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# Effects of Pituitary Stalk-transection and Type of Barrier on Pituitary and Luteal Function During the Estrous Cycle of the Ewe

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EFFECTS OF PITUITARY STALK-TRANSECTION  
AND TYPE OF BARRIER ON PITUITARY AND LUTEAL  
FUNCTION DURING THE ESTROUS CYCLE OF THE EWE

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## ABSTRACT

Effects of pituitary stalk-transection on plasma concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) prolactin (PRL) and progesterone were investigated during the estrous cycle of ewes. Pituitary stalk (SS) or sham (SH) transection was performed on day 1 (estrus = day 0) of the estrous cycle. A Teflon or Silastic barrier was placed between the cut ends of the stalk to prevent reorganization of the portal vasculature. Immediately following surgery, pulsatile administrations of gonadotropin releasing hormone (GnRH, 200ng/hr) or 0.9% NaCl was initiated and continued for the duration of the experiment. Estradiol benzoate (EB, 50 ug im) was administered to all ewes on day 3. Mean concentrations of LH were greater in SS ewes than in SH ewes ( $P < .05$ ). There was a trend ( $P = .06$ ) for the concentration of LH to be higher in ewes with Teflon compared with Silastic barriers between the cut ends of the stalk. Infusion of GnRH elevated concentrations of LH in both SS and SH ewes ( $P < .05$ ). Concentrations of progesterone were reduced ( $P < .01$ ) in saline infused SS ewes while infusion of GnRH in SS ewes maintained concentrations of progesterone similar to saline infused SH ewes. The concentrations of FSH or PRL were unaffected by SS, type of barrier or treatment with GnRH. Administration of EB failed to induce a surge of LH except in a SH ewe infused with GnRH. Ewes were more responsive to infusion of GnRH following SS than after SH as reflected by increased plasma concentrations of LH and progesterone.

## INTRODUCTION

Pituitary stalk-transection (SS) has been used to create a pituitary island devoid of hypothalamic influence. A barrier placed between the cut ends of the stalk prevented revascularization of the portal system (1). Ferin et al. (2) and Knobil (3) using Silastic barriers demonstrated that infusion of estradiol elicited surges of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in monkeys. Norman et al. (4) showed in vitro that Silastic was permeable to low molecular weight substances including gonadotropin releasing hormone (GnRH). Furthermore, when Silastic, but not Teflon, was used as the barrier in SS monkeys, systemic concentrations of estradiol were increased after 2 days of pulsatile administration of GnRH and menstrual cycles were restored. It was concluded that restoration of cyclicity (2, 3) was likely due to estradiol positive feed-back on the hypothalamus which elicited a surge of GnRH that penetrated the Silastic barrier and caused the release of gonadotropins (4). After SS of beef calves (5) or heifers (6), systemic concentrations of LH were decreased and follicular development was abolished. After infusion of GnRH, 40% of the heifers increased follicular size and one animal formed a corpus luteum (CL, 6).

Effects of lesions of specific brain areas and SS on concentrations of gonadotropins have been reported, but few of these studies have examined luteal function. Replacement of GnRH in brain-lesioned monkeys (7), raised tonic concentrations of gonadotropins and estradiol sufficiently to initiate surges of gonadotropins, ovulation and luteal function. Similar results were obtained in SS monkeys infused with GnRH and estradiol (4).

Difference between Silastic and Teflon as barriers against hypothalamic influence on pituitary function, the site of progesterone negative and estradiol positive feedback on secretion of gonadotropins and luteal function after pituitary stalk-transection were studied in ewes.

## MATERIALS AND METHODS

From October to January, 21 ewes of mixed breeding with normal estrous cycles (15-17 days) were transferred, as they were detected in estrus (day 0) by vasectomized rams, from pasture to the surgical facility. On day 1, ewes were anesthetized with sodium pentobarbital. Atropine sulphate (15 mg/ im), was administered to reduce salivation. Nitrous oxide and oxygen/carbon dioxide (95:5) were administered via an endotracheal tube at a ratio of 2:1 with the maintenance dose of halothane at 0.5 - 1.0 percent.

Transection of the pituitary stalk (SS) was by the transorbital approach of Gust (8). Briefly, following exenteration of the orbit, a dental drill was used to remove the bone between the optic foramen and the foramen orbitotundum. The dura mater was cut to reveal the pituitary stalk (Sham stalk transection, SH, n=6). The stalk was transected (n=15) with Yasargil alligator scissors (V. Mueller, Linden, NJ) close to the pituitary and a stainless steel probe was passed between the severed ends to assure transection. Silastic (n=9, 6.5 x 6.5 x .508 mm) or Teflon (n=6, 8 x 7 x 1 mm) was placed between the cut ends of the stalk to maintain separation of the pituitary and hypothalamus. Cotton was placed in the orbit against the drilled area and saturated with cyanoacrylate to form a fluid-proof seal. Gel-Foam (The Upjohn Company, Kalamazoo, MI) was packed into the orbit and the eyelids were sutured closed.

Immediately following surgery, and for the next 3 days animals received 3ml (im) penicillin G procaine, U.S.P. (200,000 IU/ml) in a solution of dihydrostreptomycin sulfate (0.25 mg/ml). After recovery from anesthesia, ewes were stanchioned and infused, via jugular vein catheters, with either hourly pulses (1 min. duration) of 200 ng GnRH (Abbott Laboratories; SS, n=7; SH, n=3) or an equal volume of physiological saline (SS, n=8; sa, n=3) throughout the experiment. Ewes were transferred from temperature controlled holding rooms to an open air barn on days 4 or 5.

All ewes received a 50 ug bolus injection of estradiol benzoate (EB) in corn oil at 20.00 h on day 3. Plasma was harvested from blood samples collected hourly, from 06.00 through 20.00 h on day 4 and daily from day 5 through day 20 then stored at -5° C until assayed for LH, FSH, prolactin (PRL) and progesterone

At necropsy completeness of transection and placement of the barriers was confirmed and ovaries were inspected for the presence of ovarian structures.

### **Radioimmunoassays**

Previously validated radioimmunoassays were used to quantify concentrations of LH (9), FSH (10), PRL (11), and progesterone (12). Standard preparations were ovine LH (NIH-6LH-S19), ovine FSH (NIAMDD-oFSH-RPI), ovine PRL (NIAMDO-oPRL-I-1) and progesterone (Sigma Corp., St. Louis, MO). Sensitivities of the assays for LH, FSH, PRL, and progesterone were 0.30 ng/ml, 0.25 ng/ml, 0.25 ng/ml and 0.10 ng/ml, respectively. Intra-assay coefficients of variation were 4.1, 5.6, 5.1 and 12.6% respectively. The interassay coefficients of variation for LH and FSH were 5.2 and 7.1%, respectively for three assays of each hormone. Samples were assayed for PRL and progesterone in single assays.

### **Statistical Analyses**

Least-squares analysis of variance for a completely randomized split plot design with repeated measures in time was used to examine hormonal data (13). Factors in the main plot

were type of barrier and treatment with GnRH or saline, while time and interactions with time were in the subplot. Data were analyzed through day 10 of the experimental period.

## RESULTS

Infusion of GnRH as compared to saline elevated mean concentrations of LH from 10 to 24 hours after the administration of EB ( $1.87 \pm .16$  vs.  $0.70 \pm .15$  ng/ml  $P < .05$ ). Concentrations of LH were increased in both SS and SH ewes by infusion of GnRH from day 5 through day 10, however mean concentrations of LH were higher in both groups of SS ewes when compared to all SH ewes ( $P < .05$ , Fig. 1).

SH ewes which received GnRH infusion exhibited an elevation of mean LH during the subsequent follicular phase (day 16 through day 20) when compared to all SS ewes. SH ewes which received saline had increased concentrations of LH during this time, but not to the extent seen in GnRH infused SH ewes. Blood samples were collected too infrequently to fully characterize a surge but peak concentrations of LH in the three GnRH infused SH ewes were 8.33, 11.21 and 12.74 ng/ml while the two saline infused SH ewes, which displayed obvious increases, had concentrations of 1.84 and 3.82 ng/ml. None of the three saline infused SS ewes nor the two GnRH infused animals which completed the 20 days, displayed an elevation of LH concentrations during the subsequent follicular phase.

Concentrations of progesterone were lower ( $P < .01$ ) in SS ewes receiving saline than in any other group. Concentrations of progesterone were elevated by day 6 then plateaued at a lower concentration than SH ewes receiving saline ( $1.06 \pm .11$  vs.  $2.20 \pm .16$  ng/ml). Pulsatile administration of GnRH to SS ewes elevated ( $P < .05$ ) mean concentrations of progesterone to values similar to SH controls. Administration of GnRH also elevated ( $P < .05$ ) concentrations of progesterone in SH ewes. At days 15 and 16, (SH,  $n=6$ ; SS,  $n=5$ ) the concentrations of progesterone declined rapidly as would be expected during the normal ovine estrous cycle (Fig.2).

There was a trend ( $P=.06$ ) for the concentrations of LH to be higher in all ewes which received Teflon as the barrier between the cut ends of the stalk when compared to ewes with Silastic barriers ( $1.22 \pm .08$  vs.  $.88 \pm .07$  ng/ml). Concentrations of FSH and PRL, were unaffected by SS, type of barrier or treatment with GnRH. Administration of 50g EB had no effect on the mean concentration of LH, with the exception that one SH ewe had a surge of LH (31 ng/ml) 17 hours after administration.

## DISCUSSION

In contrast to the study by Norman et al. (4), there were no statistically significant differences between Silastic and Teflon as barriers to hypothalamic influence in ewes. Knobil and coworkers have shown that monkeys with Silastic barriers between the cut ends of the pituitary stalk could be stimulated to exhibit surges of gonadotropins and have menstrual cycles by infusing estrogen (2, 7). No SS ewes exhibited surges of LH during the follicular phase (day 16 through 20) even when infused with GnRH, however, surges of LH were evident in SH ewes during this period. At necropsy SS ewes had ovaries with corpora albicantia and limited follicular development, while SH ewes had formed new CL.

Concentrations of L-H and progesterone were elevated in GnRH-infused ewes but concentrations of PRL were unaffected by SS or GnRH infusion. Immediately following SS or SH, plasma PRL was elevated; by 6 (8) or after 7 hours (14) post surgery concentrations of PRL returned to presurgery values. The luteotropic complex of LH and PRL is necessary for function of the CL in the ewe (15). As there was no difference in concentrations of PRL between GnRH- or saline-infused ewes, the elevated concentrations of progesterone can be accounted for by the elevated LH. Synthesis of progesterone was stimulated by administration of LH in intact (15) and hypothalamo- pituitary stalk-disconnected ewes (16).

Progesterone inhibited LH via hypothalamic mechanisms as concentrations of LH in SS ewes with GnRH treatment were higher than in SH GnRH-treated ewes with comparable concentrations of progesterone. Tonic secretion of LH during the ovine estrous cycle was inversely related to circulating progesterone (17). Ovariectomy in the ewe induced rapid increases in the secretion of LH which could be blocked by subcutaneous implants of progesterone and the hypothalamus was suggested as the site of action (18). Receptors for progesterone have been characterized in the hypothalami of rats (19,20) providing further evidence for a hypothalamic site of progesterone action.

Separate mechanisms for release of LH and FSH may exist in sheep. Plasma concentrations of LH and FSH declined following SS (21) but hourly intravenous pulses (21,22) of GnRH which mimicked endogenous secretion (23) restored LH but not FSH to pre-surgery values. In the present study, hourly infusion of 200 ng GnRH did not alter secretion of FSH but did increase concentrations of LH in SS ewes when compared to saline infused SS ewes. Increased amplitudes of pulses of exogenous GnRH raised basal LH, but had no effect on basal FSH, while decreased frequency of administration of GnRH decreased basal LH and increased basal FSH (22).

A single bolus injection of 50 ug EB elicited a preovulatory-like surge in concentrations of LH when administered to ovariectomized ewes during the breeding season (24, 25). However, only one SH ewe which received pulsatile GnRH infusions exhibited a surge of LH in response to estrogen in the present study. A group of three intact ewes on day 3 of the estrous cycle (data not included) also failed to exhibit surges of LH. At this time of the estrous cycle, the hypothalamo-hypophyseal axis may be refractory or a higher dose of estrogen may be necessary to stimulate a surge. Bolt and coworkers (26) reported that a 1 mg bolus injection of EB stimulated a surge of LH when administered on day 3 of the estrous cycle. Gust (8) demonstrated that injection of 400 ug EB 3 days after sham stalk sectioning in ovariectomized ewes elicited a surge of LH 18 hours later.

In summary, transection of the pituitary stalk of the ewes on day 1 of the estrous cycle resulted in reduced function of the CL but did not alter luteal lifespan. Function of the CL of SS ewe was enhanced by GnRH infusion as concentrations of progesterone were elevated in the GnRH infused ewes when compared to saline infused controls. This increased CL function was probably due to increased LH support for the CL, however, direct action of GnRH on the CL can not be ruled out. Lastly, progesterone apparently acts at the hypothalamus to inhibit secretion of LH in ewes.

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Figure 1: Concentration of LH for GnRH vs saline infusion in stalk (SS) and sham (SH) transected ewes from day 5 to day 10 of the estrous cycle. Degrees of Freedom Error = 69, Mean Square Error = 0.194.

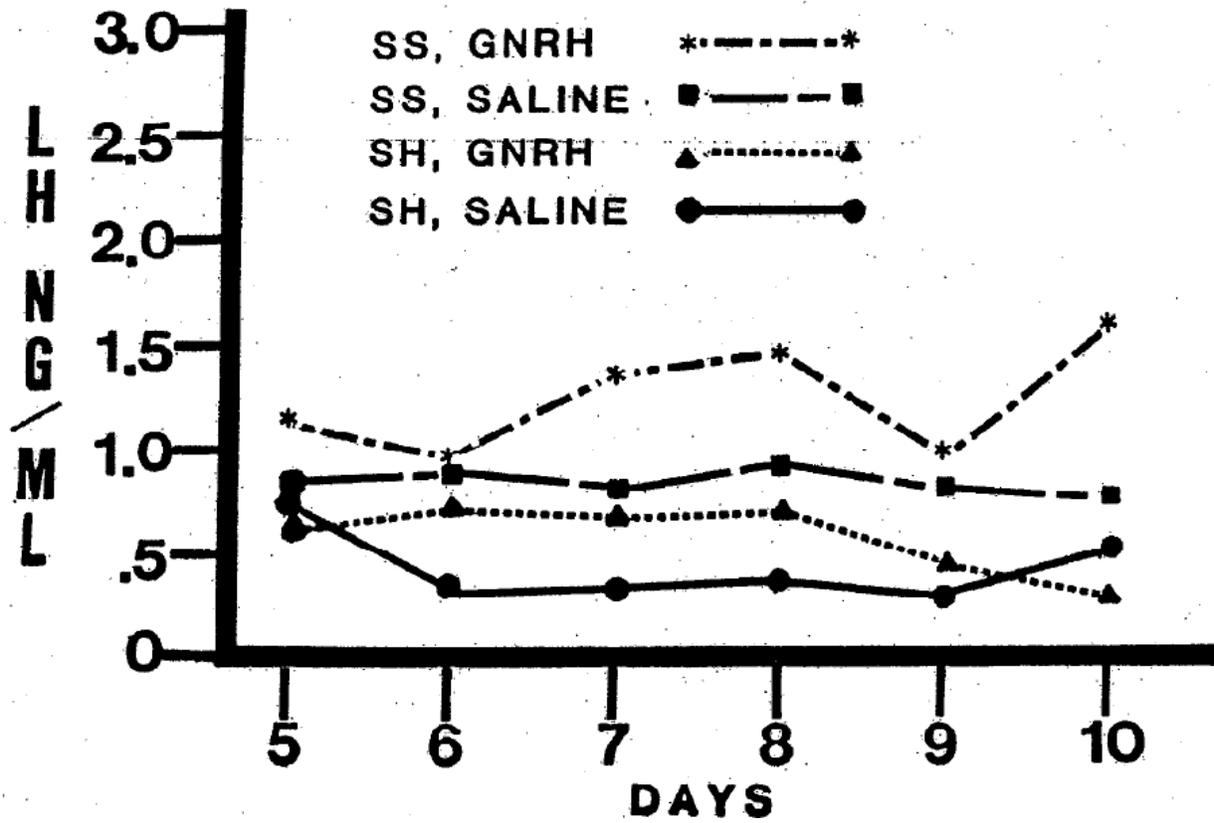


Figure 2: Concentrations of progesterone for GnRH vs saline infusion in stalk (SS) and sham (SH) transected ewes from day 5 to day 20 of the estrous cycle. Degrees of Freedom Error = 98, Mean Square Error = 0.832.

