OVULATION AND CORPUS LUTEUM MAINTENANCE IN EWES TREATED WITH 17 β -OESTRADIOL*

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Summary. Two experiments designed to measure the influence of oestradiol on ovulation and CL maintenance in cycling ewes are reported. All oestradiol-treated ewes were given a single injection of oestradiol on the 4th day of their oestrous cycle; in addition, some ewes were given continuous daily injections of progesterone or oestradiol. Ovarian condition was determined by gross observations in Exp. 1 and by gross and histological observations in Exp. 2. Oestradiol induced ovulation in 72 and 73% of the ewes in Exps. 1 and 2, respectively. Progesterone blocked the ovulatory response to oestradiol. Continued daily injections of oestradiol were effective in maintaining the CL and inhibiting follicular development. The oestrous behaviour exhibited by oestradiol-treated ewes was not associated with ovulation. Induction of ovulation by oestrogen is probably due to a release of LH. The luteotrophic nature of oestrogen in the ewe may be caused by an increase in prolactin secretion. LH as a luteotrophic factor is suggested.

INTRODUCTION

It is well established that oestrogens play an important role in regulation and control of reproductive processes in the female. However, little information is available concerning the mechanisms affected, the modifying influence of oestrogen levels, the effects of varied types of oestrogens or their interactions with progestagens on these mechanisms.

Infrequent reports indicate that oestrogen can elicit an ovulatory response in both anoestrous ewes (Hammond, Hammond & Parkes, 1942; Vandernoot, Reece & Skelley, 1949) and cycling ewes (Casida, 1946; Waite & Foote, 1962). Sufficient evidence is not yet available to draw conclusions about the mechanisms involved.

The studies reported here were designed to provide more information on the influence of oestradiol on the induction of ovulation in the cycling ewe and on corpus luteum function.

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MATERIALS AND METHODS

Experiment 1

Forty-five mature, white-faced (Columbia-Rambouillet) ewes were randomly assigned to four groups. Treatments were started in November 1963 and continued through to January 1964. Animals in Group 1 served as a control group and received no injections. Animals in Group 2 received 2 mg of 17β -oestradiol on Day 4 of their cycle (Day 0 was the first day of oestrus). The ewes in Group 3 received 2 mg of oestradiol on Day 4 and 1 mg of oestradiol daily from Day 5 to Day 24. The ewes in Group 4 received 2 mg of oestradiol on Day 4 and 5 mg of progesterone given daily from Day 3 to Day 24.

All hormones were dissolved in corn oil and given intramuscularly. Oestrus was determined with painted vasectomized rams and checks were made at approximately 12-hr intervals. Only ewes exhibiting one normal pre-treatment cycle (15 to 18 days) were used in the study.

All ewes were routinely laparotomized on Days 8 and 16, using local anaesthesia. Some ewes were laparotomized again on Day 24 and autopsied at a later date. Date of autopsy was based on the condition of the CL at laparotomy. The remaining ewes were autopsied on Day 24. At autopsy and laparotomy, the ovaries were examined and the number of CL with their location, type (natural or induced) and condition (young, mature, regressed) were noted. The naturally occurring CL were marked with India ink on Day 8. The size and number of follicles 2 mm or greater were also recorded.

Some laparotomies were performed in the oestrogen-treated group on Day 4 and the CL marked and checked again by laparotomy on Day 8 to establish differences between the naturally occurring and oestrogen-induced CL. Obvious differences in development resulting from age made them easily distinguishable.

Experiment 2

Fifty mature, cycling (Rambouillet-Columbia) ewes (selected as described in Exp. 1) were randomly assigned to six groups. The experimental period began in October 1964 and ended in December 1964. Ewes in Group 1 (control group) received no injections. Animals in Group 2 received 2 mg of 17β -oestradiol on the 4th day of the cycle. Ewes in Groups 3, 4 and 5 received the same treatment as Group 2 plus 0.5, 1.5 and 2.5 mg of oestradiol from Day 5 through to Day 22, respectively. The ewes in Group 6 were killed on the first day of their cycle, their ovaries were removed, and the regressing CL, which were approximately 17 to 19 days old, were fixed for histological analysis.

Laparotomies were performed on all ewes in Groups 1 to 5 on Days 8, 14, 18 and 22. One or both ovaries were removed surgically from some ewes beginning at Day 22 and fixed for histological analysis. The remainder of the ewes that exhibited maintained CL at the previous laparotomy were laparotomized again after an additional 15 days.

The number, location and condition of the CL and the number and size of the follicles were noted. During the laparotomy on Day 8, the induced CL were marked with India ink. The control ewes (Group 1) were slaughtered on the 10th day of the subsequent cycle and their ovaries removed and fixed for histological

analysis. All ovaries were fixed in 10% formalin and stained with Shorr's triple stain.

Painted, vasectomized rams were with the ewes continuously; checks for oestrus were made twice daily. Statistical differences were determined in both experiments using chi-square techniques.

TABLE 1

EXPERIMENT 1. THE MEAN EFFECT OF 17β -OESTRADIOL AND PROGESTERONE ON OVULATION, CL MAINTENANCE AND FOLLICULAR DEVELOPMENT

Group	Treatment	Ewes with oestrogen-induced CL (%)	Maintenance of natural and induced CL*; 24th day of cycle (%)	Follicular development at Day 16 of treatment cycle; ewes with at least one follicle > 6 mm (%)
1	Control	0 ^a	0 ^a	80 ^a
2	Oestradiol	77 ^b	8.3 ^a	53 ^a
3	Oestradiol + oestradiol daily	67 ^b	50.0 ^b	0 ^b
4	Oestradiol + progesterone daily	20 ^a	0 ^a	80 ^a

^{a,b} Values in the same column not bearing the same superscript differ significantly ($P < 0.05$).

* The patterns of regression were similar for natural and induced CL.

TABLE 2

EXPERIMENT 2. THE MEAN EFFECT OF 17β -OESTRADIOL ON OVULATION, CL MAINTENANCE AND FOLLICULAR DEVELOPMENT

Group	Treatment Day of oestrous cycle 4 5 to 22	Ewes with oestradiol-induced CL (%)	Maintenance of natural and induced CL* Days of treatment cycle 22 37 (%) (%)	Follicular development at Day 14 of treatment cycle; ewes with at least one follicle 6 mm or > (%)
1	No injection	0 ^a	0 ^a 0 ^a	62.5 ^a
2	2 mg†	70 ^{bc}	20 ^{ab} 10 ^{ab}	80 ^a
3	2 mg + 0.5 mg	44 ^b	56 ^{bc} 20 ^{ab}	0 ^b
4	2 mg + 1.5 mg	100 ^c	86 ^c 40 ^{bc}	12 ^b
5	2 mg + 2.5 mg	86 ^c	100 ^c 100 ^c	0 ^b

^{a,b,c} Values in the same column not bearing the same superscript differ significantly ($P < 0.05$).

* The patterns of regression were similar for natural and induced CL.

† Levels used and duration of 17β -oestradiol injections.

RESULTS

Results are summarized in Tables 1 and 2. Ovulation was induced in 72% of ewes injected with 2 mg 17β -oestradiol on the 4th day of the oestrous cycle (Groups 2 and 3) in Exp. 1 and 73% in Exp. 2. Oestradiol-induced ovulation was significantly inhibited by progesterone treatment (Exp. 1, Group 4, 20% versus Group 2, 77%).

Sixty-seven per cent of the ewes in Exp. 1 and 76.7% of the ewes in Exp. 2 (mean of Groups 3, 4 and 5) which received daily injections of oestradiol maintained both their natural and oestradiol-induced CL until the end of the oestradiol injection period (Tables 1 and 2). All of the ewes which received 2.5 mg of oestradiol (Day 5 to Day 22, Table 2, Group 5) exhibited active CL on the 37th day of the treatment cycle. One ewe in this group (Group 5) was ovariectomized on the 52nd day of her treatment cycle; her CL appeared grossly and histologically functional.

The oestrogen-induced CL appeared to follow the same pattern of regression as the natural CL. The CL from treated ewes taken at the 22nd, 37th or 52nd day of the cycle usually showed some regressive changes, e.g. the lutein cells and their nuclei were smaller. The well-organized cellular structure and the lack of connective tissue invasion of the oestrogen-treated and the 10-day control CL compared with the 17- to 19-day-old control CL (Pl. 1, Figs. 1 to 4) indicated that oestrogen-treated CL were functional. Recent determination of ovarian vein and CL progesterone of oestrogen-treated ewes demonstrated that these CL are capable of secreting progesterone.

Continued daily injections of oestradiol resulted in relative follicular arrest. This was demonstrated by the significant ($P < 0.05$) decrease in follicular development in these groups in both Exps. 1 and 2 (Tables 1 and 2).

With one exception in Exp. 1, those ewes receiving continued oestradiol injections (Group 3) failed to re-ovulate. The one ewe that re-ovulated did so 3 weeks or longer after the natural and induced CL had apparently regressed and 2 weeks or longer after the end of oestradiol treatment. Especially in Exp. 1, a large proportion of the ewes demonstrated a period of relative ovarian inactivity. The length of time until re-ovulation varied from several days to 3 or more weeks.

DISCUSSION

Oestrogen-induced ovulations were reported by Hammond *et al.* (1942) and Vandernoot *et al.* (1949) in anoestrous ewes. Casida (1946) reported that stilboestrol could induce ovulation in cycling ewes and Waite & Foote (1962) found that 17β -oestradiol induced ovulation during the 7th to 11th day of the oestrous cycle in sheep. Oestradiol was most effective in inducing ovulation during the early stages of the oestrous cycle (Waite, 1962). Progesterone-primed cows have been induced to ovulate with oestradiol (Ulberg & Lindley, 1960).

It has been shown that oestrogens act directly on the hypothalamo-hypophysial complex. Oestrogen-sensitive centres have been found in the median eminence region of the hypothalamus in the rat and rabbit. Oestradiol implants

EXPLANATION OF PLATE 1

Photomicrographs of sheep CL. $\times 438$.

FIGS. 1 and 2. CL of ewes receiving no treatment. Fig. 1, removed on the 10th day of the oestrous cycle. Fig. 2, a 19-day-old CL removed on the 1st day of the subsequent cycle.

FIGS. 3 and 4. CL of ewes which were receiving 2.5 mg of oestradiol daily from Day 5 to Day 22 (Exp. 2, Group 5). Fig. 3, removed on the 52nd day of the cycle. Fig. 4, removed on the 22nd day.

PLATE 1

(Facing p. 256)

in the median eminence have resulted in an initial decrease in the pituitary LH content in the rat (Ramirez, Abrams & McCann, 1964) and rabbit (Kanematsu & Sawyer, 1964). Implants of oestradiol in the hypophysis produce an initial increase in plasma LH in the rabbit. It is possible that, in the ewe, oestradiol also causes an initial release of pituitary LH. Soliman, Nasr, Abdo & Soliman (1963) found that, in ovariectomized ewes, oestrogen plus progesterone decreased pituitary LH and increased serum LH. Johnson (1964) prepared an LH-releasing factor from the sheep hypothalamus.

Oestrogen has been shown to be luteotrophic in rats (Merckel & Nelson, 1940), rabbits (Robson, 1937), horses (Nishikawa, Sugie, Onuma & Fujisaki, 1958), swine (Gardner, First & Casida, 1963) and lambs (Denamur & Mauléon, 1963); however, oestrogen is luteolytic in cattle (Wiltbank, Ingalls & Rowden, 1961; Niswender, Kaltenbach, Shumway, Wiltbank & Zimmerman, 1965) and hamsters (Greenwald, 1965). The mechanism by which oestrogens act to influence CL function is not clear. The luteotrophic factor in the ewe has not been identified. Denamur & Mauléon (1963) found that oestradiol but not prolactin was luteotrophic in intact prepuberal lambs. When they hypophysectomized and induced ovulation in prepuberal lambs, neither oestrogen nor prolactin maintained the CL. Prolactin, but not oestrogen, has been shown to be luteotrophic in hypophysectomized, hysterectomized lambs (Thibault, 1966). Exogenous oestrogen resulted in prolactin synthesis and secretion in the rat (Ramirez & McCann, 1964) and rabbit (Kanematsu & Sawyer, 1963). The luteotrophic nature of oestrogen in the ewe may be due to a release of prolactin. Short (1964) was unable to demonstrate an increase in the secretory activity of the CL on the ewes with FSH, LH, HCG, ACTH, PMSG, TSH, endometrial extracts or sheep pituitary extracts. Since oestrogen can result in LH release, the luteotrophic nature of oestrogen may be in part due to the release of LH. The reason CL were maintained for at least 15 to 30 days after oestrogen injections were stopped is not understood. Similar observations were made in the pig (Nishikawa *et al.*, 1958).

Oestrogen may have a direct luteotrophic effect on the CL as was demonstrated by Hammond & Robson in the rabbit (1951). Robson (1939) suggested that oestrogen may control the luteal function of pregnant rabbits. Keyes & Nalbandov (1967) stated that oestrogen is necessary to maintain the CL of pregnant rabbits. In addition, oestradiol may increase the sensitivity of the ovary to LH as pointed out by Bradbury (1961) in the rat. However, in a preliminary study, Le Fever, Nelms, Nolan & Stratton (1965) applied oestrogen systemically or directly to the ovaries of immature and anoestrous ewes but failed to find convincing evidence of a direct action.

Circumstantially from these data, it would appear that oestrogen initially facilitates release of LH and continued injection inhibits the release of FSH. Parlow (1964) reported that in the rat, 2 µg of oestradiol administered systemically inhibited the post-ovariectomy elevation of pituitary and serum FSH. The FSH content of male rat pituitaries was reduced by doses of oestradiol ranging from 0.004 to 0.4 mg/kg/day (Beyler & Potts, 1962). Soliman *et al.* (1963) found that in ovariectomized sheep, 20 µg of oestradiol did not significantly affect pituitary or serum FSH levels. However, 20 µg oestradiol plus 20

mg of progesterone decreased pituitary FSH but did not affect serum FSH. The depressing effect of oestradiol on follicular development may be caused by the inhibitory action of oestradiol on FSH synthesis which would depress follicular growth.

In many animals, continued daily injections of oestradiol produced recurring oestrous behaviour. The oestrous behaviour exhibited by the treated ewes was not necessarily associated with ovulation. Oestrous was probably a direct response to oestradiol injection in many cases.

Waite (1962) reported that ovulation was induced with oestradiol more frequently during the early rather than the latter part of the oestrous cycle and suggested that this might be related to differences in levels of progesterone. The inhibiting influences of exogenous progesterone described in this paper tend to bear this out.

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