Effect of charcoal-treated bovine follicular fluid on the secretion of FSH in ovariectomized and intact prepubertal heifers*

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Efeito do líquido folicular bovino tratado com carvão ativado na secreção de FSH em novilhas pré-púberes intatas e ovariectomizadas

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SUMMARY

The aim of the experiment was to determine whether charcoal-treated bovine follicular fluid (bFF) removed from visible (<22 mm) follicles altered the secretion of follicle-stimulating hormone (FSH) in intact and ovariectomized prepubertal heifers. After a 10-ml injection of bFF given at three consecutive 8-h intervals, secretion of FSH was depressed in approximately 44% of the ovariectomized heifers but there was no effect in intact heifers. When the bFF treatment ceased, there were no rebound effects on FSH concentrations above that of controls pretreatment levels. These results suggest that proteins from bFF act at the pituitary level to inhibit FSH secretion and, differently of the intact, the ovariectomized heifer is an adequate model to put this effect in evidence, particularly when the bFF have low FSH suppressing activity.

UNITERMS: Follicular fluid; FSH; Bovine.

INTRODUCTION

Charcoal-extracted bovine follicular fluid (bFF) has been shown to suppress FSH but not LH concentration when administered to ovariectomized[6,8] and intact[10,12,16,17] heifers. In intact heifers, however, several reports[7,14] were unable to detect a significant bFF-induced suppression of FSH. The authors suggested that the inhibin activity in the follicular fluid injections may was insufficient to decrease FSH or that blood samples were not taken frequently enough to detect a decrease in FSH.

In the absence of available information concerning the optimal in-vivo model system for demonstration of bFF biological activity in cattle, the objective of this study is to determine the effect of charcoal-extracted bFF injections on circulating FSH concentrations in ovariectomized or intact prepubertal heifers.

MATERIAL AND METHOD

Bovine follicular fluid was aspirated from follicles (diameter <22 mm) of ovaries collected from a local abattoir, centrifuged (700 *g* for 15 min at 4ºC) to remove cellular debris and frozen (-18ºC). After several collects, pooled bFF was thawed and incubated with 10 mg ml^{-1} activated charcoal (Norit A, Sigma Chemical Company, St Louis MO) during 2 h at 4ºC, followed by sequential centrifugation (17000 *g*, 1 h at 4ºC) and filtration through sterile 45 µm nylon tissue. Charcoal treatment removed 99.8% of estradiol and progesterone steroids and had a protein content of 80 mg ml^{-1} (Alvarez[1]).

Six 10-months crossbred heifers (210 ± 30 kg) were used. Two months before, three of them were cirurgically ovariectomized by conventional method after lateral laparotomy. Heifers received four iv injections of 10 ml bFF at 8-h intervals. Blood samples were taken at 8-h intervals (or 4 h intervals on the period of bFF injections) 48h before and 48h after the first bFF injection by jugular venipuncture into 10 ml tubes under vacuum containing 143 USP heparin (Vacutainer; Beckton Dickinson, Crowley). After centrifugation at 700 *g* for 30 min, the plasma was stored at -20ºC until the assay. Plasma concentrations of FSH were measured using validated radioimmunoassay[2]. USDA -bFSH-1-2 (bFSH) served as both reference standard and tracer. bFSH was radioiodinated by a modification of the method of Greenwood *et al.*[5]. Briefly, 5 mg bFSH was dissolved in 20 ml 0.3M phosphate buffer pH 7.5 and reacted with 800 mCi ^{125}I (Amersham Corp., Arlington Heights, IL) and 0.8 mg chloramine T for 5 min This reaction was stopped by addition of 1 mg sodium metabisulfite, chromatographed on Sephadex G100,

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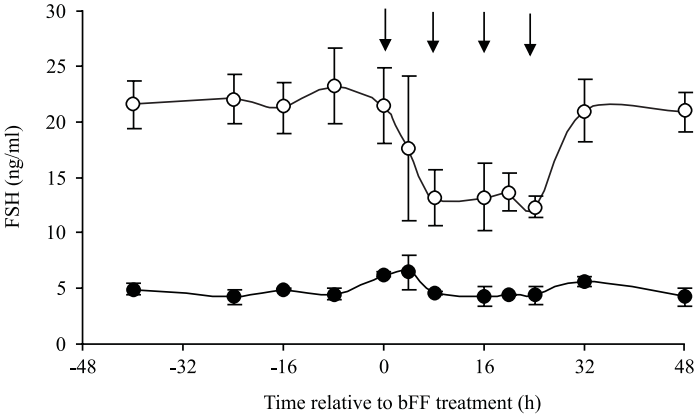

Figure 1

Mean (± s.e.m.) concentrations of follicle-stimulating hormone (FSH) in jugular venous plasma from intact (●; n = 3) or ovariectomized (○; n = 3) prepubertal heifers which received 4 injections 10 ml charcoal-treated bovine follicular fluid (arrows).

and eluted with 0.05M PBS. The peak fraction of radioiodinated bFSH was diluted to 20000 cpm/100 ml in assay buffer (PBS containing 0.1 BSA and 0.1% sodium azide).

Unknowns and standards (100 ml) were pipetted into borosilicated glass tubes and incubated with 100 ml of rabbit anti-oFSH, NIDDK-oFSH-I, dilution 1:80000 (AFP-C5288113) and 100 ml (20000 cpm) bFSH tracer for 24 h at 4ºC. After this incubation, 100 ml rabbit anti-gamma globulin sheep serum (dilution 1:10) was added, and incubation continued for 2 h at room temperature. Tubes were washed with distilled water and centrifuged at 4ºC for 10 min at 3200 *g*. Supernatants were decanted, and radioactivity in each pellet was determined using a gamma counter. Intra and interassay coefficient of variation (CV) averaged 4.2 and 12.2%, respectively. Sensitivity of the bFSH was 1.4 ng ml^{-1}.

Since there were only three animals per treatment group, statistical analysis was restricted to within-group comparison, with each animal acting as its control. Student's paired t-test was used to compare the mean plasma FSH concentrations before (control pretreatment period), during and after administration of bFF.

RESULTS

No alterations in FSH concentrations were shown in intact heifers as an effect of bFF injections. Concentrations of FSH in ovariectomized heifers dropped ($p<0.05$) as early as 4h after the first injection of bFF, reaching maximal suppression (43.9%; $p<0.01$) at the time of the second injection of bFF (Fig. 1).

There was no significant elevation in plasma FSH concentration after the final bFF injections in both, intact and ovariectomized heifers.

DISCUSSION

Charcoal treatment of follicular fluid to remove steroids and the use of ovariectomized animals with or without steroid replacement has shown that follicular fluid contains a non-steroidal substance (inhibin), produced by the granulosa cells, which operates at the pituitary level to suppress FSH secretion[3,13]. The endocrine response to bFF observed in this study confirms previous results demonstrating in vivo the selective suppressive effect of charcoal-extracted bFF on FSH in ovariectomized female cattle[6,8].

The suppression of circulating FSH in this experiment corresponds to the 30-45% maximal decrease in the FSH concentration obtained in previous experiments in intact[10,12,17], ovariectomized[6,8] and in dispersed bovine pituitary cells[11].

Differently, the dose of bFF used was unable of suppressing plasma FSH below basal concentrations in intact heifers. This and other similar results[7,14] with mature intact heifers suggest a low content of inhibin in the bFF used. Quirk; Fortune[14] suppressed plasma concentrations of FSH with 20 ml bFF injections twice daily while plasma FSH concentrations were not affected with 10 ml of bFF. Furthermore, charcoal extraction may remove inhibin as well as steroids from follicular fluid. Tsonis *et al.*[15] reported that a 10-fold greater inhibin activity was present in ovine follicular fluid (oFF) treated with 1 mg charcoal ml^{-1} of oFF compared with 10 mg charcoal ml^{-1} of oFF. In the present study, 10 mg charcoal ml^{-1} of bFF was used and may have reduced the inhibin activity of bFF. Consequently, the amount of injected bFF may have been insufficient of suppressing significantly plasma FSH concentrations in intact heifers. Moreover, since plasma FSH of ovariectomized heifers was significantly suppressed by 8[8] or 10 ml (present study) of bFF, there is the possibility that pituitary sensibility to bFF is higher in ovariectomized that intact prepubertal heifers.

There was no significant elevation in plasma FSH concentration over those of control after the final bFF injections in both ovariectomized or intact heifers. Previous studies have shown that the transient increase in plasma FSH was not associated to previous suppression of FSH by the bFF[7,9,14] but was related to the presence of the ovaries, since a rebound effect was not reported following bFF-induced suppression of FSH in ovariectomized heifers[6] or in ovariectomized ewes treated with bFF or pure bovine inhibin[4]. The absence of a transient hipersecretion of FSH of the intact heifers in the present study, may be attributed to insufficient inhibin or other not identified FSH-modulating compounds present in the follicular fluid[9].

CONCLUSION

In conclusion, these results confirmed that the suppressive activity of bFF on FSH secretion is exerced at the pituitary level and, differently of intact, the ovariectomized heifer is an adequate model to put in evidence this effect, particularly when the bFF have low FSH suppressing activity.

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RESUMO

O presente trabalho objetivou determinar o efeito do líquido folicular bovino tratado com carvão ativado (LFb) na secreção do hormônio folículo estimulante (FSH) de novilhas pré-púberes ovariectomizadas ou intatas. A aplicação de LFb (quatro injeções de 10 ml com intervalo de 8 horas) provocou uma queda de aproximadamente 44% na concentração plasmática de FSH nas novilhas ovariectomizadas, mas não teve efeito nas novilhas intatas. Não foi observada hipersecreção de FSH após o término da aplicação do LFb. Esses resultados sugerem que proteínas presentes no LFb atuam ao nível hipofisiário para inibir a secreção de FSH e, diferentemente das intatas, as novilhas ovariectomizadas constituem um modelo adequado para evidenciar esse efeito, particularmente quando o LFb possui reduzida atividade supressora do FSH.

UNITERMOS: Fluido folicular; FSH; Bovinos.

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