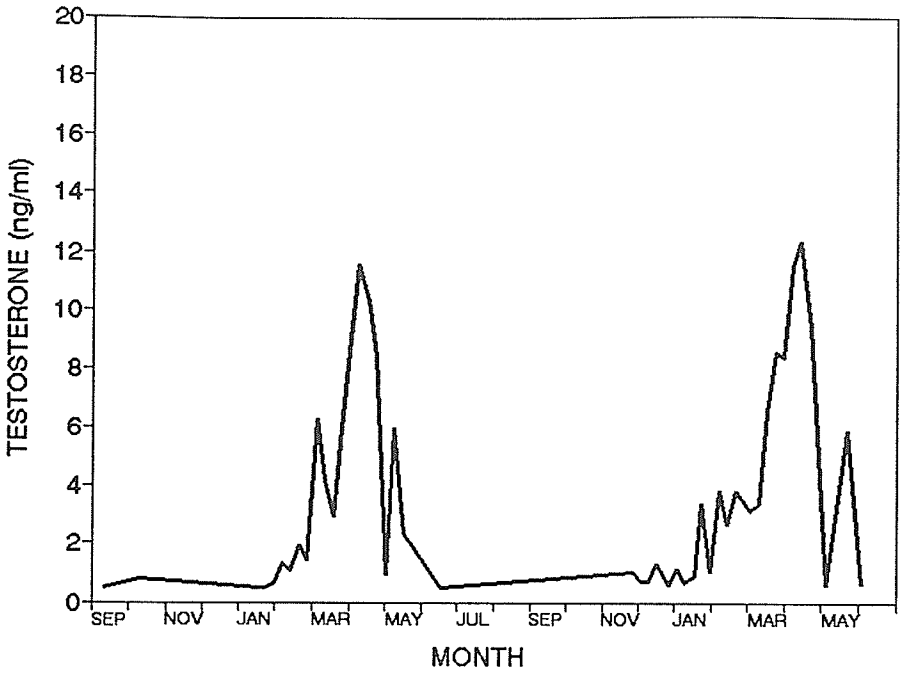
Appendix 3.n Individual progesterone profile of female fisher (Y89) over 1 year.



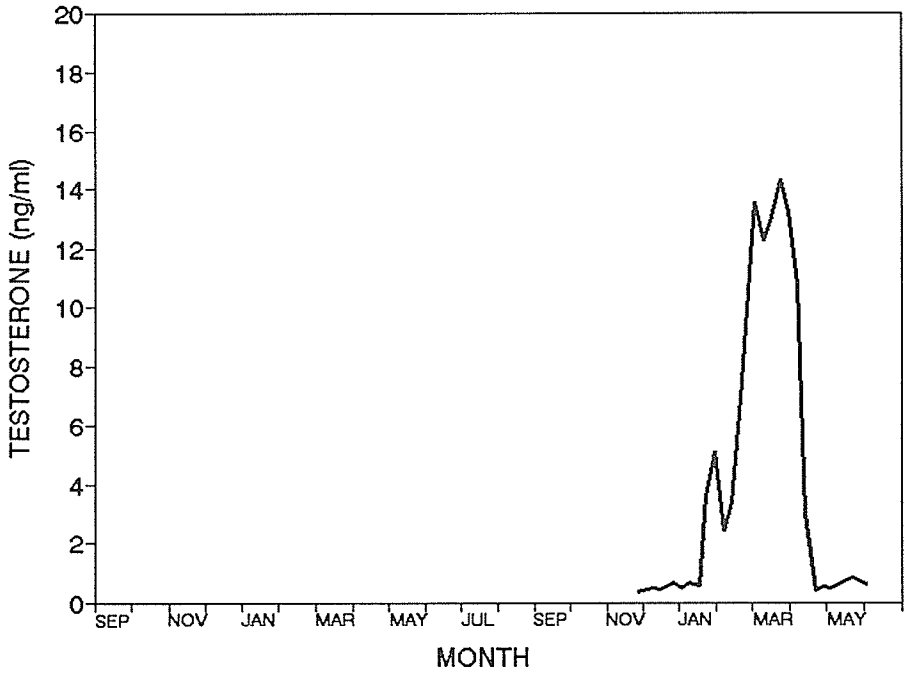
Appendix 4.a Individual testosterone profile of male fisher (Blondy) over 2 years.



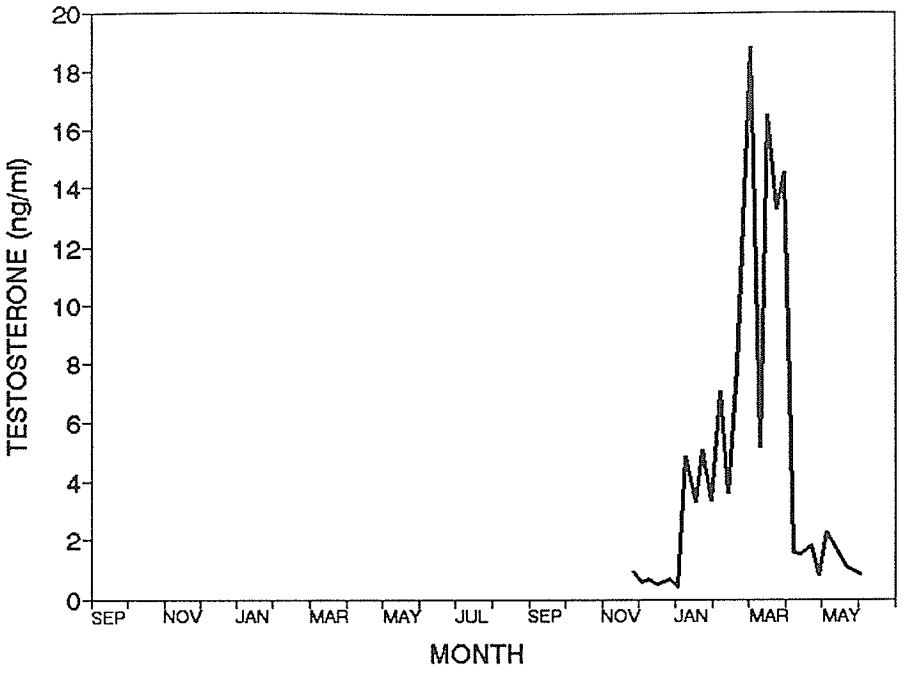
Appendix 4.b Individual testosterone profile of male fisher (Blacky) over 2 years.



Appendix 4.c Individual testosterone profile of male fisher (Junior) over 2 years.



Appendix 4.d Individual testosterone profile of male fisher (M1) over 1 year.



Appendix 4.e Individual testosterone profile of male fisher (M9) over 1 year.