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Original article

Effects of ACTH on luteinizing hormone receptors in ovine follicular wall and corpus luteum

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Summary – The effects of high systemic concentrations of ACTH during the days preceding ovulation on follicular wall and luteal tissue LH receptor concentration were studied at the expected time of ovulation and their evolution thereafter in 12 ewes. Controls (C; $n = 3$), received ten injections of sterile saline every 12 h, on days 11–16 of the estrous cycle, treated ewes (T_1, T_2, T_3; three animals each) received ten injections of ACTH (50 IU every 12 h). Ovariectomies were carried out in C and T_1 on day 16, in T_2 on day 18 and in T_3 on day 21. Relative to C, follicular concentration of LH receptors was approximately 50% in T_1 ewes ($P < 0.025$); but slightly higher (62%) in T_2, and dramatically increased (167%) in T_3. None of the animals displayed a preovulatory LH surge. ACTH treatment induced significant pathological effects on ovarian structures probably through treatment-induced increase in cortisol.

ewe / ACTH / LH receptor / atretic follicle

Résumé – Effet de l'ACTH sur les récepteurs de l'hormone lutéinisante de la paroi folliculaire et du corps jaune chez la brebis. Les effets de hautes concentrations d'ACTH sur la concentration en récepteurs à LH des follicules et du tissu lutéal au moment de l'ovulation et son évolution ultérieure ont été étudiés chez 12 brebis : témoins (C ; $n = 3$) soumises à des injections de solution saline (1,25 mL) toutes les 12 h pendant les jours 11–16 du cycle œstral ; traitées (T_1, T_2, T_3) soumises à des injections d'ACTH (50 UI) au lieu de solution saline. Tous les animaux ont été ovariectomisés en fin d'expérience : C et T_1 le jour 16 ; T_2 le jour 18 ; et T_3 le jour 21 du cycle. La concentration des récepteurs de LH était réduite à 50 % à T_1 ($p < 0,025$) et à 62 % à T_2, mais augmentée de 167 % à T_3. Aucun des animaux n'a montré de décharge préovulatoire de LH. Le traitement avec l'ACTH induit des effets pathologiques significatifs, probablement via une augmentation du cortisol.

brebis / ACTH / récepteur de LH / follicule atrétique

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INTRODUCTION

The hypothalamus–pituitary–adrenocortical (HPA) axis plays an important role in modulating the regulation of the gonadal axis, and in turn, reproduction. Stress stimulates neurosecretory neurons in the hypothalamus to secrete corticotropin-releasing hormone (CRH), which triggers pituitary release of adrenocorticotrophic hormone (ACTH) (Moberg, 1987, 1991). ACTH is released from pituitary gland under environmental and psychological stresses (Stoebel and Moberg, 1982a; Moberg, 1987, 1991).

Released ACTH acts on adrenal gland inducing the release of cortisol, which is considered to be the key adrenal factor affecting the hypothalamus–pituitary–gonadal axis (Moberg, 1991). ACTH-mediated release of cortisol has been shown to suppress preovulatory surges of LH in cows (Matteri and Moberg, 1982; Stoebel and Moberg, 1982b) and ewes (Thompson and Wise, 1978), to alter progesterone to estrogen (P/E) plasma ratio due to increased progesterone concentration (Stoebel and Moberg, 1982b) and to induce the formation of cystic follicles in cows (Liptrap and McNally, 1976) and ewes (Prezkop et al, 1984; Cooke and Benhaj, 1989). The mechanism of this effect has not been fully elucidated. A reduced number of LH receptors could lead to suppression of ovulation and in certain circumstances be responsible for cystic follicles as has been shown in chronic cystic cows (Brown et al, 1986). On the other hand, it has been suggested (Kesler et al, 1981; Inskeep et al, 1988) that a reduction in both the number of LH receptors and progesterone production could lead to a shorter luteal life span in the cow. Alternatively, glucocorticoids have been shown to have an inhibitory effect upon the concentration of LH receptors and steroidogenesis in rats (Bambino and Hsueh, 1981).

The main objective of this study was to examine the effects of high plasma concentrations of ACTH on follicular development during the days preceding ovulation. Primarily we wished to assess follicular LH binding capacity at the expected time of ovulation and observe its evolution thereafter. A secondary objective was to study associated changes in luteal LH binding capacity.

MATERIALS AND METHODS

Animals

Twelve adult Dorset Horn ewes weighing 60–71 kg were used. Each ewe had shown regular estrous cyclicity for a year. The animals were housed indoors in three separate pens, with four animals each, at Charmany Instructional Facility of the School of Veterinary Medicine, from May to September 1990. They were kept under controlled conditions of day-light (8 h light/16 h darkness) (Hafez, 1952; Ducker et al, 1970; Legan, 1979) and temperature (14°C). The ewes were fed alfalfa hay twice daily and provided with water ad libitum. During a 74-day adaptation period, they were sheared and acclimatized to frequent handling. All ewes were clinically normal at the start of the experiment.

Synchronization of estrous cycle

This was performed using progestogen implants (3 mg; Synchromate CEVA laboratories Inc, KS, USA) for 12 days followed by PMSG (500 IU; Calbiochem Co, CA, USA) im injected at implant withdrawal (Colas, 1975). The ewes were examined for estrus (estrus = 0 day) using a vasectomized ram three times daily. A ewe was considered to be in estrus when she stood for service.

Treatments, ovariectomies and blood sampling

Sheep were blocked by pen and randomly assigned to one of four groups for drug treatment and ovariectomy: C, T_1, T_2, T_3. On the assumption of a mean cycle length of 16.5 days,

control group (C, $n = 3$) received ten im injections of 1.25 mL sterile saline (0.9% NaCl) every 12 h, starting on day 11 of the estrous cycle at 2000 hours and ending at 0800 hours on day 16 of the estrous cycle. All animals in group C were ovariectomized on day 16 of the cycle immediately after the last injection; that is, immediately before the expected estrus LH surge to prevent the down regulation of LH receptors in ovulatory follicles. Animals in groups T_1, T_2 and T_3 (three animals each) were treated like the control group except that 50 IU (1.25 mL) of ACTH (Acthar gel, Rorer pharmaceuticals Co, PA, USA) were injected instead of saline. Ewes in group T_1 were ovariectomized at the same time as controls, on day 16. Ewes on T_2 and T_3 were ovariectomized on days 18 (T_2) and 21 (T_3), that is, 2 and 5 days after cessation of the ACTH treatment and beyond the normal duration of the estrous cycle, which we anticipated would be extended by the ACTH treatment.

All ewes were fitted with jugular catheters 1 day before the beginning of the treatment. Bilateral ovariectomies were performed through left flank incisions (Oehme, 1988) following sedation with Xylazine (0.01 mg/lb im) and paravertebral anesthesia (T_{13}, L_1 and L_2 nerves were blocked with 2 cc of 1% lidocaine; Lumb, 1984).

Ten milliliters of jugular vein blood were collected into heparinized tubes every 6 h during days 11–14 and every 4 h during days 15 and 16. Additional blood samples were taken 1 h after each intramuscular injection. Estrus detection was carried out every 4 h between days 15 and 21. The preovulatory plasma LH surge in the ovine is sharp (duration 8–10 h; amplitude 80–200 ng/mL) and occurs 1–12 h after the onset of estrus (Godin et al, 1969). Blood samples were to be collected hourly after detection of behavioral estrus. Blood samples were centrifuged and plasma collected and stored at –20 °C until assayed. In order not to disturb the ewes during dark hours, the management and handling of the animals were carried out using a flashlight.

Hormone assays

Plasma samples were analyzed for LH concentrations using a double-antibody radioimmunoassay (RIA). Purified ovine LH (NIADDK-oLH-I-3) iodinated using the chloramine T method, and antiserum against ovine LH (NIADDK-anti-oLH-I; 1:100) were obtained from the National Hormone and Pituitary Program at the University of Maryland, USA. The second anti-rabbit antibody was generously provided by Dr M Brownfield (School of Veterinary Medicine, U-W Madison). Two hundred microliters of standard LH prepared with stripped serum at concentrations of 0.1, 0.25, 0.5 and 1 ng/mL or plasma sample were incubated at 4 °C for 18 h, with the LH antibody (1:300 000). After a second incubation (24 h at room temperature) with labeled ovine LH, the precipitation step was carried out with anti-rabbit gamma globulin. The tubes were centrifuged at 2 800 *g* for 30 min, decanted, and counted for 1 min each in a gamma counter. Assay was validated for ovine serum by demonstrating recovery of mass and parallelism between serum pool dilutions and hormone standards. Concentrations of 0.14, 0.21, 0.57 and 1.09 ng were recovered after addition of 0.1, 0.25, 0.5 and 1 ng ovine LH and subtraction of endogenously measured hormone ($y = 2.468–0.0679x$; $r = 0.997$). Assay sensitivity (defined as 90% of maximum binding) was 0.25 ng/mL for 200 µL serum. All samples from one animal were analyzed in duplicate in one assay. The intraassay coefficient of variation of duplicate samples was less than 9.5% and the interassay coefficient of variation was 6.2%.

Cortisol concentrations in plasma were determined in unextracted serum using a ^{125}I radioimmunoassay previously validated by Peter and Bosu (1987) for bovine but not ovine serum (Diagnostic Products Corporation, Los Angeles, CA, USA). However, standard curves obtained with ovine serum were parallel to curves obtained with bovine serum. All samples from one ewe were analyzed in one assay. The intraassay coefficient of variation of duplicate samples was less than 9.1% and the interassay coefficient of variation 5.4%.

Processing of the ovaries

Following ovariectomies, the ovaries were placed in ice-cold assay buffer (0.01 M TRIS-HCl, 0.1% BSA, 0.1% NaN_3, 5 mM $MgCl_2$, 20% glycerol pH 7.4, 20 °C) and grossly examined for follicular and luteal development. All follicles > 6 mm in diameter were dissected free of the stroma and their weight and diameter recorded. Follicular fluid (FF) from each follicle was collected through a scalpel incision in the follicular wall, rather that aspiration, to prevent the gran-

ulosa cell dragging reported in numerous studies (Bellin and Ax, 1984).

The gross appearance of corpora lutea was classified into four stages according to Restall's classification (Restall, 1964): stage I: red or pink color (days 1–8 of the estrous cycle); stage II: pink color (days 7–14 of the estrous cycle); stage III: fading pink color (days 14–18 of the estrous cycle); stage IV: yellow color (days 18–25 of the estrous cycle).

Histological classification of follicular health

A piece of follicular wall (4 mm^2) was cut, fixed in Bouin's fixative, embedded in paraffin, sectioned (10 μm) and stained with H-E. The follicles were rated according to a simplified version of Hay's classification (Hay et al, 1976): 1) atretic (A) follicles with complete cellular disintegration of granulosa cells; 2) intermediate atretic (I-A) follicles with widespread cellular disintegration of granulosa cells; and 3) non-atretic (N-A) follicles with no cellular damage in granulosa layer.

Luteinization is defined as the transformation of the granulosa and the theca cells into luteal cells, involving cellular hypertrophy and hyperplasia (Dellman, 1987). Under the light microscope the luteinized areas were identified as having basophilic appearance and hypertrophied follicular cells (Grimes et al, 1987).

Measurement of LH receptor binding capacity in follicular wall

Each follicular wall was placed in a separate vial (stored in assay buffer with 20% glycerol) and frozen at –70 °C for LH radio-receptor-assay (RRA). Although degradation of LH receptors during storage was not examined, it has been shown that the loss of binding capacity of the LH receptors stored at –70 °C for 3 months using our buffer is negligible (Ireland and Roche, 1983; Brown et al, 1986). The follicular walls from all groups and corpora lutea from groups C and T_1 were placed on cold assay buffer and homogenized according to procedure described by Braden et al (1986). Purified human chorionic gonadotropin (CR-125; 11 900 IU/mg supplied by National Hormone and Pituitary Program, NHDDK, University of Maryland, USA) iodinated by the lactoperoxidase method was used as labeled ligand. Specific activity, calculated based on the percentage of radioactive iodine incorporated into the total mass of hormone, assuming a 95% recovery of the hormone after gel filtration chromatography of the iodination mixture, was 48.5 μCi/μg. Purified hCG (CR-127; 14 900 IU/mg) (NHPP and NHDDK, University of Maryland, USA) was used as unlabeled ligand. Because of the limited amount of tissue available from each follicle, binding was determined by one-point saturation analysis. Saturating concentration of labeled hCG was determined on pooled follicles from ovine ovaries collected at slaughter house. Saturation of 10 mg of tissue was obtained with 300 000 cpm of labeled hormone.

LH binding assays were performed according to the procedure described by Brown et al (1986). Briefly, aliquots of follicle wall homogenates (100 μL) were incubated with 100 μL of a saturating concentration of labeled hormone with or without 100 μL of excess unlabeled hormone (1 μg). After 16 h of incubation at room temperature the reaction was stopped with 3 mL of cold buffer (0.01M TRIS-HCl, 0.14 M NaCl, 0.1% NaN_3, pH 7.4, 4 °C) and the tubes were centrifuged at 8 000 *g* for 30 min at 4 °C. Following the aspiration of the supernatant, containing the unbound hormone, the radioactivity of bound hormone in the pellet was determined in a gamma counter. The specific binding was calculated as the difference of total binding minus non-specific binding. All follicular samples were run in two assays the intraassay coefficient of variation was less than 5.3%. Protein concentration in homogenates of follicular wall were determined by the procedure of Lowry et al (1951) using a commercial kit (Sigma Diagnostics Co, St Louis, MO, USA).

Rat testis homogenates were used to determine hormone specificity. Woolf analysis of saturation curves revealed binding to a single population of receptors.

Statistical analysis

Treatment effect on LH receptor numbers in follicular wall were analyzed as a randomized block design by one-way Anova using the general linear procedure of SAS (SAS Institute, 1988). Differences among means were tested with a pro-

tected least significant difference (LSD). Cortisol release was estimated as the area under the curve of plasma cortisol concentrations versus time. A paired *t*-test was used to determine significant differences in treated compared with control cortisol release.

The start of the preovulatory LH surge, was defined as the first abrupt increase greater than 10 ng/mL occurring within 1–12 h after the onset of behavioral estrus (Hauger et al, 1977).

RESULTS

General

Throughout the entire experiment none of the animals displayed any adverse symptoms due to the ACTH treatment. None of the sheep showed signs of estrus behavior on days 15–21 of the estrous cycle.

Hormonal profiles

None of the animals displayed a preovulatory LH surge. In control animals the cortisol concentrations remained at physiological levels throughout the experiment. The amount of cortisol released estimated as the area under the curve, was significantly lower ($P < 0.05$) in C (55.6 ± 3.7 ng/mL) than in any of the treated animals (T_1 = 363.8 ± 9.4 ng/mL; T_2 = 283.6 ± 16.5 ng/mL; T_3 = 225.8 ± 19 ng/mL). Mean daily concentrations of cortisol are illustrated in figure 1. There were significant differences ($P < 0.01$) between C and T_1, T_2 and T_3 animals on days

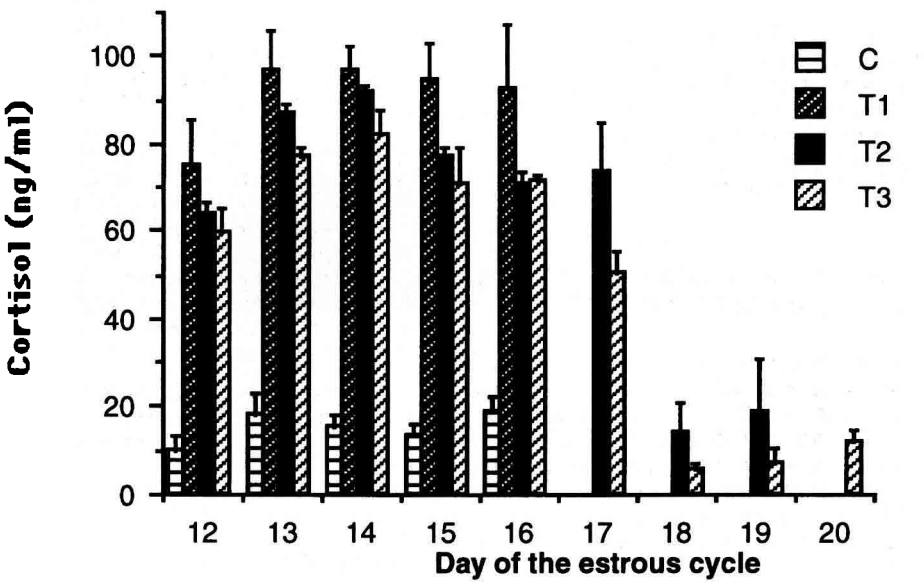
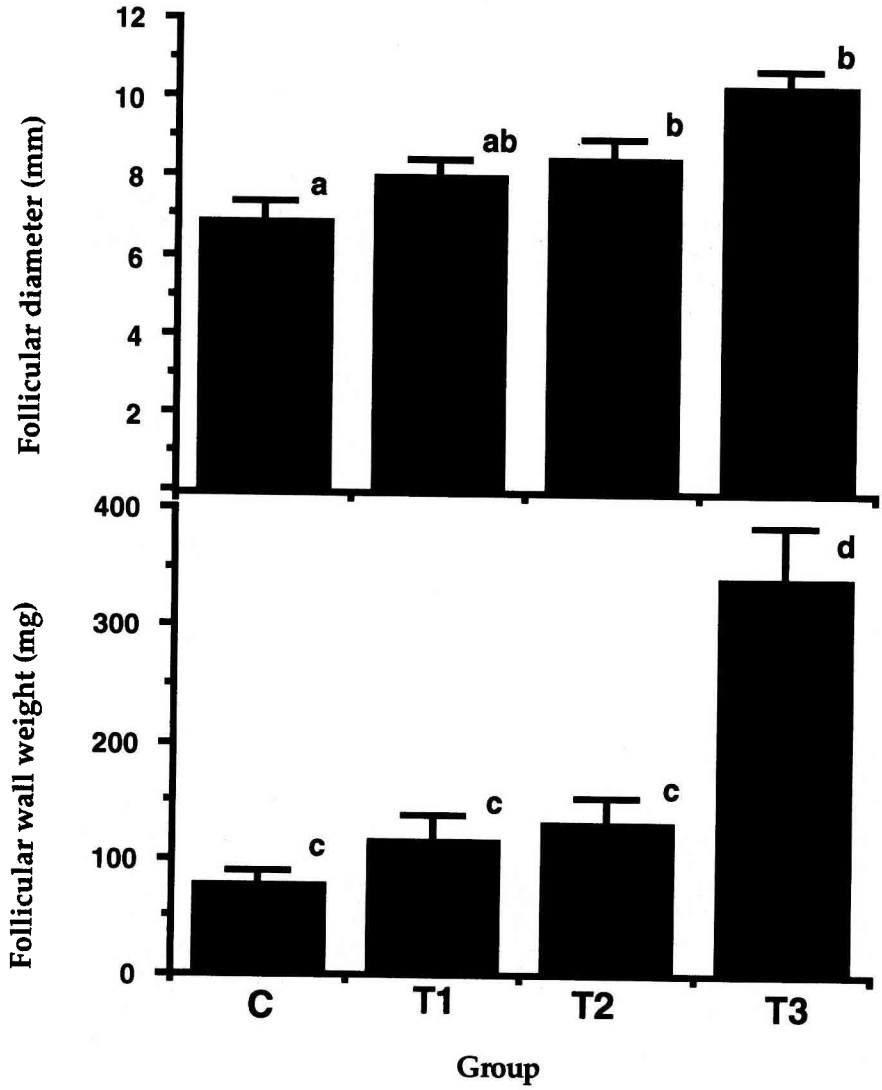


Fig 1. Daily concentration of cortisol mean (± SE) in control (C, $n = 3$; received ten injections of sterile saline intramuscularly every 12 h, on days 11–16 of the estrous cycle) and treated ewes (T_1, T_2, T_3; three animals each; received ten injections of 50 IU ACTH every 12 h). Ovariectomies were carried out in C and T_1 on day 16, in T_2 ewes on day 18 and in T_3 ewes on day 21.



a, b: different superscripts indicate significant differences (α=0.01)

c, d: different superscripts indicate significant differences (α=0.001)

Fig 2. Mean follicular diameter (mm ± SE) and mean follicular wall weight (mg ± SE) in control (C, n = 3; received ten injections of sterile saline intramuscularly every 12 h, on days 11–16 of the estrous cycle) and treated ewes (T_1, T_2, T_3; three animals each; received ten injections of 50 IU ACTH every 12 h). Ovariectomies were carried out in C and T_1 on day 16, in T_2 ewes on day 18 and in T_3 ewes on day 21.

12–16 of the estrous cycle. Concentrations in T_2 and T_3 animals returned to normal values by day 18.

Ovarian findings

The total number of follicles greater than 6 mm in diameter found in each group was similar 2.0 ± 0.0, 2.7 ± 0.6, 2.0 ± 0.0 and 2.0 ± 1.0 for C, T_1, T_2 and T_3, respectively. The mean of follicular diameter was significantly higher ($P < 0.01$) in T_2 and T_3 (8.5 ± 0.49 and 10.3 ± 0.45 mm) compared to C group (6.9 ± 0.48 mm). In group T_1 the follicular diameter (8 ± 0.45 mm) was not significantly larger than in C group ($\alpha = 0.05$; fig 2). In order to determine whether the diameter increases were due to an increase in FF volume or to follicular wall hypertrophy, the mean of follicular wall weight was measured for each group (C = 77.5 ± 11.4 mg, T_1 = 114.6 ± 22.2 mg, T_2 = 129 ± 23.4 mg and T_3 = 339.2 ± 44.4 mg; fig 2). Only follicular wall weights of T_3 ewes were significantly different from all other groups ($P < 0.005$), although there was a clear tendency for heavier follicular walls in treated animals. Therefore, 1) there was a significant increase in follicular wall mass in T_3 compared to C; 2) the increase in the mean of wall weight in T_1, T_2 and T_3 relative to C was parallel to the increase in follicular diameter, confirming that the follicles were not suffering a collapsing process.

All follicles (100%) from C group contained clear FF (N = normal); however, only two follicles out of 19 (10.5%) had clear, serous fluid with normal appearance in the treated groups (T_1, T_2 and T_3). The FF in the majority of follicles (89.5%) from treated groups had an evident haemorrhagic appearance.

The total number of corpora lutea found in each group was 1.7 ± 0.6; 3.0 ± 1.0; 1.7 ± 0.6 and 1.3 ± 0.6 for C, T_1, T_2 and T_3 groups, respectively. Externally, all corpora lutea appeared mature (stage IV) except for two ewes in T_2 group that had younger corpora lutea (stage III). Therefore, no ovulation occurred during the time in which the experiment was carried out. The mean weights of corpora lutea were similar in C (422 ± 74 mg) and T_1 (336 ± 60 mg) groups, but decreased ($P < 0.025$) in T_2 (156 ± 9 mg excluding the corpora lutea of stage III) and T_3 (128 ± 25 mg) (fig 3). That is, the majority of the corpora lutea were regressing (stage IV).

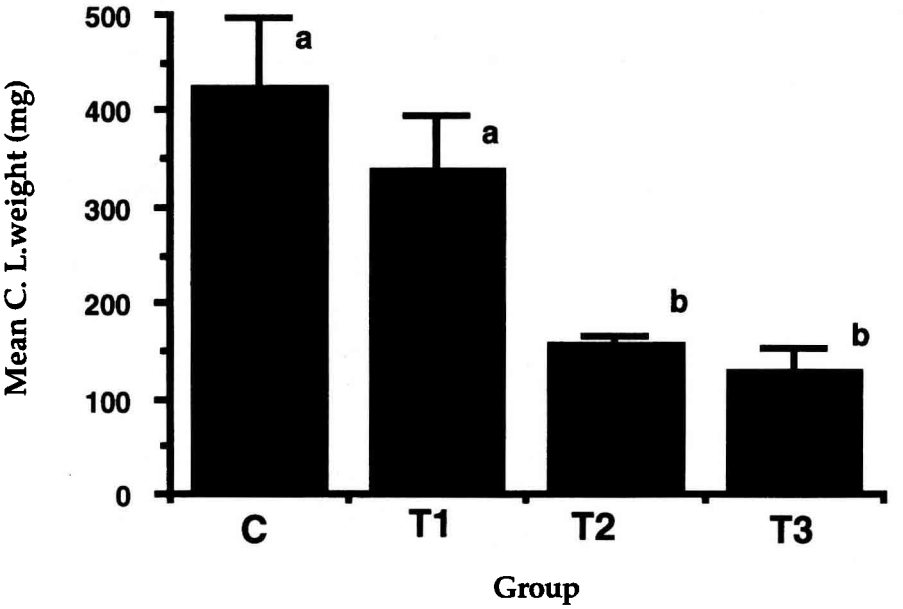
Analysis of the saturation curve of luteal tissues from C and T_1 using the Woolf model, revealed a single receptor population with high affinity constants in both groups ($K_d = 0.46 \times 10^{-10}$ M and $K_d = 0.32 \times 10^{-10}$ M, respectively) and a reduction of 34% in luteal LH receptor concentration in T_1 group relative to C (46.34 versus 70.73 fmol/mg of protein).

Analysis of the saturation curve from ovine follicles collected at slaughter house using the Woolf model, revealed a single receptor population with high affinity constant ($K_d = 0.11 \times 10^{-10}$ M).

The concentrations of follicular LH receptors for T_1 ewes was significantly lower than for C ewes (8.76 ± 2.41 versus 16.6 ± 2.1 fmol/mg of protein; $P < 0.025$, table I). Relative to group C, the concentration of LH receptors was approximately 50% in T_1 ewes; slightly higher in T_2 ewes (62%, 10.18 ± 1.76 fmol/mg of protein), and greatly increased (167%, 27.5 ± 7.9 fmol/mg of protein) in group T_3.

Histological classification of follicle health

Results of histological classification of follicles are given in table II. It must be noted that the predominant class shifts from intermediate atretic in T_1 to atretic in T_2 and T_3. The concentrations of LH receptors (fmol/mg of protein) in type A follicles from



a, b: different superscripts indicate significant differences (α=0.025)

Fig 3. Mean weight of corpora lutea (mg ± SE) in control (C, n = 3; received ten injections of sterile saline intramuscularly every 12 h, on days 11–16 of the estrous cycle) and treated ewes (T_1, T_2, T_3; three animals each; received ten injections of 50 IU ACTH every 12 h). Ovariectomies were carried out in C and T_1 on day 16, in T_2 ewes on day 18 and in T_3 ewes on day 21.

treated and control ewes were smaller than those in I-A and N-A follicles (table II). However, despite the similar histological appearance, the number of receptors in the N-A follicles was higher for the control animals than for either T_1 or T_2.

Upon histological examination three different types of large follicles were found in the ovaries from T_3 ewes: type I with presence of granulosa cells and appearance similar to normal preovulatory follicle, type II with absence of granulosa cells and appearance similar to atretic follicle and type III with absence of granulosa cells and highly luteinized theca interna layer (fig 4). This last observation is in agreement with the high concentration of LH receptors detected by RRA.

DISCUSSION

The results of the present experiment indicate that ovulation can be abolished by stress. Indications that ovulation was suppressed in all animals due to ACTH treatment were: 1) the presence of corpora lutea in clear regressing phase (luteal weight decreased from T_1 to T_3); 2) absence of estrous behavior and 3) lack of preovulatory LH surge. The inhibition of

Table I. Ovarian LH receptors in control (C, $n = 3$; received ten injections of sterile saline intramuscularly every 12 h, on days 11–16 of the estrous cycle) and treated ewes (T_1, T_2, T_3; three animals each; received ten injections of 50 IU ACTH every 12 h). Ovariectomies were carried out in C and T_1 on day 16, in T_2 ewes on day 18 and in T_3 ewes on day 21.

	C	*T_1*	*T_2*	*T_3*		
Type of structure	follicles	follicles	follicles	cystic follicles* type III	cystic follicles* type I	follicles
Number of follicles[a]	($n = 6$)	($n = 6$)	($n = 6$)	($n = 2$)	($n = 1$)	($n = 2$)
No of LH receptors (fmol/mg prot)	16.6 ± 2.1^{ab}	8.76 ± 2.4^{c}	10.18 ± 1.76^{bc}	40.40 ± 5.76	18.11	8.1±2.8
				32.96 ± 8.14 mean of cystic follicles		
				27.5 ± 7.9^{a} mean of all follicles		

* Type II large follicle broken, receptors not determined; [a] total number of follicles > 6 mm in diameter collected from three animals in each treatment group. There are significant differences between C and T_1, T_2 and T_1 and T_3 (means with different superscript are significantly different; $\alpha = 0.05$).

Table II. Number of LH receptors corresponding to three histological categories N-A (non-atretic), I-A (intermediate-atretic) and A (atretic) in control (C, $n = 3$; received ten injections of sterile saline intramuscularly every 12 h, on days 11–16 of the estrous cycle) and treated ewes (T_1, T_2, T_3; three animals each; received ten injections of 50 IU ACTH every 12 h). Ovariectomies were carried out in C and T_1 on day 16, in T_2 ewes on day 18 and in T_3 ewes on day 21.

	C			*T_1*			*T_2*			*T_3*		
Number of follicles	6			7			6			5		
Histol category	N-A	I-A	A	N-A	IA	A	N-A	I-A	A	N-A	I-A	A
Number of follicles	3		3	2	3	2	1	1	4	1	1	3
Number of LH recep (fmol/mg)	20.4 ± 0.2	–	15 ± 3.6	14.8	8.5 ± 4	5.6	12.8	10.9	5.2 ± 2	18.1	5.2	40.4 ± 6

gonadotropin secretion by glucocorticoids has been well documented in ruminants. Moberg (1991) showed that the preovulatory LH surge is especially sensitive to glucocorticoid inhibition. Ovulation failure after ACTH administration has been previously reported in cattle (Liptrap and McNally, 1976) and sheep (Cooke and Benhaj, 1989). However, this is the first time that local effects on ovarian structures are reported in domestic animals subjected to ACTH treatment.

The main objective of this experiment was to study the effects of high plasma concentrations of ACTH during the days preceding ovulation on follicular development at the expected time of ovulation and on follicular evolution thereafter. Results show that follicular concentration of LH receptors was reduced in T_1 to 50% of their concentration in C, and that it remained at a similar level 2 days later (62% in T_2 ewes). Average LH receptor concentration in T_3

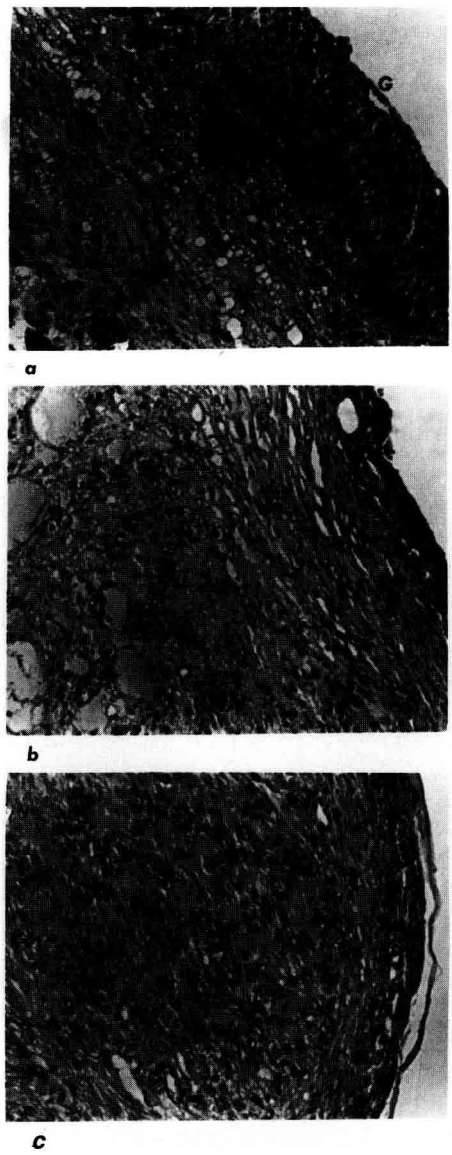


Fig 4. Photomicrographs of three different types of large follicles found in the ovaries from T_3 ewes. (a) Wall of type I follicle similar to non-atretic follicle (N-A) and characterized by presence of granulosa cells (G), H-E x 80. (b) Wall of type II follicle similar to atretic follicle category (A) and characterized by absence of granulosa cells, H-E x 84. (c) Wall of type III follicle with luteinization (L) in theca interna (TI), H-E x 80.

was higher than in T_1 or T_2 ($P < 0.05$); however when only non-cystic follicles are considered, LH receptors in T_3 are comparable to T_1 and T_2 (8.1 fmol/mg prot). Cystic follicles, on the contrary, show a very substantial increase in LH receptor content. As discussed below, these are luteinized follicles. It is therefore possible that ACTH/cortisol prevent the development of a full complement of LH receptors in the follicular wall before luteinization begins; once this process is started, the previous exposure to cortisol/ACTH does not hinder the expression of LH receptors. There is also the possibility that LH receptor expression increases as a result of ACTH/cortisol withdrawal.

The significance of reduced concentrations of follicular LH receptors at the time of expected ovulation and its effect on the fate of the follicles is not readily evident. While it is likely to be a contributing factor to ovulation failure and, possibly, to cyst development, it is not clear what degree of LH receptor reduction is necessary for the ovulation capacity to be compromised. It has been reported that cows that had borne chronic cysts for more than 10 months and had LH receptor concentrations reduced to 75% of controls, did not ovulate in response to LH surges elicited after repeated GnRH treatments (Brown et al, 1986). It was suggested in that study that the reduction in concentration of LH receptors could explain the unresponsiveness of these cysts to GnRH treatment, but the question was raised as to when and why the reduction takes place. Rajaniemi et al (1980) found a 50% reduction in LH receptor concentration in women with polycystic ovarian degeneration (POD) and hypothesized that an unusually high and/or mistimed preovulatory LH surge could down-regulate LH receptors rendering follicles unable to undergo subsequent ovulation. Alternatively, an inadequately low basal secretion of LH can also compromise the acquisition of LH receptors by preovulatory follicles (Richards et al, 1976, 1978; Webb and England, 1982; Ireland and

Roche, 1983). The reduction in binding capacity of the LH receptors in this study is likely to be due to the ACTH-induced cortisol release, which has been shown to have an inhibitory effect upon the concentration of LH receptors in rats (Bambino and Hsueh, 1981). Our results show that a reduction in the follicular concentration of LH receptors can occur in preovulatory follicles in ewes under ACTH treatment.

LH receptor concentration in luteal tissue in T_1 was reduced to 34% of C ovaries. The relevance of a reduced number of LH receptors in luteal tissue is difficult to assess, but it could affect embryo survival because the maintenance of early pregnancy depends on an adequate production of progesterone. On the other hand, there is a close relationship between the luteal production of progesterone and number of LH receptors (Diekman et al, 1978; Suter et al, 1980). Therefore, we speculate that, even if ovulation and fertilization take place, the effects of stress could compromise reproductive success by lowering the concentration of luteal LH receptors, resulting in abnormal luteal development, reduced progesterone production, and early embryonic death.

The histological classification of follicular structures revealed that, as a result of the treatment, the predominant class of follicles shifted from I-A in T_1 to A in T_2 and T_3. In both treated and control animals, the number of LH receptors was higher in N-A than in A follicles. Similar results were reported in heifers infused with E coli endotoxin (Lopez-Diaz, 1991) and other studies (Carson et al, 1979; Merz et al, 1981; Webb and England, 1982). The number of receptors in N-A follicles from control animals was higher than in N-A follicles from T_1, although only one observation was made for the treated group. Since the histological appearance of these follicles was the same, this suggests that the treatment reduced the number of LH receptors per cell, independently of other effects on cell numbers. Interestingly, similar reduction in LH receptor number in N-A follicles was observed in our heifer study (Lopez-Diaz, 1991).

Three different types of big follicles were found in T_3 ewes: type I, with presence of granulosa cells and similar to normal preovulatory follicle; type II, with absence of granulosa cells, and type III, with absence of granulosa cells and highly luteinized theca interna layer. These histological findings agree with those reported by Cooke and Benhaj (1989) 2 days after the expected ovulation in ACTH-treated ewes in an experiment similar to ours. On the other hand, these categories are similar to the histochemical cystic types described by Nakama in 1976 in cows and gilts (Nakama, 1976). According to this author, these types are evolutionary steps of the luteinized follicular cyst.

The elevated concentrations of cortisol in treated animals could favor the luteinization process since glucocorticoids increase 3β hydroxysteroid dehydrogenase (3β-HSD; enzyme responsible for the conversion of pregnenolone to progesterone) activity, while they decrease aromatase activity in rat granulosa cells (Hsueh and Erickson, 1978). Histochemically, 3β-HSD activity has been shown to be increased in the stage preceding the fully developed luteal cyst (Nakama, 1976).

Cystic and collapsing atresia are follicular degenerations that have been described in all domestic animal species. Cellular degenerations described in both classes are the same but the outcome is different (Priedkalns, 1981). Collapsing atresia is a physiological process that happens several times during an estrous cycle by which the follicles regress and disappear, while cystic atresia is a pathological process by which the preovulatory follicle grows after it fails to ovulate and persists for an abnormal period of time (Priedkalns, 1981). The follicles dissected in the present experiment became larger from the early to the late ovariec-

tomies (from 6.9 mm in diameter in T_1, to 10.3 mm in T_3). This indicates that follicles were not undergoing a collapsing but a growing process, that is, suffering cystic atresia.

Macroscopically, the appearance of follicles and FF after the ACTH treatment were striking. In 89.5% of the follicles present in treated ewes, the vessels in follicular wall were engorged with blood and FF was hemorrhagic. It is difficult to determine which factor or factors are responsible for these effects; however in one study, intrafollicular injections of indomethacin in ewes prevented ovulation, induced similar vascular changes and resulted in unovulated follicles with luteinized theca cells (Murdoch and Dunn, 1983). De Silva and Reeves (1985) reported anovulatory luteinized follicles after similar treatment in cows.

An additional explanation may be a direct effect of ACTH on the ovarian structures since strikingly similar changes in adrenal tissue have been described in women treated with ACTH for a prolonged time (4 days) (Munro and O'Hare, 1982). Among these, adrenal hyperemia and hypertrophy were described. In our experiment we found the same alterations in the follicular wall. From this experiment we cannot conclude a direct effect of ACTH and it would be interesting to explore this possibility. An important requirement to assess the ACTH effect would include the determination of ACTH receptors on the ovary. To our knowledge such a study has not been undertaken.

From our study we conclude that ACTH induces important pathological changes in follicles and corpora lutea probably through ACTH-induced cortisol release, although the role of other hormones cannot be ruled out. These changes include reduction of LH binding sites in follicular wall, inhibition of ovulation and the evolution of preovulatory follicles to luteinized cysts. LH binding sites were also reduced in corpora lutea, which could compromise luteal function.

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