EFFECTS OF CHARCOAL-EXTRACTED, BOVINE FOLLICULAR FLUID ON GONADOTROPIN CONCENTRATIONS, THE ONSET OF ESTRUS AND LUTEAL FUNCTION IN HEIFERS

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ABSTRACT

A study was conducted to determine the effect of charcoal-extracted, bovine follicular fluid (CFF) on plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations, the interval from luteolysis to estrus, and subsequent luteal function in heifers. Fifteen Angus, Simmental and Hereford heifers were allotted by age, weight and breed to a control (C, n = 8) or a CFF (n = 7) group. Heifers received injections of saline or CFF (iv, 8 ml/injection) every 12 h from d 1 (d 0 estrus) through d 5 of the estrous cycle. On d 6, each heifer was injected (im) with 25 mg of prostaglandin F2α (PGF2α). Blood samples were collected every 12 h by venipuncture starting just before the first saline or CFF injection and continuing until estrus. Thereafter, blood samples were collected every other day during the subsequent estrous cycle and assayed for FSH, LH, estradiol-17β and progesterone by radioimmunoassay. Injections of CFF had no effect (P>.05) on circulating FSH or LH concentrations from d 1 to 5 relative to the C group; however, there was a transient rise (P<.05) in FSH concentrations 24 h following cessation of CFF injections. This transient rise in FSH was not immediately followed by an increase in plasma estradiol-17β concentrations. Although CFF injections did not interfere with PGF2α-induced luteolysis, the interval from PGF2α injection to estrus was delayed (P<.05) by 5 d in the CFF group compared with the C group. Furthermore, the rise in estradiol-17β following PGF2α injection was delayed in the CFF group. The length of the subsequent estrous cycle and the plasma concentrations of progesterone were similar (P>.05) in the C and CFF groups. In summary, cessation of CFF injections resulted in a selective, transient increase in FSH concentrations. Furthermore, CFF injections preceding PGF2α-induced luteolysis delayed the onset of estrus but did not alter subsequent luteal lifespan.

(Key Words: Follicular Fluid, Follicle Stimulating Hormone, Gonadotropins, Estrous Cycle.)

Introduction

Asynchronous secretory patterns have been observed for follicle stimulating hormone (FSH) and luteinizing hormone (LH) in cattle (Dobson, 1978; Roche and Ireland, 1981), sheep (Narayana and Dobson, 1979; Goodman et al., 1981), pigs (Martin et al., 1984), hams ters (Greenwald and Siegel, 1982) and various other species. Although ovarian steroids can regulate gonadotropin secretion in cattle (Roche and Ireland, 1981; Butler et al., 1983), additional ovarian factors (e.g., inhibin) are involved in the differential regulation of gonadotropin secretion. Inhibin is a nonsteroidal molecule found in follicular fluid, which is heat and trypsin labile (Franchimont et al., 1979), and is synthesized by granulosa cells (Erickson and Hsueh, 1978). Injections of bovine follicular fluid decreased FSH but not LH concentrations in ovariectomized rats (de Jong and Sharpe, 1976), ovariectomized heifers (Ireland et al., 1983; Kiracofe et al., 1983) and intact ewes (Miller et al., 1982). Administration of follicular fluid before luteolysis might inhibit follicular maturation and consequently delay the onset of an ovulatory estrus. Decreased FSH concentrations before ovulation may result in subnormal luteal function in heifers, as previously reported in primates (Stouffer and Hodgen, 1980) and humans (Wentz, 1979).

The objectives of this study were to determine the effect of charcoal-extracted, bovine follicular fluid (CFF) injections before prostaglandin F2α (PGF2α)-induced luteolysis on

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circulating FSH and LH concentrations, the interval from luteolysis to estrus, and subsequent luteal function.

**Materials and Methods**

Bovine follicular fluid was aspirated from follicles (<20 mm in diameter) at a local abattoir. The follicular fluid was maintained on ice until transported to the laboratory. The fluid was then centrifuged (1,700 x g) to remove cellular debris and frozen at -20°C. When a sufficient amount of follicular fluid was collected to conduct the experiment, the fluid was pooled and steroids were removed by charcoal extraction. Follicular fluid was mixed with 50 mg of charcoal/ml at 4°C for 1 h. The fluid was centrifuged for 20 min at 1,400 x g and recentrifuged at 30,000 x g for 1 h to remove charcoal fragments. The CFF was frozen (−20°C) until the time of injection.

Fifteen cycling Angus, Simmental and Hereford heifers were allotted by age, weight and breed to a control-saline (C, n = 8) or CFF (n = 7) group. Heifers received injections of saline or CFF (iv, 8 ml/injection) every 12 h from d 1 (d 0 = estrus) through d 5 of the estrous cycle. On d 6, each heifer was injected (im) with 25 mg of PGF2α. Heifers were observed for estrous behavior twice daily throughout the experimental period and only heifers that stood to be ridden were considered in estrus. Jugular blood samples were collected by venipuncture into heparinized tubes before each saline or CFF injection, from d 1 to 5. Following PGF2α injection, blood samples were collected every 12 h until heifers exhibited estrus and every other day during the subsequent estrous cycle. The plasma was collected and frozen at −20°C until concentrations of FSH (Bolt and Rollins, 1983), LH (Zaied et al., 1980), estradiol-17β (Kesler et al., 1977) and progesterone (Cantley et al., 1975) were measured by radioimmunoassay. Samples from morning and evening were pooled to provide sufficient plasma for determination of estradiol-17β concentrations. The intra- and interassay coefficients of variation for FSH were 7.1 and 6.4% and for progesterone were 6.5 and 6.9%, respectively. Determinations for LH and estradiol-17β were made in one assay and the intraassay coefficient of variation was 7.3 and 22%, respectively.

Circulating concentrations of FSH, LH, estradiol-17β and progesterone were analyzed by analysis of variance in a split-plot design (Gill and Hafs, 1971). Days from PGF2α injection to the onset of estrus and the length of the subsequent estrous cycle were analyzed by Student's t-test (Steel and Torrie, 1960).

**Results and Discussion**

The charcoal-extraction procedure was an effective method of removing steroids from bovine follicular fluid. The concentrations of estradiol-17β and progesterone in the follicular fluid before charcoal extraction were 97.6 and 243 ng/ml and after charcoal extraction were 0.06 and 2.44 ng/ml, respectively. Assuming there was no clearance following an injection of 8 ml of CFF into a 350-kg heifer (blood volume = 21 liters), the circulating concentrations of estradiol-17β and progesterone would have been increased 0.2 and 8 pg/ml, respectively. These concentrations are lower than the estradiol-17β and progesterone concentrations in ovariectomized heifers (Beck et al., 1976).

As previously reported for rats (de Jong and Sharpe, 1976; Welschen et al., 1977), ewes (McNeilly, 1984) and heifers (Ireland et al., 1983; Kiracofe et al., 1983), there was no difference (P>0.05) in plasma LH concentrations between the C and CFF groups at any time period (figure 1). In addition, plasma FSH concentrations were similar (P>0.05) on d 1 to 5 in the C and CFF groups (figure 2). Previous studies reported that bovine or porcine follicular fluid decreased plasma FSH concentrations in intact rats (Marder et al., 1977), intact primates (Stouffer et al., 1981), intact ewes (Miller et al., 1982; McNeilly, 1984) and ovariectomized heifers (Ireland et al., 1983; Kiracofe et al., 1983). In the present study, blood samples may not have been collected frequently enough to demonstrate a decrease in plasma FSH concentrations because the magnitude of the decrease in FSH produced by CFF in intact primates (Stouffer et al., 1981) and intact ewes (Miller et al., 1982; McNeilly, 1984) was small. It is also possible that CFF cannot decrease plasma FSH concentrations below basal concentrations in intact heifers, even though CFF did suppress plasma FSH concentrations in intact bulls (McGowan et al., 1984). Additionally, the amount of CFF and(or) the inhibin activity of the CFF, in the present study, may not have been sufficient to suppress significantly plasma FSH concentrations. Follicle stimulating hormone concentrations were
suppressed in ovariectomized cattle when 5 (Kiracofe et al., 1983) or 20 ml (Ireland et al., 1983) of follicular fluid were injected every 6 h. In the present study, 8 ml of CFF were injected every 12 h, and may not have been sufficient to inhibit significantly FSH secretion. Furthermore, charcoal extraction may remove inhibin as well as steroids from CFF. Tsonis et al. (1983) reported that a 10-fold greater inhibin activity was present in diluted bovine follicular fluid treated with 1 mg charcoal/ml of follicular fluid compared with 10 mg charcoal/ml of follicular fluid. In the present study, 50 mg charcoal/ml of follicular fluid was used and may have reduced the inhibin activity of the CFF.

There was a transient increase (P<.01) in plasma FSH but not LH concentrations 24 h after the last CFF injection (figure 2). A similar increase in FSH concentrations following cessation of follicular fluid treatment was observed in intact ewes (Miller et al., 1982; McNeilly, 1984), intact primates (Stouffer et al., 1981) and intact rats (De Paolo et al., 1979). Furthermore, a selective rise in plasma FSH concentrations was observed after unilateral ovariectomy in rats (Butcher, 1977), hamsters (Bast and Greenwald, 1977), ewes (Findlay and Cumming, 1977) and gilts (Redmer et al., 1983).

The mechanism responsible for a selective, transient increase in plasma FSH is unclear. In
Figure 3. Circulating concentrations of progesterone (x ± SE) in heifers injected every 12 h from d 1 (d 0 = estrus) to 5 with saline (n = 8) or charcoal-extracted, bovine follicular fluid (CFF, n = 7) and injected with 25 mg PGF$_2$α on d 6. Progesterone concentrations during the subsequent estrous cycle (d 0 to 20) have been standardized to the onset of estrus.

Injections of CFF did not alter plasma progesterone concentrations before or after PGF$_2$α-induced luteolysis (figure 3); however, the interval from PGF$_2$α-induced luteolysis to estrus was delayed (P<.05) by 5 d in the CFF group compared with the C group (table 1). Injections of follicular fluid for 3 d following PGF$_2$α injection in heifers and ewes also delayed the interval from luteolysis to estrus (Miller et al., 1979; McNeilly, 1984). As previously discussed, CFF administration did not affect progesterone concentrations in the present study; consequently, the increased interval from luteolysis to estrus was not due to an inhibition of corpus luteum regression. A more likely explanation for the delayed interval to estrus is an inhibition of follicular growth by CFF.

TABLE 1. EFFECT OF SALINE OR CHARCOAL-EXTRACTED, BOVINE FOLLICULAR FLUID (CFF) ON THE INTERVAL FROM PGF$_2$α INJECTION TO ESTRUS AND SUBSEQUENT CYCLE LENGTH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. cows</th>
<th>Interval from PGF$_2$α to estrus, d$^b$</th>
<th>Subsequent cycle length, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>8</td>
<td>2.3 ± .2$^c$</td>
<td>21.4 ± .6</td>
</tr>
<tr>
<td>CFF</td>
<td>7</td>
<td>7.3 ± .7$^d$</td>
<td>18.4 ± 1.1</td>
</tr>
</tbody>
</table>

$^a$Saline or CFF injections on d 1 to 5 (d 0 = estrus).

$^b$PGF$_2$α injections on d 6.

$^c,d$Means (x ± SE) within the same column having different superscripts differ (P<.05).
creased ($P<.05$) in the C but not the CFF group (figure 4), suggesting that CFF treatment inhibited preovulatory follicular growth. It is also important to note that estradiol-17β concentrations in the CFF group did not immediately increase following the transient FSH rise. Treatment with CFF may have induced atresia, resulting in follicles that were incapable of responding to the transient FSH rise.

Injections of CFF may have prolonged the interval from PGF$_2$α to estrus by inducing atresia and(or) inhibiting follicular recruitment during d 1 to 5. A single estrogen-active follicle develops during the early luteal phase (d 3 to 7) in heifers (Ireland and Roche, 1983) and may account for the observed estradiol increase during this time (Hansel and Convey, 1983). In ewes, bovine follicular fluid treatment after PGF$_2$α injection on d 8 of the estrous cycle, decreased the size and number of follicles present (Miller et al., 1979).

The mechanism by which CFF inhibits follicular development is unclear. Bovine CFF administration may suppress FSH concentrations and(or) have a direct effect on ovarian follicles. Follicle stimulating hormone is reportedly released in a pulsatile manner during the bovine estrous cycle (Waiters et al., 1983) and CFF may interrupt the pattern of release as reported in rats (Lumpkin et al., 1984). The frequency of blood sample collection in the present study did not allow determination of the effect of CFF on pulsatile FSH release. Bovine follicular fluid also contains a FSH-binding inhibitor that inhibits FSH binding to granulosa cells (Fletcher et al., 1982; Sato et al., 1982). It has been suggested that a low molecular weight FSH-binding inhibitor may be involved in follicular atresia (Sluss et al., 1983). Therefore, CFF may inhibit follicular growth by promoting atresia.

Temporary suppression of plasma FSH concentrations during the preovulatory period was followed by subnormal luteal function in humans (Wentz, 1979) and primates (Stouffer and Hodgen, 1980). Furthermore, in early-weaned cows, plasma FSH concentrations were lower during the preovulatory period of a short vs a normal length estrous cycle (Ramirez-Godinez et al., 1982). In the present study, there was no difference ($P>.05$) between groups in the circulating progesterone concentrations or luteal lifespan during the subsequent estrous cycle (figure 3; table 1). Miller et al. (1979) also reported that follicular fluid injections following PGF$_2$α-induced luteolysis in ewes did not alter subsequent estrous cycle length. The influence of suppressed FSH concentrations on the subsequent cycle in cattle remains unclear because CFF injections did not significantly suppress FSH concentrations during d 1 to 5 in the present study. Moreover, the transient increase in FSH may have countered any preceding effect of CFF on plasma FSH concentration.

In summary, cessation of CFF treatment resulted in a selective, transient increase in FSH concentrations. Furthermore, CFF injections delayed the interval from PGF$_2$α-induced
luteolysis to estrus, but did not alter the subsequent luteal lifespan.

Literature Cited


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