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# STUDIES ON THE MAINTENANCE OF THE CORPUS LUTEUM IN THE EWE

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## SUMMARY

The possible existence of uterine factors associated with regression of the corpus luteum was studied in the sheep.

In 18 ewes the life-span of the corpus luteum was examined following excision of parts of the reproductive tract on day 4 of the oestrous cycle. At slaughter on days 23 to 25 of the cycle, total hysterectomy always resulted in the maintenance of luteal tissue but partial hysterectomy was less effective; following sham operation or removal of the Fallopian tube, the corpus luteum was not maintained.

In a second experiment with 16 ewes, bilateral ligation of all main uterine blood vessels or of uterine veins alone usually caused the maintenance of luteal tissue. Unilateral ligation of blood vessels (except in one ewe) or the bilateral ligation of uterine arteries did not prolong the life-span of the corpus luteum.

Injection of extracts of endometrial tissue obtained from ewes in the late luteal phase of the oestrous cycle failed to cause regression of luteal tissue in hysterectomized ewes.

THE MAMMALIAN OVARY has two main functions — the production of ova, and the development of the corpus luteum following ovulation. Progesterone, secreted by the luteal tissue, is concerned in the development of conditions in the reproductive tract required for pregnancy. In the functional corpus luteum of the unmated female, the luteal cells normally degenerate towards the end of the oestrous cycle and the secretion of progesterone falls. However, where mating and pregnancy occur, the life-span of the gland is prolonged, often for the greater part of gestation.

This paper is concerned with the possible mechanisms by which the ovary in the ewe "learns" that the developing blastocyst has attached itself to the uterus — *i.e.*, the animal has become pregnant. Thus the corpus luteum of the cycle continues its existence and functions as a corpus luteum of pregnancy.

## CONTROL OF THE LIFE SPAN OF THE CORPUS LUTEUM

The mechanisms which control the life-span of the corpus luteum are unknown. One widely-held theory concerning the endocrine mechanism maintaining luteal tissue

involves the production of luteotrophic hormone by the pituitary gland. This concept would seem to imply that the corpus luteum is maintained in the presence of luteotrophic hormone but that regression occurs when there is a decrease in the quantity produced or released by the pituitary gland. Nalbandov (1961) has recently discussed the existence of luteotrophic hormone. However, the identification of a luteotrophin that is effective in other than a few species has not been achieved. For instance, injections of prolactin which will prolong the life-span of the corpus luteum in the mouse and rat (Dresel, 1935; Astwood, 1941) and also in the sheep on some occasions (Moore and Nalbandov, 1956; Raeside, 1958) are without effect in the guinea-pig, rabbit, pig, cow, rhesus monkey, and in women. Likewise, gonadotrophic substances of either pituitary or uterine origin have sometimes maintained the corpus luteum but not consistently between species (Short *et al.*, 1963).

In contrast to the suggestion that a luteotrophin causes maintenance of the corpus luteum, there is other evidence to indicate that a luteolytic factor may cause its regression. Such a concept might imply that luteal tissue once formed has an inherent capacity to persist and to function for a long period. Regression of the corpus luteum might then be caused by the direct action of a luteolysin or perhaps through the non-availability of some other factor which suppresses the action of a continually-secreted luteolysin.

The existence of a luteolytic substance would offer a convenient explanation for the increase in the life-span of the corpus luteum that follows hysterectomy in the sheep (Wiltbank and Casida, 1956) and in some other species (Perry and Rowlands, 1961). Some studies suggest that such a factor may be present in the endometrium. For example, the life-span of the corpus luteum in hysterectomized and pseudo-pregnant rats has been *shortened* by injection of endometrial suspensions (Bradbury *et al.*, 1950). As well, extracts of endometrium collected near the end of the oestrous cycle in the pig have inhibited progesterone synthesis by corpora lutea *in vitro* (Duncan *et al.*, 1960). Furthermore, when portions of the uterus have been transplanted into hysterectomized rats (Hetcher *et al.*, 1946), guinea-pigs (Butcher *et al.*, 1962) and rabbits (Chu *et al.*, 1946), oestrous cycles often resumed, provided some normal appearing endometrial tissue could be later demonstrated in the successful transplant.

In view of these findings and also the failure to demonstrate in the sheep a pronounced increase in progesterone

secretion following injection of gonadotrophic hormones (Short *et al.*, 1963), the following studies were made to investigate the possible existence of a uterine luteolytic factor.

#### EXPERIMENTAL STUDIES ON THE LIFE-SPAN OF THE CORPUS LUTEUM

##### EFFECT OF PARTIAL OR TOTAL HYSTERECTOMY

Eighteen Romney Marsh ewes (six groups of three animals) were selected following laparotomy four days after oestrus (day 0). Two recently-formed corpora lutea were found in each of eight ewes but were confined to a single ovary; the remaining ewes had one corpus luteum each. All recently-formed corpora lutea were marked for later identification by injecting a small quantity of Indian ink under the *tunica albuginea*.

Various portions of the reproductive tract were removed from the ewes in five groups, following ligation of the blood supply to the particular region. Care was taken to preserve the blood vessels supplying the ovaries. Ewes of the remaining group were sham operated; loose ligatures only being applied around the blood vessels supplying the uterus and Fallopian tubes.

After recovery, all ewes were placed with raddled vasectomized rams and daily observations made for mating marks. The animals were slaughtered 23 to 25 days after the initial oestrous period and the ovaries examined histologically to determine the fate of the marked corpora lutea.

Other details of the treatments and the number of ewes in which the life-span of the corpus luteum had been prolonged are given in Table 1.

TABLE 1: MAINTENANCE OF CORPORA LUTEA IN EWES FOLLOWING PARTIAL OR TOTAL HYSTERECTOMY

Group	Part of Reproductive Tract Excised	Number of Ewes	
		Treated	With Maintained Corpora Lutea
1	Sham operation	3	0
2	Fallopian tubes	3	0
3	Uterus	3	3
4	Uterine cornu*	3	1
5	Uterine cornu, anterior half*	3	1
6	Uterine cornu, posterior half*	3	1

\* Unilateral and corresponding to the ovary with the corpus luteum.

These results show that complete removal of the uterus caused maintenance of the corpus luteum in all ewes, whereas partial hysterectomy was effective in only three of nine animals (groups 4, 5, 6). Unmarked corpora lutea, whose appearance suggested that ovulation had occurred several days before slaughter, were found in the remaining 12 animals; most were in oestrus about that time. However, oestrous behaviour was not observed in two of these ewes (groups 5 and 6) nor did it occur in any animal in which, from histological appearance, the luteal tissue was still functional.

#### EFFECT OF LIGATION OF UTERINE BLOOD VESSELS

Sixteen ewes were used to study the effect on the life-span of the corpus luteum following ligation of the uterine blood vessels, the genital tract otherwise remaining intact. Surgery was conducted four days after oestrus and the corpora lutea, always confined to a single ovary, marked as before.

Ligatures were applied to the blood vessels supplying the uterus either bilaterally or on the same side as the ovary with the corpus luteum. In eight animals both the arteries and veins were ligated. In the remainder the major arteries or veins were first isolated from the surrounding tissue by careful dissection, before ligation. The ovarian blood supply was preserved as far as possible, but interruption of vessels supplying the anterior region of the uterine horn and Fallopian tube probably affected the anastomoses with vessels to the ovaries.

The ewes were killed on days 23 to 25 and the ovaries histologically examined to determine the age of corpora lutea.

Table 2 shows details of the number of ewes treated and of ewes in which the corpora lutea were maintained.

These results show that luteal tissue was often maintained following the ligation of all uterine blood vessels, but that unilateral ligation was ineffective in causing a response. However, the data are not strictly comparable since, in the ewes where the ligatures were applied unilaterally, sham operations (loose ligatures) were not performed on the blood vessels to the opposite uterine horn. Thus, differences noted following bilateral and unilateral ligation might conceivably be owing to the different quantities of ligature material present after surgery — the effect possibly being mediated by nervous pathways.

TABLE 2: MAINTENANCE OF CORPORA LUTEA IN EWES FOLLOWING LIGATION OF UTERINE BLOOD VESSELS

Group	Blood Vessels Ligated	Number of Ewes	
		Treated	With Maintained Corpora Lutea
BILATERAL LIGATION			
7	Arteries and veins	4	3
8	Arteries	2	0
9	Veins	2	2
UNILATERAL LIGATION*			
10	Arteries and veins	4	1
11	Arteries	2	0
12	Veins	2	0

\* Corresponding to the ovary with the corpus luteum.

Most ewes in which the corpora lutea regressed returned to oestrus and ovulated several days prior to slaughter; in three other animals "silent" oestrus occurred at a similar time.

#### EFFECT OF INJECTION OF ENDOMETRIAL EXTRACTS

The uteri from three ewes were collected at hysterectomy on day 10 of the cycle; at the same time the corpora lutea were marked. After dissecting free of the underlying tissue, the endometrium was ground up with sterile sand in approximately 40 ml of normal saline. Each homogenate was centrifuged and the supernatant fluid frozen until used for intravenous injection into the original ewe. The preparations were given once daily on days 12 to 15 inclusive and the ewes were slaughtered one week later.

Saline extracts were also prepared from the endometria of about fifty ewes slaughtered at a local freezing works. From the appearance of their ovaries these animals were judged to be near the end of the oestrous cycle. The bulked extract was intravenously administered to three ewes (previously hysterectomized on day 4) twice daily on days 12 to 15 after oestrus.

At slaughter (day 22) maintained corpora lutea were found in all six ewes. Comparison of the histological appearance of these corpora lutea with those from other ewes after hysterectomy or ligation of uterine blood vessels showed the endometrial extracts had no effect — regression of luteal cells had not occurred.

## DISCUSSION

Total hysterectomy was effective in prolonging the life-span of the corpus luteum in all nine ewes subjected to this operation in this series of experiments. Although the experiments were terminated 23 to 25 days after the initial oestrus — *i.e.*, the life of the corpus luteum was prolonged by about one week — the results of Wiltbank and Casida (1956) and Kiracofe and Spies (1963) suggest that the luteal tissue would have persisted much longer — possibly for several months after hysterectomy. The hysterectomized ewe thus may serve as a convenient preparation for studies on the persistence of luteal cells and their secretion of steroid hormones. Use of this animal to test preparations of possible luteolytic factors has also been attempted in the present study.

Interruption of the blood vessels supplying both sides of the uterus prevented regression of the corpus luteum in three of four ewes. This confirms a similar result with sheep recently reported by Kiracofe *et al.* (1963). Partial hysterectomy or ligation of the blood vessels draining one side of the uterus often showed no effect on the life-span of luteal tissue. These findings appear consistent with the concept of a uterine luteolysin. However, there did not seem to be any particular region of the uterine cornu associated with the production of such a factor. Rather, it might seem that, where part of the uterus had been removed, the remainder was able to provide sufficient stimulus to cause regression of the corpus luteum.

A major problem in establishing the existence of a uterine luteolysin is to distinguish between the effects on the corpus luteum of neural stimuli and those of humoral origin. In the sheep, neural stimuli arising from within the uterus will alter the life-span of the corpus luteum (Moore and Nalbandov, 1953; Nalbandov *et al.*, 1955) and similar effects have been reported with other species (Hansel and Wagner, 1960; Donovan and Traczyk, 1962). As well, denervation of motor stimulation (parasympathetic) to the uterus in the rat will also result in the maintenance of luteal tissue (Hill and Alpert, 1961). While the present study has shown that an increase in the life-span of luteal tissue has followed hysterectomy or ligation of uterine blood vessels, this does not necessarily indicate a humoral factor to be responsible — rather it could well be due to neurogenic effects of such operations.

Attempts to shorten the life-span of the corpus luteum by injecting extracts of endometrial tissue were unsuccess-

ful. As only definite evidence of luteal regression could be taken as a positive finding, the present results, however, provide no evidence against the existence of a luteolytic factor. The difficulties associated with the extraction and testing of preparations for the presence or absence of a luteolysin are likely to be great. Even if there is such a factor, many extracts of uterine tissue may need to be examined. Possibly a more profitable approach to learning of the existence of such a factor may arise from studies on auto-transplantation of uterine tissue or the culture *in vitro* of uterine and luteal cells.

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#### DISCUSSION

DR G. R. MOULE: *Australian workers have found that stress condition led to a rapid breakdown of the corpus luteum in the ewe towards the end of the breeding season, but at the beginning, or at the height of the breeding season no effects could be produced by experimentally induced stress. What steps were taken to check on drainage from the uterus by the lymph vessels?*

DR M. F. McDONALD: No attempt was made to interrupt lymph flow from the uterus in the animals where ligatures were applied to the blood vessels.

DR D. G. EDGAR: The following comments seem pertinent to the contents of Dr McDonald's paper:

- (a) Only extracts from the uterus late in the cycle would be likely to cause regression of the corpus luteum.
- (b) Ligation of arteries would be expected to affect blood flow in the veins.
- (c) Nalbandov has shown that beads placed in the uterus of the ewe affected the life of the corpus luteum and postulated nervous transmission of the information from uterus to ovary.

DR McDONALD: Observations made at laparotomy and at slaughter indicated that the flow of blood to the uterus was greatly restricted following the bilateral ligation of arteries—yet in both ewes treated the marked corpora lutea regressed. Undoubtedly blood from neighbouring regions of the reproductive tract entered the uterus in these animals, but it seems likely that only a small venous flow could have occurred. It is interesting, therefore, that in the two ewes where only the venous drainage was interrupted, the corpora lutea persisted and presumably were functional.

DR L. R. WALLACE: *If removal of the uterus is delayed until the corpus luteum has started to regress, does it continue to regress or is this process arrested?*

DR McDONALD: I do not know.

DR D. S. HART: *Responses were obtained by ligating the venous and both the arterial and venous systems. Why, therefore, was there no response from the arterial ligatures only?*

DR McDONALD: This result might be interpreted as suggesting that a humoral stimulus from the uterus is significant in causing regression of the corpus luteum. Whether such a mechanism could operate in view of the greatly reduced venous flow after the ligation of arteries is intriguing.

DR HART: *Were any of the eggs liberated at ovulation recovered, or was any information obtained as to the position of the egg in the reproductive tract?*

DR McDONALD: No.

R. A. S. WELCH: *Could Dr McDonald comment on the observations of Nalbandov that uterine movements cause shortening of the oestrous cycle in cows by hastening the regression of the corpus luteum?*

DR McDONALD: A discussion of this question has recently been provided by Professor Nalbandov (*Rec. Progr. Hormone Res.*, 17: 119). In this he suggests that oxytocin may exert its effect by temporarily increasing the motility of the uterus, thus initiating a neural signal to the pituitary gland and modifying its pattern of secretion of gonadotrophins, or that oxytocin causes the production of a humoral substance in the uterus which may affect the life-span of the corpus luteum. Without further data little more can be said now, but, in relation to the present studies with sheep, it is interesting that oxytocin does not cause early regression of corpora lutea in hysterectomized cows (Armstrong and Hansel, 1959: *J. Dairy Sci.*, 42: 533).