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Abnormal Function of the Corpus Luteum in some Ewes with Phyto-oestrogenic Infertility

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Abstract

Ewes with permanent phyto-oestrogenic infertility show oestrus less regularly than normal ewes, and the present study examines the extent to which this results from abnormal ovarian function. Forty-nine affected ewes and 53 controls were run with rams fitted with marking crayons and harnesses, and crayon marks were recorded and laparoscopy performed at weekly intervals for 3 weeks. Fewer affected ewes showed oestrus accompanied by ovulation (28 *v.* 48, $P < 0{\cdot}001$), and four of these affected ewes had a second ovulation during the experiment. More of the ovulations observed in affected ewes were unaccompanied by behavioural oestrus than in controls (8 out of 38 *v.* 2 out of 50; $P < 0{\cdot}05$). Six affected ewes had no corpus luteum or oestrus, and five of these had adhesions over the genitalia. Hydrops uteri in five other affected ewes was accompanied by prolonged maintenance of the corpus luteum. Some other abnormalities were also observed.

In a second study, plasma progesterone concentrations were measured twice daily in 12 affected ewes which were run with rams. Five ewes had oestrous cycles of abnormal duration (two of more than 23 days, two of 21 days, and one of 11 days), and these were accompanied by plasma progesterone patterns different from those of the ewes with an oestrous cycle duration of 16–18 days.

It is concluded that the irregular oestrous cycles in affected ewes are due mainly to abnormal life span and progesterone secretion by the corpus luteum, which in turn largely result from changes in the uterus.

Introduction

Ewes which have been exposed to oestrogenic pasture for a long period develop pathological changes in the reproductive tract (Bennetts *et al.* 1946) and can remain permanently infertile (Schinckel 1948). This permanent phyto-oestrogenic infertility (PPI) is due mainly to a failure of sperm transport through the cervix (Lightfoot *et al.* 1967). While the incidence of behavioural oestrus is not greatly impaired (Underwood and Shier 1952), affected ewes come into heat less frequently during the first part of the natural breeding season (Adams 1979), the time at which sheep are normally mated in Western Australia. The duration of the oestrous cycle is also somewhat more variable in ewes with PPI (Adams *et al.* 1975).

The present studies were carried out to determine whether the changes in the occurrence of oestrus as detected by the ram reflect changes in the ovarian cycle in ewes with PPI. The growth and regression of the corpus luteum (CL) was observed by laparoscopy (Oldham and Lindsay 1980), and the functioning of the CL was studied by measuring progesterone levels.

Materials and Methods

Laparoscopic Observations

Merino ewes originating from the same flock were used, but those with PPI had grazed a highly oestrogenic pasture of Yarloop subterranean clover for 3 years and only 11% had lambed to their last mating. The control ewes had grazed non-oestrogenic pasture during this period and were of normal fertility. After treatment the ewes were run together again as a group on non-oestrogenic pasture for 2 years before the study.

Laparoscopic observations on the ewes before the experiment revealed that many of the affected ewes had severe abnormalities of the genital tract, which prevented visualization of their ovaries. Accordingly, this study was carried out on 49 of 89 available ewes with PPI, and 53 of 55 control ewes, selected on the criterion that they had ovaries which were accessable to laparoscopic study.

Three successive, weekly, laparoscopic observations were carried out under local anaesthesia (Oldham and Lindsay 1980) on each ewe during the middle of the normal breeding season in April. No attempt was made to synchronize the occurrence of oestrus in the ewes. At each observation, the position in the ovary of any CL present was recorded, and its approximate age estimated from the description of Oldham and Lindsay (1980). In order to detect the occurrence of oestrus, the ewes were run as a group with vasectomized rams fitted with marking harness and crayons (Radford *et al.* 1960), commencing 1 week before observations, and the colour of the crayons was changed the day before each laparoscopic observation, when marks on the ewes were recorded.

Progesterone Study

The Merino ewes in this study came from a group described previously by Findlay *et al.* (1973) which had grazed Yarloop subterranean clover for 7 years, and fewer than 10% had lambed after their last mating. They were run on non-oestrogenic pasture for 2 years before study.

Two vasectomized rams, fitted with harness and crayons, were run with a group of 40, 10-year-old ewes, all affected with PPI. The first 12 ewes marked by the rams were selected for the experiment and blood samples were obtained by venipuncture from the jugular vein in heparinized syringes twice daily at 0800 and 1600 h beginning on the day of oestrus. The blood was immediately centrifuged at room temperature, and the plasma stored at −15°C until assay. The final blood sample was taken after the ewes exhibited a second oestrus. Two of the 12 ewes failed to be marked a second time by the rams and the sampling of these ewes was concluded on the 23rd day. Plasma progesterone concentrations were determined by the method of Obst and Seamark (1975), in their laboratory. Dog plasma was used as the source of binding protein. Standards containing 0, 0·2, 0·4, 1·0, 2·0 and 4·0 ng progesterone in 0·5 ml oophorectomized ewe plasma were run with each assay. Progesterone values of 0·2 ng were readily detectable and significantly different from zero. Estimates of the coefficient of variation at different points on the standard curve ranged from 2·5 to 6·5%. The interassay coefficient of variation for 10 assays was 11·8%.

Results

Laparoscopic Observations

Forty-eight of the 53 control ewes had a normal oestrus in which the period when the CL formed was consistent with the period in which the ewe was marked by the ram. In three ewes the CL did not regress during the 21-day observation period, and two ewes had a 'silent heat', in which a new CL formed, but the ewe was not marked by the ram.

Fewer (only 28 out of 49; $\chi_1^2 = 14{\cdot}98$, $P < 0{\cdot}001$) ewes with PPI had a normal oestrus accompanied by ovulation. In eight affected ewes the CL did not regress and five of these were observed to have hydrops uteri, as did two ewes which showed a normal oestrus. Six affected ewes had only a silent heat. Another six ewes had no CL and were not marked by the ram, and were therefore considered to be in anoestrus. Five of these ewes in anoestrus had adhesions over the genitalia, but adhesions were seen in five other affected ewes, four of which had a normal oestrus while the

remaining ewe had a silent heat. The remaining affected ewe was marked by the ram after her CL regressed, but no new CL was formed.

Four of the 28 affected ewes classified above as having a normal oestrus also had an additional oestrus in which there was premature regression of the CL and maturation of a new follicle, because the ewe was marked and a new CL formed at two successive weekly observation periods. Two of these ewes were not marked by the ram when the second CL was formed. As described above, six other affected ewes and two controls also had a silent heat. Thus the formation of a new CL was less commonly associated with being marked by the ram in ewes with PPI (8 out of 38 *v.* 2 out of 50 silent heats; $\chi_1^2 = 4{\cdot}66$, $P < 0{\cdot}05$). Another ewe with PPI appeared to develop a new CL without regression of the previous CL and without being marked by the ram. No such abnormalities were seen in the control ewes.

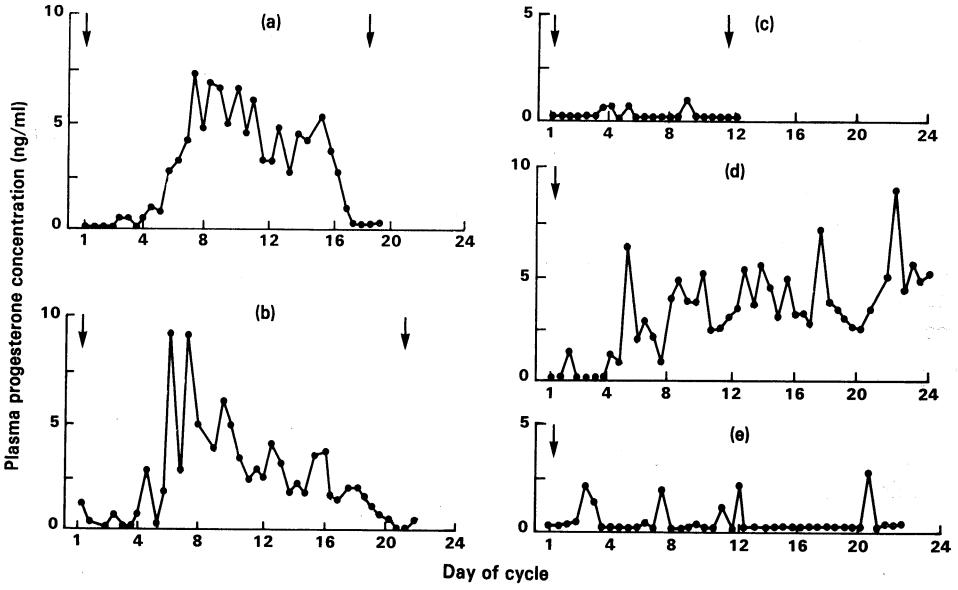

Fig. 1. Means of twice daily plasma progesterone concentrations from a PPI ewe (*a*) with a normal oestrous cycle, (*b*) with a 21-day cycle, (*c*) with an 11-day cycle, (*d, e*) from two ewes respectively which did not return to oestrus within 23 days. Vertical arrows indicate day of onset of oestrus.

Progesterone Concentrations

A variation in ovarian activity similar to the above was noted in the affected ewes in which progesterone levels were monitored. Seven of the 12 ewes had a normal oestrous cycle of 16–18 days duration, accompanied by normal progesterone levels (Obst and Seamark 1970). In these ewes, progesterone concentrations began to rise 4–5 days after mating to reach a maximum value of about 9 ng/ml at around 11 days, and began to fall about day 14 so that by day 17–18, progesterone was again low or undetectable (Fig. 1*a*).

Two ewes had oestrous cycles of 21 days duration, and the period of decreasing progesterone concentration was longer than the other ewes (Fig. 1*b*). One ewe had an abnormally short oestrous cycle (11 days), with consistently low levels of progesterone (Fig. 1*c*), while the remaining two ewes did not show a second oestrus

within the 23-day sampling period. One of these (Fig. 1*d*) had continuously elevated progesterone concentrations, while the other (Fig. 1*e*) had very little detectable progesterone at any stage. At slaughter both these ewes were found to have hydrops uteri.

Discussion

It is possible that the stress of repeated laparoscopy decreased the frequency of oestrus in the laparoscopic study (Adams 1979). As has been reported previously (Adams *et al.* 1975; Adams 1979) ewes with PPI displayed oestrus less often than normal ewes. A large part of the irregularity in behavioural oestrus could be accounted for by changes in the occurrence and persistence of the CL, and changes in the ability of the CL to secrete progesterone. Ewes with a normal oestrous cycle also had normal progesterone levels, which is consistent with Smith's (1975) report that progesterone secretion is normal in ewes with PPI, because in that study the length of the oestrous cycle was normal. Obst and Seamark (1970) recorded reduced plasma progesterone concentrations in ewes grazing oestrogenic clover, but this may have been due to a pharmacological effect of the concurrent intake of phyto-oestrogen.

The increased proportion of silent heats in ewes with PPI may be due to the reduced behavioural responsiveness of these ewes to oestradiol (Adams 1978), but another possible explanation is that the affected ewes did not have adequate circulating levels of progesterone in the cycle preceding the ovulation. Robinson *et al.* (1956) have shown that pretreatment with progesterone for up to 12 days is necessary for an optimal oestrous behavioural response to oestradiol, but this did not occur in several ewes with PPI. In fact, ewes affected with PPI have considerable variability in the occurrence and duration of the progestational phase of the oestrous cycle, and this should be considered in the interpretation of any future experiments which are carried out on such ewes during the breeding season.

The data given indicate the abnormal function of the ovary was related to pathological changes in the genitalia. Thus, of the 43 affected ewes with a CL, the CL failed to regress in 5 out of 7 ewes with hydrops uteri, and 3 out of 36 ewes without hydrops uteri. This difference is statistically significant ($\chi_1^2 = 11{\cdot}52$, $P < 0{\cdot}01$). Similarly, of the total 49 affected ewes, failure to cycle at all occurred in 5 out of 10 ewes with adhesions over the genitalia and 1 out of 39 ewes without adhesions. This difference is also statistically significant ($\chi_1^2 = 12{\cdot}54$, $P < 0{\cdot}01$). The selection against ewes with gross physical damage of the genitalia in the laparoscopic study, and the selection of ewes coming into heat for the progesterone study, probably excluded many potentially abnormal ewes from this work. Thus, this work may be considered to underestimate the prevalence of abnormalities in these flocks.

An association between absence of oestrous behaviour and abnormalities of the genital tract has been reported previously (Fels and Neil 1968; Adams *et al.* 1975). It is probable that changes not visible by laparoscopy, including cystic hyperplasia and endometritis, were present in the uterus of many of the laparoscopically normal ewes (Adams 1975) and that these changes were responsible for the other abnormalities of ovarian activity, such associations having been described, for example, by Brinsfield and Hawk (1968). Thus, it appears that most of the variability in the occurrence of oestrus that has been described in ewes with PPI may be attributed to pathological changes in the uterus which prevent it from exercising its normal

control over the life span of the CL. However, it is possible that changes in the hypothalamo–pituitary–ovarian axis in ewes with PPI, as shown by the failure of ewes with PPI to secrete LH in response to oestradiol (Findlay *et al.* 1973), the decreased ovarian compensatory hypertrophy in PPI ewes after hemiovariectomy (Adams 1976), and the reduced behavioural sensitivity to oestradiol (Adams 1978) may also affect the regularity of behavioural oestrus.

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