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Effects of gonadotrophin concentration on hormone production by theca interna and granulosa cells from bovine preovulatory follicles

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Introduction

Mammalian preovulatory follicles produce primarily oestradiol and androgens before the LH and FSH surges. The production of these steroids by the follicle requires both theca and granulosa cells, since theca cells synthesize androgens from progestins but lack aromatizing capacity, whereas granulosa cells aromatize exogenous androgens to oestrogens but lack the ability to convert progestins to androgens (Fortune and Quirk, 1988). In ruminants, the gonadotrophin surge triggers a rapid decrease in oestradiol and androgen production and a marked increase in progesterone and oxytocin biosynthesis (reviewed in Fortune, 1994). It is unclear how changes in gonadotrophin concentrations *in vivo* regulate this follicular to luteal phase shift in hormone production. Theca and granulosa cells obtained from bovine preovulatory follicles before the gonadotrophin surge 'spontaneously luteinize', as evidenced by increased production of progesterone and oxytocin; high concentrations of gonadotrophins accentuate these changes (Aladin Chandrasekher and Fortune, 1990; Voss and Fortune, 1991). In contrast, similar concentrations of gonadotrophins do not stimulate or maintain thecal androgen production or granulosa oestradiol secretion *in vitro* (Tsonis *et al.*, 1984; Fortune and Quirk, 1988; Saumande, 1991).

The failure of gonadotrophins to sustain androgen or oestradiol secretion by follicle cells from domestic species *in vitro* has hampered studies on the regulation of hormone production by developing preovulatory follicles in these species. Oestradiol secretion by granulosa cells from pregnant mares' serum gonadotrophin (PMSG)-treated immature calves is stimulated by very low doses of FSH (Saumande, 1991). The objective of this study was to test the hypothesis that low doses of gonadotrophins maintain follicular secretion of androgens and oestradiol by cultured theca and granulosa cells, whereas higher doses induce luteinization (i.e., progesterone and oxytocin secretion). Therefore, theca interna and granulosa cells were isolated from bovine preovulatory follicles before the gonadotrophin surge and cultured with various doses of LH or FSH.

Materials and Methods

Holstein heifers ($n = 5$) with normal and regular oestrous cycles were injected i.m. with 25 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Lutalyse: Upjohn Co., Kalamazoo, MI) on day 7 of the oestrous cycle (day 0 = day of oestrus) to initiate luteal regression and the next follicular phase (Voss and Fortune, 1991). The ovary bearing the preovulatory follicle (determined by transrectal ultrasonography) was removed per vaginam 24 h after $PGF_{2\alpha}$ injection (i.e., 24–36 h before the expected time of the preovulatory gonadotrophin surge).

The preovulatory follicle was identified and dissected from the ovary; theca interna and granulosa cells were isolated as described in a previous report (Voss and Fortune, 1991). The theca interna was cut into 72 pieces and the pieces transferred into 24-well Costar dishes (three pieces per well); granulosa cells were distributed into 24-well Primaria plates (2×10^5 cells per well). Both theca interna and granulosa

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Table 1. Hormone secretion in the absence of gonadotrophins by theca interna and granulosa cells from bovine preovulatory follicles

	Theca interna[a]		Granulosa cells[b]		
Time of culture (h)	Androstenedione (ng per culture)	Progesterone (ng per culture)	Oestradiol (ng per culture)	Progesterone (ng per culture)	Oxytocin (pg per culture)
0–24	24.2 ± 7.8	0.4 ± 0.1	10.4 ± 0.5	1.3 ± 0.2	0.0 ± 0.0
24–48	6.1 ± 2.7[c]	1.7 ± 0.3[c]	1.2 ± 0.4[c]	7.0 ± 1.3[c]	0.0 ± 0.0
48–72	3.1 ± 1.7[c]	2.4 ± 0.5[c]	0.5 ± 0.2[c]	13.0 ± 2.5[c]	7.4 ± 1.1[c]
72–96	1.9 ± 1.3[c]	4.6 ± 0.8[c]			

[a]Means ± SEM of ten theca interna cultures, two from each of five follicles.
[b]Means ± SEM of twelve granulosa cell cultures, four from each of three follicles.
[c]Significantly different relative to 0–24 h ($P < 0.01$).

cells were cultured in 0.5 ml of defined medium as described by Voss and Fortune (1991). Theca interna pieces were cultured with or without ovine LH (0.5, 1, 2, 4, 8, 16, 32, 64, 128 or 256 ng ml^{-1}; NIH-LH-S23) and granulosa cells were cultured with or without ovine FSH (1, 2, 4, 8, 16, 32, 64, or 128 ng ml^{-1}; NIH-FSH-S17) at 37°C in a humidified incubator gassed with 5% CO_2 and 95% air. Media were withdrawn and replaced completely with fresh media every 24 h for 72 h (granulosa) or 96 h (theca interna). The collected media were stored at −20°C and later assayed by radioimmunoassay for androstenedione and progesterone in theca interna cultures and oestradiol, progesterone and oxytocin in granulosa cultures (Fortune and Eppig, 1979; Voss and Fortune, 1991).

Hormone concentrations are expressed as ng androstenedione, oestradiol or progesterone per culture ± SEM and pg oxytocin per culture ± SEM. If heterogeneity of variance was present, hormone concentrations were log transformed before statistical analysis. The data from the first day of culture (0–24 h) were subjected to two-factor analysis of variance (ANOVA) with experiment (heifer) and treatment (concentration of LH or FSH) as the two factors. Data from the remaining days of culture were summed over time and the cumulative data were analysed by two-factor ANOVA. Duncan's multiple-range test was used to make comparisons among the means when the ANOVA showed a significant treatment effect.

Results

Androstenedione secretion by theca interna cultured without LH decreased throughout the culture period, with the greatest decline ($P < 0.01$) occurring between the first and second day (Table 1). In contrast, basal progesterone production by theca interna increased ($P < 0.01$) throughout the culture period (Table 1). During the first 24 h of culture, androstenedione production by theca interna cultures was stimulated ($P < 0.05$) by a wide range of LH concentrations (4, 8, 16, 32, 64, 128, and 256 ng ml^{-1}; Fig. 1a). However, on days 2–4 of culture, only lower doses of LH (2 and 4 ng ml^{-1}) stimulated ($P < 0.05$) androstenedione secretion above control values (Fig. 1b). Although progesterone production was stimulated ($P < 0.05$) by most concentrations of LH throughout the culture period (Fig. 1), the highest doses of LH were much more effective than the lower stimulatory doses during days 2–4 of culture (Fig. 1b).

Oestradiol production by granulosa cells cultured without FSH decreased ($P < 0.01$) throughout the culture period, with the largest decline occurring between the first and second day of culture (Table 1). In contrast, progesterone secretion by control cultures increased ($P < 0.01$) throughout the culture period, with the greatest increase occurring between the first and second day of culture (Table 1). Basal oxytocin secretion from granulosa cells was negligible for the first 2 days of culture and increased markedly by day 3 (Table 1). During the first 24 h of culture, FSH had no effect on oestradiol production

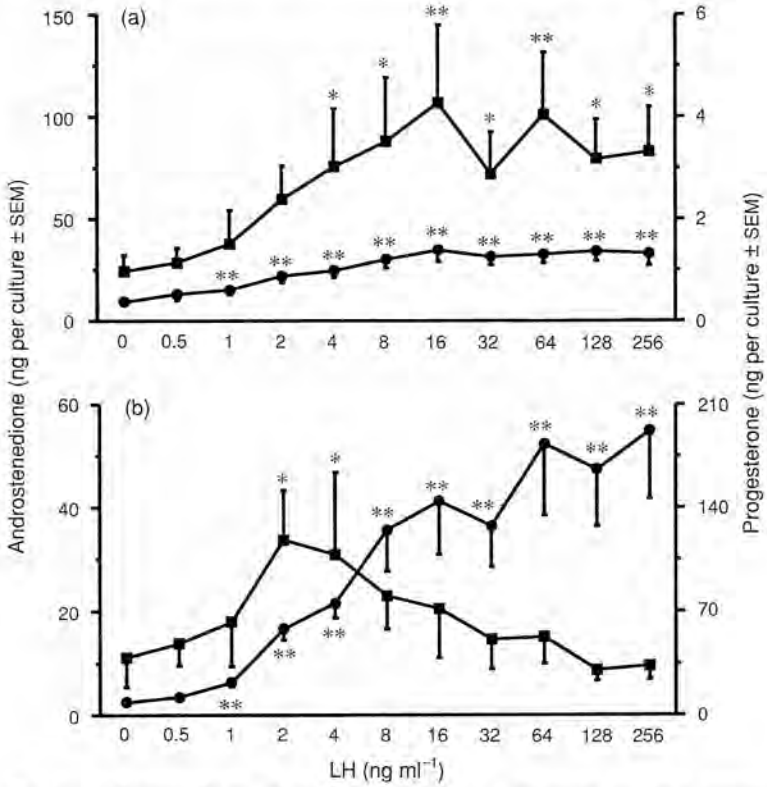

Fig. 1. Androstenedione (■) and progesterone (●) secretion by theca interna isolated from bovine preovulatory follicles before the LH surge and cultured for 96 h in defined medium alone or with graded doses of LH ($n = 10$ cultures, two from each of five follicles). (a) Steroid secretion during 0–24 h of culture; (b) cumulative steroid secretion during 24–96 h of culture. Data were subjected to ANOVA and Duncan's multiple-range test; significant differences from controls (no LH) are indicated: $^{*}P < 0.05$, $^{**}P < 0.01$. Note difference in scale of y axis between panel (a) and (b).

by granulosa cells (Fig. 2a). However, during days 2–3 of culture, the lowest doses of FSH (1 and 2 ng ml^{-1}) enhanced ($P < 0.01$) oestradiol secretion, whereas higher concentrations of FSH (8–128 ng ml^{-1}) inhibited ($P < 0.05$) oestradiol accumulation (Fig. 2b). The doses of FSH (1 and 2 ng ml^{-1}) that stimulated oestradiol production had little effect on progesterone production by granulosa cells (Fig. 2). In contrast, during days 2–3, the higher doses of FSH (32–128 ng ml^{-1}) consistently stimulated progesterone accumulation above controls (Fig. 2b). Oxytocin secretion was low during the first day of culture (data not shown). However, during the last day of culture, oxytocin production was dramatically enhanced ($P < 0.01$) by FSH in a dose-dependent fashion (Fig. 2b).

Discussion

The results reveal that only very low doses of LH and FSH maintain functions typical of the follicular phase in bovine theca and granulosa cells, respectively. In contrast, higher doses of gonadotrophins accentuate the tendency of each cell type to 'luteinize' *in vitro*. These findings are important because they explain why high concentrations of gonadotrophins fail to maintain oestradiol and androstenedione production by cultured follicle cells of domestic species. In addition, these findings mimic the responses

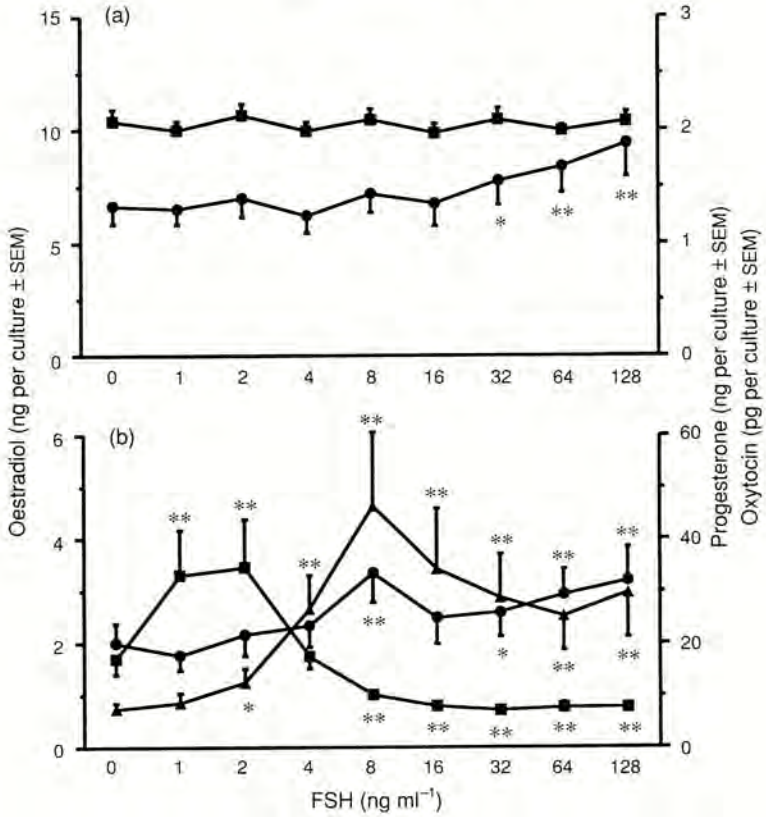

Fig. 2. Oestradiol (■), progesterone (●), and oxytocin (▲) secretion by granulosa cells (2×10^5 cells per culture) isolated from bovine preovulatory follicles before the LH surge and cultured for 72 h in defined medium alone or with graded doses of FSH ($n = 12$ cultures, four from each of three follicles). (a) Oestradiol and progesterone secretion during 0–24 h of culture; (b) cumulative oestradiol and progesterone during 24–72 h of culture and oxytocin secretion during 48–72 h of culture. Data were subjected to ANOVA and Duncan's multiple-range test; significant differences from controls (no FSH) are indicated: $^*P < 0.05$, $^{**}P < 0.01$. Note difference in scale of y axis between panel (a) and (b).

of bovine preovulatory follicles to basal versus surge concentrations of gonadotrophins *in vivo*. Therefore, our culture system will provide an experimental model for studying *in vitro* the cellular and molecular mechanisms that regulate hormone production in follicular cells before and after the preovulatory gonadotrophin surge.

The results show that after the first 24 h of culture, only a very narrow range of LH (2–4 ng ml^{-1}) and FSH (1–2 ng ml^{-1}) concentrations stimulate androstenedione and oestradiol production in bovine theca interna and granulosa cells, respectively. Furthermore, our results demonstrate that higher concentrations of gonadotrophins not only stimulate follicular progesterone and oxytocin production, but also inhibit oestradiol production by granulosa cells. These findings suggest that high and low doses of gonadotrophins regulate hormone production differentially in bovine preovulatory follicles. In contrast, a wider range of gonadotrophin concentrations can sustain androstenedione (Bogovich *et al.*, 1986) and oestradiol (Dorrington *et al.*, 1975; Erickson and Hsueh, 1978; Fortune and Armstrong, 1978; Fortune and Hilbert, 1986) secretion in thecal and granulosa cell cultures from proestrous follicles of rats.

Surge concentrations of LH may activate multiple signal transduction pathways to regulate changes in steroid, prostaglandin and protease production by granulosa cells from preovulatory follicles of rats (Morris and Richards, 1993; Shimamoto *et al.*, 1993). It is possible that the differential effects of low

versus high doses of gonadotrophins on hormone production in bovine preovulatory follicles could be achieved through activation of different signal transduction pathways. Therefore, our experimental model will be suitable for dissecting *in vitro* the signal transduction pathways regulated by basal concentrations of gonadotrophins during the follicular phase and those that are activated in response to surge concentrations.

Conclusions

Very low doses of LH (2–4 ng ml^{-1}) and FSH (1–2 ng ml^{-1}) stimulate androstenedione and oestradiol secretion above control concentrations in theca interna and granulosa cell cultures, respectively. In contrast, high concentrations of gonadotrophins promote a shift to progesterone and oxytocin production, characteristic of luteinizing cells. These results explain previous failures, in numerous studies, to achieve sustained stimulation of follicular phase-type functions in cultured cells from domestic animals and provide an experimental system for further study of interactions between gonadotrophins and follicular cells.

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