The effect of exogenous oxytocin on estrous cycle length and corpus luteum lysis in ewes

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Summary. The effect of exogenous oxytocin on estrous cycle length and corpus luteum lysis was investigated. A total of 111 ewes was used in 4 separate experiments. The results of experiment 1 showed no significant reduction of estrous cycle length of oxytocin-treated ewes ; however, 2 out of 8 ewes experienced new estrus 6 days after the initial one. The results of experiment 2 clearly showed ($P < 0.05$) the luteolytic property of 50 IU of oxytocin when administered at days 1 to 7 of the cycle. The results of experiments 3 and 4 showed that the destruction of the endometrium or uterine ablation (hysterectomy) negated the luteolytic property of oxytocin. It was concluded that the administration of 50 IU of oxytocin daily from days 1 to 7 of the cycle resulted in luteal lysis in ewes and that the luteolytic action of oxytocin was mediated through the endometrium.

Introduction.

Armstrong and Hansel (1959) originally reported that oxytocin administered during the first third of the estrous cycle in cows reduced the cycle length to 8 to 12 days. Afterwards many investigators (Hansel, 1959 ; Cameroun and Fosgate, 1964 ; Hansel and Wagner, 1960 ; Labhsetwar *et al*, 1964 ; Anderson *et al*., 1965 ; Ginther *et al*., 1967 ; Donalson and Takken, 1968 ; Carlson and Black, 1969 ; Donalson, 1969 ; Harms and Malven, 1969 ; Hoppe *et al*., 1969 ; Auletta *et al*., 1972 ; Milvae *et al*., 1976 ; Newcomb *et al*., 1977) studied the luteolytic effect of exogenous oxytocin in cattle to determine the mechanism of this hormonal action. Most of these authors reported that the uterus implicated as a target organ of oxytocin action, thus suggesting a local utero-ovarian mechanism (Armstrong and Hansel, 1959 ; Hansel, 1959 ; Hansel and Wagner, 1960 ; Labhsetwar *et al*., 1964 ; Anderson *et al*., 1965 ; Black and Duby, 1965 ; Ginther *et al*., 1967 ; Auletta *et al*., 1972 ; Newcomb et al, 1977), although some investigators (Cameroun and Fosgate, 1964 ; Donalson *et al*., 1965 ; Donalson and Taken, 1968 ; Carlson and Black, 1969 ; Donalson, 1969 ; Harms and Malven, 1969) found indications of hypophyseal-pituitary-gonadal axis involvement by altering the function of pituitary and ovary. In general, most of the work so far has been carried out on cattle, and it seems that cows are the only domestic animals in which the administration of oxytocin early in the estrous cycle inhibits corpus luteum deve-

lopment and shortens the estrous cycle. The object of the present investigation was to determine the effect of exogenous oxytocin on the length of the estrous cycle and on lysis of the corpus luteum in ewes and to interpret the possible mechanism of this hormonal action.

Material and methods.

Four separate experiments were carried out in the present study.

Experiment 1. — Twenty-four cycling ewes of a local milking breed (Karagou-niko) were synchronized subcutaneous silicone rubber implants containing 375 mg of progesterone and left in place for 14 days. As the ewes came into estrus, they were individually identified by numbered plastic eartags and randomly assigned to 3 experimental groups of 9 animals each. Estrus was detected by vasectomized rams and the presence of corpora lutea was ascertained by laparotomy on day 2 (day 0 = estrus) of the cycle. The ewes in group 1 received an intramuscular injection of 50 IU of oxytocin daily from days 1 to 7 of the estrous cycle ; the animals in group 2 received the same injection daily from days 8 to 14. The ewes in group 3 received no treatment and served as controls. All ewes were kept with 2 vasectomized rams and checked for estrus 3 times daily for a period of 25 days. Table 1 shows the overall experimental design.

TABLE 1

Experimental design

Treatment	Days injected [1]	Day of laparotomy [2]	No ewes [3]
Oxytocin 50 IU [4].	1-7	2	8
Oxytocin 50 IU [4].	8-14	2	8
Controls [5]	—	2	8

[1] Day 0 = day of estrus.
[2] For ascertaining ovulation.
[3] All ewes were synchronized using progesterone implants for 14 days.
[4] Intramuscular daily injections.
[5] No treatment.

Experiment 2. — A total of 30 ewes were used in this trial to further examine the luteolytic effect of exogenous oxytocin on the corpus luteum itself. As in experiment 1, the ewes were synchronized and divided into 3 equal groups. The ewes in groups 1 and 2 were treated with oxytocin, as in the first experiment, while the ewes in group 3 received no treatment and served as controls. Estrus was detected by vasectomized rams and ascertained by laparotomy at day 2 of the estrous cycle. All the ewes of

the 3 groups were autopsied on day 13 of the cycle and the corpora lutea were care-fully dissected and weighed. Table 2 shows the overall experimental design.

TABLE 2

Experimental design

Treatment	Days injected [1]	Day of laparotomy [1]	Day of slaughter [1]	No ewes [2]
Oxytocin 50 IU [3].......	1-7	2	13	10
Oxytocin 50 IU [3].......	8-14	2	13	10
Controls [4]............	—	2	13	10

[1] Day 0 = day of estrus.
[2] All ewes were synchronized using progesterone implants for 14 days.
[3] Intramuscular daily injections.
[4] No treatment.

Experiment 3. — This experiment investigated corpus luteum functioning under the action of exogenous oxytocin and the possible luteolytic mechanism of this hor-mone. A total of 33 ewes were synchronized using progesterone implants for 14 days and 1 000 IU of PMS on the day of implant removal ; they were divided into 3 equal groups as they came into estrus. Vasectomized rams were used to detect estrus. The ewes of groups 1 and 2 were given an intramuscular injection of 50 IU of oxytocin daily from days 1 to 7 of the estrous cycle, while ewes of group 3 received no treatment and served as controls. All ewes were laparotomized on day 3, reproductive tracts were exteriorized and the uteri of group 1 ewes were infused with 20 cc of a 10 p. 100 phenol solution (endometrium-modified group) ; the uteri of group 2 ewes infused with 20 cc of physiological saline solution (endometrium-intact group). Control ewes received no intrauterine infusion. The intrauterine infusion of a 10 p. 100 phenol solution was found (Alifakiotis, 1973 ; Foote *et al.*, 1974 ; Alifakiotis, 1977) to destroy the secretory portion of the endometrium in sheep and to cause luteal maintenance. All ewes were autopsied on day 12 of the cycle. The genital tracts were dissected and specimens from the two uterine horns of each ewe were removed and further pro-cessed using the standard hematoxylin-eosin staining method for determining the degree of endometrial destruction. The corpora lutea were removed, weighed and frozen for subsequent progesterone analysis. The progesterone content of the corpora lutea was extracted and measured using radioimmunoassay.

Extraction and radioimmunoassay of progesterone.

We used the basic procedure described by Louis and Kittok (1973) with some minor modifications of corpus luteum progesterone extraction (Svejda and Foote, 1973). Each corpus luteum was homogenized in 3 ml of physiological saline solution using a glass grinder. To account for procedural loss, 25 000 cmp (40 000 dmp) of [3]H-1a, 2a, -progesterone were added for recovery. The luteal emulsion was extracted twice using 5 ml of analytical grade diethyl ether for 30 sec. each time, and the pooled

extracts were washed with 1 ml of deionized water. The tubes containing the washed extracts were then stored at — 20 °C for 1 hr. to freeze the aqueous phase. The extracts destined for radioimmunoassay were decanted into 10 ml volumetric flasks and dried down under nitrogen at 40 °C ; the flasks were then made up to 10 ml by adding methyl alcohol. Aliquots of 1 ml volume were counted for procedural loss. A 10 ml aliquot taken from the first volumetric flask placed in a second 10 ml volumetric flask. The extract in the second flask was made up to 10 ml by again adding methyl alcohol. Aliquots of 0.1 ml were taken from these flasks, placed in triplicate glass culture tubes (12 × 75 mm) and then dried down under nitrogen at 40 °C.

Normal rabbit sera, diluted 1 : 400 with 0.05 M EDTA (or 0.1 p. 100 Knox gelatin) in 0.01 M phosphate buffered saline, were used to dilute the antibody to 1 : 4 500. 0.2 ml of the antibody was added to each tube, wortexed for 10 sec. and allowed to incubate at room temperature for 30 min. Two hundred microliters of 0.1 p. 100 gelatin in 0.01 M phosphate buffered saline, containing 45 000 dpm of ^{3}H- 1a, 2a, -progesterone (New-England Nuclear, 96 c/m) were added to each tube. The contents of the tubes were mixed for 5 sec. and incubated for 12 to 18 hrs. at 5 °C.

To separate bound and free progesterone, 1.0 ml of 0.5 p. 100 dextran T70 (Pharmacia, Uppsala, Sweden) and 0.25 p. 100 carbon decolorizing neutral norit (Fisher Scientific Co.) in deionized water were added to each tube. The contents were mixed, incubated in an ice bath for 10 min. and then centrifuged at 2 500 g for 10 min. at 5 °C. A 0.5 ml aliquot of the supernatant fluid was diluted with a liquid scintillation fluid (Omnifluor) for quantification of radioactivity in a liquid scintillation spectrometer (Beckman, model 133). For comparison among assays, standard sera with high and low progesterone, and extracts from blank extraction tubes were assayed with each set of unknown luteal extracts. The data from the assay, based on standard curves, were reduced by computer. Table 2 shows the overall experimental design.

TABLE 3

Experimental design

Treatments		Day of injection (²)	Day of laparotomy and infusion (²)	Day of slaughter (²)	No. ewes (³)
Injection	Infusion (¹)				
Oxytocin 50 IU (⁴)	10 p. 100 phenol.	1-7	2	12	11 (⁵)
Oxytocin 50 IU (⁴)	Phys. saline solution	1-7	2	12	11 (⁶)
Controls (⁷).......	—	—	2	12	11

(¹) Intrauterine 20 cc.
(²) Day 0 = day of estrus.
(³) All ewes were synchronized using progesterone implants for 14 days and 1 000 IU PMS at day of implant removal.
(⁴) Intramuscular daily injections.
(⁵) Endometrium-modified ewes.
(⁶) Endometrium-intact ewes.
(⁷) No treatment, endometrium-intact ewes.

Experiment 4. — A total of 24 ewes was used in this experiment to further elicit the pathway of oxytocin as a luteolytic substance. The ewes were allotted to 3 equal groups as they came into estrus after synchronization. Vasectomized rams were used for estrus detection. Ewes in group 1, serving as controls, received 50 IU of oxytocin from days 1 to 7 of the estrous cycle and were laparotomized on day 10. The ewes in group 2 also received 50 IU of oxytocin from days 1 to 7 and were hysterectomized on day 3 of the cycle. The ewes in the third group were hysterectomized on day 8 and received 25 mg of $PGF_{2\alpha}$-tham on day 10 of the estrous cycle. All ewes were autopsied on day 13 of the cycle and the corpora lutea were removed and weighed. Table 4 shows the overall experimental design.

TABLE 4

Experimental design

Treatment		Day of injection [1]	Day of surgery [1]	Day of slaughter [1]	No. ewes [2]
Injection	Surgery				
Oxytocin 50 IU [3].....	Laparotomy	1-7	10	13	8 [5]
Oxytocin 50 IU [3].....	Laparotomy and hysterectomy	1-7	3	13	8 [6]
$PGF_{2\alpha}$-tham 25 mg [4]..	Laparotomy and hysterectomy	10	8	13	8 [6]

[1] Day 0 = day of estrus.
[2] All ewes were synchronized using progesterone implants for 14 days.
[3] Intramuscularly daily injections.
[4] One intramuscular injection.
[5] Endometrium-intact ewes.
[6] Hysterectomized ewes.

The results of all the experiments were statistically analyzed using analysis of variance ; the treatments were compared by the Duncan method (Duncan, 1955).

Results.

Experiment 1. — The effect of exogenous oxytocin on estrous cycle length is presented in figure 1. All the control ewes and 6 of the 8 oxytocin-treated animals during the first (days 1 to 7) and the second (days 8 to 14) halves of the estrous cycle had a mean estrous cycle length of 19.8 days (16 to 21 days), which is considered normal for this breed (Karagouniko milking breed), while 2 ewes from group 1 (oxytocin days 1 to 7) had an estrous cycle length of 6.0 days and 2 ewes from group 2 (oxytocin days 8 to 14) had an estrous cycle lasting 15 and 24 days, respectively. Although the results of this trial showed no significant reduction of estrous cycle length in oxytocin-treated and control ewes, the 2 ewes of group 1 (oxytocin days 1 to 7), in which new estrus was detected 6 days after the initial one, tended to have a shorter estrous cycle. This trend becomes evident when comparing the interestrous periods between

the oxytocin-treated ewes during the first half of the cycle (15.13 days) and the oxytocin-treated ewes during the second half of the cycle (18.63 days), as well as the controls (18.75 days).
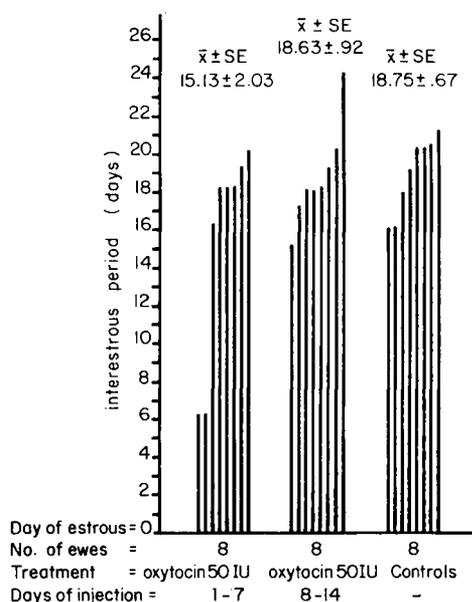

FIG. 1. — *The effect of oxytocin on estrous cycle duration in ewes.*

Experiment 2. — The luteolytic effect of exogenous oxytocin was evidenced during this experiment, as shown in table 5. The administration of 50 IU of oxytocin daily resulted in a significant ($P < 0.05$) reduction of luteal weight as compared to that of the controls (518.4 $\pm$ 41.4 and 564.6 $\pm$ 49.9 vs 758.8 $\pm$ 50.7 mg) on day 14 of the estrous cycle. A tendency for greater luteal reduction was observed when oxyto-

TABLE 5

The effect of exogenous oxytocin on corpus luteum weight in ewes

Treatment	Days injected [1]	No. ewes [2]	Corpus luteum weight ($x \pm$ SE, mg) [3]
Oxytocin 50 IU [4]......	1-7	10	518.4 $\pm$ 41.4 (a)
Oxytocin 50 IU [4]......	8-14	10	564.6 $\pm$ 49.9 (a)
Controls [5]...........	—	10	758.8 $\pm$ 50.7 (b)

[1] Day 0 = day of estrus.
[2] All ewes were synchronized using progesterone implants for 14 days.
[3] Corpora lutea removed from autopsied ewer at day 14 of estrous cycle.
[4] Intramuscular daily injections.
[5] No treatment. *$P < 0.05$ for comparison a to b.

cin was administered during the first half (days 1 to 7) as compared to ewes treated during the second half (days 8 to 14) of the estrous cycle (518.4 ± 41.4 vs 564.6 ± 49.9 mg).

Experiment 3. — The luteolytic effect of exogenous oxytocin was further confirmed in this experiment by investigating the anatomical degeneration (weight) of the corpora lutea and their functional regression (luteal progesterone content) (Stormshak *et al.*, 1963). The results of this experiment are summarized in table 6. Thus, oxytocin demonstrated a significant (P < 0.05) luteolytic effect in oxytocin-treated ewes with intact endometrium (1 594.2 ± 145.4 mg) as compared to untreated controls also having an intact endometrium (2 458.6 ± 314.6 mg). However, the luteolytic property of oxytocin was negated in oxytocin-treated ewes with modified endometrium (2 514.8 ± 272.7 mg), as shown by comparing the oxytocin-treated ewes with intact endometrium (1 594.2 ± 145.4 mg ; P < 0.05) to the untreated controls with intact endometrium (2 458.6 ± 314.6 mg). The progesterone content of the corpora lutea tended to follow the same patterns as luteal weight. Thus, the corpora lutea from oxytocin-treated ewes with intact endometrium had lower progesterone content (9.55 ± 1.24 μg/mg) than the corpora lutea from untreated controls with intact endometrium (13.22 ± 2.31 μg/mg) or from oxytocin-treated ewes with modified endometrium (11.72 ± 1.46 μg/mg).

TABLE 6

The effect of exogenous oxytocin on corpora lutea in ewes with intact and modified endometrium

Treatment	Days injected [1]	No. ewes [2]	State of endometrium	Corpora lutea [3]		
				Weight ($\bar{x}$ ± SE, mg)	Progesterone content ($\bar{x}$ ± SE, mg)	Progesterone concentration ($\bar{x}$ ± SE, μg/mg)
Oxytocin 50 IU [4]	1-7	10	modified [6]	2,514.8 ± 272.7 (a)	27.07 ± 5.55	11.72 ± 1.46
Oxytocin 50 IU [4]	1-7	10	intact [7]	1,594.2 ± 145.4 (b)	14.14 ± 3.04	9.55 ± 1.24
Controls [5]......	—	10	intact	2,458.6 ± 314.6 (a)	32.23 ± 10.58	13.22 ± 2.31

[1] Day 0 = day of estrus.
[2] All ewes were synchronized using progesterone implants for 14 days and 1 000 IU PMS on day of implant removal.
[3] Corpora lutea removed from autopsied ewes on day 12 of estrous cycle.
[4] Intramuscular daily injections.
[5] No treatment.
[6] Intrauterine infusion of 20 cc of 10 p. 100 phenol solution at day 2 of estrous cycle.
[7] Intrauterine infusion of 20 cc of physiological saline solution at day 2 of estrous cycle.
* P < 0.05 for comparison a to b (same column).

Experiment 4. — The luteolytic effect of exogenous oxytocin was tested in hysterectomized ewes to determine the possible route of action of oxytocin as a luteolytic agent. The results of this trial are summarized in table 7. Thus, the administration of 50 IU of oxytocin from days 1 to 7 of the estrous cycle in ewes with intact uterus resulted in luteolysis, while in hysterectomized ewes the luteolytic property of oxytocin

was negated (806.4 $\pm$ 86.8 vs 1 380.0 $\pm$ 189.6 mg ; P $<$ 0.05). The substitution of the uterus, the source of the luteolytic agent (Goding, 1974), by $PGF_{2\alpha}$-tham caused corpus luteum lysis as compared to hysterectomized ewes (446.1 $\pm$ 112.3 vs 1 380.0 $\pm$ 189.6 mg ; P $<$ 0.05) and to oxytocin-treated ewes with intact uterus (446.1 $\pm$ 112.3 vs 806.4 $\pm$ 86.8 mg).

TABLE 7

The effect of oxytocin and $PGF_{2\alpha}$ on corpus luteum in intact and hysterectomized ewes

Treatment	Days injected [1]	Day of hysterectomy [1]	Corpus luteum weight ($\bar{x} \pm$ SE, mg)	No. ewes [3]
Oxytocin 50 IU [4].....	1-7	—	806.4 $\pm$ 86.8	8
Oxytocin 50 IU [4].....	1-7	3	1,380.0 $\pm$ 189.6	8
$PGF_{2\alpha}$-tham 25 mg [5]..	10	8	446.1 $\pm$ 112.3	8
				8

[1] Day 0 = day of estrus.
[2] Corpora lutea removed from autopsied ewes on day 12 of estrous cycle.
[3] All ewes were synchronized using progesterone implants for 14 days.
[4] Intramuscular daily injections.
[5] One intramuscular injection.

Discussion.

The administration of 50 IU of oxytocin to ewes resulted in corpus luteum regression whether injection was carried out during the first (days 1 to 7) or the second (days 8 to 14) half of the estrous cycle. However, a tendency for greater luteal regression was observed when oxytocin was injected during the first half of the cycle. It is somewhat surprising that cycle shortening was observed in only 2 out of 8 ewes. However, a comparable observation was reported by Milne (1963), who found that neither the dosage level of oxytocin (15 to 50 IU) nor the time of commencement of treatment after estrus (1 to 4 days) affected estrous cycle length within the normal limits of 14 to 19 days in 7 ewes of the Merino breed, although the corpora lutea on day 8 of the cycle appeared to be degenerate upon histological examination. Contrary to this report, Dobrowolski (1973) observed that 50 IU of oxytocin injected on days 3 to 5 of the cycle in 11 Merino ewes shortened the estrous cycle by 0.96 $\pm$ 0.21 days ; this was regarded as significant. Experiments in cows treated with oxytocin during the first halft of the cycle demonstrated evident luteal regression (Armstrong and Ha nsel, 1959 ; Harms and Malven, 1969 ; Auletta *et al.*, 1972), although the estrous cycle length was sometimes normal (Simmons and Hansel, 1964). The explanation of the normal estrous cycle length with regressed corpora lutea in cattle was that luteal progesterone concentration was higher than the critical level (20 $\mu g/mg$), below which the cows showed precocious estrus (Simmons and Hansel, 1964). Therefore, it seems possible that precocious estrus in oxytocin-treated ewes could be related to the degree of luteal regression.

There may be several modes of oxytocin action responsible for luteal regression when a high dose of the hormone (50 IU) is administered. However, the fact that oxytocin loses its luteolytic effect in endometrium-modified ewes or in hysterectomized ewes strongly suggests that the pharmacological dose of this hormone exerts its luteolytic action through the endometrium, probably by influencing the synthesis and/or the release of uterine luteolytic agent, rather than directly on the ovary and/or the hypothalamo-pituitary axis. This is in agreement with recent reports by Sharma and Fitz-patrick (1974) and Roberts *et al.* (1975) showing that exogenous oxytocin in ewes caused a pronounced release of $PGF_{2\alpha}$ from the uterus. Recently, Chan (1977) demonstrated that exogenous oxytocin induces enhanced PG synthesis and release from the non-pregnant as well as from the parturient uterus of rats. Moreover, the amount of released $PGF_{2\alpha}$ appeared to depend more on the priming dose of estrogen, which sensitizes the uterus to oxytocin action, than on the challenging dose of oxytocin. Further experiments in cows (Newcomb, 1977) indicate that the release of PGF from the uterus, after oxytocin treatment, is not the result of myometrial contractions, but that it might be of metabolic origin (such as synthesis from precursors) because of the prolonged release of PGF from the uterus after a single oxytocin infusion.

Conclusions.

The conclusions draw from the results of the present study are the following :
1. the administration of 50 IU of oxytocin daily does not seem to apparently decrease the length of the estrous cycle in milking ewes ;
2. the administration of 50 IU of oxytocin daily from days 1 to 7 or from days 8 to 14 results, however, in luteal regression, appraised from the reduction of luteal weight and luteal progesterone content ;
3. endometrial destruction or uterine ablation negates the luteolytic effect of exogenous oxytocin.

The last conclusion strongly supports the hypothesis that the luteolytic effect of an exogenous pharmacological dose of oxytocin is exerted through the uterine-ovarian axis, probably by inducing synthesis and /or release of the uterine luteolytic agent from the endometrium.

Résumé. Nous avons étudié l'effet de l'ocytocine exogène sur la longueur du cycle estrien et la lyse du corps jaune, en utilisant 111 brebis au cours de 4 expériences. L'expérience 1 montre que la longueur du cycle estrien n'est pas réduite significativement dans le lot des animaux traités à l'ocytocine ; cependant, 2 sur 8 présentèrent un nouvel estrus 6 jours après le premier. L'expérience 2 montre clairement (p < 0,05 la propriété lutéolytique de 50 UI d'ocytocine si on les administre du 1er au 7e jour du cycle. Les expériences

3 et 4 montrent que la destruction de l'endomètre ou l'ablation de l'utérus annulent la propriété lutéolytique de l'ocytocine. On peut conclure que l'administration quotidienne de 50 UI du 1er au 7e jour du cycle entraîne la lyse du corps jaune chez les brebis et que l'action lutéolytique de l'ocytocine s'exerce par l'endomètre.

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