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THE MEASUREMENT OF PLASMA PROGESTERONE LEVELS IN THE NORMAL MARE AND ITS APPLICATION TO SOME EQUINE BREEDING PROBLEMS

A thesis submitted in partial fulfilment
of the requirements for the degree of
Master of Veterinary Science
at Massey University

CHRISTOPHER MORTON KELLY
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A radioimmunoassay system was developed to measure plasma concentrations of endogenously produced and exogenously administered progesterone in non-pregnant and pregnant mares in normal and some abnormal reproductive states. Assay sensitivity was 0.5 ng/ml, with a between assay coefficient of variation of 16.8% for a high progesterone sample, estimated over 24 assays, and 8.5% for a low progesterone sample, estimated over 15 assays; the within assay coefficient of variation was 7.3, 10.1 and 6.9% respectively for six replicates of one sample, estimated in three separate assays.

Plasma progesterone concentrations of six normal non-pregnant cycling mares followed regular cyclic changes, with levels less than 0.5 ng/ml during oestrus and ranging between 8-22 ng/ml at peak values in dioestrus. The first oestrus following the winter non-breeding period was longer than the following oestrus and the period from ovulation to progesterone decline tended to be less variable than the rest of the cycle.

There was a large between-mare variation in plasma progesterone concentrations in mares at both early and late stages of gestation with levels varying from 4.9 to 15 ng/ml in the former and 5.2 to 16.9 ng/ml in the latter group. No significant effect was noted between stage of gestation and progesterone concentration.

A group of five mares all had plasma progesterone concentrations greater than 9.5 ng/ml within 24 hours prior to parturition; two of these mares sampled within eight hours prior to parturition had plasma progesterone concentrations of 4.3 and 3.9 ng/ml. The first *post partum* sample was taken within 24 hours of foaling; by this time plasma progesterone concentrations had fallen to less than 0.5 ng/ml and remained low until sampling ceased at the first *post partum* oestrus.

Prostaglandin F2α (THAM salt) was effective in causing luteolysis in 13 mares with active corpora lutea before treatment. By three days post-injection 12 of the 13 mares had plasma progesterone concentrations of less than 0.5 ng/ml and by five days post-injection 12 of the 13 mares were exhibiting oestrus. Of the ten mares bred at the induced oestrus, seven became pregnant to that mating.

Plasma progesterone concentrations were measured on 16 non-pregnant mares in anoestrus. Six of eight mares sampled early in the breeding season (September) had plasma progesterone concentrations of less than 0.5 ng/ml, the other two mares had plasma progesterone concentrations of 0.6 ng/ml. Eight of eight mares sampled later in the breeding season (November and December) had plasma progesterone concentrations greater than 0.5 ng/ml, the levels ranging from 6.2 to 13.1 ng/ml.

Concentrations of plasma progesterone in normal dioestrous mares were measured half and one hourly (three mares) for 24 hours and four hourly (two mares) for 120 hours. There were large apparently random variations, with more than 100% differences being recorded between a number of consecutive samples. Plasma progesterone concentrations varied from 7.8-23.0, 3.2-21.9 and 4.2-12.9 ng/ml for the three mares sampled half hourly and hourly, and from 6.8-24.6 and 0.8-11.0 ng/ml for the two mares sampled four hourly.

Radioactive progesterone, administered by venepuncture to a mare with no detectable endogenous plasma progesterone, disappeared from the plasma within 40 minutes; 85% of the injected steroid had left the plasma by 2.5 minutes post-injection.

Two and 25 mg of progesterone in 16% alcohol in saline was administered by venepuncture to mares with plasma progesterone concentrations of less than 0.5 ng/ml. For the mare given 2 mg, the plasma half life of injected progesterone was 1.75 minutes for the initial "fast" component, and for the mare given 25 mg the plasma half life was 2.75 minutes. There was a second peak of plasma progesterone at from 8 to 19.5 minutes for the former and from 9.5 to 17 minutes for the latter mare. A third much smaller peak was recorded at about 50 minutes post-injection for the mare given 2 mg progesterone.

A mare with no detectable endogenous plasma progesterone was administered by intramusuclar injection a total of 600 mg progesterone

in arachis oil over a period of seven days. Plasma concentrations of the steroid reached a maximum of 4.3 ng/ml at one day post-treatment and were maintained at this level for only a maximum of 24 hours. A second mare, again with no detectable plasma progesterone, was administered by intramuscular injection a total dose of 2 g of hydroxyprogesterone capronate in castor oil over a period of ten days. Maximum plasma progesterone concentrations of 1.2 ng/ml, maintained for less than 24 hours, were reached nine days after the first injection.

Wide variation in plasma progesterone levels within and between mares over relatively short time periods suggest that there are many difficulties in identifying "progesterone insufficiency" as a cause of embryonic absorption or abortion in this species. Moreover the short half life of this steroid in the plasma of the mare, together with the sustained high dose levels that would be required to elevate plasma concentrations of progesterone to a level equivalent to that produced by normal secretory corpora lutea, indicate that current levels of administration of this drug are likely to have little effect in overcoming such a breeding problem unless the progesterone is acting at a local level. A definitive answer in respect to this vexed question concerning the existence or not of "progesterone insufficiency" as a cause of prenatal loss in the mare, together with an appropriate method of treatment, still remains to be found.

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"THE MEASUREMENT OF PLASMA PROGESTERONE LEVELS IN THE NORMAL MARE AND ITS APPLICATION TO SOME EQUINE BREEDING PROBLEMS"

INTRODUCTION

The progestogens are a group of steroid hormones, the most important of which is progesterone (Jones, 1968). These compounds are largely produced and secreted by the corpus luteum and in many species, the placenta (McDonald, 1975), although small quantities are produced by the adrenal cortex (Williams, 1968). Together with other hormones associated with reproduction, progesterone plays an integral part in regulating the oestrous cycle and development and growth of the mammary gland; the most dramatic role of this steroid however is in the maintenance of pregnancy (McDonald, 1975).

The development of new ultramicro measurement techniques has enabled research workers to accurately measure most hormones associated with reproduction, including progesterone in the plasma of mares (Evans and Irvine, 1975).

The purpose of the study reported in this thesis was to develop a radioimmunoassay technique for detecting plasma progesterone and to apply it to the measurement of this steroid in mares in both normal and abnormal reproductive states. Plasma progesterone concentrations for these mares, together with the effects of administration of an exogenous source of luteolysin in selected cases, are described in the first two experimental sections of the thesis.

During the later phases of the investigations attention was directed to establishing the pattern of variation in plasma progesterone concentration observed throughout 24 and 120 hour sampling periods with individual mares in dioestrus since an appreciation of such variation was essential to any interpretation that could be placed on the measurement of plasma progesterone *per se*. In the concluding stage of the study efforts were made to

measure the half life of exogenously administered progesterone and to determine the plasma concentrations that developed following repeated administration by injection of this steroid.

As a result of these experiments an attempt has been made to rationalise the diagnosis and treatment of 'progesterone deficiency' as a cause of prenatal loss in the mare.

LITERATURE REVIEW

A. ENDOCRINE CONTROL OF REPRODUCTION IN THE MARE

It has been said of the mare that "the only consistent thing about her reproductive performance is its inconsistency". Whether that statement is true or not, in very recent years, following the development and use of microanalytical techniques, it has become clear that endocrine control of reproduction in the mare is much less simple than was originally thought.

Most of the steroid and protein hormones involved in reproduction of the mare have now been measured in various tissues and fluids. This has enabled research workers to characterise hormone secretion patterns in the cycling and pregnant mare. Currently research is proceeding in an effort to elucidate answers to questions raised by results of reproductive hormone analyses carried out on blood plasma.

1. The Oestrous Cycle

a. Puberty

Since most mares are seasonally polyoestrous, onset of puberty occurs during the spring and summer. Fillies generally come into oestrus for the first time in the spring following 16 to 17 months of age (Nishikawa, 1959; McDonald, 1975). Because of variations in birth dates in relation to the spring period some fillies will not show first oestrus until 22 to 26 months of age. Of the many factors that influence the onset of puberty, the level of nutrition and inherited characteristics appear to be most important (Roberts, 1971).

b. Hypothalamus

Hypothalamic extracts, when incubated with the pituitary of many species, have been shown to cause luteinizing hormone (LH) and follicle stimulating hormone (FSH) release (Lamming, 1973). On the basis of these and similar experiments it was thought that there were two so called "releasing factors" produced in the

hypothalamus, one for LH and another for FSH. Shally, Arimura, Baba, Nair, Natsuo, Redding and Debeljuk (1971) however, postulated that only one releasing factor exists and characterised it as having a decapeptide structure. With this substance they were able to stimulate LH and FSH release from porcine pituitaries, both in vivo and in vitro.

This decapeptide elevates plasma LH and FSH concentrations when given to beef heifers (Kaltenbach, Dunn, Kisen, Corah, Akbar and Niswender, 1974) and Ginther and Wentworth (1974) have shown that administration of a single intravenous injection of 400 µg of gonadotrophin releasing hormone (GnRH) gives a transient rise in plasma LH in both anoestrous mares and mares on day two of oestrus. The response was temporary and did not alter onset of oestrus in the former group, or ovulation time in the latter group. FSH was not measured. Subsequently, Garcia and Ginther (1975) were able to cause a sustained increase in plasma LH with 2.37 µg/kg of GnRH infused over a 24 hour period. They also found that the decapeptide did not alter ovulation time.

W.R. Allen (pers.comm.) and Heinze and Klug (1975) on the other hand consider this substance is a potent ovulator, particularly where follicles are undersized. Moreover, Irvine, Downey, Parker and Sullivan (1975) were able to shorten the duration of oestrus and the time from onset of oestrus until ovulation in mares by administration of 1 mg GnRH on day two of oestrus.

Evans and Irvine (1976) have been able to detect plasma levels of FSH 3.7 times the pretreatment levels after administration of 1 mg GnRH to anoestrous mares. These workers also detected a rise in plasma LH after GnRH administration not unlike that seen in the normal cycling mare and they suggest this supports the theory of the existence of a single hypothalamic releasing hormone.

Of the factors influencing initial release of GnRH from the hypothalamus, changes in the external environment are the most important. It is well known that the equine hypothalamus responds to increasing light and that maximum

ovarian activity is usually a few weeks after the longest day in the year (Osborne, 1966).

The onset of oestrus in the spring may be accelerated with the use of artificial light (Burkhardt, 1947; Sharp, Kooistra and Ginther, 1975) by as much as 3½ months (Nishikawa, 1959). Local irradiation with ultra-violet light is ineffective (Burkhardt, 1947), while blinding mares to block natural daylight increase will delay oestrus (Nishikawa, 1959) indicating that the effect is truly a photoperiodic one. Loy (1967) suggested the photoperiod may regulate cycles by governing or regulating an already existing, inherent although crude annual rhythm, or it may actually initiate a completely new rhythm. He indicated that there was some evidence that the former is the case in the ewe and mare. The induced cycles are fertile (Loy, 1968).

The level of nutrition affects reproduction (Day, 1939; Pashen and Allen, 1976) possibly via the hypothalamus. Van Niekerk and Van Heerden (1972) showed that only 25% of a group of mares kept at a constant weight ovulated in a given time during the spring while 100% of the same number of mares fed supplementary feed to produce increases in weight (a rising nutritional plane) ovulated during the same period of time. Environmental temperature may also affect onset of oestrus in the spring (Roberts, 1971). It would appear likely that these environmental factors mediate their effect via the release of GnRH from the hypothalamus.

The onset of anoestrus in the autumn may be delayed by boosting falling daylight hours using artificial lighting (Nishikawa, 1959), so presumably those factors responsible for initiation of GnRH release, acting in reverse, are also responsible for GnRH suppression in autumn as mares pass into anoestrus.

c. Pituitary

That there is a definite relationship between the pituitary and ovary has been known for many years and the anterior lobe or adenohypophysis is a major endocrine area controlling the reproductive organs. The two

pituitary gonadotrophins LH and FSH are water soluble glycoproteins with a molecular weight of approximately 30,000, and produced by the basophilic cells; a third reproductive hormone prolactin, is secreted by specific lactotrophic cells in the rat at least (Williams, 1968). Prolactin is luteotrophic in rats (McDonald, 1975) and possibly sheep (Denamur, 1974), but whether this hormone is luteotrophic or even a gonadotrophin in the horse is not known.

Hellbaun (1933) demonstrated by injection of pituitary extract into 21 to 23 day old rats and weighing the ovaries that equine pituitaries have about a four fold greater gonadotrophic activity than sheep pituitaries. He also demonstrated that equine pituitaries had more FSH like activity than sheep pituitaries; the latter possessed a predominantly LH like action.

i. Follicle Stimulating Hormone

Classically, FSH is thought to cause growth of graafian follicles and oestrogen production by those follicles (Roberts, 1971). While the former is probably true, whether FSH by itself will cause oestrogen production is open to question, since in hypophysectomized females FSH causes multiple follicular growth without oestrogen production, and many workers believe that this gonadotrophin alone cannot cause oestrogen production in the intact female (Williams, 1968; McDonald, 1969). In the mare, there is a time lag of some eight days between the mid-dioestrous FSH surge (with follicle development) and a rise in plasma oestradiol 17β concentrations (Evans, 1976).

Evans and Irvine (1975) measured FSH throughout the oestrous cycle and early pregnancy and found that FSH concentrations were raised five fold by "surges" rather than "spikes"; these "surges" recurred at 10 to 11 day intervals during the cycle and early pregnancy. The late oestrous - early dioestrous surge of FSH appeared to initiate development of up to twenty follicles while the mid-dioestrous surge was considered important for the further development of follicles likely to ovulate

10 to 13 days later. FSH together with LH may also play a role in the final maturation of the graafian follicle(s) destined to ovulate.

The sensitivity of the mare to FSH has yet to be determined. Certainly, attempts to stimulate follicular growth with pregnant mare serum gonadotrophin (PMSG) have generally been unrewarding (Burkhardt, 1947; Nishikawa, 1959; McDonald, 1969; Baier, Berchthold and Brummer, 1973) even in dose rates in excess of those needed to superovulate cattle, sheep (Roberts, 1971) and does (Nishikawa, 1959). PMSG, however, probably has an LH like action in mares (Irvine, 1976) rather than the FSH like action observed in other species.

Douglas, Nuti and Ginther (1975) using purified equine pituitary extracts and not PMSG were able to induce multiple ovulation in five of eight mares without affecting the duration of oestrus.

Follicular growth appears to be independent of gonadotrophin stimulation until the stage at which the primary oocyte attains its mature size and is associated with early antral formation. Thus, while hypophysectomy causes ovarian atrophy, follicles develop to the antral stage (Williams, 1968). The exact mechanisms governing the maturation of a select number of follicles, while the vast majority become atretic, are not understood. Nalbandov (1953) explained the process as being based on hormone dilution rather than the more common theory of oestrogen inhibition of FSH, stating that as the follicles increase in size there is insufficient FSH to maintain all of them, hence most become atretic. In superovulation studies on sheep and cattle, it is thought that the amount of FSH released is closely related to the number of follicles that ovulate (Roberts, 1971), indicating gonadotrophin control is probably a major factor in this mechanism.

As mentioned earlier, LH and FSH are thought to be released from the pituitary under the influence of GnRH from the hypothalamus, although whether the single GnRH of Schally _et al._, (1971) is the naturally

occurring factor for both LH and FSH remains to be finally proven (Lamming, 1973). Since LH and FSH are released from the pituitary at different stages of the oestrous cycle some factor must be responsible for secretion of one or both differentially. The most likely source of regulation is from the ovarian steroids, either directly or via the hypothalamus. Oestrogen for example has been shown to cause LH release, and administration of oestrogen will cause ovulation in rats, cattle, sheep and rabbits (McDonald, 1975) but not humans (Williams, 1968). Apparently progesterone in low doses may also cause LH release in some species (McDonald, 1969); however Evans and Irvine (1975) found an inverse relationship between progesterone and LH levels in the mare suggesting a negative feedback system. No such relationship between FSH and progesterone concentrations could be demonstrated in this species. The effects of the steroid hormones on gonadotrophin release are dose-dependent and large doses of oestrogen may completely inhibit this response creating a "physiological hypophysectomy" (McDonald, 1969).

Gorski and Barraclough cited by McDonald (1969) proposed a theory of dual hypothalamic control of pituitary gonadotrophins. Based on a number of experiments in rats, they suggested that the arcuate ventromedial nucleus just dorsal to the pituitary median eminance region regulates the first level of hypothalamic control or "tonic" discharge of LH and FSH. This discharge, although sufficient to maintain oestrogen secretion is not sufficient to initiate ovulatory surges of LH. A higher or "cyclic" centre located in the anterior hypothalamus just dorsal to the optic chiasma regulates the second level of control in the ovulatory process. Further, they postulated that the "cyclic" centre exerts an integrating function on the "tonic" centre during the oestrous cycle such that certain critical gonadal steroid concentrations, neurogenic stimuli, and other external

environmental stimuli (e.g. light) are integrated.

Although not proven, a similar situation could exist in the mare (Sponseller and Raker, 1969); there is evidence suggesting gonadotrophin is released in a cyclical manner whether the mare is pregnant or not (Van Rensburg and Van Niekerk, 1968; Allen, 1974; Evans and Irvine, 1975; Nett, Holtan and Estergreen, 1973).

ii. Luteinizing Hormone

It appears to be well established that FSH together with LH causes follicular maturation, oestrogen production and ovulation. Pineda and Ginther (1972) treated mares with an antiserum produced against an equine pituitary fraction; this blocked oestrus and ovulation and caused degeneration of the largest follicle when given on days two through to six of oestrus. In late dioestrous oestradiol 17β peaks before the LH surge, thus indicating that this steroid may be involved in facilitation of the ovulatory release of LH (Patterson, Chen and King, 1972; Noden, Oxender and Hafs, 1975).

Undoubtedly LH is luteotrophic at least during and soon after ovulation in most species. Thereafter the role the pituitary plays in corpus luteum (CL) maintenance varies with the species. In the cow for example LH is luteotrophic during most stages of the cycle, and may prolong CL lifespan even in the presence of the uterus (McDonald, 1969). LH can augment progesterone synthesis from ovine luteal tissue _in vitro_, and from the intact CL in hypophysectomized sheep (Denamur, 1974). This worker also noted that following hypophysectomy on day two of the oestrous cycle and simultaneous administration of antiserum to LH and prolactin, the development of the CL was impaired only after the effect of the ovulatory surge of LH has worn off. Thus the trophic stimulus initiated by the hormonal discharge at ovulation (primarily FSH and LH) continues to exert its effect well into the luteal phase in sheep at least.

Pineda and Ginther (1972) used anti-sheep equine pituitary antiserum and treated mares on days three through to seven of dioestrus, which lead to significantly reduced CL weights. The results suggested that the pituitary gland of mares contains a substance necessary for CL maintenance at least in the early to mid-dioestral period. In the mid-luteal phase of the oestrous cycle however administration of human chorionic gonadotrophin (HCG) to mares caused a transient reduction in plasma progesterone levels while doses of 2,000 i.u. of HCG may be luteolytic from 10 to 35 days of pregnancy, since administration of this hormone consistently caused pregnancy failure (Allen, 1975). Although HGC has LH like properties in the mare, in that it will induce premature ovulation, whether it is anti-gonadotrophic or not in the pregnant animal is not known.

Whitmore, Wentworth and Ginther (1973) developed an anti-PMSG antibody for use in radioimmunoassay estimations of LH in mares. They showed that levels of LH peaked at 4.79 ng/ml (using equine LH 13MGIDOLHF2 as standard with an activity of 2.3 units/mg) the day after ovulation and fell to values found in mid-dioestrus, by approximately five days after ovulation. Similarly, Noden, Oxender and Hafs (1974) using an anti-ovine LH antiserum showed LH levels rose from mid-dioestrous levels of 53 to 70 ng/ml, (using equine LH LER1138-1 with a potency of 0.27 U NIH LH - S_1/mg) two days prior to oestrus and peaked at about 800 to 1000 ng/ml, just after ovulation. Levels fell gradually over the following five days. Evans and Irvine (1975) also noted LH peaked after ovulation at about 40-50 ng/ml (using equine LH, potency 3.98 mg NIH - LH - 51/mg). In contrast to the above findings, Pattison et al. (1972) found the LH surge peaked 24 hours before ovulation at 129.4 ng/ml (using ovine LH LFR - 1052 C2 as standard) and returned to dioestrous levels of 21 ng/ml in five to eight days.

The sustained plasma level of LH throughout oestrus in the mare is in contrast to the brief surge (6 to 8 hours) seen in other species (Noden *et al.*, 1974). Geschwind, Dewey, Hughes, Evans and Stabenfeldt (1975) suggested this may be due to the long half-life of endogenous LH in the equine species which may in turn be responsible for the relatively large number of double ovulations and the relatively long duration of oestrus in the mare.

That maximum levels of LH may occur after ovulation is also in contrast to other species including the cow, ewe, sow (Hansel and Echternkamp, 1972) and human (Neill, Johansson, Datta and Knobil, 1967; Williams, 1968).

LH release is not only related to oestrogen but perhaps even more importantly to progesterone. Thus rising levels of progesterone in the blood exert a negative feedback on the pituitary resulting in a decline in LH secretion in the mare (Noden *et al.*, 1974; Evans and Irvine, 1975).

iii. Thyroid Stimulating Hormone

It is well known that abnormalities in the levels of thyroxine affects reproduction in most species (McDonald, 1975) but the effects of different levels of thyroid stimulating hormone (TSH) in the mare are not known. TSH is a glycoprotein with a molecular weight of between 10,000 and 28,000 and is secreted by the basophilic beta cells of the adenohypophysis. The functional effect of TSH on the thyroid is to increase the production and output of thyroxine and triiodothyronine. Thyroxine and TSH regulate one another via a classic negative feedback mechanism. Release of TSH from the pituitary is mediated via the hypothalamus which discharges thyroid releasing factor via the portal system into the adenohypophysis (McDonald, 1969).

Kelley, Oehme and Brandt (1974) measured serum thyroxine, resin triiodothyronine uptake and thyroxine resin T3 index in normal cycling mares. Although serum thyroxine decreased after ovulation

in these mares, there were no significant differences in thyroid gland function test results during the different stages of the oestrous cycle. While favourable responses have been reported in treatment of mares for obese anoestrus (Roberts, 1971; Lieux, 1972), when Lowe, Foote, Baldwin, Hillman and Kallfelz (1974) thyroidectomized some mares, they continued to have regular oestrous cycles, despite showing signs of clinical hypothyroidism. Since obese mares *per se* are often poor breeders (Day, 1939) it may well be that any effect gained by thyroxine therapy is secondary to the slimming effect associated with increased basal metabolic rate of mares treated in this manner.

d. Ovary

The equine ovary has a gametogenic function and also under the influence of the pituitary gland and uterus, acts as a temporary endocrine gland producing and secreting oestrogens and progestogens.

i. Anatomy

At birth females have their full complement of oocytes in primordial (or older) follicles. These are never replenished and only decrease in number during the female lifespan. Primordial follicles consist of an oocyte surrounded by a single layer of epithelial cells with no thecal cells present (McDonald, 1975). These primary follicles are arranged in rows tangential to the ovulation fossa and between the connective tissue stroma of the ovarian cortex in the mare (Van Niekerk, Gerneke and Van Heerden, 1973).

The tunica albuginea separates the cortical and medullary areas in the embryonic gonad, and eventually covers most of the external surface of the adult equine ovary except for the ovulation fossa (Stabenfeldt, Hughes, Evans and Geschwind, 1975).

ii. Follicular Phase

In the southern hemisphere, ovarian activity is at its lowest in August (18% active) and at its highest in January (91.5% active) (Osborne, 1966). Van Niekerk et al. (1973) noted that the quiescent ovaries of mares in deep anoestrus were 2.4 x 1.6 x 1.6 cm in size, weighing 35 g, with the primary follicles in rows. These small ovaries had a total content of only about 1 μg oestradiol 17β (Van Rensburg and Van Niekerk, 1968). As ovarian activity begins under the influence of small quantities of pituitary gonadotrophins, the ovaries increase in size (3.5 x 2.9 x 2.6 cm) and weight (75.6 g); these increases are due almost entirely to an increase in the number of developing follicles present (Van Niekerk et al., 1973). Although it is generally accepted that ovarian activity is initiated in early spring by the pituitary gonadotrophins, treatment of anoestrous ovaries in mares with pituitary gonadotrophins (Burkhardt, 1947; Roberts, 1971) and GnRH (Allen W.R., pers comm. 1974; Betteridge and Mitchell, 1974) has generally been unrewarding. Evans and Irvine (1975a) however, using a series of 1 mg injections of GnRH at ten day intervals together with exogenous steroid hormone therapy, were able to induce anoestrous mares to ovulate; six being served and five became pregnant to the ovulation. This experiment was conducted in early spring however and no controls were used. It may be that either the doses of gonadotrophins and releasing hormones used were inadequate in the earlier experiments, that the local conditions within the ovaries render them sensitive to the action of gonadotrophins only at the correct time or that the exogenous steroid therapy in the latter experiment had a "priming" effect.

Since as many as 20 follicles per ovary may develop simultaneously (Van Rensburg and Van Niekerk,

1968) many must undergo atresia. Van Niekerk et al. (1973) distinguished between two types of atresia. The first, associated with small follicles of less than 5 mm in diameter, was characterised by an initial absence of mitotic divisions in the granulosal cells and a shrinking of the cytoplasm especially of the basal cells. As a result, the cells lost their regular arrangement and contact with the basement membrane. Finally the upper layers of the stratum granulosum sloughed off into the antrum of the follicle, while the fibroblasts of the undifferentiated thecal layers surrounding the follicle proliferated, grew inwards and obliterated the antrum to form a nodule of scar tissue. Atresia of larger follicles on the other hand (i.e. those with a clearly defined stratum granulosum, theca interna and theca externa) started by death and desquamation of the granulosal cells into the follicular fluid. Simultaneously the basement membrane between the theca interna and granulosal cells hypertrophied, thickened and formed a distinct hyaline membrane. Some thecal cells penetrated this membrane and formed a layer of phagocytic cells on its distal (inner) surface. This hyaline membrane contracted, causing collapse of the follicle and cutting off the blood supply to the phagocytic layer. Eventually the antrum became obliterated forming a corpus fibrosum atreticum of scar tissue.

The mechanism by which atresia of most follicles occurs at the expense of a minority which eventually ovulate, is unknown. Nalbandov (1953) suggested a theory of "hormonal dilution". He hypothesised that as follicles increased in size there was insufficient FSH to maintain all of them, hence most became atretic. In contrast, Evans and Irvine (1975) consistently noted an FSH surge preceding ovulation by 10 to 13 days. They believe this mid-dioestrous surge exerts a priming or inductive effect so that follicles may undergo changes necessary for the subsequent production of fertile ova.

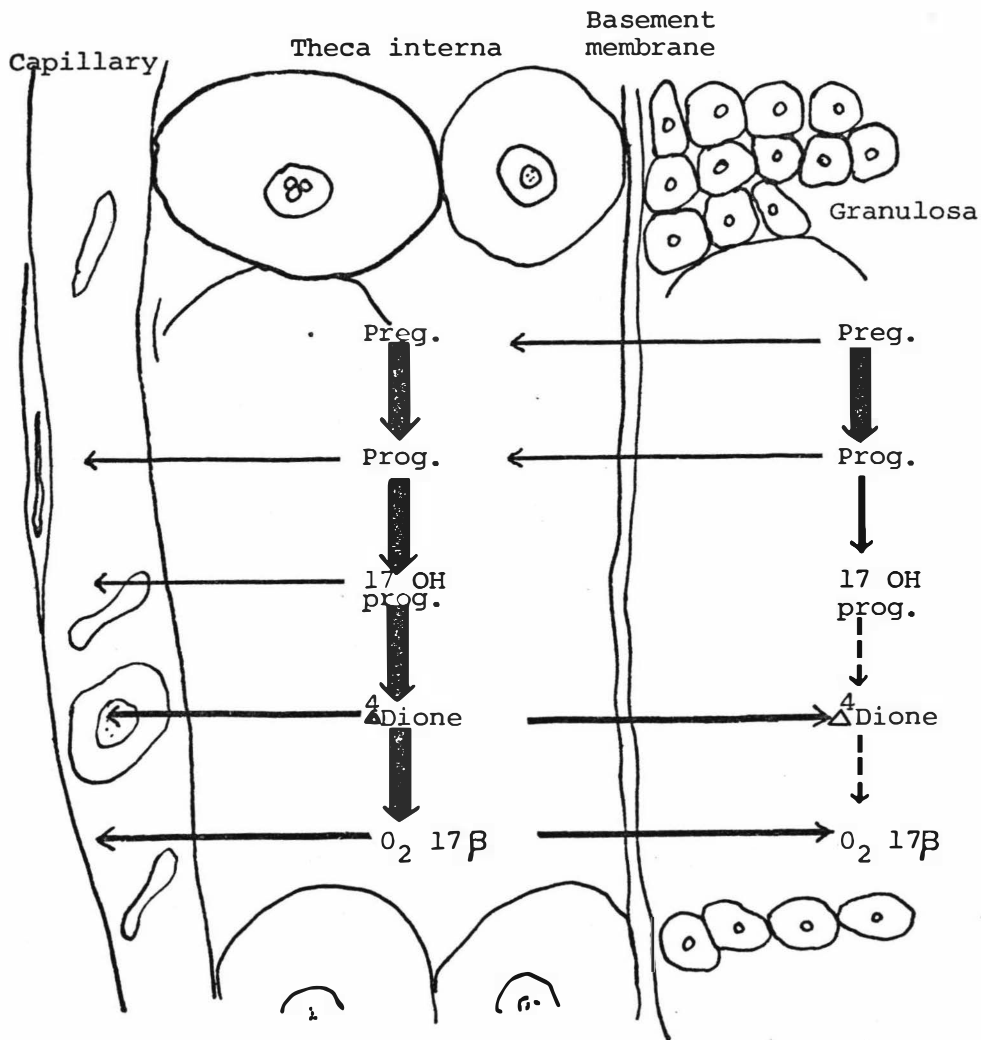


FIGURE 1: STEROID SYNTHESIS IN THE EQUINE GRAAFIAN FOLLICLE

KEY: Preg = pregnenolone; Prog = progesterone; 17αOH prog = 17 hydroxy-progesterone; Δ^4-dione = androstenedione; O_2 17β = oestradiol (Short, 1964)

Ovaries of mares become more active as daylight length increases. It is well known that early in the breeding season, mares tend to have long oestrous periods (Roberts, 1971); these may be as long as 63 (Van Niekerk _et al_., 1973) or even 80 (Caslick, 1937) days (see also review by Witherspoon, 1971). Usually the ovaries of these mares are knobbly and contain several atretic and developing follicles up to 3 cm in diameter (Van Niekerk _et al_., 1973). Few if any reach maturity and ovulate. The latter workers suggested that these long oestrous periods early in the breeding season were due to an imbalance of FSH and LH secretion. The fact that follicles grow all the time suggests a continual supply of FSH, while the failure of these follicles to mature and ovulate suggests an inadequate supply of LH.

Follicles destined to ovulate, mature under the influence of FSH and LH; the latter begins to rise significantly on the day prior to oestrus. At the start of oestrus the maturing follicle is an average 20 mm in diameter (Squires, Douglas, Staffenhagen and Ginther, 1974) and contains about 25 ml of fluid (Van Rensburg and Van Niekerk, 1968).

Follicular fluid is essentially a filtrate of blood which also contains high concentrations of steroids, mucopolysaccharides and a heparinoid anticoagulant, all of which are secretory products of the follicle. Short (1964) observed histologically that these maturing follicles consist of an outer fibrous theca externa surrounding the vascular and granular theca interna, which in turn is bounded on the inside by a basement membrane through which no blood vessels penetrate. Within the lumen of the follicle itself there are many granulosal cells loosely attached to the basement membrane and to each other, but without a blood supply. The oocyte itself rests in a "nest" of granulosal cells (McDonald, 1975). Macroscopically those follicles destined to ovulate when the mare is in oestrus have a very vascular orange-pink lining, with the granulosal

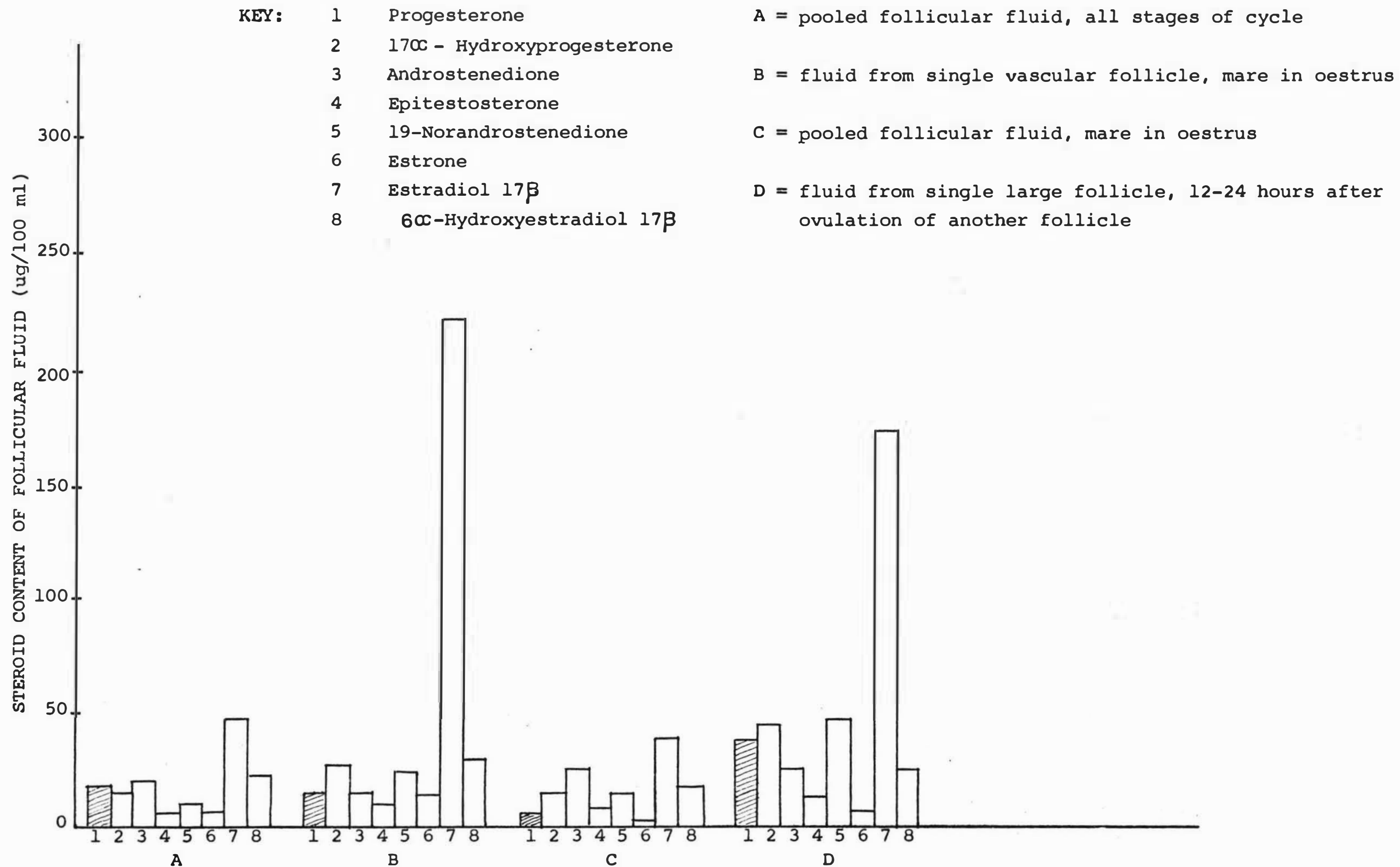


FIGURE 2: HORMONE CONCENTRATIONS OF FOLLICULAR FLUID IN VARIOUS REPRODUCTIVE STATES (SHORT, 1964)

cells having a characteristic mucoid appearance not seen at any other stage of the cycle (Short, 1964; Younglai, 1971).

Figure 1 facing page 15 summarises the probable pathways of steroid synthesis in the equine graafian follicle, while Figure 2 facing page 16 summarises the hormonal concentrations of the follicular fluid during various reproductive states.

The major steroid in the follicular fluid of the mature graafian follicle is oestradiol 17β (Short, 1964, Younglai and Short, 1970) with levels of around 2 μg/ml in mid-oestrus (Van Rensburg and Van Niekerk, 1968).

Van Rensburg and Van Niekerk (1968) considered that hyperaemia of the follicle wall and follicular size up to a volume of 20 ml was proportional to the oestradiol 17β content; over 20 ml volume was not related to oestradiol 17β content. Short (1964) also noted that only vascular follicles have high levels of steroid synthesis at oestrus; he reported that follicular content of oestrogen did not interfere with ovarian oestrogen secretion, indicating that follicular fluid was not an important reservoir of this hormone. Further, Van Rensburg and Van Niekerk (1968) noted that follicular fluid oestrogen content decreased from the commencement of oestrus and also indicated that there was some evidence to suggest that oestradiol 17β production in one follicle may affect the concentration in others. When a low concentration was found in a large follicle the concentration in the smaller follicles tended to be higher, and when two large follicles were present, only one contained large amounts of oestradiol 17β. Such observations raise the possibility of a local intra-ovarian feedback system.

Nett _et al_. (1973) measuring plasma oestrone and oestradiol 17β by radioimmunoassay (RIA) found levels of from 25 to 50 pg/ml in post partum mares in oestrus, while levels of mares in dioestrus were from 5 to 15 pg/ml. Noden _et al_. (1975) found peak plasma levels of oestradiol 17β and androstenedione occurred two days before ovulation and were declining on the day of ovulation, while plasma oestrone remained constant (between 9 and 12 pg/ml) throughout the cycle. Hillman and Loy (1969), measuring urinary oestrogens, found levels of 15 - 20 μg/ml of oestrone about a week before ovulation. Oestrone levels peaked at 60 μg/ml the day before ovulation. A marked and significant drop in urinary oestrone was seen on the day of ovulation and this decline continued to luteal phase levels of about 20 μg/ml, by one to two days after ovulation. Oestrous behaviour usually preceded the marked elevation of oestrogen excretion by one to three days, and ceased one to two days following the ovulatory decline in oestrogen excretion. Interestingly, urinary oestrone levels were consistently about twice those of oestradiol 17β whereas blood and follicle levels of oestrone are about one tenth those of oestradiol 17β (Short, 1964; Van Rensburg and Van Niekerk, 1968). It may be that most of the oestradiol 17β is metabolised before being excreted. This also would explain why signs of oestrus occur before, and continue after urinary oestradiol 17β rises.

Levels of progesterone are very low in preovulatory follicles (Short 1964; Van Rensburg and Van Niekerk, 1968).

The principle natural oestrogens produced by the ovary in decreasing order of potency are oestradiol 17β, oestrone and oestriol (Jones, 1968). Probably oestriol is a metabolite of oestrone and oestradiol 17β, the latter two being interconvertible - all are lipid soluble (Williams, 1968).

The roles of the oestrogens are many and varied. Their main actions are in inducing feminization of the growing young female, with increase in genital tissue size and volume (Williams, 1968), and the clinical and nervous manifestations of oestrus (Roberts, 1971). Non-specific actions of oestrogens include an epitheliotrophic action (McDonald, 1969), growth retardation, subcutaneous fat deposition, and an anabolic affect not only to specific target tissues, but also a generalized effect on protein (Williams, 1968).

iii. Oestrus

Length of oestrus in the mare is very variable, being longer in the early part of the breeding season. Trum (1950) found that the length of oestrus varied from 2 to 40 days, with an average of 5.5 days. Oestrous periods are significantly more variable than dioestrous periods (Witherspoon, 1971); however for a particular mare length of oestrus is significantly more repeatable than length of dioestrus (Ginther, Whitmore and Squires, 1972). The latter authors recorded split oestrus occurring in 4.9% of oestrous periods. One of the most constant periods is the time from ovulation until the end of oestrus. Hughes, Stabenfeldt and Evans (1972) observed that 46% of mares ovulated within 24 hours and 78% within 48 hours of the end of oestrus, while 10% ovulated when out of oestrus.

Oestrous behaviour is a complex phenomenon, being modified by season and by individual animal differences (Hughes _et al_., 1972). High levels of circulating oestrogens are not necessarily associated with visible oestrus (Van Rensburg and Van Niekerk, 1968). On the other hand, it appears that the mare is very sensitive to the suppressive effects of progestogens (Loy and Swan, 1966; Evans, Hughes and

Stabenfeldt, 1971). The net effect of this is that the luteal phase is much more constant than the follicular phase of the cycle, irrespective of the time of year.

iv. Ovulation

Some authors claim that impending ovulation is almost invariably associated with a detectable softening of the mature follicle (Fallon, 1967; Butterfield and Mathews, 1970; Roberts, 1971; Witherspoon, 1971), while others consider softening prior to ovulation occurs only inconsistently (Witherspoon and Talbot, 1970; Hughes et al., 1972). A similar state of confusion exists as to the time of ovulation. Hughes et al. (1972) studying 42 ovulations and Ginther et al., (1972) studying 30 ovulations, found no significant difference in time of ovulation (day versus night), whereas Witherspoon and Talbot (1970) studying 25 ovulations found 23 occurred between 11 p.m. and 7 a.m. with only two ovulations occurring outside those hours.

Double ovulation occurs not uncommonly in mares. Figures of from 1.5% (Ginther et al., 1972) to 42.8% (Warszawsky, Parker, First and Ginther, 1972) have been reported. The incidence of double ovulations may be affected by:

- type of horse: in pony mares, multiple ovulation is uncommon (Arthur and Allen, 1972)
- season; multiple ovulation is more common in the beginning (Hughes et al., 1972) of the breeding season
- whether the study was carried out on post mortem specimens or on the basis of rectal examinations (Hughes, Stabenfeldt and Evans, 1972a)

Thoroughbreds probably have about 18% multiple ovulations (Arthur, 1969). Since the recorded incidence of twin births, or twin abortions is only 1 - 2% (Jeffcott and Whitwell, 1973) then twin

ovulations may be an important cause of infertility in mares.

The mare is a "left" ovulator, about 60% of ovulations being from the left ovary (Osborne, 1966; McDonald, 1969).

Generally there is a remarkable within-mare consistency regarding ovulation time and follicle size (Day, 1939; Trum, 1950; Witherspoon and Talbot, 1970), presence of multiple ovulations (Hughes _et al_., 1972a), length of oestrus (Ginther _et al_., 1972) and general reproductive pattern from year to year (Irvine, 1974).

The mare is similar to the bitch in that when ovulation occurs the ovum is extruded as a primary oocyte before the first polar body has been expelled (McDonald, 1969; Webel, Ellicott, Dziuk, 1970; Roberts, 1971). Ageing of the ovum continues in the oviduct. Interestingly in the mare in contrast to the bitch, the fact that the ovum is shed from the ovary in an "immature" state does not seem to affect its rapid ageing since equine ova, as with ova of most other species, probably are viable in the oviduct only for a few hours (Allen, 1972). Another idiosyncrasy of the equine ovum is that the majority of those that are unfertilized are retained in the oviducts for at least two months (Betteridge and Mitchell, 1974) and may be up to seven months (Van Niekerk and Gerneke, 1966). The site of retention is mainly in the middle third of the oviducts (Steffenhagen, Pineda and Ginther, 1972). These latter authors noted that fewer ova were retained from bred than from nonbred mares, suggesting that some unfertilized ova were carried into the uterus by the fertilized ovum. Further, in the bred mares more unfertilized ova were in the distal third of the oviduct further supporting the theory of fertile ova "carrying" unfertilized ova towards the uterus.

Roberts (1971) reported that intrafollicular pressure *per se* is not a cause of ovulation - when intrafollicular pressures have been measured, no increase just prior to ovulation has been demonstrated. Collapse of the follicle wall does not occur until after the cumulus has passed through the small stigma on the surface of the follicle. Observed ovulations are not violent eruptions, but may take from a few seconds to several minutes. Although the mechanism of ovulation is not known there is some evidence to suggest that LH induces proteolytic enzyme synthesis, an activity which weakens the follicle wall leading to rupture (McDonald, 1975).

Ovulation occurs only through the ovulation fossa in mares (Warszawsky *et al*., 1972) probably because this is the only area which is not covered by thick tunica albuginia and mesovarium (Bergin and Shipley, 1968). Immediately following ovulation blood vessels of the theca interna rupture through the basement membrane resulting in haemorrhage into the follicle - no doubt aided by the anticoagulant action of the follicular fluid. As ovulation occurs, the fimbriae and proximal oviduct must be bathed in follicular fluid with a high concentration of oestrogens (Short, 1964) - this may be important in ova transport (Roberts, 1971). The follicle wall is thrown into folds or trabeculae and granulosal cells increase in size and penetrate the blood clots. The thecal cells swell then regress, and do not contribute significantly to the formation of the CL (Harrison, 1946).

Short (1968) noted that manual rupture of large follicles caused luteinization with progesterone production. Since granulosal cells have the ability to produce progesterone before ovulation, he suggested that progesterone production by granulosal cells after ovulation may be due to the development of a blood supply and the consequent availability of nutrients. Van Rensburg and Van Niekerk (1968) were

able to detect progesterone in the follicle wall as soon as eight hours after ovulation, even though there were no macroscopic signs of luteinization; these signs are not seen until 18 hours post ovulation (Harrison, 1946). After ovulation a large blood clot, which may be palpated per rectum, fills the lumen of the follicle with bleeding occurring from the follicle wall and not at the site of rupture (Roberts, 1971).

v. Luteal Phase

Progesterone metabolism and reported plasma progesterone levels in the mare are considered in a later section of this literature review.

The main physiological roles of progesterone are the preparation of the uterus for the nutrition and implantation of the zygote, development of the blastocyst and maintenance of the uterine environment until term.

Classically progestogens have a negative feedback on the pituitary causing a decreased secretion of both FSH and LH, but this is by no means absolute. Stabenfeldt, Hughes, Wheat, Evans, Kennedy and Cupps (1974) hysterectomized mares with active corpora lutea; while these CL remained functional (producing progesterone) follicle production and even ovulation was not entirely suppressed, although the high levels of progesterone did block overt oestrus. While Evans and Irvine (1975) found an inverse relationship between progesterone and LH, no relationship could be found between progesterone and FSH. Again, this suggests the possibility of inherent cyclic gonadotrophin discharge by the pituitary.

The CL of the mare, unlike that of the bovine, does not protrude greatly from the ovarian surface. However it may be palpated per rectum for a variable time after ovulation. Allen (1974) using small Welsh Mountain pony mares, was able to palpate the CL for an average of 8.7 days (1 - 14) after ovulation. He commented that the recently formed CL was very

easy to confuse with a follicle. Hughes _et al_. (1972a) were able to palpate the CL for 8.9 days (range 1 - 18) after ovulation in larger breeds.

The life of the CL in the non-pregnant cycling mare is terminated at about 12 days (range 5 - 18) after ovulation (Hughes _et al_., 1972). The question is - what causes this CL regression? Goding (1974) suggested the following alternatives:

- the CL may regress because it has reached the end of its biological lifespan
- it may regress because of withdrawal of hormones necessary for its continued functioning, or
- it may regress because of the action of a luteolysin.

In most physiological situations the secretory activity of the CL is the net result of an interplay between trophic and lytic factors (Denamur, 1974). Although early in the life of the CL luteotrophin may be necessary, there is increasing evidence that CL regression in the mare, as in many other animal species, is caused by a luteolysin. Whereas in cattle exogenous progesterone (100 mg daily) given early in the luteal phase of the cycle causes an early return to oestrus, (probably due to the progesterone causing a decrease in the luteotrophin, LH) (Ginther, 1970), in mares similar doses do not cause a decrease in gonadotrophin release (Loy, Hughes, Richards and Swan, 1967). LH does however appear to be luteotrophic in the mare at least in the early stage of the luteal phase (Pineda and Ginther, 1972).

The uterus affects CL lifespan in most species, including the mare, although the unilateral influence of the uterine horn on the corresponding ovary, which is seen in cattle and sheep, probably is not present in the equine species (Ginther and First, 1971; Stabenfeldt _et al_., 1974). There is now abundant evidence supporting the concept that CL regression may be caused by prostaglandin $F2\alpha$ ($PGF2\alpha$) in most species (McCracken, Baird, Carlson, Goding and

Barcikowski, 1973) and that in the sheep at least the uterus releases PGF2α at the correct time of the cycle to cause luteolysis (Goding, 1974). It is likely that PGF2α is the natural luteolysin in most if not all domestic animals although, as Lamond (1974) indicated evidence for this is only indirect and incomplete in the mare. A "counter-current" theory has been proposed to explain how PGF2α reaches the ovary from the uterus by McCracken and his co-workers (McCracken et al., 1973). They suggested that PGF2α diffuses actively against the pressure gradient from the ovarian vein (draining the uterus) to the ovarian artery, a mechanism which of course necessitates close apposition of both blood vessels. To support this theory Delcampo and Ginther (1973) observed that in those species in which the uterine horn has a direct unilateral luteolytic effect there is indeed a close anatomical relationship between the ovarian artery and vein, whereas those species in which no unilateral effect has been demonstrated (the mare in particular) do not have this close association between the ovarian artery and vein. In these species the luteolytic effect probably is mediated systemically rather than locally.

There are several reports indicating that PGF2α (or synthetic analogues) given to mares after about day 4 of dioestrus results in oestrus in three to five days (Douglas and Ginther, 1973; Berwyn-Jones and Irvine, 1974; Allen, Stewart, Cooper, Crowhurst, Simpson, McEnery, Greenwood, Rossdale and Ricketts, 1974) and that this response is associated with a precipitous drop in plasma levels of progestogens (Allen and Rossdale, 1973; Oxender, Hafs and Noden, 1973; Allen and Rowson, 1973).

It would appear then that the CL of the mare, like that of other domestic animals, has its lifespan terminated abruptly by the release of the luteolysin PGF2α. How this effect is brought about is not certain, although McCracken et al., (1973) suggested

that $PGF2\alpha$ may cause luteolysis either by altering the blood flow and arterio-venous shunts within the ovary, or by an antigonadotrophic effect. Under the influence of the luteolysin, a hyaline like material accumulates between the luteinized granulosal cells, and eventually the CL is reduced to a scar called a corpus albicans.

The ovary produces a third hormone, relaxin. Relaxin is a non-steroid water soluble protein hormone, probably produced by the CL (Roberts, 1971). While this hormone causes softening of the epiphyses of rodents and is of importance in facilitation of birth in these species, its role in reproduction of higher mammals has not yet been delineated (Williams, 1968).

e. Uterus

The equine uterus is very responsive to the steroid hormones. Van Niekerk and Van Heerden (1972) found that the uterus of the mare in anoestrus (i.e. minimal hormonal stimulation) was small (width of body 5.4 cm, width of horns 3.8 cm, thickness of walls 0.2 cm) with no tone, the mucosa being dry and sticky. Histologically there was a general impression of cytological inactivity, the epithelial cells being small with scant cytoplasm and lightly staining nuclei. The gland cells were few and small. In contrast these authors found that the uterus of the mare in oestrus at the beginning of the breeding season was larger (uterine body diameter 6.5 cm, uterine horn diameter 5.0 cm, thickness of wall 0.5 cm) with increased tone. The mucosa was bluish-red in colour and was moist and glistening. The epithelial cells were of a tall columnar type with much cytoplasm, while the gland cells were greater in number and more coiled. Thus the mare's uterus under the influence of oestrogens has more tone than that of the mare in anoestrum. However the progestogen-dominated uterus has even greater tone since Van Niekerk (1965) reported that uterine tone, when measured on a scale from zero (no tone) to ten (maximum tone), was zero when the mare was in oestrus, rose to two as

progestogens were produced by the CL and, if the mare became pregnant, increased to a maximum of ten at about 25 days of pregnancy. If the mare was not pregnant then the tone after 10 - 12 days decreased (in parallel with CL regression) to the next oestrus.

As mentioned earlier, the uterus has a direct effect on ovarian progestogen production, possibly by producing and secreting PGF2α. Thus uterine distension induces premature oestrus in mares (Neely, Hughes, Stabenfeldt and Evans, 1974) while hysterectomy will prolong the CL beyond its normal lifespan (Ginther and First, 1971).

2. Pregnancy

a. Blastocyst, Embryo and Foetus

Pregnancy must be recognized in the mare as early as two to six days after ovulation (Allen, 1969) since all fertilized ova, but few non-fertilized ova, pass into the uterus. Betteridge and Mitchell (1974) suggested that the mechanism for selective ovum transport of only fertile ova must be associated with fertilization properties and not just sperm penetration; such changes probably being surface differences which must precede emergence from the zona pellucida. By 6 days after ovulation, the fertilized ovum has reached the uterus (Roberts, 1971).

The growing blastocyst initially derives its nutrition from the "uterine milk" secreted by the endometrial glands of the progestogen dominated uterus (McDonald, 1969). By 17 days of gestation the blastocyst is about 6.1 cm in diameter and deriving nutrition from the yolksac. Van Niekerk (1965) noted that nidation occurred at about 25 to 30 days, at which time he said placentation changed from yolksac to allanto-chorial. By day 45 of gestation rudimentary villi begin to develop over the entire surface of the allanto-chorion and these villi interdigitate with corresponding crypts in the maternal endometrium as precursors of microcotyledons (Van Niekerk and Allen, 1975).

As noted by Roberts (1971) and Bergin, Gier, Frey and Marion (1967) true nidation or implantation does not occur in horses. The latter authors, in contrast to

Van Niekerk (1965), found that direct attachment between maternal and foetal placentae did not exist until the chorionic villi become highly developed at about 80 days. It is probable that the "nidation" observed by Van Niekerk is in fact endometrial cup formation, for as mentioned by Bergin et al. (1967), the blastocyst may change its relative position in the uterus up to 35 to 40 days of gestation.

The concentration of oestrone, equilin, equilenin and oestradiol 17β were all less than 20 pg/ml in blood of mares until 90 days of pregnancy, after which time, the former three rose, reaching a peak of 828 pg/ml at 210 days. Oestradiol levels peaked at 71 pg/ml at 240 days, then all oestrogens declined to low levels just before parturition (Nett et al., 1973).

An association between urinary oestrogen rise and foetal gonad size has been known for a long time (Allen, 1972). From about the fourth or fifth month to the ninth month of pregnancy the interstitial cells of the foetal gonads increase in size until they may weigh 150 g, after which time they reduce markedly to about an eighth that size at parturition (Roberts, 1971). MacArthur, Short, O'Donnell (1967) isolated six radiochemically pure steroids (cholesterol, dehydroepiandrosterone, testosterone, androstenedione 17α - hydroxyprogesterone and oestrone) from hypertrophied foetal gonads, indicating that these gonads possess enzyme systems capable of synthesizing large numbers of steroids. Then, when Raeside, Liptrap and Milne (1973) performed foetal gonadectomies on mares in advanced pregnancy and studied urinary oestrogen secretion in these mares, they found that bilateral foetal gonadectomy caused a rapid decrease in urinary oestrogen excretion to very low concentrations, whereas in mares where only one foetal gonad was removed, the urinary excretion was reduced by about one half. No decrease in urinary oestrogen excretion was noted in sham-operated mares. It seems clearly established therefore, that the foetal gonads are involved in oestrogen production. These same authors noted that absence of foetal gonads was probably implicated in most of the abortions which invariably occurred following

foetal gonadectomy. This adds some support to the findings of Nishikawa (1959) who found that the use of stilboestrol injections reduced the incidence of abortion in 536 mares from 12% (untreated) to 3.3% (treated) in the middle and last trimester of pregnancy. Because of the different conditions under which each group of mares were seen in this trial however, differences in abortion rate due to environmental and management conditions could not be ruled out.

b. Placenta and Ovary

The presence of endometrial cups in the uterus of the pregnant mare has been noted for many years. Rowlands (1948) observed that these structures were arranged in a horse-shoe like manner along the outer curvature of the uterus with the open ends toward the uterine body. He reported that they contained extremely active secretory cells. The hormone produced, PMSG, is primarily FSH-like in activity but with some LH-like activity, depending on the species in which it is used. Day and Rowlands (1948) measured PMSG in pony serum from the 45th to the 100th day of pregnancy and found levels of around 100 iu/ml, although there was a high individual variation between mares. Rowlands (1949) noted that mares carrying twins had higher than normal levels of PMSG. More recently Allen (1969b) developed a haemagglutination inhibition assay which measures PMSG in plasma and can detect it at 35 days of gestation.

W.R. Allen and others have carried out an extensive series of investigations on endometrial cup formation. They noted the cups develop from 30 days onward, opposite the transitory although well defined allanto-chorionic girdle (Allen and Moor, 1972). Structurally they are composed of a discreet, very densely packed mass of large epithelial decidual like cells which develop before the allantochorion becomes attached to the endometrium.

Until recently it was thought the endometrial cups were maternal in origin. However following the observations of Allen (1969a) who noted that plasma PMSG levels varied with the different hybrids even though the endometrial cups all appeared identical histologically, and

after Allen and Moor (1972) demonstrated that allantochoriol girdle cells could be grafted into a nongravid uterus *in vitro* and produce PMSG, these authors suggested that endometrial cup life in the pregnant mare (and different lifespans in hybrids) could be explained by a maternal immunological defence mechanism, and that the endometrial cups (and hence PMSG production) could arise from cells which really were foetal in origin. Spincemaille, Bouters, Vandeplassche and Bonte (1975) have added support to this hypothesis by noting that matings of mares with a full brother results in high levels of PMSG production with detectable levels persisting until 250 days of gestation, presumably as a result of some degree of immunological tolerance. For further information on endometrial cups the reader is referred to a review by Moor, Allen and Hamilton (1975).

Follicle development and growth has been reported in the pregnant mare at the time of expected first oestrus (Van Rensburg and Van Niekerk, 1968) and at about 25 days (Van Niekerk, 1965; Bain, 1967). Allen (1974) and Van Rensburg and Van Niekerk (1968) reported waves of follicular activity approximately every ten days. Whereas Squires *et al*. (1974) noted maximum ovarian follicular size at 60 days of pregnancy, Rowlands (1949) considered that ovarian activity decreased from days 46 to 74, suggesting that the ovaries become less responsive due to the sustained action of oestrogens from former follicles. Thus it would appear that the ovary of the pregnant mare has waves of follicular growth even in the presence of high levels of progestogens. Both Van Rensburg and Van Niekerk (1968) and Nett *et al*., (1973) found low levels of oestradiol 17β in follicles and peripheral blood respectively, after 40 days of pregnancy although the former authors noted waves of oestradiol 17β production in follicles prior to 40 days of pregnancy. It is well known that pregnant mares may occasionally show oestrous behaviour, particularly in the first few weeks of gestation.

As the name suggests PMSG has classically been thought of as a gonadotrophin in the mare. This may not be entirely true. Rowlands (1949) first noted a negative association between ovarian activity and PMSG production. While this in itself may be doubtful, he also first noted that a mare producing PMSG which subsequently resorbs an embryo continues to produce PMSG and does not exhibit overt oestrus. Subsequently Van Niekerk (1965) noted that mares which resorb embryos after 36 days of pregnancy did not come into oestrus for 40 to 80 days, and although the ovaries were large and well developed, there was little or no follicular activity. Allen (1972), after surgically removing the conceptus at various stages of pregnancy, found that those mares in which the conceptus was removed at less than 35 days of pregnancy took about one month to cycle. The delay probably was due to the presence of an active CL. When the conceptus was removed between days 40 and 63 the mare would take up to three months to return to oestrus. The endometrial cups which had already been formed continued to secrete PMSG in this latter group. Even if a luteolysin is used to destroy CL tissue these mares will not exhibit oestrous behaviour in contrast to the former group of mares. Thus the presence of PMSG seems to inhibit follicular development, or at least oestrogen secretion. If the conceptus is removed between 85 and 100 days of pregnancy, oestrus follows in one to four weeks, presumably because PMSG production has ceased.

Although Van Rensburg and Van Niekerk (1968) contended that maximum ovarian activity and ovulation occurred mainly from 40 to 50 days of gestation, Allen (1974) and Squires et al. (1974) found maximum ovarian activity to be later than this (roughly coinciding with maximum PMSG production). The former author noted a marked seasonal variation, there being very little activity in winter. These authors and Allen (1975) concluded that the primary stimulus to the equine ovary during pregnancy is from pituitary gonadotrophin with which PMSG is synergistic. Similarly Van Rensburg and Van Niekerk (1968) suggested that ovarian activity is due to a combination of both pituitary gonado-

trophin and PMSG. Further support to this concept comes from the observations of Evans and Irvine (1975) who noted that the regular 10 to 13 day surges of FSH seen during the oestrous cycle continue at least until 25 days of gestation (after which time it was not measured).

Squires and Ginther (1975) reported both similarities and differences in ovarian function between pregnant and hysterectomized mares and suggested that, while PMSG did not appear to stimulate follicular development, it did prolong the life-span and stimulate the secretory activity of the primary CL and induce ovulation and/or luteinization of secondary follicles of pregnant mares. However the exact role of PMSG in equine pregnancy is still unknown (Spincemaille et al., 1975).

The primary CL of pregnancy was thought to last until about 45 to 50 days of gestation and Rowland (1949) apparently could not detect the primary CL in mares 74 days pregnant. However, Squires et al., (1974) marked the primary CL of pregnancy via laparotomy and found it was still functional up to 160 to 180 days. Despite this, secondary corpora lutea are formed in the pregnant mare from ovulated follicles, or from occasional luteinization without ovulation of follicles (Short, 1968). Van Rensburg and Van Niekerk (1968) considered these secondary CL were all formed at around 40 days gestation. Allen (1974) detected 17 ovulations in nine Welsh Mountain pony mares in the first fourth months of pregnancy - the time to first ovulation was 52.5 days. Squires et al. (1974) also noted secondary CL first being formed at about 40 to 60 days, with the numbers increasing gradually until day 160, after which time both the primary and secondary corpora lutea appeared to regress together.

After about 160 days of pregnancy the ovaries atrophy and cease to play a role in maintenance of pregnancy (Short, 1956; Amoroso and Finn, 1962); the role of progesterone production is then taken over by the placenta.

c. Initiation of Parturition

Little enough is known of the hormonal control of parturition in sheep and cattle, but even less is known

in the horse.

Plasma progestogen levels drop precipitously after parturition in the mare (Ganjam, Kenney and Flickinger, 1975; Lovell, Stabenfeldt, Hughes and Evans, 1975) to less than 0.5 ng/ml by two days _post partum_ (Smith, 1974). Lovell _et al_. (1975) noted that plasma progestogen levels fell dramatically during the last few days before parturition, although birth occurred in the presence of significant levels in the plasma. This dramatic fall in plasma progestogens _post partum_ probably was due to placental separation, since Lovell _et al_. (1975) noted similar endocrine patterns before and after parturition between normal mares and an ovariectomized mare. As these authors indicate the mare appears similar to women and guinea-pigs, but different from the cow and ewe in that plasma progestogen levels remain elevated at the time of delivery. Both Nett _et al_. (1973) and Lovell _et al_. (1975) observed that plasma oestrogens are elevated during the last few weeks of gestation, dropping to low levels by one day _post partum_. It would appear that parturition in the mare proceeds in spite of an increasing progesterone: oestrogen ratio.

Although the uterus of the human and mare in late pregnancy is very sensitive to oxytocin, in the human at least labour can proceed without this hormone.

Recently, attention has swung to the foetus as a "triggering" cause of parturition. As Roberts (1971) mentions, the foetus can regulate its own length of intra-uterine existence. Liggins and his co-workers have convincingly shown that the foetus plays an important role in this way, probably via altered corticosteroid levels (Liggins, 1977). The mare is likely to be similar to other species in this respect (Nathanielsz, Rossdale, Silver and Comline, 1975). Although it is well known that parturition may be initiated in ruminants by exogenous corticosteroids, similar dose rates of corticost-eroids do not alter gestation length in horses (Burns, 1973) although about ten times the dose rate that is effective in ruminants, given daily for four days, will induce

parturition in the mare (Alm, Sullivan and First, 1974). In this respect the mare is similar to the sow, and since they have a similar type of placentation, it would appear that the ruminant placenta allows easier diffusion of corticosteroids than does that of the mare or sow.

Prostaglandin F2α has a marked stimulatory effect on the uterus and it may also play a part in parturition. Although Alm, Sullivan and First (1975) were unable to consistently induce parturition in mares in late pregnancy with 12 mg of PGF2α (Tham salt), Rossdale (1976) was regularly able to induce parturition in mares in late pregnancy using from 250 μg to 1000 μg of a synthetic analogue of PGF2α (ICI-fluprostenol). Interestingly Alm _et al_. (1975) were able to induce parturition in pregnant ovariectomized mares with PGF2α .

B. METABOLISM OF PROGESTERONE IN THE MARE

Although it was known as early as 1910 that destruction of the CL caused abortion in pregnant rabbits (Frankel, 1910), progesterone, the steroid hormone produced by the CL was not isolated until 20 years later by Allen (1930). This first isolate was made from the CL of a sow and, noting that as well as being chemically different from oestrone it was essential for maintenance of pregnancy, he called the new compound progestin.

Progesterone is derived from cholesterol, which yields pregnenolone, the common substrate for all of the steroid hormones (Williams, 1968).

1. Synthesis of Progesterone

a. Ovary

i. Follicular Phase

It is well accepted that the graafian follicle secretes oestrogen and the CL secretes progesterone. The exact pathways of synthesis of the various steroids by the different cell types however are not so well defined.

Channing and Grieves (1969) demonstrated that *in vitro*, equine granulosal cells from follicles at all stages of the oestrous cycle are able to "luteinize" and produce both progesterone and oestradiol 17β from acetate; the metabolic pathways being the same as for conversion of acetate to progesterone by luteal tissue (Channing, 1969). *In vitro*, mid-oestral granulosal cells are stimulated by neither LH nor HCG to produce progesterone whereas granulosal cells from follicles in the mid-dioestral phase are (Channing, 1969*a*). Similarly, Younglai (1971) demonstrated that *in vitro*, thecal cells produce oestradiol 17β and smaller amounts of progesterone, while granulosal cells secrete progesterone and smaller amounts of oestradiol 17β.

The difference in steroid production by the different cell types would appear then to be quantitative rather than qualitative, and *in vivo* some mechanism must operate allowing differential product-

ion of steroids depending on the particular phase of the oestrous cycle. Considering the above evidence, it may be that the gonadotrophins exert this effect.

Equine granulosal cells can readily convert androstenedione and testosterone to oestradiol 17β and oestrone in short term incubations (Channing, 1969). Since, in the follicular fluid there is a large amount of androstenedione formed from the theca interna (Short, 1964) one might expect the granulosal cells to produce oestradiol 17β _in vivo_. This would explain the situation measured _in vivo_ where high concentrations of oestradiol 17β are found in follicular fluid (Van Rensburg and Van Niekerk, 1968) and where the androstenedione:oestradiol 17β ratio is 1:10 (Younglai and Short, 1970) with much lower concentrations of oestradiol 17β being found in ovarian vein blood, where the androstenedione:oestradiol 17β ratio is 1:1 (Short, 1964). However Younglai and Short (1970) measured uptake and synthesis of 16-^{3}H-pregnenolone and 4-^{14}C-androstenedione and showed that the granulosal cells in the intact follicle have very weak aromatizing ability - only a very small percentage of radioactive androstenedione was converted to oestradiol 17β. Perhaps then as these authors suggest, follicular fluid preferentially takes up oestradiol 17β, at the expense of androstenedione.

Short (1964) proposed a "two cell type" theory of steroid synthesis. He pointed out that the theca interna cells have the enzymes necessary for the synthesis of oestradiol 17β from cholesterol (namely 17α hydroxylase and 17 desmolase) whereas the granulosal cells have very weak activity for these two enzymes. He suggested that in the intact follicle at oestrus, where there is a very rich blood supply, the theca interna cells synthesize oestrone and oestradiol 17β, and liberate them into the ovarian interstitial fluid from where they may diffuse into the follicular fluid, ovarian blood supply and

lymphatics. The granulosal cells have a very limited blood supply before ovulation however, and so probably have very restricted ability to synthesise steroids.

ii. Luteal Phase

After ovulation, the granulosal cells of the newly formed CL produce both progesterone and 20α-hydroxy pregnenolone (Short, 1964). Progesterone content is about 4 to 6 μg by 24 hours after ovulation, while at 12 days after ovulation progesterone and 20α -hydroxy pregnenolone levels are 300 and 5 to 12 μg/g respectively (Van Rensburg and Van Niekerk, 1968). Because granulosal cells have little or no 17α -hydroxylase or 17 desmolase activity, no oestradiol 17β, oestrone or androstenedione is synthesised (Short, 1964).

Van Rensburg and Van Niekerk (1968) were able to detect progesterone in the follicle wall as soon as 8 hours after ovulation, although there was no macroscopic signs of luteinization. Thereafter the concentration in luteal tissue continued to increase linearly throughout the 14 day luteal phase.

Van Niekerk, Morgenthal and Gerneke (1975) noted that thecal cells regressed very quickly after ovulation, while hypertrophy and luteinization of granulosal cells commenced 10 hours after ovulation. The luteal tissue enlarged mainly through hypertrophy of the lutein cells. These workers note maximum plasma progesterone levels in cycling mares were reached 5 days after ovulation, and maintained until day 11, whereafter regression of these levels coincide with regression of the luteal cells.

b. Placenta

Although Squires and Ginther (1975) noted that the placenta or uterus contributed to the plasma progesterone pool of the pregnant mare by the third month of gestation, it is generally accepted that it is not until about 200 days of gestation that the placenta has replaced the ovary

as a source of production of steroids (Allen, 1972). Because Short (1957), by U.V. absorption, was able to isolate both 20β-hydroxy pregn-4en-3one and progesterone from the equine placenta after 200 days of gestation, but not from the peripheral blood, he proposed that the epithelio-chorial placentation stopped its passage into the maternal circulation. In contrast to this Smith (1974) found progesterone concentrations in the plasma of late pregnant mares to be quite high. Since he used a radioassay technique however, it is possible he was measuring a compound immunologically (but not necessarily biologically) identical to progesterone.

Placental metabolism of progesterone gives rise to a number of metabolites. Thus Savard, Andrec, Brooksbank, Reyneri, Dorfman, Heard, Jacobs and Soloman (1958) using radiochemical techniques on a 9 month pregnant pony concluded that:

(i) All neutral urinary steroids are direct metabolites of progesterone.

(ii) Very little pregnane 3β-diol and pregnane 3, 20α-dione is present in the urine.

(iii) Acetate is assimilated into allopregnenolone and thence progesterone and oestrone.

(iv) Circulating cholesterol is not very important in the synthesis of oestrone.

(v) Oestrone is synthesised via pathways similar to progesterone (from acetate via cholesterol).

(vi) Equilin and equilenin (unsaturated oestrogens) are derived from acetate via a different pathway to that of progesterone and oestrone.

(vii) The cholesterol used is mainly local and derived from the foetal side of the placenta.

In agreement with Short (1957), these authors concluded that by 200 days of gestation all the progesterone production was localised in the placenta and not surprisingly they found higher levels of radioactivity on the foetal rather than on the maternal side of this structure. Uranediol, 5α-pregnane-3β:20β-diol, 5α-pregnane 3β:20α-

diol and 7 new steroids were all isolated from the urine of mares in advanced pregnancy (Brooks, Klyne and Millar, 1952).

Thus it appears that the high levels of oestrogens found in the blood and urine of mares in advanced pregnancy at least in part come from the metabolism of progestogens, primarily progesterone.

Oestrogens may be detected in the urine of mares as soon as 50 days of pregnancy by biological tests but the commonly used chemical tests will not normally detect oestrogens until after about 120 days (Cox, 1971).

MacArthur _et al_. (1967) extracted 6 steroids from 110-220 day old hypertrophied foetal gonads (both male and female), indicating that foetal gonads have enzyme systems capable of synthesising many steroids. It appears that the presence of foetal gonads are necessary for the high level of urinary oestrogens seen in the pregnant mare since foetal gonadectomy results in urinary oestrogens falling to near zero (Raeside _et al_., 1973).

Although progesterone is also produced by the adrenal cortex and testis, with minute quantities escaping into the peripheral circulation, it is probably only a precursor for further steroid production by these organs (Williams, 1968).

While progesterone is the most important progestogen there are other naturally occurring biologically active progestogens including 20α hydroxy- Δ^4 pregnene-3-one; 20β hydroxy- Δ^4-pregnene-3-one and 17-hydroxy Δ^4-pregnene-3, 30-dione (Stabenfeldt, 1974).

2. Transport and Breakdown of Progesterone

Like the other steroid hormones progesterone circulates in a "bound" state by forming a complex with plasma proteins. The "tightness" of the binding is important in regulating the action and metabolism of the steroid (Williams, 1968). In the mare however, binding of plasma progesterone may at best be rather tenuous. Evans, Faria, Hughes and Stabenfeldt (1975) for example measured the plasma half life of progesterone in mares and found it to be 15.9 minutes and 65.6 minutes for the fast and slow component

respectively while Ganjam, Kenney and Flickinger (1975), using radioactive progesterone, estimated that the plasma half life in this species was 2.5 minutes and 20 minutes for the fast and slow components. The latter authors observed a much slower third component which they were unable to accurately measure. They considered the different 'components' to be due to differences in binding to plasma proteins, a conclusion which agrees with the finding of Mitchell, Ganjam, Kenney, Khaleel and Reynolds (1972) who found that equine plasma lacks any specific progesterone-binding proteins.

Evans et al. (1975) estimated the production rate of progesterone in the cycling mare at 1.24 mg/min/Kg which is of the order of 700-800 g per day per horse. In order to establish the quantities of exogenous progesterone required to significantly raise blood levels, Ganjam et al. (1975) administered 150 mg progesterone in ethanol by daily intramuscular injection to ovariectomized mares, and noted that it took 21 days for plasma levels of progesterone to reach those normally present in dioestrous mares. These results suggest that the mare is capable of producing large quantities of progesterone; presumably this may be related to the apparently very short plasma half life of this steroid.

Most of the steroid inactivation takes place in the liver with the rate being largely dependent on factors influencing the availability of the steroid molecule to the hepatic cell, including the "binding" to plasma proteins (Williams, 1968). Most of the byproducts of steroid metabolism are probably excreted in the bile (McDonald, 1975).

C. MEASUREMENT OF PLASMA PROGESTERONE IN THE MARE

Methods for estimating progesterone in biological samples have closely followed the development of techniques for analysis of steroids in general. Prior to 1950 bioassays of crude extracts were used. The advent of spectrophotometric techniques and development of chromatographic separation methods for steroids, resulted in chemical techniques being used almost exclusively. Further refinement, and the development of competitive protein binding (CPB) and radioimmunoassays (RIA) has resulted in several highly sensitive and specific methods of measurement of plasma progesterone concentration becoming available.

1. Non-Pregnant Mare

The best known of the bioassays which measures plasma progesterone concentration is that of Hooker and Forbes (1947). The test relies on guinea-pig endometrial cell nuclei change, from the normal fusiform dense stromal structure to plump oval nuclei in the presence of progesterone. This assay is not specific for progesterone (Edgar, 1953; Short, 1958) and the levels recorded are higher than those found with chemical tests on similar samples (Edgar, Flux and Ronaldson, 1959).

Of the chemical tests for estimation of plasma progesterone concentration, spectrophotometry as developed by Haskins (1950) was the technique initially used to measure concentrations of progesterone in the plasma of mares. Thus Short (1959), using spectrophotometry after extraction and chromatographic separation of the various steroids, measured levels of 0.008 µg/ml of progesterone in the plasma of non pregnant mares in the luteal phase of the cycle. The test required 500 ml of blood and probably lacked specificity since it was likely to have measured all progestogens rather than progesterone alone.

Collins and Summerville (1964), when measuring plasma progesterone concentrations in women were the first workers to use isotope labelling - they used flame ionization detection, achieving a sensitivity of 0.01 to 0.1 µg; however it was not until the establishment of CPB and radioimmuno methods of microanalysis that great

advances in sensitivity of assays were achieved.

Plasma proteins will reversibly bind many substances, including hormones. For example corticosteroid-binding globulin (CBG) will bind with steroids (Murphy, 1964). This phenomenon is utilized in CPB assays. The principle of CPB assays depends on the fact that if a radioactively labelled form of a substance, S^+, is added to plasma containing unlabelled S and a limited amount of its specific binding protein, P (e.g. CBG), then if a dynamic equilibrium exists between S and P, S will evenly distribute itself and displace S as shown in the equation: -

$$SP + S^+ \rightleftharpoons S^+P + S$$

thus if S^+ or S^+P is measured, and the amount of added S^+ is known, S can be deduced (Murphy, 1964).

Murphy (1967) was the first worker to measure progesterone in the plasma of humans using a CPB assay and she found levels of less than 20 ng/ml in non pregnant females, 22.8 ng/ml in pregnant females, and less than 8 ng/ml in normal males. Subsequently many workers have measured plasma progesterone concentrations in our own species (Johansson, 1969a; Israel, Mishell, Stone, Thornecroft and Moyer, 1972), monkeys (Neill, Johansson and Knobil, 1967), sheep (Bassett, Oxborrow, Smith and Thorburn, 1969; Thorburn, Bassett and Smith, 1969), cattle (Cain, Cerini, Cerini, Chamley, Cumming and Goding, 1972), guinea pigs (Challis, Heap and Illingworth, 1971), and mares (Smith, Bassett and Williams, 1970; Plotka, Witherspoon and Goetsch, 1971; Allen and Hadley, 1973; Van Neikerk, Morgenthal, Sanders and Malan, 1973a; Evans and Irvine, 1975). Sensitivity of the CPB assay is reported to vary from 0.1 ng (Stone, Nakamura, Mishell and Thornecroft, 1971), to 0.25 ng (Morgan and Cooke, 1972). Human (Johansson, 1969), guinea pig (Cain *et al.*, 1972), chick (Morgan and Cooke, 1972) and dog (Neill, Johansson, Datta and Knobil, 1967) plasma have been used as a source of CBG plasma; it has been established that the use of heterologous plasma gives greater sensitivity (Murphy, 1967).

TABLE I: PLASMA PROGESTERONE LEVELS IN NON PREGNANT MARES MEASURED BY RIA

Reproductive State	Plasma Progesterone ng/ml	Reference
Oestrus before ovulation	0.7	Noden *et al*. (1974)
Mid dioestrus	14-16	
Oestrus	0.9-0.5	Ganjam *et al*. (1975*a*)
Mid dioestrus	7-8	
Oestrus (early)	0.4	Noden *et al*. (1975)*
Oestrus (late)	0.8	
Mid dioestrus	13.6	
Oestrus	0.5	Palmer and Jousset (1975)
Dioestrus	4-10	
Oestrus	0.5	Nett, Pickett, Seidel and Voss (1976)
Dioestrus	4-10	

* Measured progestogens

Many workers using CPB assays, have now measured plasma progestogens (Stabenfeldt, Hughes and Evans, 1972; Ganjam *et al*., 1975; Lovell *et al*., 1975) and plasma progesterone (Smith *et al*., 1970; Plotka *et al*., 1971; Allen and Hadley, 1973; Van Neikerk *et al*., 1973*a*; Evans and Irvine, 1975) during the oestrous cycle of the mare. In general, levels are less than 0.5 ng/ml during oestrus, begin rising 24 to 48 hours after ovulation to reach a peak at about 12 days post ovulation of from 4 to 15 ng/ml and decline rapidly to less than 1 ng/ml within 2 days, at about 13 to 18 days post ovulation. Although Van Neikerk *et al*. (1973*a*) noted a diurnal variation of plasma progesterone with differences of up to 3 ng/ml between the high morning samples and low evening samples, other workers have not reported this. Certainly plasma progestogen levels vary enormously during the luteal phase of the oestrous cycle both within and between mares and Smith *et al*. (1970) noted this, as well as the relatively long late dioestral period of low progestogen levels, and suggested that the pattern was more like that seen in primates (Neill *et al*., 1967*a*) than sheep (Thorburn *et al*., 1969) with the low concentrations during prolonged oestrus being comparable to those found during the follicular phase of the primate menstrual cycle.

Since all steroids will competitively bind with CBG (Murphy, 1967) elaborate, time consuming extraction procedures are necessary to ensure removal of extraneous steroids. If some extraction procedures are omitted, the assay is quicker and more convenient (Johansson, 1969) but less specific (Morgan and Cooke, 1972).

The development of RIA techniques has enabled workers to measure a wide range of compounds in biological fluids with even more sensitivity and accuracy than was the case with CPB techniques. Abraham (1969) was the first worker to develop an RIA to measure a steroid hormone, oestradiol 17β.

Table I facing page 42 summarises levels of plasma progesterone found in non pregnant mares using RIA.

Ganjam et al. (1975a) measuring plasma progesterone concentrations in the mares using both CPB and RIA techniques found as would be expected, that levels obtained from the more specific RIA were consistently lower than those obtained using CPB.

2. Pregnant Mare

Edgar (1953a) using spectrophotometry could not detect progesterone in the peripheral plasma of pregnant mares with a test sensitive to 0.1 μg/ml while Short (1959), using a similar method but with a sensitivity of 0.002 μg/ml, found levels of 0.014 μg/ml in a mare 64 days pregnant but could not detect any progesterone in the peripheral plasma of a 170 day pregnant mare.

Allen and Hadley (1973) were the first workers to measure progestogen levels in the blood of pregnant mares using a CPB assay. They found levels of from 9.4 to 17.4 ng/ml in mares from 7 to 17 days of pregnancy. Van Neikerk et al. (1973) noted a slight decrease in plasma progesterone concentration in mares pregnant between 10 to 14 days and found major peaks at 20 to 23 days, 30 to 32 days, and again 6 days after formation of the secondary corpora lutea, at an average of 42 days of pregnancy. They noted that the periods of low plasma progesterone concentrations corresponded with the respective waves of follicular activity reported by Van Rensburg and Van Neikerk (1968).

Smith (1974) measured progesterone in the plasma of mares throughout pregnancy and found that there were considerable differences between mares at any one stage of gestation, with levels rising during the first 60 days, falling to a minimum at 180 days and reaching the highest level from 270 days until term. Since Smith's report, Barnes, Nathanielsz, Rossdale, Comline and Silver (1975), Burns and Fleeger (1975), Ganjam et al. (1975) and Lovell et al. (1975) have all recorded high plasma progestogen levels in mares in advanced pregnancy with values as high as 60 ng/ml (Burns and Fleeger, 1975). The variation in results between the earlier workers, such as those reported by Short (1959) and Stabenfeldt

TABLE II: PLASMA PROGESTERONE AND PROGESTOGEN LEVELS IN PREGNANT MARES BY RIA

Gestation length (days)	Plasma (ng/ml) progesterone	Plasma (ng/ml) progestogens	Reference
10+		14	Barnes *et al.* (1975)
30		13	Burns and Fleeger (1975)
90		28	
70+		40	
10+		60	
180	1.6	4.7	Ganjam *et al.* (1975a)
235	2.5	7.3	
328	4.2	15.2	
60+	5	6	Lovell *et al.* (1975)
10+	6.5	10	

\+ Days prior to parturition

(1969), and latter workers may be largely accounted for by the marked differences in detection techniques.

Two peaks of plasma progestogens in pregnant mares have been reported by Ganjam *et al*. (1975). The first occurred during the third month of pregnancy, coinciding with high levels of PMSG, while the second peak occurred in the eleventh month. These workers identified at least two unknown progesterone metabolites, which cross reacted with the CPB and RIA systems, and concluded that these metabolites probably contributed to the high progestogen values obtained in late pregnancy when chromatography was omitted from the assay systems.

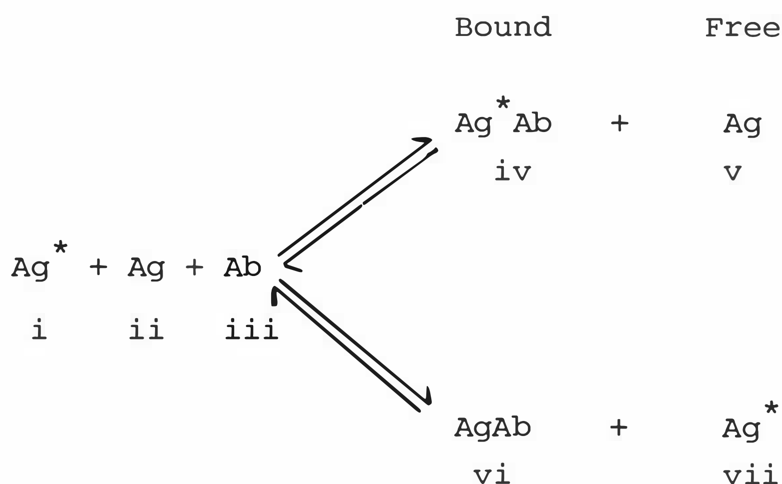
Table II facing page 44 summarises plasma progesterone levels obtained by RIA reported by various authors.

Progesterone concentrations in the plasma of mares fall dramatically after parturition, to less than 1 ng/ml by 24 hours *post partum* (Ganjam *et al*.,1975; Lovell *et al*., 1975). Although plasma progesterone levels begin to fall immediately prior to parturition, foaling proceeds in the presence of significant plasma levels of this steroid (Barnes *et al*.,1975; Lovell *et al*.,1975). Ganjam *et al*. (1975) found plasma progesterone concentrations of 1.7 ng/ ml in the plasma of 24 hour old foals. This, together with an umbilical arterio-venous difference of about 4 ng/ml in favour of the umbilical artery suggests that the foetus contributes to the progesterone pool. Further, Barnes *et al*., (1975), who measured high steroid hormone levels in the near-term foetal umbilical artery and vein and a large arterio-venous difference of these steroids, suggested that there may be a metabolic cycle of progestogens whereby they are converted to a metabolite in the foetus and then back to progesterone in the placenta.

D. RADIOIMMUNOASSAY OF PLASMA PROGESTERONE

The technique of RIA, first developed for the measurement of hormones, has been expanded during the past decade to include the detection of many biological agents (see review by Skelley, Brown and Besch, 1973).

All RIA procedures are based on the original observation by Berson and Yalow (1958) that low concentrations of antibodies to the antigenic hormone insulin could be detected by their ability to bind radiolabelled (^{131}I) insulin. Unknown concentrations of antigen could therefore be determined by taking advantage of the observation that the radiolabelled hormone molecules ("tracer") compete physicochemically with nonlabelled hormone molecules (either standards or unknowns) for binding sites on the antibodies. The principle of the RIA is illustrated below (after Skelley *et al.*, 1973).



i. Ag* Labelled antigen (e.g. hormone)
ii. Ag Unlabelled antigen (e.g. hormone standard or unknown)
iii. Ab Antibody to Ag and Ag*
iv. Ag*Ab Labelled antigen bound to antibody
v. Ag Free unlabelled antigen
vi. AgAb Unlabelled antigen bound to antibody
vii. Free labelled antigen

The RIA has several advantages over CPB assays:

(i) For CPB assays deproteinization of the sample is necessary.

(ii) Although it is cheaper and quicker to set up a CPB assay for a few analyses, it remains less costly to perform hundreds or thousands of assays for RIA, even though development of a suitable antiserum might take time.

(iii) The main advantage of RIA over CPB assay is that RIA is potentially more sensitive owing to higher affinity constants and the nature of the antibody in RIA offers greater specificity.

Morgan and Cook (1972) and Ganjam et al. (1975) measured plasma progesterone concentrations in women and mares respectively using CPB and RIA. There was good agreement between the results of the two methods, both groups of workers concluding that the RIA was more sensitive and in the case of the former authors, more robust.

Steroids and other low molecular weight compounds are not inherently antigenic but will act as haptens when conjugated to proteins (Goodfriend and Sehon, 1970). Consequently, the first substances to be measured by RIA were large molecular weight compounds, capable of being antigenic in a heterologous system (Murphy, 1964). Berson and Yalow (1958) observed that low concentrations of antibodies to the antigenic hormone insulin could be detected by their ability to bind (^{131}I) insulin and could detect less than 1 µg of insulin-binding antibody. These workers subsequently developed the first RIA to measure plasma insulin in man (Yalow and Berson, 1960).

Erlanger, Borek, Beiser and Lieberman (1959) were able to conjugate bovine serum albumin (BSA) to progesterone, desoxycorticosterone and estrone and found the conjugates were antigenic in rabbits. Further, Beiser, Erlanger, Agate and Lieberman (1959) showed the percent cross reactivity of antibodies developed to the conjugate, depended on the site of BSA conjugation. These latter two discoveries have meant that with a little judicious biochemistry and patience all those substances which will act as haptens (which includes most hormones) may now be measured by RIA. BSA or human serum albumin (HSA) are the commonest

proteins used for conjugation (Thornecroft, Tillson, Abraham, Scaramuzzi and Caldwell, 1970). Niswender (1973) concluded that steroid hormones should be conjugated to protein at sites on the B or C ring of the molecule for maximum specificity and minimum cross reactivity.

The ultimate requirement for any RIA is a reliable antiserum (Skelley et al., 1973). Such reliability exists when there is an equal competition between the standard or unknown antigen and the labelled antigen for binding sites on the antibody. The five main criteria used to assess the reliability of RIA are recovery, sensitivity, specificity, accuracy and precision (Furr, 1973).

Abraham (1969) was the first worker to develop an RIA to measure a steroid hormone when he measured plasma oestradiol-17β in women, achieving a recovery of 82.2% and a sensitivity of 10 pg. The first report of progesterone measurement by this technique was by Abraham, Swerdloff, Tulchinsky and Odell (1971). They used 11-desoxycorticosteroid-HSA to form antibody, the free and bound hormone being separated with a dextran coated charcoal suspension; a recovery of 84.2% was achieved with a sensitivity of from 10 to 25 pg. Since then many further reports of plasma progesterone measurements using this technique have come forward e.g. in humans (Furuyama and Nugent, 1971; de Villa, Roberts, Wiest, Nikhail and Flickinger, 1972; Morgan and Cooke, 1972; Youssefnejadian, Florensa, Collins and Summerville, 1972), domestic fowl (Furr, 1973), cattle (Lewis, Hafs and Seguin, 1973) and mares (Noden et al., 1974; Burns and Fleeger, 1975; Ganjam et al., 1975a; Noden et al., 1975; Palmer and Jousset, 1975).

Progesterone has been extracted using petroleum ether (Abraham et al., 1971; de Villa et al., 1972; Morgan and Cooke, 1972), light petroleum (Furr, 1973), hexane (Furuyama and Nugent, 1971), and 1 benzene:2 hexane (Lewis et al., 1973). Recovery rates varied from 66.1% (Furuyama and Nugent, 1971) to 84.2% (Abraham et al., 1971). Sensitivity varied from 0.25 ng/ml (Abraham et al., 1971; Furuyama and Nugent, 1971; Furr, 1973) to 1.0 ng/ml (Morgan and Cooke, 1972). Specificity, as expected, varied with the position

of the BSA conjugation. de Villa *et al*., (1972) and Youssefnejadian *et al*., (1972), using 11-hydroxyprogesterone hemisuccinate to form antibodies, reported excellent specificity. With microchromatographic separation of duplicate samples, there was no statistical difference in the values obtained for plasma progesterone concentrations in luteal phase females compared with those values found in samples where chromatographic separation was not undertaken. Morgan and Cooke (1972) used antisera formed against progesterone conjugated at both the 20 and 11 positions. The antibody formed against progesterone-11-BSA showed more specificity and less cross reactivity than that formed against the progesterone-20-BSA. Furuyama and Nugent (1971), on the other hand, using progesterone-3-BSA to form antibody, found plasma progesterone levels approximately twice as high without chromatography compared with when chromatography was used. Ganjam *et al*., (1975) utilizing an antiserum against 11-hydroxy-progesterone-hemisuccinate noted that the only compounds that cross reacted with this antibody besides progesterone (100%) were 5α-pregnane-3, 20-dione (15%) and 5β-pregnane-3, 20-dione (6%).

The accuracy of assays is assessed by estimating the percent difference between the values of progesterone measured and the expected values. This varied from 16% (de Villa *et al*., 1972) to 20% (Abraham *et al*., 1971). The coefficient of variation of the values found gives an estimate of precision. Values of from 4.4% (n=24 Furuyama and Nugent, 1971) to 15% (n=10 Morgan and Cooke, 1972) have been quoted.

GENERAL MATERIALS AND METHODS

A. ANIMALS

A total of 72 mares whose ages varied from 3 to 18 years were used. Sixty two of the mares were thoroughbreds pastured on two studs in the Manawatu area and 10 were of mixed breeding, mainly pony mares.

The thoroughbred mares were fed good quality grass *ad libitum* and the majority were in good bodily condition, except for 10 mares which at the beginning of the breeding season were in poor bodily condition. All the non-pregnant mares were run together and every second day were driven into a yard and teased. The mares were teased twice daily when in oestrus.

Teasing was carried out using a standardbred stallion on one stud and a pony stallion on the other stud. Each mare was caught and teased for approximately half a minute if the mare showed no interest, or longer if the mare appeared to be in oestrus. Mares in oestrus often approached the teaser as he walked around the yard. A mare was considered to be in oestrus if she displayed two or more of the following signs: - "winking" (eversion of the vulvar labia), squatting, tail raising, urinating, lack of dioestrous behaviour. Mares in dioestrus displayed one or more of the following characteristics; laying the ears back, biting, squealing, kicking and striking. Mares were recorded as being in oestrus, "indifferent" or not in oestrus. The "indifferent" group were teased again after about 10 minutes. If these mares were due to be in oestrus about that time, and the behaviour was a change from that when in dioestrus, they were considered to be coming into oestrus and were examined.

Mares were examined by the author at the first observed oestrus of the breeding season. The external genitalia were inspected, and a rectal examination performed, attention being paid to the cervix, uterus and ovaries. A vaginal examination was carried out, both manually and with a speculum. If abnormalities were observed these mares were precluded from the experimental groups. Ovarian, uterine and cervical changes were recorded at

this initial and at all subsequent examinations.

The non-thoroughbred mares were part of the Massey University herd. These mares were grazed on good pasture, supplemented with hay if the grass became scarce. All were in good bodily condition. When serial blood samples were taken, mares were stabled for periods of up to 4 days in constant light (natural light, with fluorescent lighting between sunset and sunrise). They were fed 3 Kg of a commercial preparation of horse nuts with lucerne chaff twice daily and hay _ad libitum_ while stabled.

B. BLOOD COLLECTION

When single samples of blood were to be collected, they were obtained by left jugular venepuncture using a 10 ml heparinized vacutainer. When serial samples were to be taken, an indwelling jugular cannula consisting of approximately 200 mm x 2 mm silicon rubber ("Silastic", Dow Corning Corporation, USA) was inserted via a 12 gauge needle into the vein, the free end being sutured to the skin. Cannula patency was maintained by flushing with 10 ml of 0.9% normal saline containing 20 iu sodium heparin/ml. Blood samples were collected into a 10 ml disposable syringe and immediately transferred to a 10 ml test tube containing 143 units of sodium heparin.

As soon as possible after collection, and always within 90 minutes, samples were centrifuged at 3,000 rpm for 4 minutes. The plasma was separated and stored in 10 x 75 mm disposable culture tubes at -18° C until required. Any special method used during the collection of samples is detailed in the appropriate section of the results.

C. RADIOIMMUNOASSAY

1. Reagents

Phosphate buffered saline (PBS) contained 0.01 M phosphate buffer and 0.14 M sodium chloride, with 0.01% (w/v) sodium merthiolate as a preservative; pH was adjusted to 7.3.

0.02 M EDTA-PBS was a solution of 0.02 M ethylene diamine tetra acetic acid, disodium salt (EDTA), in PBS.

0.1% gelatin-PBS was a solution 0.1% Knox gelatin in PBS.

Organic solvents (mainly technical grade) were redistilled before use. Ethanol was purified by refluxing for 8 hours over m-phenylenediamine then redistilled three times before use.

Dextran coated charcoal consisted of 0.5% (w/v) dextran T70 (Pharmacia, Sweden) and 0.25% (w/v) charcoal (Darco G60 Atlas Chemical Industries, U.K.) in glass distilled water.

Scintillation fluid consisted of toluene-triton X-100 (2:1) containing 3 g 2,5-diphenyloxazole (PPO) and 100 mg 1,4-bis 2-(5-phenyloxazolyl) -benzene (POPOP) per litre.

Standard progesterone (Sigma Chemical Co., USA) in ethanol, at a concentration of 1 ng/ml was prepared fresh for each assay from a stock solution containing 10 ng/ml of progesterone in ethanol.

Radioactive progesterone (H^3 -1α,2α, progesterone, 58 ci/mmol, The Radiochemical Centre, Amersham, U.K.) was stored as a stock solution containing approximately 900,000 dpm (disintegrations per minute)/μl in ethanol. After drying the ethanol under air, a stock solution of radioactive progesterone was prepared with 0.1% gelatin in PBS to a working solution of approximately 252 dpm/μl for each assay.

Antiserum prepared in sheep against 11-progesterone-BSA was supplied by courtesy of Dr R. Fairclough, Ruakura Agricultural Research Centre, Hamilton.

Freeze-dried antiserum was reconstituted with 0.02 M EDTA-PBS. Normal sheep serum, 1 in 400 was added to

provide an additional protein source. The reconstituted antiserum was stored in 1 ml aliquots in glass sealed vials at -18° C at a concentration of 1:200, while the final working concentration of 1:2,000 was prepared by further diluting the serum with 0.02 M EDTA-PBS containing 1:400 normal sheep serum.

2. Extraction Procedure

Triplicate, 100 µl plasma samples were extracted with 2 ml of benzene-hexane (1:2) by vortex mixing for 30 seconds in 16 x 100 mm glass tubes. The aqueous layer was frozen then the solvent was decanted into 10 x 75 mm glass tubes and evaporated to dryness. The walls of the tubes were rinsed with 0.1 ml dichloromethane and again evaporated to dryness.

Aliquots of progesterone-free gelding plasma, containing appropriate additions of standard progesterone were extracted in the same manner as the samples to provide a standard curve over the range 0 to 15 ng/ml.

3. Radioimmunoassay Procedure

The radioimmunoassay procedure was a slight modification of that described by Lewis, Hafs and Seguin (1973).

After extraction of unknowns, two plasma samples of known progesterone levels, and standards, all in triplicate, then evaporation of solvents under air; 0.2 ml of antiserum diluted 1:2,000 was added to each tube and vortexed for 10 seconds. Tubes were stood at room temperature for half an hour to allow reaction of antibody and progesterone, then 0.2 ml of radioactive progesterone was added to each tube which was vortexed for 5 seconds, and allowed to incubate for between 12 and 18 hours at 5° C.

After incubation, 0.5 ml of dextran coated charcoal was added to each tube, which was then vortexed for 10 seconds, incubated in an ice bath for 10 minutes and centrifuged at 3,000 rpm at 4° C in a refrigerated centrifuge (MSE Mistral 6L) for 10 minutes. An 0.5 ml aliquot of supernatant was then removed from each tube and placed in a scintillation vial (Packard) to which was added 6 ml of scintillation fluid. The radioactivity was counted in a liquid scintillation counter (Packard Tri-

curb Model 2002) for two minutes.

4. Calculation of Results

An IBM 1620 computer was used to determine plasma progesterone concentrations by the method of Burger, Lee and Rennie (1972). With this technique a best-fit expression for the standard curve was calculated, then values for the samples (mean of triplicates, standard deviations and 95% fiducial limits) were computed. A typical standard curve is shown in Figure 3 facing page 57.

5. Validation of Assay

a. Antibody specificity

The cross reactivity of the antibody with other steroids was tested by Dr R. Fairclough (see below).

Steroid	Percent cross reactivity
progesterone	100
estradiol	0
pregnenolone	0.04
corticosterone	1.25
17α -hydroxy progesterone	1.88
cortisol	1.80
20α -hydroxy progesterone	4.2
4 androstene 11β ol, 3, 17, dione	1.2
4 androstene 17β ol, 3, one	1.5
11 desoxycortisol	0.4
androstenedione	1.43
testosterone	0.61
cholesterol	0.8

At a dilution of 1:2,000, binding by antiserum of the radioactive progesterone was 49.1 ± 6% (N=20).

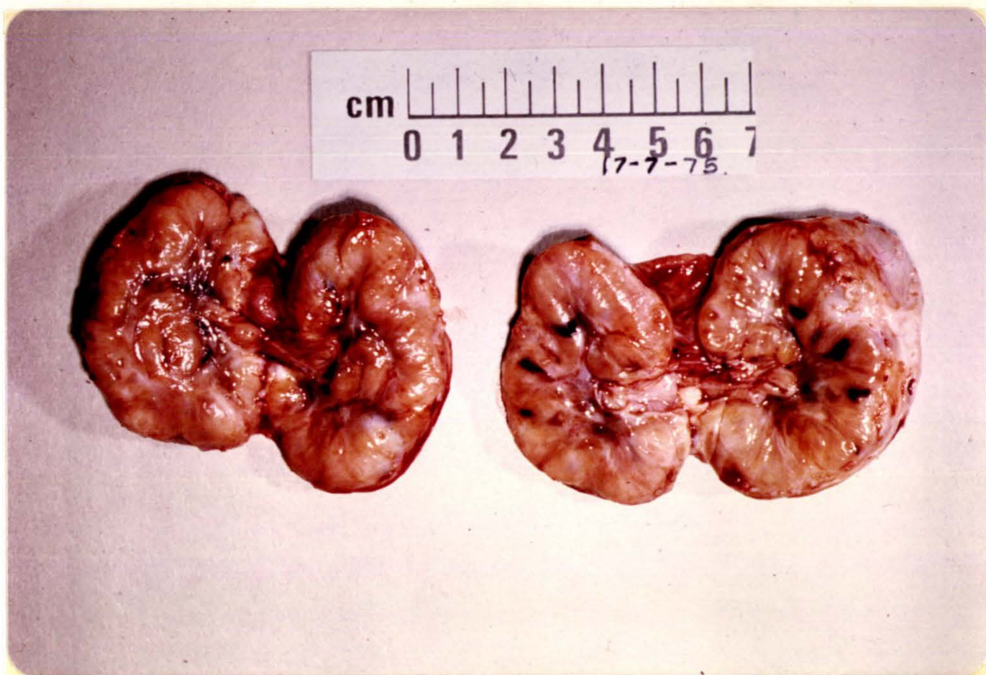
b. The dextran coated charcoal absorbed 96.1 ± 2.4% (N=10) of the free progesterone.

c. Using benzene hexane (1:2), 77.7 ± 3.8% (N=18) of progesterone (10 ng/ml) was extracted from plasma.

Recovery of hormone added to plasma was not checked since the progesterone standards in this method were added to tubes containing 100 µl of gelding plasma and then processed as with the unknowns.

FIGURE 3: OVARIES FROM A MARE IN ANOESTRUS

FIGURE 4: OVARIES FROM A MARE IN OESTRUS


cm
0 1 2 3 4 5 6 7
17-7-75

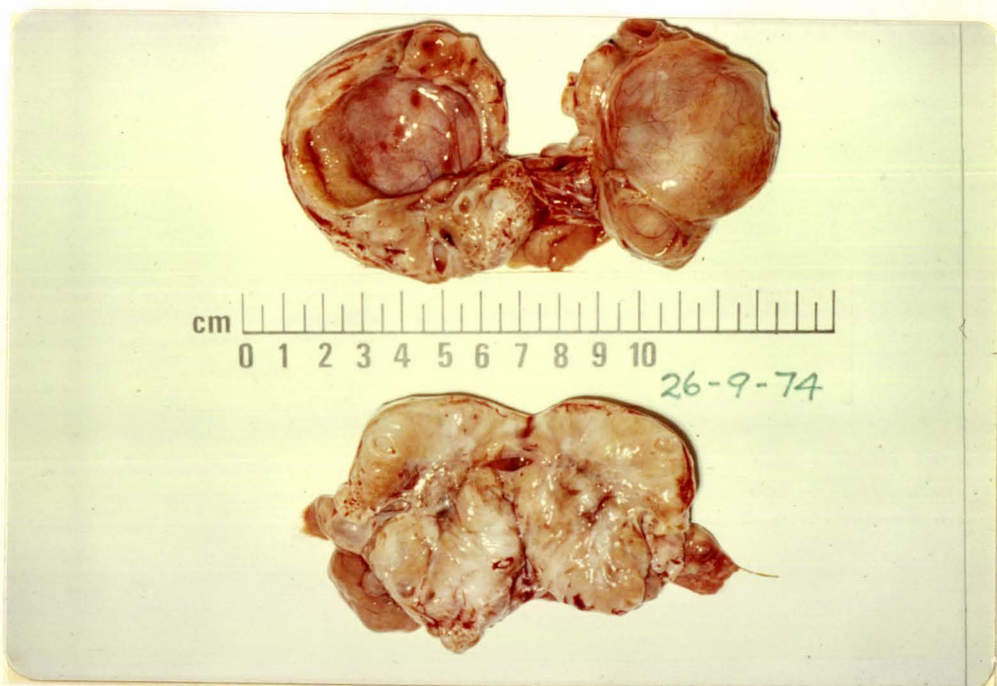
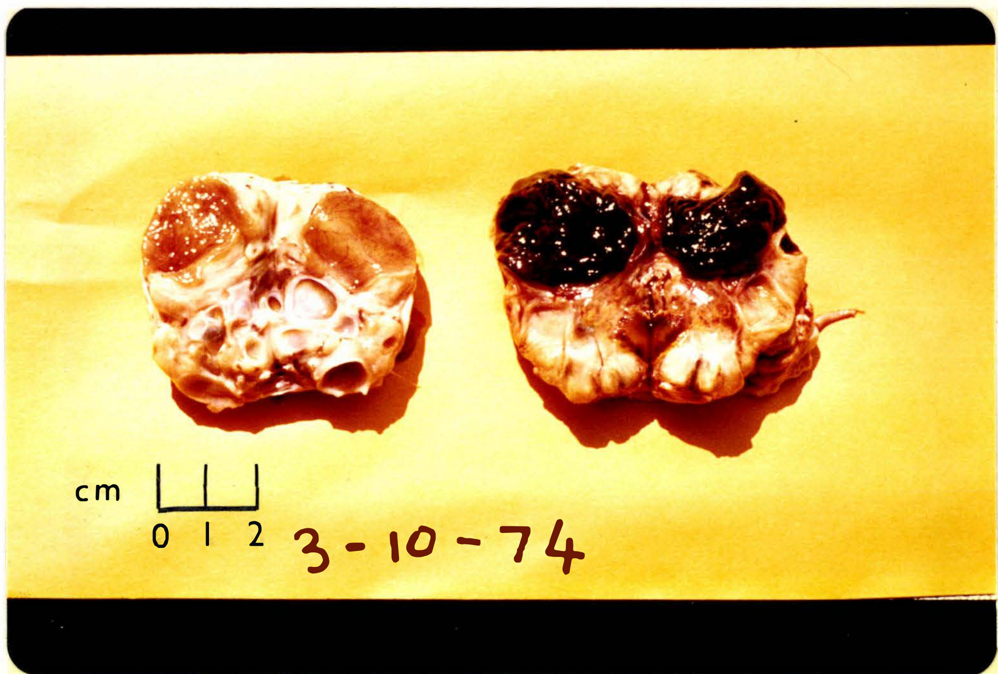

cm
0 1 2 3 4 5 6 7 8 9 10
26-9-74

FIGURE 5: OVARIES FROM A MARE IN DIOESTRUS


cm
0 1 2
3-10-74

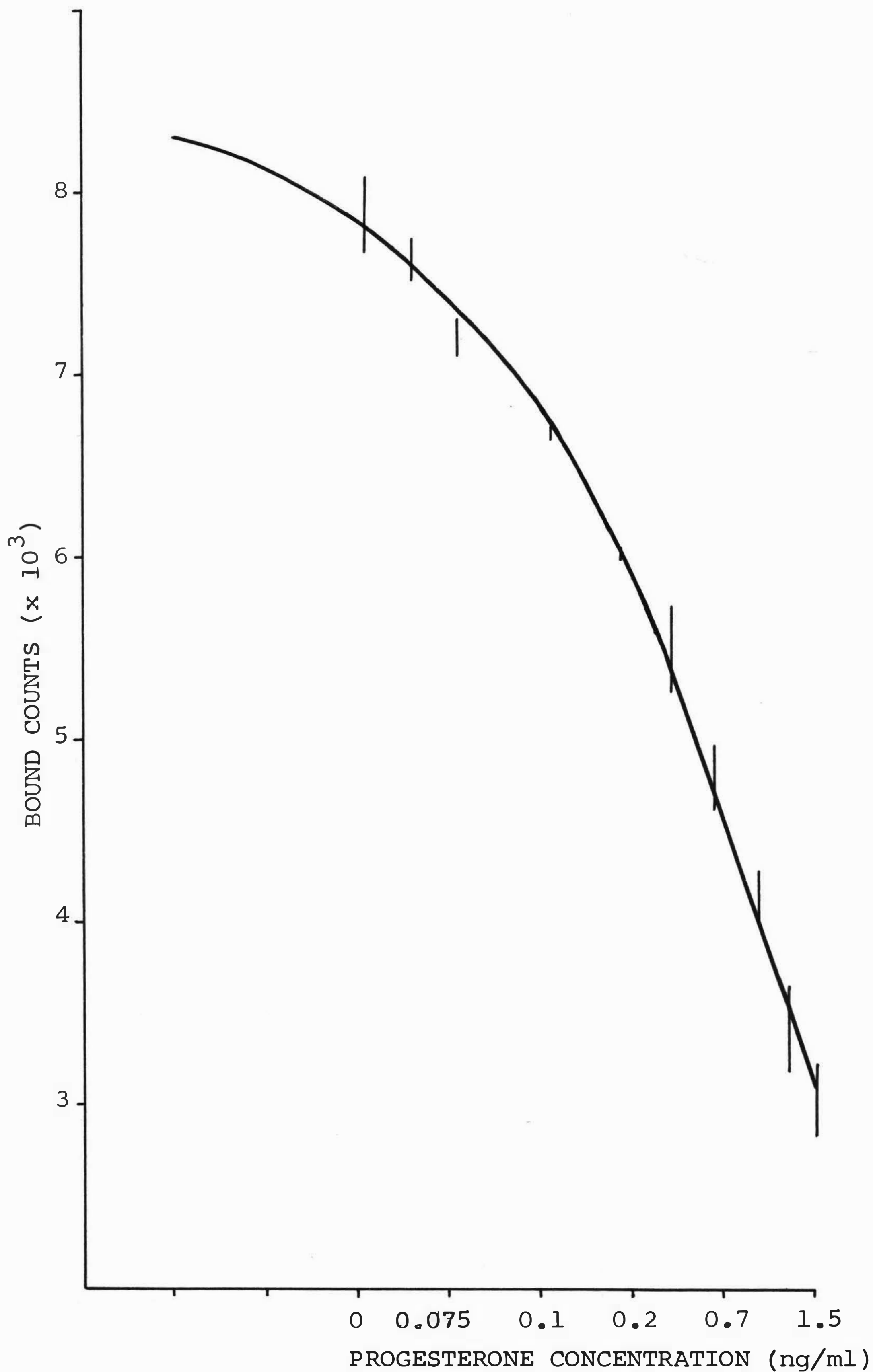


FIGURE 6 : A TYPICAL SENSITIVITY CURVE (± S.E. MEAN COUNTS) SHOWING "BEST-FIT" OF PROGESTERONE STANDARDS

d. Parallelism of the standards and a curve obtained by assaying varying quantities of progesterone extracted from a plasma sample was checked by assaying the sample undiluted (14.5 ng/ml), diluted 1:1 (6.8 ng/ml) and diluted 1:3 (3.8 ng/ml) in gelding plasma (N=3).

e. Assay sensitivity, defined as the lowest point on the standard curve with a coefficient of variation equal to 50% (Burger, Lee and Rennie, 1972) ranged from 0.1 to 0.45 ng/ml. This range of values corresponded with the lowest plasma progesterone concentrations with 95% fiducial limits which did not overlap zero. The overall assay sensitivity was taken as 0.5 ng/ml, since this concentration of plasma progesterone is probably the absolute minimum value likely to reflect a secretory CL (Hughes *et al*., 1972).

f. Between assay precision was estimated by repeated assay of plasma samples in different assays. A plasma sample with a high progesterone content (mean 10.5 ng/ml) had a between assay coefficient of variation (CV) of 16.8% for 24 assays; a plasma sample with a low progesterone content (mean 1.1 ng/ml) had a between assay CV of 8.5% for 15 assays.

g. For a plasma sample with a mean progesterone concentration of 11 ng/ml, within assay CVs of 7.3%, 10.1% and 6.9% were estimated when the sample was assayed 6 times in each of 3 assays.

h. Biological validation of the assay was assessed by estimating plasma progesterone levels in mares whose ovarian function was known. In 7 mares with inactive ovaries, (see example in Fig.3 on page 55), plasma progesterone levels were from 0.00 ng/ml to 0.32 ng/ml.

In two mares which were in oestrus when sampled, (see example in Fig.4 on page 55) plasma progesterone concentrations were 0.26 and 0.32 ng/ml respectively.

In two mares which were in early to mid dioestrus when sampled, (see example in Fig.5 on page 56) plasma progesterone concentrations were 13.1 and 3.2 ng/ml respectively.

On the basis of the validation tests performed, it was considered that this assay provided reliable estimates of equine plasma progesterone levels.

TABLE III: **PLASMA PROGESTERONE LEVELS OF MARES LESS THAN 70 DAYS PREGNANT**

Mare	Plasma progesterone (ng/ml)	Estimated stage of pregnancy when sampled (days)	Sex of embryo
21/5	10.9	32	female
13/3	13.9	36	female
24/2	4.9	36	male
D 5/11	9.1	40	male
3/12	8.0	42	female
G 5/11	13.1	42	male
15/4	9.3	48	female
5/4	8.3	46	female
B131/7	10.8	50	female
B431/7	15.0	64	female

RESULTS AND DISCUSSION

Plasma progesterone levels were measured in mares in both normal and abnormal reproductive states. This section of the thesis outlines the results of the plasma progesterone levels detected in these mares and concludes with a consideration of the results obtained when exogenous progestogens were administered.

A. PLASMA PROGESTERONE LEVELS OF NORMAL MARES IN VARIOUS REPRODUCTIVE STATES

1. Cycling, Non-pregnant Mares

The plasma progesterone levels of 6 non-lactating mares are summarised in Figs. 7 and 8 on and facing page 60 . The sampling dates were from the 1st September to the 31st of October, 1974. Mare "MO" had a split oestrus extending over 22 days; her first oestrus was of 12 days duration. Excluding the split oestrus for mare "MO" length of oestrus over 11 cycles for the 6 mares was 9.2 days with a range from 6.5 to 14 days. Length of dioestrus over 6 cycles was 17.6 days with a range from 11 to 22 days.

2. Pregnant Mares, Gestation Length Less Than 70 Days

a. Special methods

Plasma was collected from 10 mares immediately before they were anaesthetised for surgical removal of their blastocysts. The embryos were removed, sexed and aged by measuring crown-rump length (Bergin, Gier, Frey and Marion, 1967).

b. Results

Table III (facing page 59) summarises the plasma progesterone levels in these mares. Plasma progesterone levels had a mean of 10.3 ng/ml with a range of from 4.9 to 15.0 ng/ml. Mean stage of pregnancy when samples were taken was 43.6 days with a range of from 32 to 64 days.

There were no significant differences between plasma progesterone levels related to either stage of gestation or sex of embryo in this group of mares.

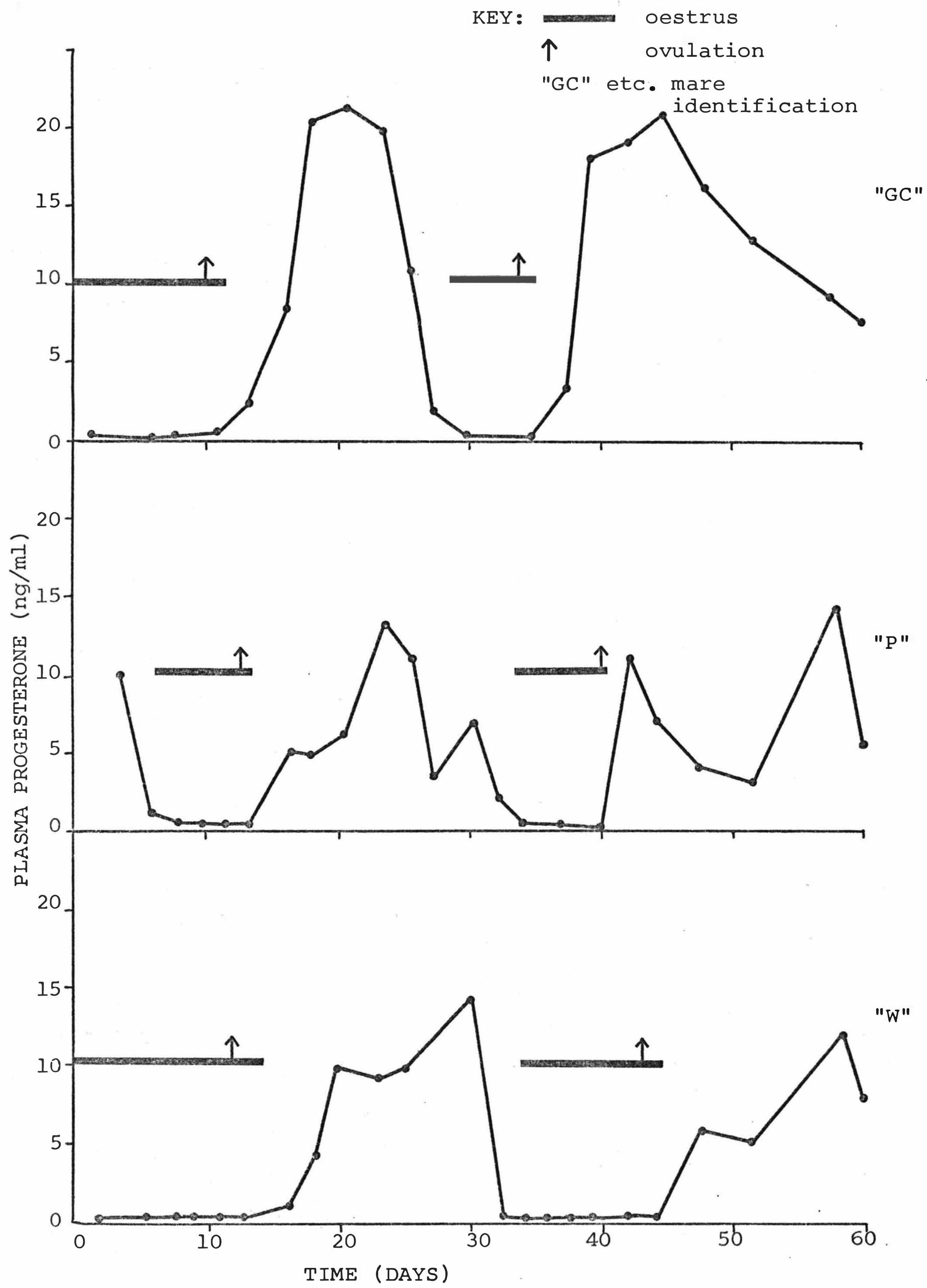


FIGURE 7: CONCENTRATION OF PLASMA PROGESTERONE IN NORMAL CYCLING MARES

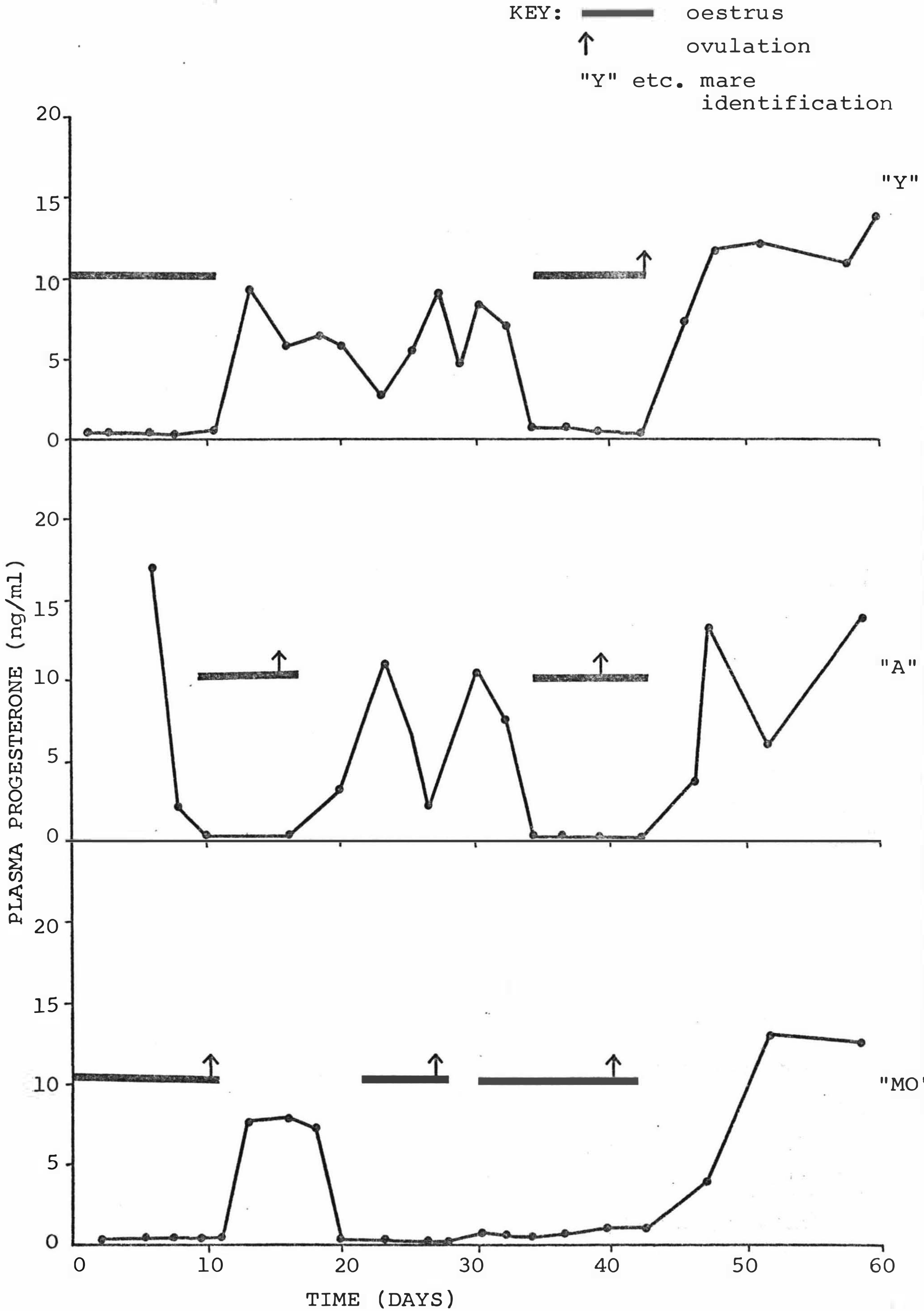


FIGURE 8: CONCENTRATION OF PLASMA PROGESTERONE IN NORMAL CYCLING MARES

TABLE IV: PLASMA PROGESTERONE LEVELS OF MARES GREATER THAN 240 DAYS PREGNANT

Mare	Plasma progesterone (ng/ml)	Stage of pregnancy when sampled (days)	Gestation length (days)	Sex of foal
Bi	11.2	292	351	female
Lo	7.0	261	338	male
Su	12.3	262	345	male
L.L.	12.2	287	363	male
M.L.	9.8	266	340	female
We	16.8	259	339	male
B.A.	11.6	324	354	female
Pl.	14.3	267	342	female
L.R.	12.9	290	352	female
Sc.	16.9	276	346	female
L.Q.	8.8	240	343	female
Pi	7.7	299	355	female
B.N.	7.4	249	330	male
Ku	5.2	267	344	male
M.S.	5.9	276	332	female
M.B.	10.5	255	341	male
J.M.	10.9	317	346	female
C.B.	7.6	272	349	male
Gl	6.9	256	344	female
Kg	10.2	317	351	female
Sg	9.9	262	338	male

3. Mares, 240 Or More Days Pregnant

a. Special methods

Plasma was collected from 21 mares, all 240 or more days pregnant when sampled. Each mare subsequently produced a normal, single live foal to the pregnancy.

b. Results

Table IV facing page 61 summarises the plasma progesterone levels of these mares. The mean plasma progesterone level was 10.3 ng/ml with a range of from 5.2 to 16.9 ng/ml.

Mean stage of pregnancy when samples were taken was 275.9 days with a range from 240 to 324 days. Mean gestation length was 345 days with a range of from 330 days to 363 days for these pregnancies.

As with the former group, there was no statistical difference between plasma progesterone levels related to either stage of gestation or sex of embryo in this group of mares.

4. Plasma Progesterone Levels In Mares From Immediately Prior to Parturition Until The First Day of Oestrus Post Partum

Table V, facing page 62 summarises the plasma progesterone levels for the 5 mares sampled in this group.

Mean plasma progesterone concentrations of 11 samples from these 5 mares measured either during the 3 days before or on the day of parturition was 9.4 ng/ml (range 3.9-13.7 ng/ml). By the first day *post partum* plasma progesterone levels were all less than 0.5 ng/ml and remained so until the first *post partum* oestrus.

5. Discussion

Concentrations of progesterone in the plasma of normal cycling mares in this study are similar to those reported by other workers (Plotka *et al.*, 1971; Stabenfeldt *et al.*, 1972; Evans and Irvine, 1975) with peak dioestrous levels being higher than some reports (Van Niekerk *et al.*, 1973*a*; Smith *et al.*, 1970). Differences in type of assay (RIA *versus* CPBA), extraction techniques, antibody formation and separation methods probably account for the differences

TABLE V: PLASMA PROGESTERONE LEVELS (ng/ml) IN MARES FROM IMMEDIATELY PRIOR TO PARTURITION TO FIRST *POST PARTUM* OESTRUS

	Days from Parturition													
Mare	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10
Ma	13.3	11.3	9.6	-	ns	-	ns	-	-	ns	-	oe		
Co	-	3.9	-	4.3	ns	ns	ns	ns	-	ns	-	ns	ns	oe
Re	-	-	13.7	3.9	ns	ns	-	ns	-	ns	ns	ns	oe	
Tc	8.1	-	9.7	-	ns	-	ns	-	ns	-	ns	ns	oe	
Am	13.2	-	12.6	-	ns	-	ns	-	ns	-	ns	-	ns	oe

Day 0 = day of foaling. Sample collected prior to foaling

\- = not sampled

ns = plasma progesterone < 0.5 ng/ml

oe = oestrus

observed by different workers.

It is recognised that mares in oestrus are sensitive to low concentrations of plasma progesterone (Hughes *et al*., 1972). In this study oestrus was only displayed by the mares when plasma progesterone concentrations were less than 1 ng/ml and usually less than 0.5 ng/ml, an observation in agreement with most other workers (Evans and Irvine, 1975; Palmer and Jousset, 1975; Nett, Pickett, Seidel and Voss, 1976).

As Noden *et al*. (1975) suggest oestrus is probably terminated by rising levels of plasma progesterone secreted by the newly formed CL, although this is by no means absolute since Hughes *et al*. (1972) noted that at different times, 10 of 11 mares studied for a year ovulated when out of oestrus. There were 3 mares in the current study which for short periods did not display oestrus but had plasma progesterone concentrations of less than 1 ng/ml. This occurred immediately before or after oestrus, only for up to 48 hours at the most, and probably reflected low endogenous plasma oestrogen levels at this time.

Excluding the split-oestrus displayed by mare "MO", length of the first oestrus in this study (mean 10 days) was longer than the next oestrus (mean 8 days); however this first oestrus was early in the breeding season when longer oestrous periods are expected (Trum, 1950; Hughes *et al*., 1972).

Oestrous cycles in this study were quite variable in length and in agreement with Ginther *et al*. (1972) length of dioestrus, which ranged from 11 to 22 days was less variable than length of oestrus which ranged from 6.5 to 14 days (excluding the split oestrus). Further, in common with observations of Palmer and Jousset (1975), the single most variable portion of the oestrous cycle was from the time of progesterone decline to ovulation (6 to 12 days *versus* 16 to 20 days for the period from ovulation to progesterone decline; excluding the mare "MO" which had a split oestrus).

Of 13 observed periods of oestrus, 12 were accompanied by a detectable ovulation. The oestrus for which no ovulation was detected was followed by a rise in plasma progesterone, and since oestrus without ovulation is

uncommon (Hughes *et al.*, 1972), and rectal examination may not be an infallible method of detecting ovulation (Palmer and Jousset, 1975), it is likely that this mare ovulated, with the ovulation going undetected.

Mare "MO" was unusual in that after a very short dioestrus (11 days) she had an extended "split" oestrus (22 days) with a double ovulation as determined by rectal examination, the first ovulation probably not resulting in CL formation since there was no significant rise in plasma progesterone until after the second ovulation 13 days later. Although apparently normal when selected for the study this mare was later found to have a chronic uterine infection and she exhibited a series of short oestrous cycles, a characteristic of some mares with endometritis (Allen and Hadley, 1972). This mare did however, become pregnant later in the season and delivered a healthy foal the following year.

There was a wide variation in plasma progesterone levels in the normal mares in early pregnancy, a finding similar to that reported by Smith (1974). The results of this study also are in agreement with those of Allen and Hadley (1973), but in contrast with those of Holtan, Nett and Estergreen, (1975), in that between the sampling times of 32 and 64 days of pregnancy there was no significant trend, upward or downward in plasma progesterone concentrations in the mares.

Plasma progesterone levels in the normal mares in late pregnancy showed wide variation similar to that found in the mares in early pregnancy. The levels recorded in this investigation were higher than those reported by Lovell *et al.*, (1975), similar to those reported by Ganjam *et al.*, (1975), and lower than the levels reported by Burns and Fleeger (1975).

Recently it has been established that progesterone-like compounds are present in the plasma of mares in advanced pregnancy and react with CPBAs (Holtan *et al.*, 1975) and at least some RIAs (Ganjam *et al.*, 1975). Measurement of some or all progesterone metabolites in different assay systems may explain the differences in

values obtained by different workers for plasma progesterone levels in mares in advanced pregnancy.

The anti-progesterone antibody used in the investigation carried out by the author is similar to that used by Ganjam *et al*. (1975) so that the plasma steroids measured in late pregnancy as progesterone probably were progestogens together with some progesterone. Despite the fact that a number of workers have reported high plasma progesterone concentrations in late pregnant mares it might be that Short (1959) and Stabenfeldt (1969) were correct when they suggested that from 200 days of gestation onwards, progesterone metabolism is largely localised with progesterone being produced and metabolised in the foeto-placental unit and little or no progesterone escaping into the peripheral circulation of the dam.

Plasma progesterone levels of mares from immediately prior to parturition to the "foal" oestrus again highlighted the great variation in levels of this steroid (or steroids) in normal mares. While the plasma progesterone concentrations of the two mares "CO" and "Re" sampled within 24 hours of parturition were 4.3 and 3.9 ng/ml respectively, which was lower than the values recorded for nine measurements taken between 24 and 72 hours prior to parturition (mean 10.6 ng/ml), there were insufficient samples to determine whether there is a significant fall in plasma progesterone concentrations at this time or not.

Barnes *et al*., (1975); Burns and Fleeger (1975) and Lovell *et al*., (1975) all recorded a decrease in plasma progesterone concentrations immediately prior to parturition, in contrast to the findings of Ganjam *et al*.,(1975). Burns and Fleeger (1975) suggested that this *pre partum* drop in plasma progesterone might have been due to a sudden increase in foetal metabolism of progestogens to glucocorticoids which in turn may have sensitised the uterus to oestrogen. Most workers (Barnes *et al*.,1975; Burns and Fleeger, 1975; Ganjam *et al*., 1975; Lovell *et al*., 1975) agree that the mare, unlike most other species, commences parturition in the presence of significant amounts of progesterone, an observation also suggested

by this study, since the 2 mares sampled within 12 hours of parturition both had significant concentrations of plasma progesterone at that time.

By 24 hours *post partum* plasma progesterone levels had fallen below detectable levels, a finding in common with those of other workers (Barnes *et al.*, 1975 and Burns and Fleeger, 1975). Ganjam *et al*. (1975) measured plasma progestogens immediately *post partum* while Lovell *et al*. (1975) measured these substances within five hours of parturition; both noted that plasma progestogens had dropped to less than 1 ng/ml by this time. The precipitous *post partum* drop in steroid levels could be explained by the sudden removal of the source (foetus and placenta) and the short half life of progesterone in this species.

As expected *post partum* plasma progesterone levels remained low until measurements ceased at the "foal" oestrus (see Table V).

TABLE VI: RESULTS OF TREATMENT OF MARES WITH PROSTAGLANDIN F2α

Mare	Reproductive State	Plasma progesterone (ng/ml)					Day first in oestrus			Day served after treatment			Pregnant
		0*	1	2	3	4	3	4	5	4	5	6	
Br	Mid dioestrus, cycling normally and not lactating	4.6	+	1.6	ns+	ns		yes			not		
LLB	Mid dioestrus, cycling normally and not lactating	8.9	+	1.2	+	2.2					not		
SP	Mid dioestrus, cycling normally and not lactating	11.8	2.2	ns	ns		yes				yes		yes
LQ	Mid dioestrus, 16-18 days <u>post partum</u>	8.6	+	ns	ns	+		yes			yes		yes
Ka	Mid dioestrus, 16-18 days <u>post partum</u>	6.2	1.2	+	ns	+	yes					yes	no
Aj	Mid dioestrus, 16-18 days <u>post partum</u>	9.2	+	ns	+	ns	yes				yes		yes
EJ	Anoestrus, 4 weeks after ceasing training	11.3	2.2	ns	+	ns		yes			not		
Ku	No oestrus after 'foal heat', 34-36 days <u>post partum</u>	11.1	+	ns	+	+	yes					yes	yes
C	No oestrus after 'foal heat', 34-36 days <u>post partum</u>	6.2	1.1	+	ns	+	yes			yes			yes
J	Previously cycling, but no cycles for 28-36 days and had not been served	9.2	+	ns	+	+	yes					yes	yes
A	Previously cycling, but no cycles for 28-36 days and had not been served	6.3	1.3	+	ns	+			yes			yes	no
Pl	Not cycling for 34-38 days, served but not pregnant	8.9	+	ns	+	ns		yes				yes	no
LL	Not cycling for 34-38 days, served but not pregnant	9.1	+	ns	+	+	yes				yes		yes

* Sampling day, day 0 is day of treatment with samples being taken before administration of PGF2α.

\+ Not sampled

ns Plasma progesterone <0.5 mg/ml

B. PLASMA PROGESTERONE LEVELS IN MARES WITH MODIFIED REPRODUCTIVE ACTIVITY

1. Use of Prostaglandin F2α To Induce Luteolysis

a. Special methods

Plasma was collected from 13 mares which were thought to have active CL, both before and after treatment with PGF2α. These included both lactating and non-lactating mares in normal mid-dioestrus and prolonged dioestrus. A diagnosis of prolonged dioestrus was made when the mare had not shown overt oestrus for more than 28 days but was not pregnant, and examination of the tubular genitalia gave the impression of the genital tract being under the influence of progestogens, i.e. tight pale cervix, reasonable to marked uterine tone and large active ovaries with or without a palpable follicle.

These mares were injected with 5 mg Prostin (Trometh-amine, THAM 5 mg/ml, Upjohn, USA) intramuscularly.

b. Results

The results of treatment of the 13 mares with PGF2α are summarised in Table VI facing page 66.

Before treatment plasma progesterone concentrations of all mares were high (mean 8.6 ng/ml, range 4.6 to 11.8 ng/ml). For the 5 mares sampled the day after treatment the mean plasma progesterone concentration was 1.6 ng/ml with a range of from 1.1 to 2.2 ng/ml.

By day 2 post injection, 8 of the 10 mares sampled had plasma progesterone concentrations less than 0.5 ng/ml, and all the mares sampled on day 3 had plasma concentrat-ions of this steroid less than 0.5 ng/ml. Mare LLB continued to have significant concentrations of plasma progesterone during the sampling period; not unexpectedly this mare failed to display oestrous behaviour for the duration of the experiment.

Excluding mare LLB the mean time to first display of overt oestrus after treatment was 3.5 days with a range of from 3 to 5 days. Ten of the mares were mated after treatment; average time from treatment to service was 5.4 days with a range of from 4 to 6 days. Seven of these

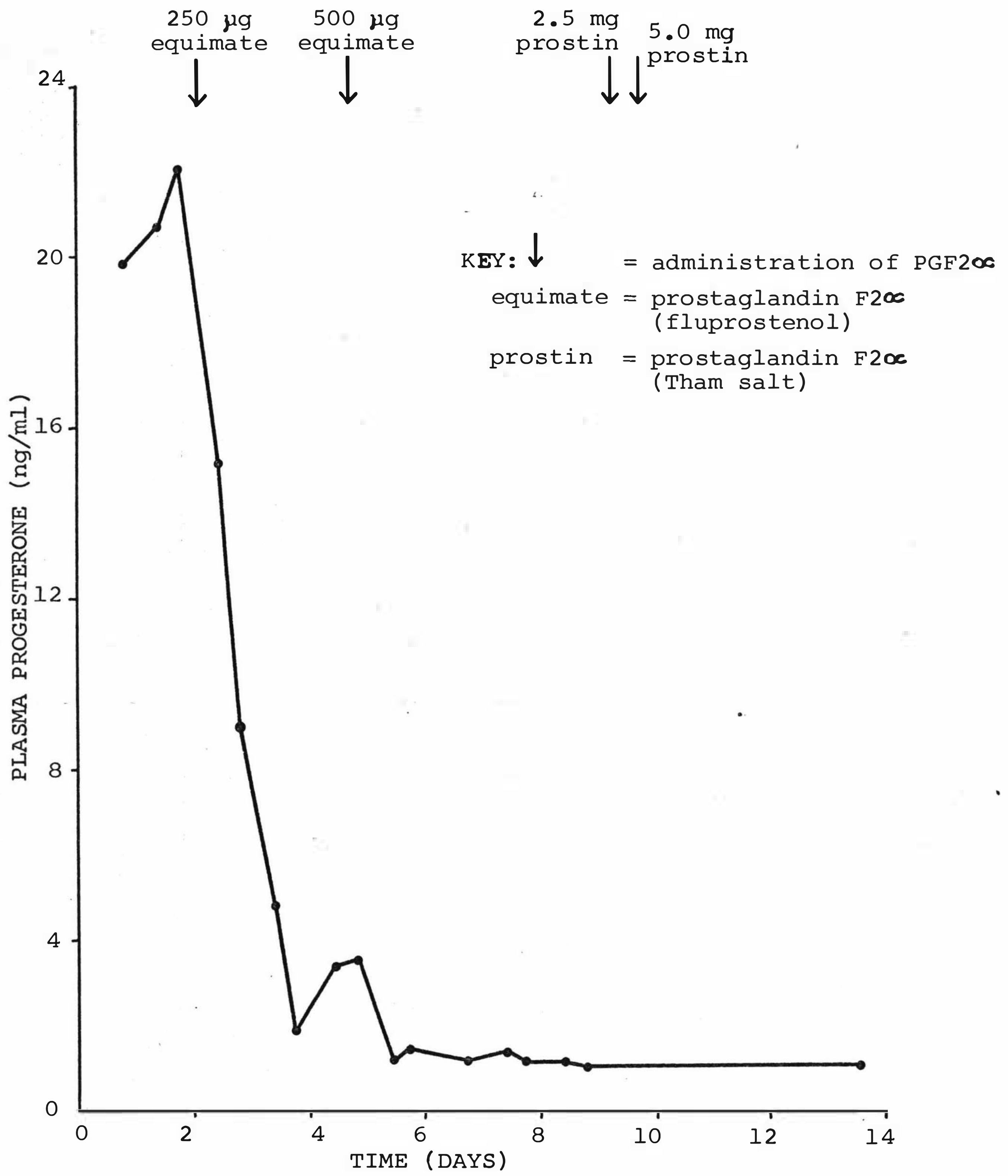


FIGURE 9: CONCENTRATION OF PLASMA PROGESTERONE IN A PREGNANT MARE GIVEN INTRAMUSCULAR PROSTAGLANDIN F2α

mares became pregnant to that mating.

The mare LLB which apparently failed to respond to the PGF2α exhibited overt oestrus 9 days after injection, was mated 3 days later and became pregnant to the mating.

Of the 5 mares not pregnant to the induced oestrus, 4 were mated at a subsequent oestrus, an average of 22.75 days (range 19 days to 24 days) after the first day of the induced oestrus, and 3 became pregnant to that mating. The fifth mare of this group showed oestrus 19 days after the first day of the induced oestrus but since it was toward the end of the breeding season, the owner requested that she was not to be mated.

2. Termination Of Pregnancy Of An Approximately Eighty Five Day Pregnant Mare

A mare, approximately 380 kg in weight and assessed as being about 85 days pregnant (estimated by crown-rump length of foetus - see Bergin *et al.*, 1967) was treated with intramuscular injections of PGF2α to induce an abortion. Figure 9, facing page 67 summarises progesterone concentrations in the plasma of this mare, and the treatment schedule of PGF2α used. The reproductive changes which were observed are summarised on the following page:

Date	Treatment	Changes Observed On Reproductive Examination
22.11.76	250 μg Fluprostenol	
24.11.76		
25.11.76	500 μg Fluprostenol	Uterine tone decreased in non-pregnant horn. Decreased pressure of uterine fluid, cervix relaxing.
28.11.76am	2.5 mg PGF2α (THAM)*	No change in uterus, cervix more relaxed and dilated to 3 cm.
28.11.76pm	5 mg PGF2α (THAM)	Uterine tone decreased further, cervix dilated to 5 cm.
3.12.76		No change, cervix was manually dilated to a diameter of 10 cm and a live foetus physically removed. Placenta passed within 3 hours.
9.12.76		Uterine body involuted to 15 cm in diameter. Cervix contracted.

* NOTE: Since there appeared to be little clinical response to the synthetic analogue fluprostenol, treatment was continued using the semi-synthetic Tham salt, Prostin.

3. Measurement Of Plasma Progesterone In An Ovariectomized Mare

a. Special methods

Four plasma samples, taken at weekly intervals during November, were obtained from a mare which had been ovariectomized for 3 years.

b. Results

A total of 20 estimations of plasma progesterone levels were carried out - 5 per sample. Every estimation was less than 0.5 ng/ml of plasma progesterone.

TABLE VII: PLASMA PROGESTERONE LEVELS OF 16 ANOESTROUS MARES

Mare	Age	Plasma progesterone (ng/ml)		Sample date
Y	10	ns		5.9.75
F	12	0.6		5.9.75
MQ	7	ns	"Early	5.9.75
A	8	ns	"Anoestrous"	5.9.75
T	17	ns	Group	15.9.75
W	4	ns		15.9.75
C	5	ns		15.9.75
AG	3	0.6		15.9.75
FC	18	13.1		8.11.75
EJ	4	11.3		9.12.75
K	16	11.1	"Late	12.11.75
CB	10	6.2	"Anoestrous"	12.11.75
JM	7	9.2	Group	16.11.75
P	12	8.9		16.11.75
LL	15	9.1		22.11.75
BA	12	6.3		22.11.75

ns = plasma progesterone concentrations < 0.5 ng/ml

4. Measurement of Plasma Progesterone In Anoestrous Mares

a. Special methods

Mares were considered to be anoestrus if they were not pregnant but exhibited no oestrous behaviour for more than 30 days between September and December when other mares under the same management conditions were exhibiting cyclic oestrous behaviour. They were examined as two groups: anoestrous mares in September (early in the breeding season) and anoestrous mares in November and December (late in the breeding season).

b. Results

Table VII facing page 69 summarises the plasma progesterone levels of 16 anoestrous mares. Plasma progesterone levels of anoestrous mares in September were all less than 1 ng/ml and 6 of the 8 mares sampled had steroid levels less than 0.5 ng/ml. The mean age of this group of mares was 8.25 years, less than the mean age of the mares that were anoestrus late in the breeding season (11.75 years) and of all non-pregnant mares under the same management conditions (11.0 years). Mares in the "early anoestrous group" were generally in poor nutritional condition and all still retained their"winter coat".

Plasma progesterone levels of the "late anoestrous group" were all high (mean 9.4 ng/ml). Body condition and hair length of these mares were comparable to that of the other non-pregnant mares on the stud.

5. Plasma Progesterone Concentrations In Mares In Advanced Pregnancy Prior To Abortion Of Twins Or Premature Parturition Of Dead Foals

Four of 25 mares in advanced pregnancy in which plasma progesterone levels were measured for an earlier study (see Table IV) subsequently aborted twins or had still-born foals.

Table VIII facing page 70 summarises the plasma progesterone levels of these mares.

Mean plasma progesterone concentrations of these mares was 11.5 ng/ml, not significantly different from that measured

TABLE VIII: CONCENTRATION OF PLASMA PROGESTERONE IN MARES IN ADVANCED PREGNANCY HAVING STILLBORN FOALS

Mare	Plasma progesterone (ng/ml)	Time from sampling to foaling (days)	Gestation length (days)	Result
FA	16.2	15	336	Filly born dead
MA	11.4	78	306	Twins born dead
HL	11.7	61	320	Filly born dead
MR	6.3	82	292	Twins born dead

in mares which delivered normally (see Table IV facing page 61).

6. Discussion

A number of workers have used PGF2α and its analogues to control the oestrous cycle of the mare (Douglas and Ginther, 1972; Allen and Rossdale, 1973; Allen and R wson, 1973; Berwyn-Jones and Irvine, 1974; Noden et al., 1974; Douglas and Ginther, 1975; Kenney, Ganjam, Cooper and Lauderdale, 1975; Oxender, Noden and Hafs, 1975 ; Spincemaille et al., 1975).

A rapid fall in plasma progesterone levels occurs when PGF2α (or its analogues) is either infused into the mare's uterus (Allen and R wson, 1973), or is given by intramuscular injection (Allen and Rossdale, 1973; Berwyn-Jones and Irvine, 1974; Noden et al., 1974), by subcutaneous injection (Douglas and Ginther, 1972), and by direct injection into the CL (Douglas and Ginther, 1975). Neither Douglas and Ginther (1975), who compared the luteolytic effect of different doses of PGF2α when administered by all the above routes, nor Spincemaille et al. (1975) who compared the intramuscular and intra-uterine routes of administration at different dose rates, found significant differences in effectiveness of luteolysis between either dose rate or route of administration. This is an understandable observation, since in the mare there is neither a unilateral nor a local uterine luteolytic effect on CL life (Ginther and First, 1971), and the lack of an intimate association of uterine and ovarian vasculature seen in some other species (Del Campo and Ginther, 1973) suggests that any endogenous uterine luteolysin would probably have to reach the equine CL via the systemic circulation anyway. The minimum effective dose in mares is 1.25 mg of PGF2α - Tham salt (Douglas and Ginther, 1975) per 450 kg liveweight and 125 µg for the ICI analogue Fluprostenol (Allen, et al., 1974) per 450 kg liveweight.

The CL of the mare is refractory to the action of luteolytic agents for the first 4 days of its lifespan (Allen, 1977). Thus length of dioestrus is not shortened in mares treated with PGF2α between ovulation and day 5

of dioestrus.

Response of mares in dioestrus (normal or extended) treated with PGF2α is quite constant and, based on a number of clinical trials throughout the world, some 85% of mares responded positively (Allen, 1977). Mares that respond, return to oestrus in from 2.2 days (Oxender et al., 1975) to 4.4 days (Kenney et al., 1975) post treatment. Failure of response to PGF2α treatment by mares in clinical trials may be due to either the absence of an active mature CL, or the presence of a mature follicle in the presence of a CL. In this latter situation the PGF2α probably causes luteolysis, but, if the mature follicle ovulates and a new CL is formed before the mare has time to develop a follicle and display oestrus in response to the treatment, she will appear to not respond to the treatment.

Allen (1975b) named 4 clinical conditions in which PGF2α might be used in non-cycling mares: -

(i) Mares which had not returned to oestrus after mating but which were found to be not pregnant.

(ii) Mares which had resorbed or aborted their conceptus during the first 40 days of pregnancy.

(iii) Mares which were not bred at the "foal heat" and subsequently failed to return to oestrus at the expected time.

(iv) Barren and maiden mares which were not showing regular oestrous cycles.

In this study all mares treated with PGF2α responded as evidenced by a post treatment drop in plasma progesterone levels. The mean time from treatment to oestrus and ovulation, as well as the conception rate of 70% obtained at the induced oestrus, were all comparable with figures reported elsewhere e.g. Allen et al. (1974). Mare LLB was unusual in that she appeared to respond to the PGF2α since there was a fall in plasma progesterone levels; however in neither sample taken after treatment did the plasma steroid levels fall below 0.5 ng/ml. This mare may have had a mature follicle or a newly formed CL, together

with an active mature CL at the time of treatment, since rectal examination 2 days after treatment detected an apparently recent CL.

Of the 13 mares treated in this investigation, 12 showed some evidence of sweating a few minutes after treatment with 6 mares affected enough to have sweat running down their legs and dripping off the ventral abdomen. Sweating usually commenced about 15 minutes after injection and this was the only untoward reaction observed. Millar, Lauderdale and Geng (1977) reported sweating as a common reaction in mares treated with the natural or semi-synthetic forms of PGF2α and the response is due to the stimulant action of the drug on smooth muscle. These workers also noted transient diarrhoea and colic in a few cases.

One mare, approximately 85 days pregnant, was treated with PGF2α , both the THAM salt and the synthetic analogue fluprostenol, to assess their effectiveness as abortifacients. Treatment was effective in inducing luteolysis, as reflected by a decrease in plasma progesterone levels, and in causing cervical relaxation and dilation; however it was necessary to manually remove the foetus 11 days after the first injection. Placental separation had not taken place at this time since the foetus was alive at the time of delivery. It appeared that in this case the problem was one of uterine inertia. PGF2α causes collagen breakdown in the cervix (Liggins, 1977) and enhances myometrial contractility in the human (Eliasson, 1973; Liggins, 1977). However its use as an abortifacient in women in the first, second (Lichtman, Bremmer, Mishell, 1974) and third trimesters (Eliasson, 1973) is questionable due to its unpredicatability in expulsion of the conceptus and serious side effects in some cases. Its use as an abortifacient in mares similarly appears to meet with mixed success. While a single subcutaneous injection of 1.5 mg of PGF2α on day 32 of pregnancy will consistently cause abortion (Kooistra and Ginther, 1976), single injections of up to 2.25 mg of the drug fail to consistently induce abortion in mares from 80 to 300 (Douglas, Squires

and Ginther, 1974) and from 300 to 321 (Alm et al., 1975) days of gestation, although Rossdale, Jeffcott and Allen (1976), using the synthetic PG analogue fluprostenol, were able to consistently induce apparently normal parturition with a single injection in mares at term. It may be that PGF2α will only consistently terminate pregnancy in early pregnant mares before the endometrial cups have developed; PMSG may have some "protective" action - certainly this gonadotrophin will prevent mares from displaying oestrus in the absence of significant plasma progesterone concentrations (Allen, 1972). In mares in late pregnancy, when progesterone is synthesised and metabolised by the placenta, PGF2α probably terminates pregnancy by virtue of its oxytoxic-like effect on the myometrium. The apparently more predictable response obtained in inducing labour in late pregnant mares using the synthetic PG analogue fluprostenol compared with the semi-synthetic Tham salt may be due to the different effects of the two drugs on smooth muscle (Rossdale et al., 1976).

Plasma progesterone levels in the two groups of "anoestrous mares" reflected the ovarian state of each group and demonstrated the use of plasma progesterone measurements to differentiate between two important forms of non-cyclicity seen in non-pregnant mares. Plasma progesterone levels in the group of mares examined during September were all very low, suggesting a state of true anoestrus. Generally these mares were in poor bodily condition and, in contrast to mares which were cycling on the stud at the same time, none had shed their winter hair coat, an observation in agreement with that of Kooistra and Ginther (1975). These workers noted a close correlation between loss of winter coat and establishment of reproductive cyclicity in the spring. The group of non-cycling mares examined in November and December, in contrast to the former group, had, without exception, high levels of plasma progesterone, indicative of an actively secreting CL. This condition of spontaneous prolongation of the life of the CL, although apparently unique to mares, is an important cause of infertility

since as many as 25% in a group may undergo one or more periods of prolonged dioestrus during any one breeding season (Allen, 1977).

The plasma progesterone concentrations of two pregnant mares in advanced gestation that were carrying twin foetuses (mares "MA" and "MR", Table VIII) was 11.4 and 6.3 ng/ml respectively, which was not significantly different from a group of mares in advanced pregnancy that were carrying normal single foetuses (mean 10.3 ng/ml, range 5.2 to 16.9 ng/ml, see Table IV). Expectedly, gestation length in the mares carrying twin foetuses was significantly shorter than in the normal group of foaling mares.

Plasma progesterone levels in the 2 mares which had still-born foals (16.2 and 11.7 ng/ml for mares "MR" and "HL" respectively, see Table VIII) were not significantly different from those of the normal foaling mares (Table IV); this is not surprising since there was a time difference of 15 and 61 days between sampling and birth of the dead foals. Although Allen, W.R. (1972) and Allen, W.E. (1974) recorded low plasma progesterone levels in some early pregnant mares which later aborted or resorbed, as Ganjam et al. (1975) records since, there is only at best inconclusive evidence that abortion in mares may be caused by a progesterone deficiency. The concept of low progesterone levels being a cause of abortion thus continues to be only a theoretical possibility.

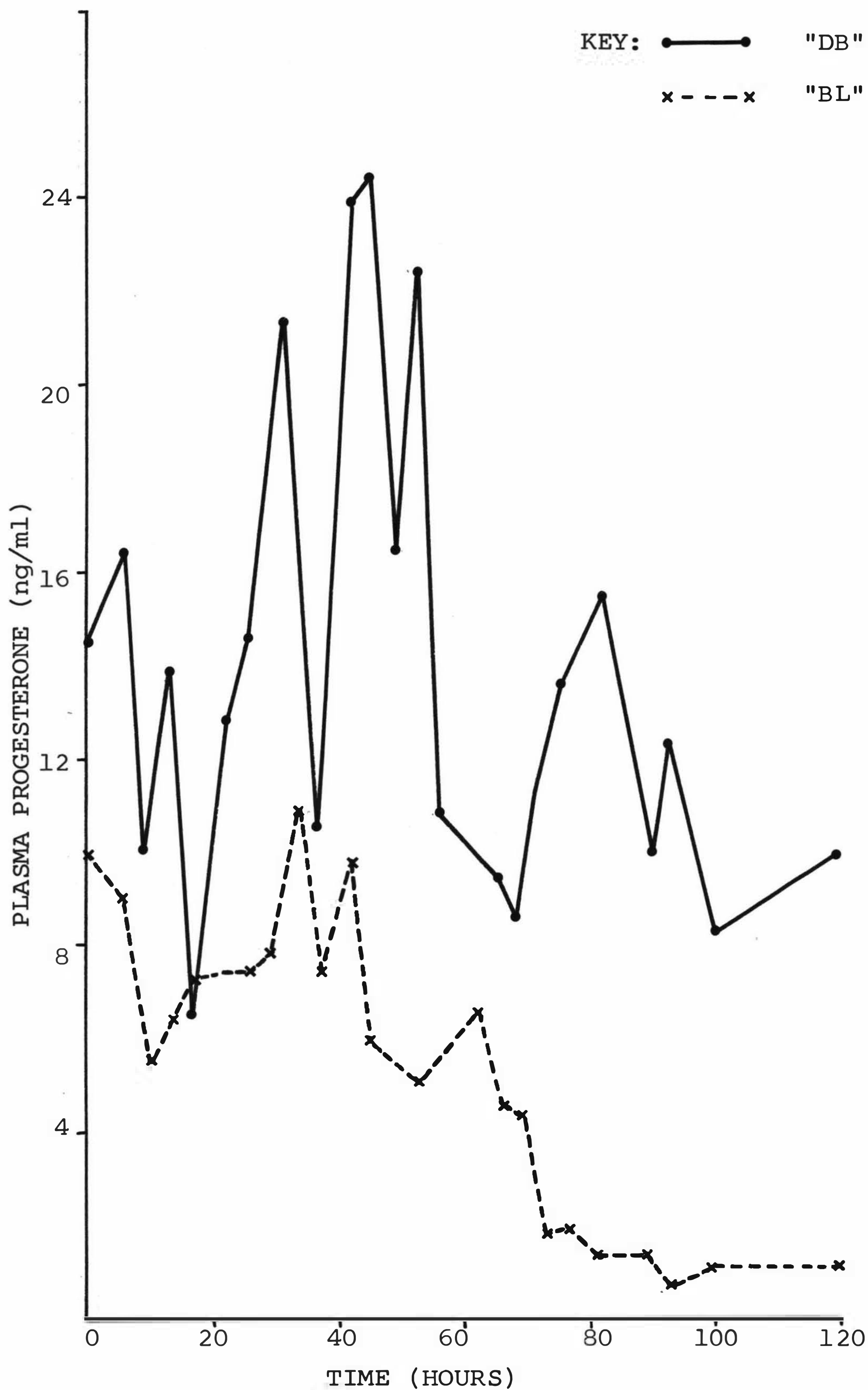


FIGURE 10: CONCENTRATION OF PLASMA PROGESTERONE IN TWO MARES SAMPLED FOUR HOURLY

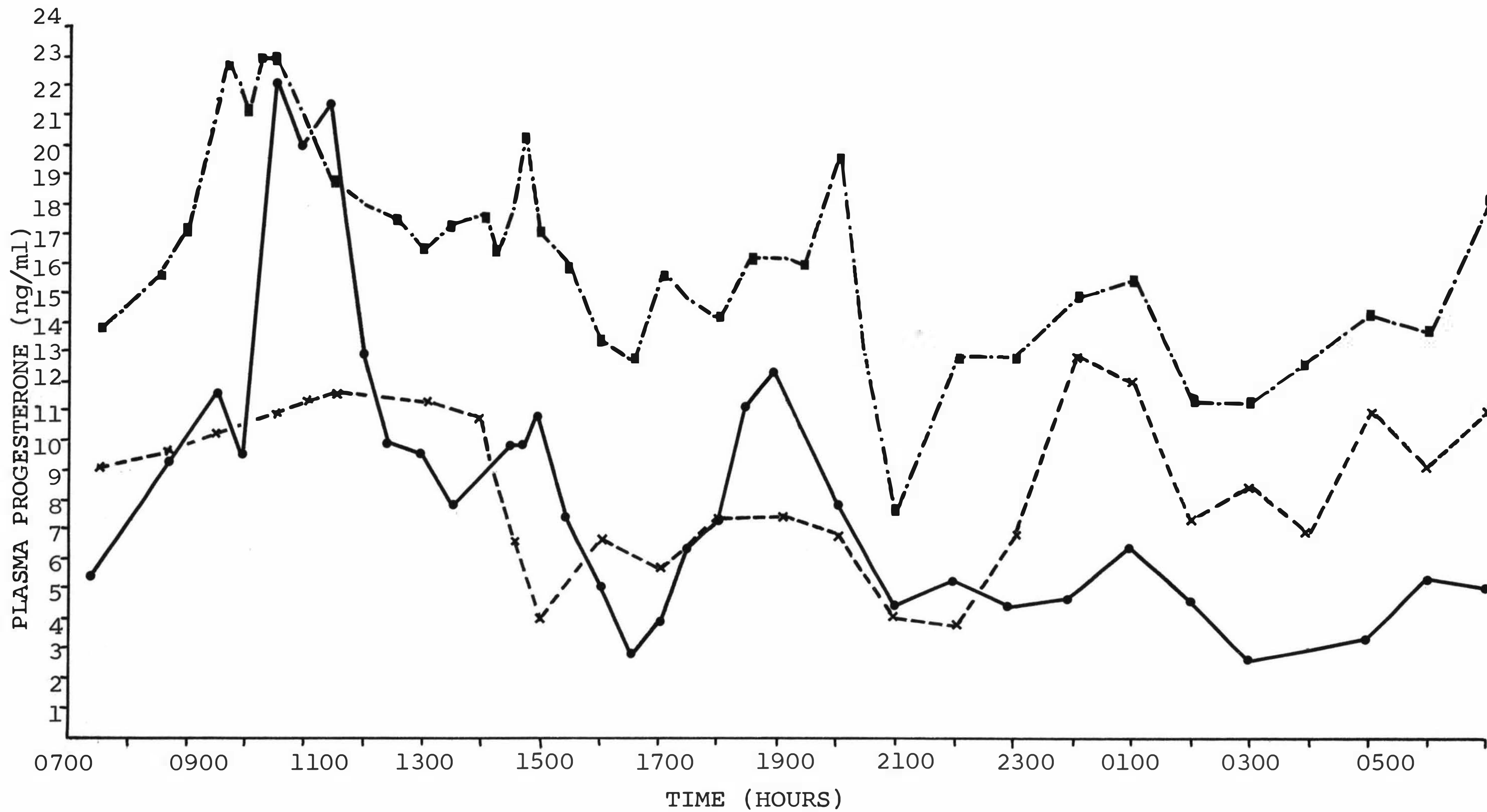


FIGURE 11: CONCENTRATION OF PLASMA PROGESTERONE IN THREE MARES SAMPLED AT 30 OR 60 MINUTE INTERVALS

C. REPEATED MEASUREMENTS OF PLASMA PROGESTERONE CONCENTRATION IN NORMAL MARES AND IN MARES GIVEN EXOGENOUS PROGESTERONE

Two series of investigations were undertaken during this final section of the study. In the first group of experiments repeated measurements of plasma progesterone concentrations were made on mares in a relatively stable reproductive state (mid-dioestrus). Two mares were sampled at 4 hourly intervals for 120 hours and a further 3 mares at ½ or 1 hourly intervals for 24 hours. Plasma samples were obtained using indwelling catheters (see Chapter 2).

In the second group of experiments exogenous progesterone was given to anoestrous mares and regular measurements of plasma progesterone made to try and determine when peak levels were reached as well as the metabolic clearance rate of progesterone from the plasma.

1. Four Hourly Plasma Progesterone Measurements On Normal Dioestrous Mares

Figure 10 facing page 75 summarises the plasma progesterone concentrations of 2 mares, sampled 4 hourly. Mare "BL" displayed oestrous behaviour 3 days after the last plasma sample was taken.

2. Plasma Progesterone Measurements At Hourly Or Half-hourly Intervals On Normal Dioestrous Mares

Figure 11 page 75 summarises plasma progesterone levels in 3 mares sampled half-hourly and hourly. Although visual examination suggests a diurnal pattern of plasma progesterone levels, examination of the values within the 95% fiducial limits of the assay showed that the differences were not statistically significant. Similarly, comparison of four hourly sample estimates from these 3 mares together with those of the 2 mares in Figure 10 did not reveal any consistent variations in plasma progesterone levels between mares.

3. Measurement Of Radioactive Progesterone Administered To A Mare In Anoestrus

a. Special methods

An anoestrous mare with a very low concentration of

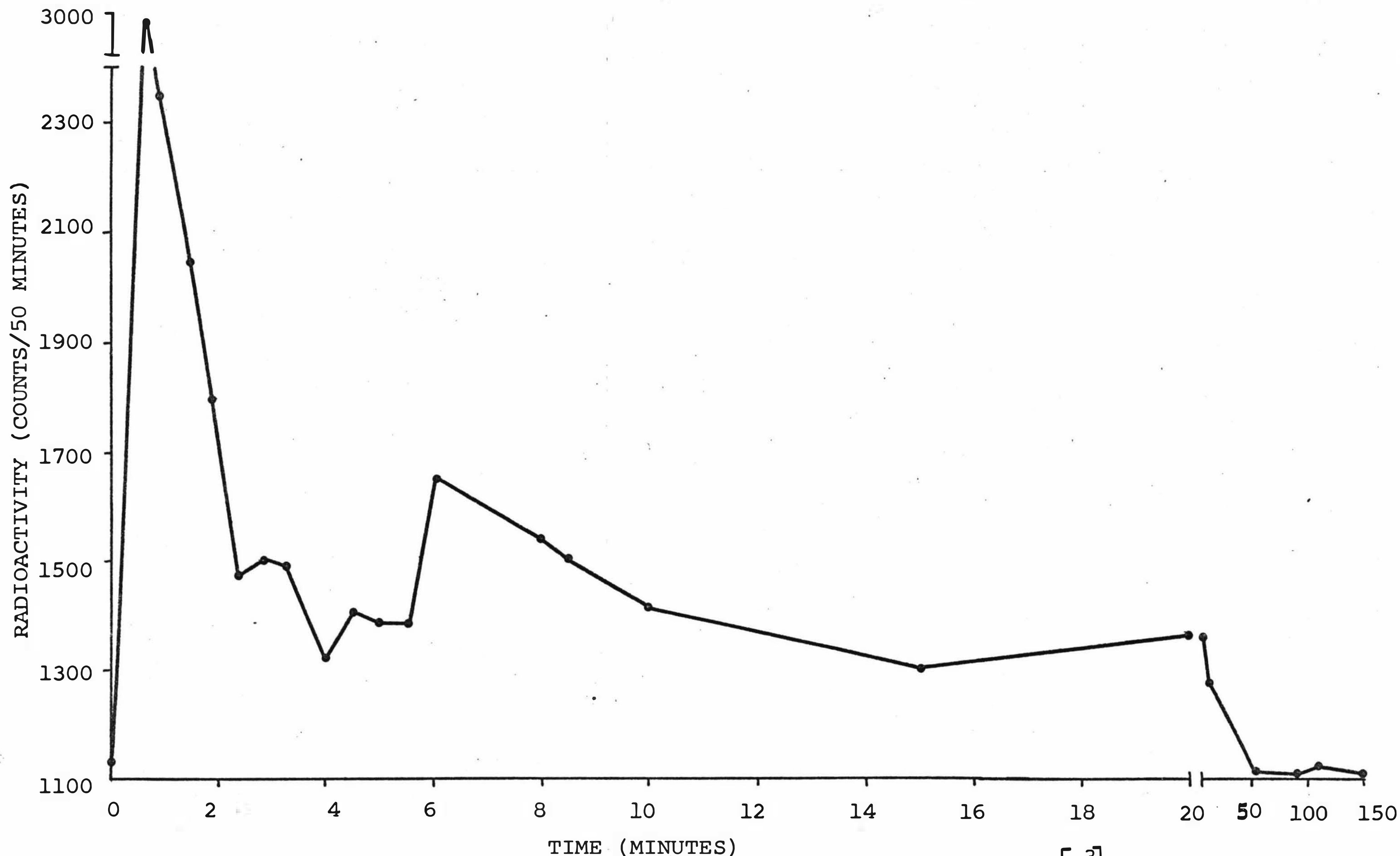


FIGURE 12: DISAPPEARANCE OF RADIOACTIVITY FROM THE PLASMA OF A MARE GIVEN [H^3] 1,2, PROGESTERONE INTRAVENOUSLY

endogenous plasma progesterone (less than 0.5 ng/ml) was injected slowly, over a period of 30 seconds, with radioactive progesterone ((H^3)-1, 2 progesterone) in ethanol via right jugular venepuncture, and blood samples were taken at regular intervals via left jugular venepuncture. The weight of this mare was 256 kg, with an estimated blood volume of 23 l (Dukes, 1955). A total of 52,698,175 counts per 50 minutes (c/50 m) of radioactive progesterone was injected which would give a theoretical peak concentration of 2290 c/50 m/ml of plasma.

One ml of plasma from each sample was extracted as for the RIA procedure, evaporated to dryness, resuspended in scintillation fluid, vortexed and counted. The aliquot of radioactive progesterone used as the total to calculate total radioactivity injected was added to gelding plasma and extracted as with the plasma samples before counting. This eliminated the need to correct for extraction losses. A blood sample taken immediately before administration of the radioactive progesterone was used as a blank to establish background radioactivity (1120 c/50 m). No attempt was made to isolate progesterone from the samples.

b. Results

Figure 12 facing page 77 summarises the radioactivity of the plasma, sampled over a period of 150 minutes.

Highest total radioactivity of 2998 c/50 m (1878 c/50 m due to infused radioactive steroid) was recovered from the sample obtained 75 seconds after injection of the radioactive progesterone. This represented an estimated recovery of 82% of injected steroid.

Most (greater than 80%) of the tritiated steroid had disappeared from the plasma 2½ minutes after injection. Six minutes post injection there was a second peak, which flattened out, the levels remaining elevated until 20 minutes post injection after which time values fell to pre-injection levels within 50 minutes.

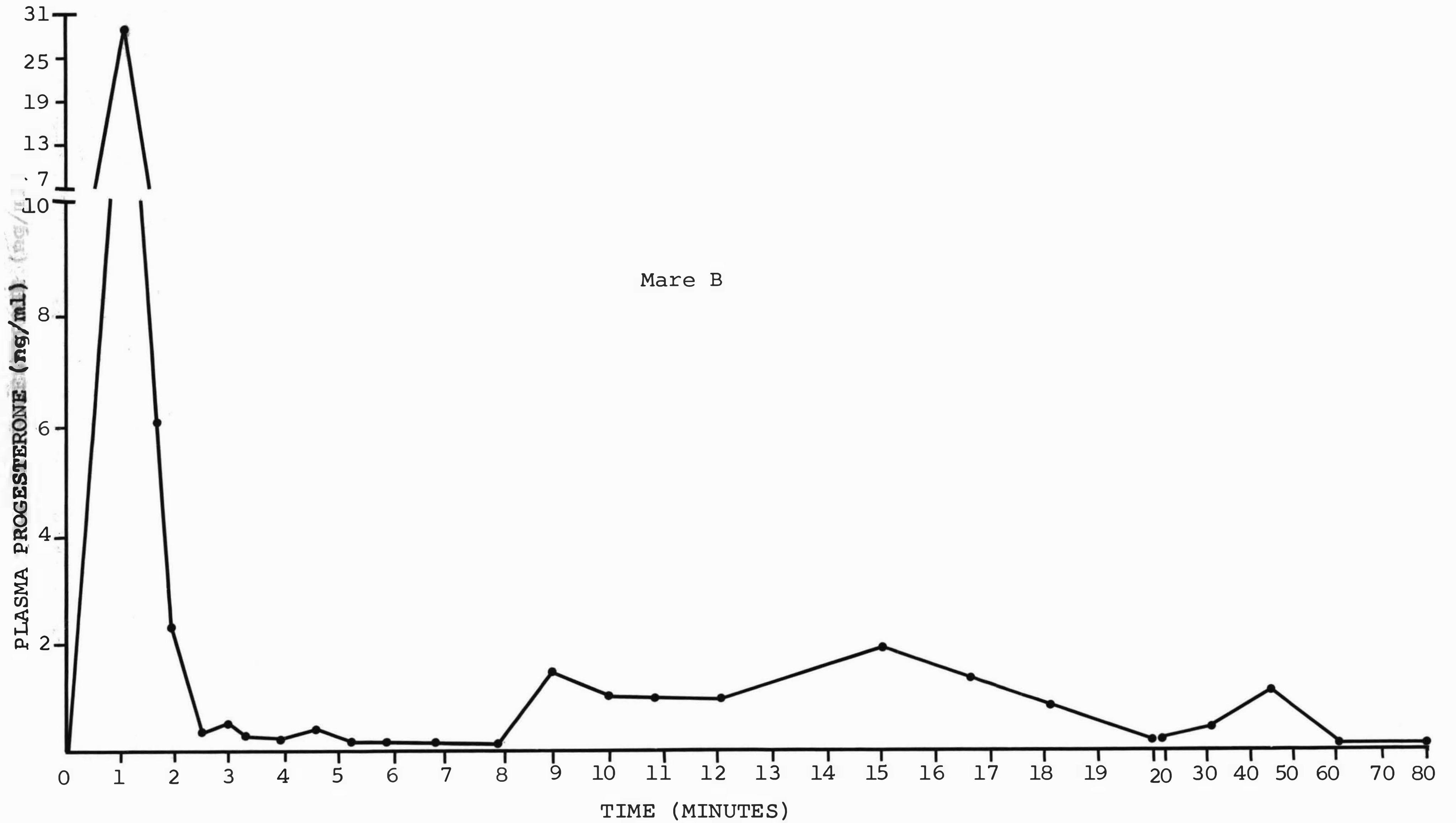
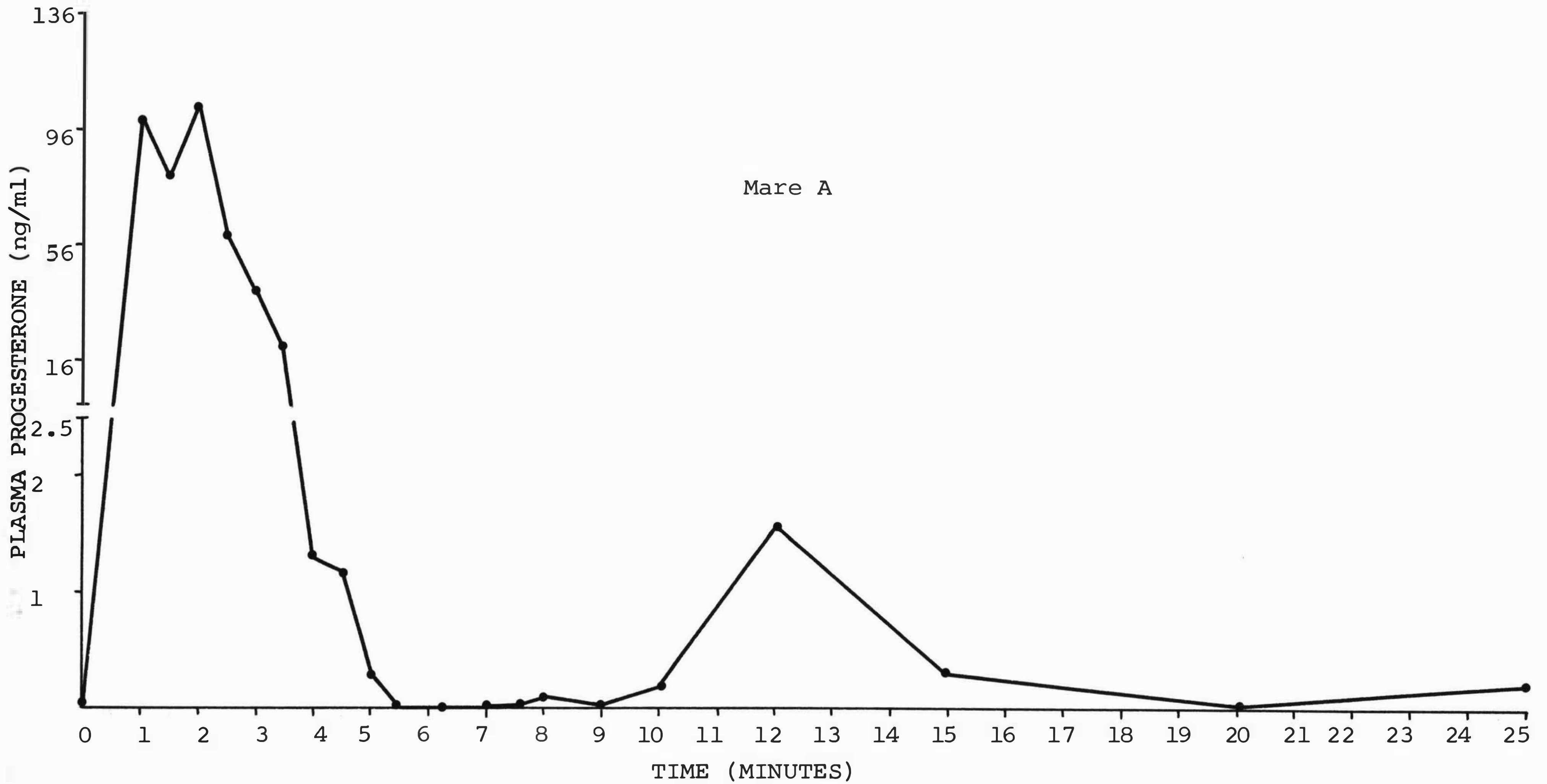


FIGURE 13: CONCENTRATION OF PLASMA PROGESTERONE IN A MARE GIVEN 2 mg PROGESTERONE INTRAVENOUSLY



<u>FIGURE 14</u>: CONCENTRATION OF PLASMA PROGESTERONE IN A MARE GIVEN 25 mg PROGESTERONE INTRAVENOUSLY

4. Plasma Progesterone Levels In Two Anoestrous Mares Given Exogenous Progesterone

a. Special methods

Two mares were established to be in winter anoestrus with minimal plasma progesterone levels (0.2 and 0.0 ng/ml respectively). The mares, weighing 263 kg (Mare "B") and 463 kg (Mare "A") were injected over 30 seconds by slow left jugular venepuncture with 2.0 mg and 25 mg of progesterone (Sigma, USA) in 6 ml of 16.7% ethanol in saline respectively. Assuming no loss of steroid from the plasma until it is evenly distributed in the blood, theoretical peak plasma concentrations could be 83 ng/ml in mare "B", with an estimated blood volume of 24 l, and 595 ng/ml in mare "A", with an estimated blood volume of 42 l (Dukes, 1970). Blood for plasma progesterone estimation was collected in 10 ml heparinised vacutainer tubes by right jugular venepuncture.

b. Results

The plasma progesterone concentrations in the two mares are shown in Figures 13 and 14 facing and on page 78.

Maximum concentrations of plasma progesterone were obtained by 2 and 1 minutes post injection in mares "A" and "B" respectively, with maximum plasma levels of 100 ng/ml in the former case and 30 ng/ml in the latter case. Plasma levels of the steroid reached non-significant levels by 5.5 minutes post-injection in mare "A", and by 3.5 minutes in mare "B". A second peak of plasma progesterone was observed, from 9.5 to 17 minutes post-injection in mare "A" and from 8 to 19.5 minutes in mare "B". A third and much smaller peak of plasma progesterone was recorded in mare "B" at about 45 minutes after injection.

5. Plasma Progesterone Levels In A Mare Given Progesterone In Oil

a. Special methods

The animal used in this experiment was an 8 year old unilaterally ovariectomized mare, whose remaining ovary measured approximately 1 cm x 0.5 cm x 0.5 cm and which

TABLE IX: PLASMA PROGESTERONE LEVELS IN A MARE GIVEN PROGESTERONE IN ARACHIS OIL

Date	Plasma progesterone (ng/ml)	Dose of progesterone (mg)
8.8.75	0	100
9.8.75	0	200
10.8.75	2.1	0
11.8.75	1.1	0
12.8.75	0	0
13.8.75	0	200
14.8.75	0	0
15.8.75	3.7	100
16.8.75	4.3	0
17.8.75	0	0
18.8.75	0	0

had never been observed to be in oestrus. Six-weekly plasma progesterone levels measured over the 10 months before this experiment all were less than 0.4 ng/ml.

Two separate injection regimes were undertaken. In the first, progesterone in arachis oil (Progesterone 25 mg/ml, Intervet, USA) was given intramuscularly. In the second, 7 weeks later, hydroxyprogesterone capronate in castor oil (Primulot depot, 500 mg/ml, Schering, N.Z.) was administered intramuscularly.

Blood samples and administration of steroid were carried out at 10.00 hours daily, with blood sample always being withdrawn before steroid was administered. Details of the injection regimes are shown in Tables IX and X facing pages 80 and 81 .

b. Results

Table IX facing page 80 summarises the plasma progesterone levels obtained in the mare given progesterone in oil, while Table X facing page 81 summarises the plasma steroid levels obtained after administration of hydroxyprogesterone capronate.

Elevations of plasma progesterone levels for short periods were obtained 24-48 hours after administration of progesterone in oil (Table IX); an even smaller effect was noted with hydroxyprogesterone capronate in spite of the higher dose administered.

6. <u>Discussion</u>

Van Niekerk <u>et al</u>. (1973<u>a</u>) recorded large within-mare diurnal variations in levels of plasma progesterone when mares were in dioestrus, with afternoon samples showing significantly higher levels of progesterone than morning samples. In the present study, although there was a similarly large within-mare variation (6.8 to 24.6 ng/ml and 0.8 to 11.0 ng/ml for mares "DB" and "BL" respectively, sampled 4 hourly) there was no evidence of any consistent diurnal variation in plasma progesterone concentrations. The fact that mare "BL" displayed oestrus behaviour would explain the low plasma levels of progesterone obtained in this animal toward the end of the sampling period.

TABLE X: PLASMA PROGESTERONE LEVELS IN A MARE GIVEN HYDROXYPROGESTERONE CAPRONATE IN OIL

Date	Plasma progesterone (ng/ml)	Dose of hydroxy-progesterone capronate (mg)
1.11.75	0	500
6.11.75	0	500
10.11.75	1.2	500
11.11.75	0	500
12.11.75	0.6	0
14.11.75	0.5	0
16.11.75	0	0

In the second series of investigations, where a more intensive sampling procedure was followed (sampling at hourly and half-hourly intervals), again both within and between mare plasma levels of progesterone varied markedly, in an apparently random manner (see Figure 11).

The effects of stress, including stress of venepuncture, on the release of some steroid and protein hormones has been reported; Hart (1973) for example reported changes in prolactin release due to stress in goats while J.E. Cox (pers.comm.) considers stress due to venepuncture may cause changes in plasma testosterone concentrations in some stallions. Attempts were made to keep the stress of blood collection in the 24 hour plasma progesterone concentration experiment to a minimum. Indwelling intravenous cannulae were used to collect blood samples, with environmental conditions including lighting being kept as constant as possible.

Although plasma concentrations of another steroid hormone, testosterone, fluctuate quite widely in the stallion (Cox, Williams, Rowe and Smith, 1973) ram and bull (Katongole, Naftolin and Short, 1971) and boar (Flor Cruz, 1977), this is probably due to the trophic effect of LH surges in the bull (Katongole et al., 1971) ram (Pervis, Illius and Haynes, 1974) and boar (Flor Cruz, 1977). Plotka, Foley, Witherspoon, Schmoller and Goetsch (1975) measuring hourly plasma progesterone and oestrogen concentrations in mares found large variations between progesterone samples. These measurements were confined to the 24 hours before and after ovulation and plasma concentrations of progesterone were consequently very low. Except for the latter 16 hours when plasma concentrations rose significantly, these workers probably were measuring extra-luteal progesterone, possibly of adrenal origin. Although there are no reports of frequent sampling of mares in the luteal phase for plasma progesterone concentrations, Ridley and Greenwald (1975) measuring plasma progesterone concentration every 2 hours in the luteal phase of the cyclic hamster recorded large fluctuations between samples.

The reasons for the observed fluctuations of plasma progesterone concentrations in the mare are speculative, but must be associated with variations of secretion of progesterone into the blood, variations in removal and breakdown of progesterone from the blood, or a combination of the two. Progesterone is largely produced and secreted into the plasma by the CL which itself in some species might be influenced by luteotrophic factors such as LH (Denamur, 1974). Although Allen (1973) was able to produce a temporary reduction in plasma progesterone concentrations in mares in the luteal phase by administration of the LH-like substance HCG, plasma concentrations of LH in normal dioestrous mares are very low (Noden et al., 1975) and probably an inverse relationship between plasma LH and progesterone exists in the mare (Evans and Irvine, 1975).

Since a mature equine CL only contains about 200 µg of progesterone at any one time (Van Rensburg and Van Niekerk, 1968; Younglai, 1971) and the ovary produces greater than 4 mg/hour (Short, 1964) and probably of the order of 30 mg/hour (Evans et al., 1975) luteal cells must be producing progesterone at a prodigious rate in a mature secretory CL. Because in the mare plasma progesterone has a very short half life (Ganjam et al., 1975) small variations in the output of the steroid would quickly be reflected in a change in peripheral plasma progesterone concentrations. If luteal cells are being constantly formed then destroyed as the CL gradually matures and finally undergoes luteolysis, with each cell secreting significant amounts of the steroid, then the observed fluctuations in the plasma concentrations of progesterone might be explained by the constant turnover and change in number of luteal cells of the CL. In support of this concept, Van Rensburg and Van Niekerk (1968) demonstrated that concentrations of progesterone in the equine CL varied with the stage of the cycle, the highest concentrations being in mid-dioestrus, while Harrison (1946) showed that this increase in progesterone concentration probably was due to an increase in luteal cells. Evans et al.

(1975) have also recorded a marked increase in progesterone synthesis per gram of luteal tissue 5 days after ovulation.

While there appeared to be a periodicity of plasma progesterone concentrations with peaks and troughs occurring about every 5 hours in the experiment in which plasma steroid concentrations were measured hourly, these changes were not significantly different. No diurnal or circadian rhythms were evident in the plasma steroid concentrations in this experiment to explain the variations observed.

Progesterone is primarily metabolised in the liver (Williams, 1968); thus after intra-peritoneal injection of C^{14} progesterone into rats and mice Barry, Eidinoff, Dobriner and Gallagher (1952) were able to recover almost all of the radioactive steroid from the intestinal tract and faeces, having been excreted via the bile. While it is possible to explain fluctuations in plasma progesterone concentrations by changes in rates of plasma clearance and metabolism of the steroid, there seems to be no useful explanation as to why such changes should occur in the normal healthy mare.

Although these experiments were undertaken on non-pregnant dioestrous mares the results are likely to have been the same in pregnant mares, at least before the development of secondary corpora lutea, since the primary CL of the pregnant mare, for the first 14 days, is formed and functions in the same manner as that of the non-pregnant mare (Van Niekerk _et al_., 1975); moreover the large between-mare variations in plasma progesterone concentrations observed in non-pregnant mares also applies to pregnant mares (see earlier results in this thesis).

Whilst it has been suggested that "progesterone deficiency" may be a cause of abortion in mares (Roberts, 1971; Allen, 1972; Lieux, 1972) and women (Williams, 1968), treatment of threatened or habitual abortion by progestogens in women at least, has been unrewarding (Shearman and Garnett, 1963; Moller and Fuchs, 1965). From the experiments reported in this thesis it seems clear that

although a single plasma progesterone concentration estimation on a mare will establish the presence or otherwise of secreting luteal and/or placental tissue, the large variation in the normal values for plasma progesterone concentrations that occur would preclude any likelihood of a single or even 2 or 3 plasma progesterone concentration estimations indicating a condition of luteal and/or placental insufficiency. Thus if the condition of "progesterone deficiency" does in fact exist as a cause of abortion or embryonic resorption in mares, these experiments, although limited to only a few mares do suggest that the diagnosis of such a condition would be very difficult to establish.

In the first of the final series of experiments, intravenous administration of radioactive progesterone in alcohol to an anoestrous mare caused a marked but very transient rise of radioactivity in the plasma. At peak plasma concentration, there was a recovery rate of 82% indicating that the majority of the injected steroid was in the peripheral plasma. A "bolus" effect of injected radioactive steroid was unlikely since it was administered slowly into the right jugular vein and blood for estimation of radioactivity was collected from the left jugular vein. No attempt was made to isolate radioactive progesterone from the plasma samples; the total radioactivity was counted. If any radioactive products of progesterone metabolism were present in the plasma, the effect would be to apparently delay the clearance of radioactive progesterone from the plasma.

The result of this experiment indicated that the intravenously administered progesterone was very rapidly cleared from the plasma, and in the mare progesterone probably has a very short plasma half life.

Ganjam _et al_. (1975) in agreement with the present study, found that intravenous injection of a single injection of radioactive progesterone to ovariectomized mares was followed by a very rapid clearance from the plasma, with at least 3 components to the disappearance curve. The major 'fast' component had a half life of

2.5 minutes, the second slower one a half life of about 20 minutes with a third much slower component not accurately measurable. Evans *et al*. (1975) however, after obtaining a steady state of intravenously infused tritiated progesterone, ceased infusion and found that peripheral plasma levels took about 16 minutes to fall to near pre-infusion levels. This latter study differed from that of Ganjam *et al*. (1975) and the present study in that the experimental mares were in dioestrus and so already had high levels of circulating progesterone, a "steady state" of infused radioactive steroid was obtained before cessation of administration for estimation of the plasma half life and significantly higher levels of infused radioactive steroid were infused. Thus, in Evans *et al*. (1975) study, the presence of high levels of endogenous and exogenous progesterone could have contributed to the disparity in the results (Pearlman, 1957).

To further investigate the apparently very short plasma half life of progesterone, a second experiment was carried out where 2 and 25 mg of progesterone in 16.7% alcohol in saline were administered to 2 anoestrous mares. Plasma half life for the first component of the administered progesterone was 1.5 and 2.5 minutes respectively; a second peak of plasma progesterone concentration was then recorded at 8 to 18 minutes after the initial injections, while a third smaller and shorter peak at about 50 minutes post administration was recorded. These findings are in agreement with those of Ganjam *et al*. (1975) who suggest that the different components of progesterone clearance from the plasma might be due to the loose binding of progesterone to high-capacity low-affinity proteins.

The differences in the plasma half life of progesterone in the two mares in the present study may have been related to the dose of the injected steroid since the longer plasma half life was observed in the mare given the greater quantity of progesterone. Further experiments would be necessary to establish such a dose response effect.

Imora (1967) measuring the biological half life of progesterone in cows found that there was a two phase

clearance of the steroid from the plasma; it was 2.2-3.3 minutes in cows with active CL but was 11.1 minutes in cows with regressing CL. These differences in plasma half life of progesterone are interesting and have not been recorded by other workers in non-pregnant animals, although Heap, Bedford and Linzell (1975) recorded different metabolic clearance rates of progesterone from the blood of goats, depending on whether the animals were pregnant or not. In women, Pearlman (1957) recorded a turnover rate of 3.3 minutes, and reported that it was not established if this turnover rate varied with the endocrine status of the individual. Hughes *et al*. (1975) recorded that neither the ability of the equine CL to produce progesterone nor the plasma metabolic clearance rates of the steroid altered markedly with the age or number of functional CL. The short biological plasma half life of progesterone in the mare recorded in the present and other studies could be explained by the lack of binding of progesterone to plasma proteins in the mare (Mitchell *et al*., 1972).

Treatment of habitual abortion in mares with progestogens has been reported to be successful by some authors. Lieux (1972) using 200-500 mg of a depot-type progestogen weekly from the time the pregnancy is diagnosed until the last month of gestation reported good results, while Allen (1972) using 500-1,000 mg of hydroxyprogesterone capronate weekly for from 20 to 80 days of pregnancy produced some evidence that the procedure was of use. In view of the findings in this thesis, and reports in respect to the half life of this steroid in the mare it would seem that very large doses of progesterone would need to be administered to maintain effective plasma concentrations.

To obtain further information a final experiment was conducted in which plasma progesterone concentrations were measured in anoestrous mares following administration of both progesterone in oil and a depot-type progestogen at recognised "normal" dose rates. At no stage did plasma progesterone concentrations reach those recorded in either

dioestrous or pregnant mares. It took at least 24 hours after administration of the steroid for plasma concentrations to rise significantly and there appeared to be a "residual" effect since the plasma concentrations remained elevated for 48 hours after administration of the steroid. Ganjam et al. (1975) noted that it took 21 days of daily administration of 150 mg of progesterone for plasma concentrations to reach levels of 6.7 ng/ml, although with administration of a higher dose of 300 mg progesterone daily, plasma concentrations typical of those seen in dioestrous mares were reached in 14 days.

The failure of daily 500 mg doses of hydroxyprogesterone capronate to elevate plasma progesterone concentrations beyond 1.2 ng/ml, with this concentration being maintained for only a maximum of 24 hours, could be explained by the failure of the RIA system used in this study to significantly cross react with hydroxyprogesterone. Although hydroxyprogesterone capronate has been used in the treatment of habitual abortion in mares (Allen, 1972), and is available commercially for treatment of the condition in women, this steroid is a breakdown product in the metabolism of progesterone (Short, 1964; Williams, 1968) and whether it has the same biological activity as progesterone in the mare is not known.

While these experiments were, of necessity, conducted on only a limited number of anoestrous mares, there is no evidence to suggest that the metabolism of luteal progesterone differs between pregnant and non-pregnant mares. Thus exogenous administration of progesterone in pregnant mares should be expected to produce similar plasma concentrations to those seen in anoestrous mares. If this is the case then it appears that currently used doses of exogenous progesterone if given to mares to overcome suspected luteal (or placental) insufficiency, are not capable of providing sustained physiological plasma progesterone concentrations.

It is arguable that plasma progesterone concentrations do not accurately reflect events at the cellular level.

For example, in this study although daily doses of 100 and 200 mg of progesterone in oil failed to elevate plasma concentrations to those seen in mares in mid-dioestrus, similar doses will suppress oestrus but not ovulation (Loy and Swan, 1966). Despite this, since conclusive evidence of progesterone deficiency as a cause of abortion has yet to be produced, and in the light of the findings in this study, the whole area of exogenous progesterone administration at any stage of pregnancy in the mare needs re-evaluation.

SUMMARY AND CONCLUSIONS

An RIA system for measuring plasma progesterone concentrations in pregnant and non-pregnant mares in various reproductive states was developed. Sensitivity of the assay was 0.5 ng/ml: the between assay coefficient of variation was 16.8% over 24 assays for a high progesterone sample and 8.5% over 15 assays for a low progesterone sample while the within assay coefficient of variation for 6 replicates of a sample, measured in 3 assays was 7.3, 10.1 and 6.9%.

When normal cycling mares were sampled plasma progesterone levels varied in a regular cyclic manner according to the presence or otherwise of an active secreting CL, and the values obtained were consistent with those reported overseas (Noden, et al., 1975) and in New Zealand using a CPB assay (Evans and Irvine, 1975). Plasma progesterone concentrations, measured by other workers for mares in early (Burns and Fleeger, 1975) and late (Ganjam et al., 1975) pregnancy, were similar to those obtained in this study for mares of equivalent gestation length. Large between-mare variations in the levels of this steroid were noted in mares at similar stages of pregnancy.

Plasma samples from a group of non-pregnant non-cycling mares indicated that there was a clear relationship between time of sampling (early versus late in the breeding season) and plasma progesterone concentration. Mares sampled early in the breeding season had without exception low or non-significant levels of plasma progesterone, whereas those mares sampled late in the breeding season had concentrations of steroid indicative of an actively functioning CL. These results clearly demonstrate two main types of anoestrus observed in this species; the first where mares continue to be anoestrus with small inactive ovaries well into the artificial breeding season imposed by man (Pashen and Allen, 1976) and the second, supporting the claim by Hughes et al. (1972) and more recently other workers, where a state of anoestrus (or more correctly prolonged dioestrus) exists due to the presence of an active secretory CL retained beyond its normal lifespan.

In this study plasma progesterone levels were measured in a number of mares with mature CL before and after treatment with PGF2α. Plasma progesterone concentrations fell precipitously after treatment, confirming reports by others such as Allen _et al_. (1974) and Berwyn-Jones and Irvine (1974) that adequate doses of PGF2α are luteolytic in mares with mature CL.

Both the synthetic and semisynthetic forms of PGF2α were used as an abortifacient in an approximately 85 day pregnant mare. Since only one animal was treated no definite conclusions could be drawn from the result. Nevertheless the PGF2α was effective in causing luteolysis, as judged by a fall in plasma progesterone concentrations, yet despite this and cervical relaxation, a live foetus had to be physically removed 12 days after the first injection of PGF2α. Other workers have similarly reported failure of PGF2α to consistently cause abortion in mares pregnant more than 24 days (Alm _et al_., 1974; Kooistra and Ginther, 1976).

The latter section of this thesis was concerned with the serial measurement of plasma progesterone concentrations in dioestrous mares, measurement of the plasma half-life of this steroid, and measurement of plasma progesterone concentrations in mares given exogenous progesterone. Four, one and half hourly measurements of plasma progesterone concentrations in dioestrous mares revealed surprisingly large fluctuations and plasma levels varied as much as 100% within 30 minutes. Similar variations in plasma progesterone levels were reported by Ridley and Greenwald (1975) in the cyclic hamster. If a similar degree of variation exists in the pregnant mare, and this does not seem unreasonable (see results and discussion, page 83), it is unlikely that a single or even two or three samples would be sufficient to diagnose a problem of 'relative progesterone deficiency'. Furthermore it is possible that plasma levels of this steroid do not accurately reflect either use or requirements at a local level (e.g. the gravid uterus).

Radioactive progesterone and progesterone in 16% alcohol in saline was administered to a mare with very low endogenous levels of this steroid in an effort to measure its plasma half life. Results demonstrated that radioactive progesterone disappeared very rapidly from the plasma after a single injection, an observation similar to that of Ganjam *et al*. (1975) who recorded a plasma half life of 2.5 minutes. Following the intravenous administration of 2 and 25 mg of progesterone in 16% alcohol in saline to a further two anoestrous mares, the plasma half life was measured as 2.5 and 3.5 minutes respectively for the initial 'fast' component. A second peak of plasma steroid at from 10 to 15 minutes was obtained for the mare given 2 mg, and from 8 to 18 minutes for the mare given 25 mg of progesterone.

Administration of progesterone in arachis oil, and of hydroxyprogesterone capronate in castor oil, to a mare with no measurable endogenous plasma progesterone resulted in surprisingly little elevation of plasma progesterone levels. Thus a total dose of 600 mg progesterone in arachis oil administered over 8 days only raised plasma progesterone levels to 4.3 ng/ml, a level which was maintained for less than 24 hours, while a total dose of 2 g of hydroxyprogesterone capronate in castor oil administered over 12 days raised plasma progesterone levels to a maximum of 1.2 ng/ml, again for only a 24 hour period. It is probable that levels measured in the second instance were falsely low since the antiserum used in this RIA did not cross react significantly with hydroxyprogesterone. These results support the findings of Ganjam *et al*. (1975) who found that it took 21 days of daily administration of 150 mg progesterone for plasma progesterone concentrations to reach physiological levels.

Although the mare in oestrus is sensitive to small amounts of progesterone (Hughes *et al*., 1972), and 100 mg progesterone administered daily will successfully postpone this event (Loy and Swan, 1966), the results reported in this thesis suggest that doses of this order given daily are unlikely to elevate plasma levels to

1 ng/ml, the concentration generally regarded as being indicative of the presence of a secretory CL (Berwyn-Jones and Irvine, 1974). It is possible therefore, that exogenously administered progesterone exerts a local effect to suppress oestrus, while plasma levels remain low.

Although a number of the experiments reported in this thesis were, of necessity, conducted on small numbers of mares several conclusions can be drawn:

1. Normal non-pregnant cycling mares exhibit regular rhythmical changes in plasma progesterone concentrations, with oestrus being manifested only when levels of this steroid were less than 1 ng/ml, and being terminated generally by rising plasma concentrations of progesterone.

2. There is a large between-mare variation in plasma progesterone concentrations between normal, early and late pregnant mares, and a large, apparently random within and between mare variation in plasma steroid levels of mares in dioestrus.

3. Unlike most species mares apparently proceed with parturition in the presence of significant amounts of plasma progestogens; these levels fall precipitously immediately *post partum* and remain low at least until the first *post partum* oestrus.

4. At adequate dose rates, intramuscular administration of PGF2α is luteolytic, causing a rapid decline in plasma progesterone levels in mares with mature secreting CL.

5. Measurement of plasma progesterone concentrations in mares will enable differentiation between anoestrus due to relative ovarian inactivity and prolonged dioestrus due to the presence of an active CL, maintained beyond its normal lifespan.

6. Intravenously administered progesterone is rapidly cleared from the plasma in mares with low endogenous steroid levels.

7. Administration of commonly used doses of progesterone in oil to mares failed to elevate plasma levels in a mare with low endogenous levels of the steroid to those measured in mares which have secretory CL.

8. The diagnosis of the condition of "progesterone insufficiency" as a cause of embryonic resorption or abortion is unlikely to be able to be made on a single or even two or three estimations of plasma progesterone.

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