GONADOTROPIN CONCENTRATIONS, FOLLICULAR DEVELOPMENT, AND LUTEAL FUNCTION IN PITUITARY STALK-TRANSECTED EWES TREATED WITH BOVINE FOLLICULAR FLUID[1,2]

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ABSTRACT

Two experiments, each arranged as a 2 × 2 factorial, were conducted in ewes to examine direct effects of bovine follicular fluid (bFF) on follicular development and luteal function and to further characterize follicular development and luteal function after pituitary stalk transection (SS). In Exp. 1, ewes were sham-operated or SS on d 6 of an estrous cycle and received 5 ml of saline or bFF three times daily on d 5 through 11 of the same cycle. In Exp. 2, all ewes were SS on d 6 of an estrous cycle and treated with saline or bFF three times daily on d 5 through 11 and with ovine FSH (60 μg; NIADDK-oFSH-16) or saline (1.2 ml) from d 7 to 11. In Exp. 2, ewes were ovariectomized on d 11 to assess effects of treatments on follicular development and luteal function. In both experiments, concentrations (ng/ml) of FSH on d 7 were suppressed ($P \leq .005$) by bFF compared with saline (.50 ± .17 vs 1.63 ± .15) and remained suppressed ($P \leq .005$) through d 11 (.46 ± .12 vs 1.54 ± .12). Replacement therapy (oFSH) restored concentrations of FSH. Concentrations of LH were not affected by bFF but were elevated ($P \leq .05$) 1 d after SS (d 7; .88 ± .09 vs .56 ± .09) and remained elevated ($P \leq .05$; 1.31 ± .20 vs .65 ± .11) from d 6 through 11. Concentrations of progesterone were unaffected by SS. The number of follicles ≥ 2 mm on d 11 was reduced by SS and by bFF. Growth of follicles even with oFSH supplementation, which restored follicular development in SS ewes receiving saline, was inhibited by bFF, and no follicle exceeded 2 mm after treatment with bFF. Administration of bFF in vivo did not alter circulating progesterone or LH-stimulated secretion of progesterone in vitro. These results indicate a direct action of bFF at the pituitary to reduce secretion of FSH and provide evidence that bFF acts at the ovary to reduce follicular development.

Key Words: Follicular Fluid, Pituitary-Gonadal Axis, Follicles, Corpus Luteum, Ewes

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Introduction

Exogenous bovine (b), ovine (o), and porcine (p) follicular fluid (FF) reduced ovarian follicular development either by reduced secretion of FSH (e.g., Hoak and Schwartz, 1980) or by direct action on the ovary (e.g., Cahill et al., 1985). Luteal function was reduced in ewes by bFF in one study (Larson et al., 1987) but not in another (Wallace and McNeilly, 1985). Pituitary stalk

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transection (SS) frees the pituitary of hypothalamic input. Follicular growth was reduced after SS in cattle (Anderson, 1977; Anderson et al., 1981; Awotwi et al., 1984), and in ewes luteal function was decreased after interruption of hypothalamic input to the pituitary was performed 1 d (Mallory et al., 1986), but not 5 d, after estrus (Niswender et al., 1986).

The present study examined the effects of treatment with bFF on secretion of FSH, LH, and progesterone in SS and intact ewes. Pituitary stalk transection provided an in vivo model to evaluate alterations in FSH and LH produced by bFF and to examine subsequent effects on follicular development and on in vivo and in vitro luteal function. Treatment with bFF and FSH allowed examination of direct ovarian effects of bFF alone.

Materials and Methods

Surgical Procedures

Vasectomized rams with painted briskets were penned with ewes twice daily for detection of estrus. Mature ewes of mixed breeding that had shown an estrous cycle of normal duration (≈ 17 d) were used. Surgical procedures, including cannulation of a jugular vein, were initiated on d 5 after estrus (estrus = d 0). After exenteration of the left orbit and exposure of the dura mater between the optic and orbitorotundal foramina (Mallory et al., 1986), Gel-Foam[6] was packed into the orbit and the upper and lower eye lids were sutured closed. The following day (d 6), ewes were immobilized with gallamine triethiodide (14 mg, infused i.v. at a rate of approximately 3 mg/min) and the orbit and palpebral margins were desensitized with 2% lidocaine by injecting 1 ml (s.c.) into the upper and lower eye lids and by instilling 2 ml into the orbit for 2 to 3 min. The dura mater was reflected in 19 sham (SH) ewes, whereas the pituitary stalk was transected and a Teflon barrier was inserted between the cut ends in 17 ewes. Penicillin G procaine, USP (20,000 IU/ml) in a solution of dihydrostreptomycin sulfate (.25 ng/ml) was injected (i.m., 3 ml/d) for 3 d after surgery. Completeness of transection and proper placement of the Teflon barrier were confirmed at necropsy.

[6]The Upjohn Co., Kalamazoo, MI.

Experiments

Two experiments (Figure 1), each with a 2 × 2 factorial arrangement of treatments, were conducted sequentially. In Exp. 1, the first factor was surgery (sham operation or pituitary stalk transection), and the second factor was treatment with 5 ml of .9% NaCl (saline; SH, n = 9; SS, n = 8) or charcoal-extracted bFF (SH, n = 10; SS, n = 9). Treatments were injected (s.c.) into the axillary region at 8-h intervals beginning at 0800 on d 5 and continuing through d 11. This regimen reduced circulating FSH and prevented any rebound in concentrations of FSH between bFF injections (Larson et al., 1987). Bovine follicular fluid was obtained and processed as described by Larson et al. (1987) and included culture to confirm the absence of the bacterium *Serratia liquifaciens*, which has been associated with an inhibition of binding of FSH to its receptors (Sluss and Reichert, 1984).

Stalk-sectioned ewes from Exp. 1 were used for Exp. 2. In addition to injections of saline or bFF (first factor), these ewes received treatments with saline (1.2 ml) or ovine FSH (60 μg; NIDDK-oFSH-16; second factor). Saline or oFSH was administered (s.c.), in the axillary region on the opposite side of that given bFF, at 8-h intervals beginning at 0800 on d 7 and continuing through d 11. This dose and route

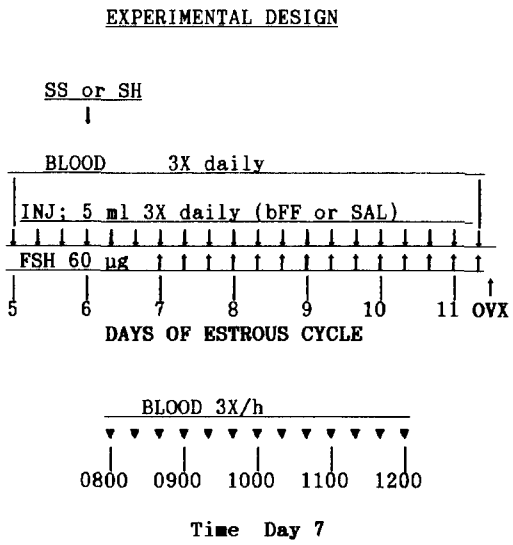

Figure 1. Experimental design detailing time of treatment injections, blood collection, and surgery for Exp. 1 and 2.

of administration was based on the half-life of FSH and the approximate total fluid volume of the ewes; it was as follows: an attempt to produce circulating levels of FSH similar to those during the luteal phase. The resulting groups were 1 = saline-saline, n = 4; 2 = bFF-saline, n = 5; 3 = saline-FSH, n = 4; or 4 = bFF-FSH, n = 4.

All ewes were ovariectomized during the afternoon of d 11, allowing 4 d between the beginning of treatment with oFSH (d 7) and ovariectomy (d 11). This is sufficient time for oFSH to stimulate growth of ovulatory follicles (Coleman and Dailey, 1983) after treatment with bFF. Treatment of ewes with bFF from d 1 through 15 of the estrous cycle increased the length of the follicular phase by 3 d (Larson et al., 1987) and the estrous cycle by 4 d (Miller et al., 1982), presumably by inhibiting folliculogenesis.

Corpora lutea (CL) were dissected from the ovary on ice and placed in ice-cold vials containing TC Medium 199[7]. Numbers and diameters of visible follicles (≥ 2 mm) and mass of each CL were recorded. Processing and incubation of luteal tissue were modifications of procedures described by Gross et al. (1985). Briefly, each CL was sliced into six equally thick pieces (.5 to 1.0 mm). Each piece was weighed and placed in an individual vial (6 × 2.5 mm) containing 5 ml of fresh TC Medium 199. Two vials were incubated on ice, and four vials (two containing no LH and two with 500 ng of LH; NIH-oLH-S22) were incubated at 38°C in a closed chamber under O_2:CO_2 (95:5) while agitating on a shaking platform. After 4 h of incubation, media were decanted and stored frozen. Ovaries, exclusive of CL, were placed in 10% buffered formalin and subsequently embedded in paraffin using an automated system. Serial sections (15 μm) were mounted and stained with hematoxylin-eosin (Humason, 1962). Follicular populations were characterized microscopically using every seventh section. Assuming that follicles were spherical, diameters of follicles were measured in the section containing the maximal circumference using a digital image analyzer. Measurements could not be made on 11 of 72 ovaries across all treatments because of inadequate fixation of the tissue.

[7]DIFCO Laboratories, Detroit, MI.
[8]Sigma Chemical Co., St. Louis, MO.

Blood Collection and Hormone Assays

Blood (6 ml) was collected via indwelling jugular catheters every 8 h beginning at 0800 on d 6 and continuing through d 11 after estrus. Blood samples were always collected before injection of treatments and placed into heparinized tubes held on ice and centrifuged within 10 min of collection. In addition, the short-term effects of SS on concentrations of FSH and LH were evaluated in samples collected every 20 min from 0800 to 1200 on d 7. Plasma was stored frozen until it was assayed by RIA for FSH (Keisler et al., 1985) and LH (Fogwell et al., 1977). Samples taken at 1600 from d 6 through 10 were assayed for progesterone (Sheffel et al., 1982). Standard preparations were ovine FSH (NIAMDD-oFSH-RP1), ovine LH (NIH-oLH-S19), and crystalline progerstone.[8] Least detectable amounts of LH, FSH, and progesterone were .25, .17, and .20 ng/ml, respectively. Intra- and interassay CV were 2.9 and 20.7% for LH (n = 6), 4.4 and 24.0% for FSH (n = 6), and 9.9 and 13.4% for progesterone (n = 5). Efficiency of extraction of progesterone exceeded 90%. Samples from incubations of CL were diluted 1:100 or 1:500 with fresh medium, and progesterone was determined by RIA without extraction. Minimum sensitivity was 10 pg/tube, and intra- and interassay CV were 7 and 14%, respectively. Assay data were computed as nanograms of progesterone per milliliter of media per gram of incubated tissue. Mean values for progesterone from 4°C incubations were considered to be passive release because synthesis and active release were inhibited. The values from 38°C incubations were corrected by subtracting the corresponding passive release values. Control incubations were considered baseline. Therefore, effects of added LH on secretion of progesterone were expressed as percentage changes from the nonstimulated baseline.

Statistical Analysis

Data for plasma concentrations of FSH, LH, and progesterone were examined by ANOVA for a split-plot design with repeated measures in time (Gill and Hafs, 1971; SAS, 1986). Variables in the main plot were type of surgery and treatment (bFF or saline; Exp. 1) or treatment with bFF or saline and FSH or saline (Exp. 2). Time and interactions with time were

TABLE 1. CONCENTRATIONS OF FOLLICE-STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH) FOR STALK-(SS) OR SHAM-(SH) TRANSECTED EWES RECEIVING SALINE (SAL) OR BOVINE FOLLICULAR FLUID (bFF)

	FSH		LH	
Treatment	D 7	D 6 to 10	D 7	D 6 to 10
SH	1.29 ± .15[a]	1.76 ± .12	.56 ± .09	.65 ± .11
SS	.84 ± .14	1.32 ± .22	.88 ± .09*	1.31 ± .20*
SAL	1.63 ± .15	1.54 ± .12	.74 ± .10	.98 ± .10
bFF	.50 ± .17*	.46 ± .12*	.70 ± .08	.74 ± .09

[a]Mean ± SEM (ng/ml)

*$P \leq .05$

in the subplot. Main plot sums of squares were partitioned into planned contrasts. Data for progesterone were examined by analysis of covariance (SAS, 1986); luteal mass was used as the covariate to adjust for differences in mass among ewes. Because treatments were initiated after luteal formation, differences in luteal mass would be due to individual ewe variation, principally the number of CL. Data for concentrations of progesterone from incubations of CL, visible follicles (≥ 2 mm in diameter), and for number of follicles (≥ .2 to 2.0 mm) per ovary observed histologically, were examined by ANOVA.

Results

Gonadotropin Concentrations

Short-term effects of SS were examined on d 7 (Table 1). Concentrations of FSH were unaltered 1 d after SS; however, FSH was lower ($P \leq .005$) in ewes treated with bFF than in those treated with saline. Concentrations of LH were increased ($P \leq .05$) 1 d after SS but were not affected by treatment with bFF.

Concentrations of FSH were lowered ($P \leq .0001$) on d 6 through 11 in ewes given bFF compared with those given saline (Figure 2), and FSH was increased by administration of FSH three times daily (Figure 3). Stalk transection had no effect on the concentrations of FSH during d 6 through 11. Concentrations of LH during d 6 through 11 were not affected by treatment with bFF; however, SS ewes had higher ($P \leq .05$) concentrations of LH than SH ewes (Figure 4). No surgery × bFF interactions were significant for FSH or LH. In bFF-treated ewes receiving replacement therapy with oFSH, concentrations of FSH equaled those observed in control (SS-saline-saline) ewes (Figure 3).

Follicular Development

Number of visible (≥ 2 mm) follicles (Figure 5) was reduced after SS ($P \leq .01$) and during treatment with bFF ($P \leq .01$). The number of follicles tended to be greater ($P \leq .06$) during treatment with oFSH than during treatment with saline. The interactions between SS and bFF and between oFSH and bFF were not significant. No follicle in ewes treated with bFF exceeded 2 mm, although ewes that were SH, SS, or SS and treated with oFSH had at

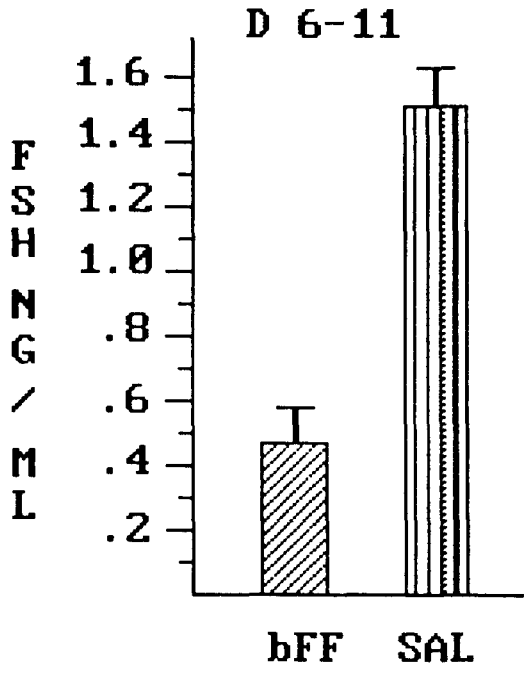

Figure 2. Concentrations of follicle-stimulating hormone (FSH) for bovine follicular fluid (bFF) vs saline (SAL) administration pooled for stalk- and sham-transected ewes ($P \leq .05$).

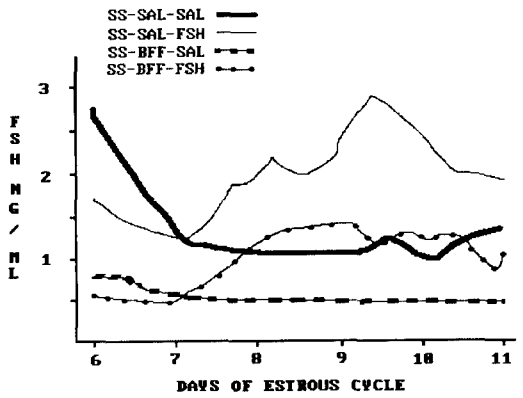

Figure 3. Concentrations of follicle-stimulating hormone (FSH) in stalk-transected (SS) ewes treated with saline (SAL, n = 4), FSH (n = 4), bovine follicular fluid (bFF, n = 5), or FSH and bFF (n = 4) three times daily from d 6 through 11 of the estrous cycle. Mean squares for error A and B were 2.62 and .15, respectively.

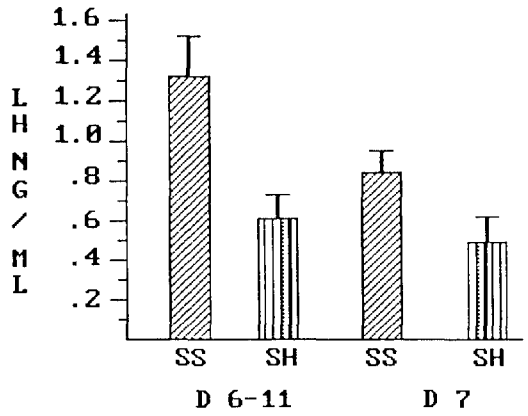

Figure 4. Concentrations of luteinizing hormone (LH) for stalk-(SS) and sham-(SH)transected ewes for d 6 through 11 and d 7 after estrus. (mean ± SEM)

least one follicle ≥ 2 mm; average diameter of the largest follicle was 4.9 ± .5, 5.3 ± 1.7, and 3.8 ± .8 mm, respectively. Histologically, the number of follicles ≥ .2 but < 2.0 mm were not altered by SS or treatment with bFF, FSH, or their interaction.

Luteal Function

Circulating concentrations of progesterone were not affected by administration of FSH, bFF, SS, or their interactions (overall mean = 3.6 ± .25 ng/ml). Weights of CL did not differ among treatments. Percentage of change in LH-stimulated concentrations of progesterone in media from incubations of CL was not altered by treatment with bFF or oFSH (overall mean response = 139 ± 20%).

Discussion

Administration of bFF three times daily reduced concentrations of FSH, but not of LH, in intact ewes (Larson et al., 1987). Because concentrations of FSH were not differentially suppressed in SS and SH ewes, we conclude that the site of inhibitory action of bFF on secretion of FSH must be the anterior pituitary, in support of previous studies in ovariectomized ewes (Clarke et al., 1986; Li et al., 1989). In contrast to the effects of FSH, concentrations of LH were not affected by bFF, but they were greater in SS than in SH ewes, which support previous results from this laboratory (Mallory et al., 1986; Gust et al., 1987) and from studies using median eminence-lesioned (Bishop et al., 1972) and SS male rats (Ching et al., 1986). Progesterone is the dominant circulating ovarian steroid inhibiting secretion of LH during the luteal phase in ewes (Hauger et al., 1977) and its inhibitory action is at the level of the hypothalamus (Kaynard and Karsch, 1988). The increased concentrations of LH seen after SS may be due to an interruption of an hypothalamic signal to the pituitary induced by progesterone. For example, intravenous dopamine inhibited GnRH-induced secretion of LH in rabbits (Dailey et al., 1978) and dopamine inhibited the LH response to GnRH in SS ewes (Donnelly and Dailey, 1991). Progesterone receptors have been found in hypothalamic areas containing dopaminergic neurons (McEwen, 1981).

The number of follicles was reduced by SS, but SS did not affect mean diameter of the largest follicle. A low FSH:LH ratio, such as that after SS in the current study, led to altered follicular development in primates (for review see diZerega and Hodgen, 1981). Injections of oFSH tended to promote follicular growth after SS. Alternatively, the increase in mean LH after SS may have induced widespread atresia, as observed after administration of hCG to ewes (Turnbull et al., 1977), rats (Selye et al., 1933), and guinea pigs (Reed and Hounslow, 1971).

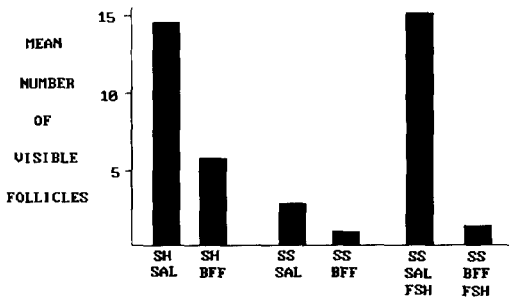

Figure 5. Number of follicles (≥ 2 mm in diameter) on the ovaries of ewes that were sham- (SH) or stalk-transected (SS) and received saline (SH-saline, n = 9; SS-saline, n = 4), bovine follicular fluid (bFF; SH-bFF, n = 10; SS-bFF, n = 5), follicle-stimulating hormone (FSH; SS-saline-FSH, n = 4) or bFF and FSH (SS-bFF-FSH, n = 4). The respective standard errors of the mean were 2.3, .6, 2.3, .6, 7.2, and .6.

In contrast to SS, treatment with bFF restricted follicular growth to a size ≤ 2 mm, suggesting inhibition of the movement of follicles into a pool of larger follicles. Treatment with bFF for 3 d reduced the diameter of the largest follicle and the number of follicles ≥ 3 mm for 8 d after prostaglandin-induced luteolysis in ewes (Miller et al., 1979). Treatment of mares with equine FF for 4 d after prostaglandin-induced luteolysis reduced concentrations of FSH and growth of the largest follicle for at least four more days (Bergfelt and Ginther, 1985). Administration of bFF prevented the rise in FSH and compensatory ovarian hypertrophy after unilateral ovariectomy in prepuberal heifers (Johnson et al., 1985), whereas bFF treatment in heifers within 12 h after estrus reduced the secondary surge of FSH and delayed follicular growth (Turzillo and Fortune, 1990). Treatment with bFF from d 5 through 10 after calf removal at 24 h postpartum reduced concentrations of FSH and diameter of the largest follicle (Hinshelwood et al., 1987).

Because replacement therapy with exogenous oFSH increased concentrations of FSH in SS ewes treated with bFF to levels observed in SS ewes treated with saline but did not restore the number of follicles or the diameter of the largest follicle, bFF would seem to reduce follicular development directly at the ovary. Similarly, treatment with pregnant mare serum gonadotropin (PMSG) failed to restore follicular growth from d 12 through 15 of the estrous cycle after electrocautery of follicles and treatment with oFF in ewes (Cahill et al., 1985). In anestrous ewes, treatment with bFF, concurrently with GnRH, increased the number of small follicles (≤ 2 mm) but reduced the number of large follicles (Hunter et al., 1987). Injections of FF into gilts impaired the stimulatory effect of exogenous pFSH in recruitment of medium-sizes follicles (Guthrie et al., 1987). In hypophysectomized, diethylstilbestrol-treated immature rats, follicular response to gonadotropins (human menopausal gonadotropin; PMSG) was suppressed by human FF (diZerega et al., 1983a), pFF (Kling et al., 1984), and media from human granulosal cell cultures (diZerega et al., 1983b). In contrast, infusion of oFSH concurrently with bFF increased concentrations of FSH 10- to 20-fold, prevented the delay to onset to estrus, increased ovulation rate, and increased secretion of progesterone in ewes (McNeilly, 1985). Administration of bFF for 4 d did not inhibit pFSH-induced follicular growth in unilaterally ovariectomized, prepuberal heifers. However, concentrations of LH increased after injection of pFSH and remained elevated, possibly due to contamination (1.9%) of exogenous pFSH with LH (Moser, 1988, 1989).

Circulating concentrations of progesterone were not affected by any treatment in this study, which supported previous reports of no effect of bFF on plasma concentrations of progesterone from d 1 through 11 of the estrous cycle of ewes (Wallace and McNeilly, 1985). Although Larson et al. (1987) demonstrated an effect of bFF on concentrations of progesterone during d 11 through 15, this effect was attributed to an early decline in progesterone. Concentrations of progesterone before d 11 were not affected by bFF. In the present study, LH-stimulated secretion and(or) synthesis of progesterone from CL in vitro was not altered by treatment with bFF in vivo on d 5 through 11.

Formation of functional CL requires LH in ewes (Niswender et al., 1986, 1972). Although hypophysectomy 5 d after ovulation resulted in regression of partially formed CL (Kaltenbach et al., 1968), interruption of the hypothalamopituitary portal vasculature on d 5 (Niswender et al., 1986) or 6 (present study) did not disrupt secretion of progesterone. Disruption of hypothalamic connections to the pituitary prior to formation (d 1) of the CL (Mallory et al., 1986) altered secretion of

progesterone, possibly by altering the bioactivity or the pattern of luteotropic support. Induction of hourly pulses of LH with hourly infusion of GnRH restored progesterone to values similar to those of controls (Mallory et al., 1986). Therefore, secretion of progesterone after d 5 or 6 may be resistant to alteration in the quality of luteotropic support. However, the formative stages of the CL seem to require precise luteotropic input.

Implications

By using treatments with bovine follicular fluid (bFF) and(or) ovine follicle-stimulating hormone (oFSH) and pituitary stalk transection, different paradigms were established for plasma concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Stalk transection increased concentrations of LH without affecting FSH, treatment with bFF decreased FSH without affecting LH, and treatment with oFSH increased circulating FSH without affecting LH. These results confirm that bFF acts at the pituitary to preferentially reduce FSH secretion and at the ovary to decrease follicular development independent of the ratio of FSH:LH or absolute concentrations of FSH. Decreasing the ratio of FSH:LH by pituitary stalk transection reduced follicular growth because exogenous oFSH restored follicular growth. In contrast, luteal function was not affected by these treatments.

Literature Cited

Anderson, L. L. 1977. Development in calves and heifers after hypophysial stalk transection or hypophysectomy. Am. J. Physiol. 232:E497.

Anderson, L. L., D. L. Hard, L. S. Carpenter, E. K. Awotwi and M. A. Diekman. 1981. Neuroendocrine regulation of luteinizing hormone secretion in beef calves. Biol. Reprod. 24:795.

Awotwi, E. K., D. S. Keeney, D. L. Hard and L. L. Anderson. 1984. Effect of pulsatile infusion of luteinizing hormone-releasing hormone on luteinizing hormone secretion and ovarian function in hypophysial stalk-transected beef heifers. Biol. Reprod. 31:989.

Bergfelt, D. R. and O. J. Ginther. 1985. Delayed follicular development and ovulation following inhibition of FSH with equine follicular fluid in the mare. Theriogenology 24:99.

Bishop, W., C. P. Fawcett, L. Krulich and S. M. McCann. 1972. Acute and chronic effects of hypothalamic lesions on the release of FSH, LH, and prolactin in intact and castrated rats. Endocrinology 91:643.

Cahill, L. P., M. A. Draincourt, W. A. Chamley and J. K. Findlay. 1985. Role of intrafollicular regulators and FSH in growth and development of large antral follicles in sheep. J. Reprod. Fertil. 75:599.

Ching, M. C., M. S. Blank, K. J. Catt, A. Negro-Vilar and M. L. Dufau. 1986. Effects of acute hypophyseal transection on anterior pituitary hormone secretion. Soc. Neurosci. Abstr. 12:1411 (Abstr.).

Clarke, I. J., J. K. Findlay, J.T. Cummins and W. J. Ewens. 1986. Effects of ovine follicular fluid on plasma LH and FSH secretion in ovariectomized ewes to indicate the site of action of inhibin. J. Reprod. Fertil. 77:575.

Coleman, D. A. and R. A. Dailey. 1983. Effects of repeated removal of large ovarian follicles and treatment with progestin on ovarian function in the ewe. Biol. Reprod. 29:586.

Dailey, R. A., R. C. Tsou, G. T. Tindall and J. D. Neill. 1978. Direct hypophyseal inhibition of luteinizing hormone release by dopamine in the rabbit. Life Sci. 22:1491.

diZerega, G. S. and G. D. Hodgen. 1981. Luteal phase dysfunction infertility: A sequel to aberrant folliculogenesis. Fertil. Steril. 35:489.

diZerega, G. S., R. P. Mars, J. D. Campeau and O. R. Kling. 1983a. Human granulosa cell secretion of protein(s) which suppress follicular response to gonadotropins. J. Clin. Endocrinol. & Metab. 56:147.

diZerega, G. S., R. P. Mars, J. D. Campeau and O. R. Kling. 1983b. Identification of proteins in pooled follicular fluid which suppress follicular response to gonadotropins. J. Clin. Endocrinol. & Metab. 56:35.

Donnelly, P. J. and R. A. Dailey. 1991. Effects of dopamine, norepinephrine and serotonin on secretion of luteinizing hormone, follicle-stimulating hormone and prolactin in ovariectomized, pituitary stalk-transected ewes. Domest. Anim. Endocrinol. 8:87.

Fogwell, R. L., G. S. Lewis, R. L. Butcher and E. K. Inskeep. 1977. Effects of ovarian bisection on response in intrafollicular injections of $PGF_{2\alpha}$ and on follicular development in ewes. J. Anim. Sci. 45:328.

Gill, J. L. and H. D. Hafs. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331.

Gross, T. S., W. F. Williams, C. S. Galina, J. Manspeak, M. A. Haaland and R. Russel. 1985. Functional status of the peri-partum bovine corpus luteum. Anim. Reprod. Sci. 8:193.

Gust, C. M., E. K. Inskeep and R. A. Dailey. 1987. Concentrations of luteinizing hormone, follicle stimulating hormone and prolactin following transection of the pituitary stalk in ovariectomized ewes. Domest. Anim. Endocrinol. 4:103.

Guthrie, H. D., D. J. Bolt, G. H. Kiracofe and K. F. Miller. 1987. Effect of charcoal-extracted porcine follicular fluid and 17β-estradiol on follicular growth and plasma gonadotropins in gilts fed a progesterone agonist, altrenogest. J. Anim. Sci. 64:816.

Hauger, R. L., F. J. Karsch and D. L. Foster. 1977. A new concept for control of the estrous cycle of the ewe based on the temporal relationship between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. Endocrinology 101:807.

Hinshelwood, M. M., D. J. Dierschke and E. R. Hauser. 1987. The effect of bovine follicular fluid on follicle-stimulating hormone (FSH) concentrations in beef cows in the early postpartum period. Biol. Reprod. 36(Suppl. 1):153.

Hoak, D. C. and N. B. Schwartz. 1980. Blockade of recruitment of ovarian follicles by suppression of the secondary surge of follicle-stimulating hormone with porcine follicular fluid. Proc. Natl. Acad. Sci. USA 77: 4953.

Humason, G. L. 1962. In: Animal Tissue Techniques. p 124.

W. H. Freeman and Co., San Francisco, CA.

Hunter, M. G., J. E. Hindle, A. S. McNeilly and B. J. McLeod. 1987. Treatment with bovine follicular fluid (bFF) suppresses follicular development in control and GnRH treated anestrous ewes. Soc. Study Fertil. Annu. Conf. (Abstr. 116). Cambridge, UK.

Johnson, S. K., M. F. Smith and R. G. Elmore. 1985. Effect of unilateral ovariectomy and injection of bovine follicular fluid on gonadotropin secretion and compensatory ovarian hypertrophy in prepuberal heifers. J. Anim. Sci. 60:1055.

Kaltenbach, C. C., J. W. Graber, G. D. Niswender and A. V. Nalbandov. 1968. Effect of hypophysectomy on the formation and maintenance of corpora lutea in the ewe. Endocrinology 82:753.

Kaynard, A. H. and F. J. Karsch. 1988. Hypophyseal actions of pulsatile gonadotropin-releasing hormone in the ewe: Development and application of a new experimental model. Neuroendocrinology 48:287.

Keisler, D. H., E. K. Inskeep and R. A. Dailey. 1985. Roles of pattern of secretion of luteinizing hormone and the ovary in attainment of puberty in ewe lambs. Domest. Anim. Endocrinol. 2:123.

Kling, O. R., P. C. Roche, J. D. Campeau, K. Nishimura, R. M. Nakamura and G. S. diZerega. 1984. Identification of a porcine follicular fluid fraction which suppresses follicular response to gonadotropins. Biol. Reprod. 30: 564.

Larson, G. H., P. E. Lewis, R. A. Dailey, E. K. Inskeep and E. C. Townsend. 1987. Follicle stimulating hormone pattern and luteal function in ewes receiving bovine follicular fluid during three stages of the estrous cycle. J. Anim. Sci. 64:1491.

Li, J., H. Francis and I. J. Clarke. 1989. Effects of bovine follicular fluid on pulsatile secretion of gonadotropin-releasing hormone and gonadotropins in ovariectomized ewes. J. Neuroendocrinol. 1:61.

Mallory, D. S., C. M. Gust and R. A. Dailey. 1986. Effects of pituitary stalk-transection and type of barrier on pituitary and luteal function during the estrous cycle of the ewe. Domest. Anim. Endocrinol. 3:253.

McEwen, B. S. 1981. Neural gonadal steroid actions. Science (Washington, DC) 211:1303.

McNeilly, A. S. 1985. Effect of changes in FSH induced by bovine follicular fluid and FSH infusion in the preovulatory phase on subsequent ovulation rate and corpus luteum function in the ewe. J. Reprod. Fertil. 74:661.

Miller, K. F., J. K. Critser and O. J. Ginther. 1982. Inhibition and subsequent rebound of FSH secretion following treatment with bovine follicular fluid in the ewe. Theriogenology 18:45.

Miller, K. F., J. K. Critser, R. F. Rowe and O. J. Ginther. 1979. Ovarian effects of bovine follicular fluid treatment in sheep and cattle. Biol. Reprod. 21:537.

Moser, M. T. 1988. Effect of bovine follicular fluid and follicle-stimulating hormone on follicular growth in prepuberal heifers. Anim. Reprod. Sci. 18:227.

Moser, M. T. 1989. Effect of bovine follicular fluid and follicle-stimulating hormone on follicular growth in unilaterally ovariectomized prepuberal heifers. J. Dairy Sci. 72:2170.

Niswender, G. D., C. E. Farin, F. Gamboni, H. R. Sawyer and T. M. Nett. 1986. Role of luteinizing hormone in regulating luteal function in ruminants. J. Anim. Sci. 62(Suppl. 2):1.

Niswender, G. D., K.M.T. Menon and R. B. Jaffe. 1972. Regulation of corpus luteum during the menstrual cycle and early pregnancy. Fertil. Steril. 23:432.

Reed, M. and W. F. Hounslow. 1971. Induction of ovulation in the guinea pig. J. Endocrinol. 49:203.

SAS. 1986. SAS User's Guide: Statistics. SAS Inst., Inc., Cary, NC.

Seyle, H., J. B. Collip and D. L. Thomson. 1933. Further studies on production of thecal luteinization by means of A.P.L. Proc. Soc. Exp. Biol. Med. 30:780.

Sheffel, C. E., B. R. Pratt, W. L. Ferrell and E. K. Inskeep. 1982. Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. J. Anim. Sci. 54:830.

Sluss, P. M. and L. E. Reichert, Jr. 1984. Secretion of an inhibitor of follicle stimulating hormone binding to receptor by the bacteria Serratia, including a strain isolated from porcine follicular fluid. Biol. Reprod. 31: 520.

Turnbull, K. E., A.W.H. Braken and P. E. Mattner. 1977. The pattern of follicular growth and atresia in the ovine ovary. Aust. J. Biol. Sci. 30:229.

Turzillo, A. M. and J. E. Fortune. 1990. Suppression of the secondary FSH surge with bovine follicular fluid is associated with delayed ovarian follicular development in heifers. J. Reprod. Fertil. 89:643.

Wallace, J. M. and A. S. McNeilly. 1985. Increase in ovulation rate after treatment of ewes with bovine follicular fluid in the luteal phase of the estrous cycle. J. Reprod. Fertil. 73:505.