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Steroid Synthesis by Ovarian Follicles in Response to LH and $PGF_2\alpha$ (39334)

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Shemesh and Hansel (1) reported that changes in the concentration of testosterone in bovine jugular vein plasma are quite similar to those that occur in plasma estrogen concentrations during the estrous cycle. These changes include a marked rise in plasma testosterone concentration during proestrus immediately following the decline in plasma progesterone levels. Evidence that the mammalian ovary secretes androgens was first provided by Hill (2). Hilliard *et al.* (3) reported that rabbit ovaries can produce androgens *in vivo*, as has been shown *in vitro* (4). The secretion of androgen, estradiol, and progesterone into the blood of the ovarian vein of sheep is significantly increased by injection of gonadotropins; the steroid levels begin to rise within 30 min of injection (5). It was also found by Shemesh and Hansel (6), that arachidonic acid injected into the bovine corpus luteum *in vivo* was converted into prostaglandin F and that the large amounts of prostaglandin F produced in this way resulted in elevated plasma estrogen concentrations.

The present investigation was undertaken to measure testosterone production by the isolated bovine follicular tissue and to determine the effects of exogenous LH and $PGF_2\alpha$ on this production. Estrogen and progesterone production were also examined to ascertain whether changes in androgen biosynthesis are accompanied by changes in synthesis of these steroids.

Materials and methods. Animals and tissues. Ovaries were removed from four normal Holstein heifers at the 16th or 17th day of their estrous cycles through flank laparotomies made under local anesthesia. All follicles, ranging in size from 0.4 to 1 cm in diameter, were dissected free, minced, pooled between ovaries, and incubated in Krebs–Ringer bicarbonate buffer at 37° for 2 hr as described by Hansel (7). Most of the follicles minced were small (approx 0.4–0.6 cm in diameter). However, ovaries from each animal contained one or more large (approx 1.0 cm) follicles. Five incubation vessels were used for each treatment for each animal. Samples were frozen on Dry Ice at the end of each incubation. Each vessel contained 50 mg of follicular tissue and the stimulatory levels of LH (LH-B13, 5 μg/ml) or $PGF_2\alpha$ Tham salt (5 μg/ml). Previous work has shown that these levels of LH and $PGF_2\alpha$ will cause maximum stimulation of progesterone biosynthesis in bovine luteal tissue slices (8).

Extractions. The samples were thawed, homogenized, and methanol was added to a concentration of 60%. Approximately 5000 cpm each of tritiated estradiol, estrone, progesterone, and testosterone (80–110 Ci/mmole; New England Nuclear) were added to each vessel. The homogenate was then transferred to stainless steel centrifuge tubes with repeated washings of 60% ethanol. The homogenate was shaken for 3 hr and stored overnight at −18°. The tubes were then spun in a refrigerated centrifuge at approx 20,000 *g* for 45 min to remove precipitated protein and lipid. The supernatant was collected and taken to dryness on a rotary evaporator. The residue was dissolved in 1 ml of ethanol and transferred with repeated 50-ml washings of benzene and light petroleum (1:1) to a separatory funnel. The benzene:light petroleum solution was extracted twice with 25 ml of 0.4 NaOH to yield a phenolic fraction. The combined alkali extracts were acidified with HCl and extracted with ether (3 × 25 ml). The ether was washed with water (2 × 1/20th vol) to neutrality. After removal of the organic solvents the residues were each transferred with 4 ml of ethanol into small test tubes and dried under N_2 at 45°. The remaining benzene:light petroleum solution was washed with water and the organic phase was dried in a rotary evaporator. The resi-

due was transferred to a test tube with ethanol and dried under N_2 at 45°.

Chromatography. Column chromatography of the estrogens was accomplished on Sephadex-LH 20 with benzene:methanol (90:10). The neutral steroids, progesterone, and testosterone were also separated by column chromatography on Sephadex-LH 20 using hexane:benzene:methanol (90:10:10), as described by Carr *et al.* (9). The average recoveries of known amounts added to 20 replicates were (mean ± SE) 91 ± 8% for estrone, 72 ± 13% for estradiol, 87 ± 7% for progesterone, and 76 ± 9% for testosterone.

Radioimmunoassays. The method of Echternkamp and Hansel (10) was employed for the estrogen assays. The method described by Hixon *et al.* (11) was employed for progesterone radioimmunoassay and that of Shemesh and Hansel (1) was used for testosterone radioimmunoassay.

Statistics. The data were subjected to analysis of variance and least significant differences were determined by Tukey's hsd method (12).

Results and discussion. the effects of adding LH or $PGF_2\alpha$ to the incubation media on synthesis of testosterone by bovine follicular tissue are shown in Fig. 1. Incubation alone failed to cause an increase in testosterone ($P > 0.05$). Additions of LH and $PGF_2\alpha$ each caused increased testosterone synthesis ($P < 0.01$ and $P < 0.01$, respectively). Estradiol synthesis (Fig. 1) was also stimulated by addition of LH ($P < 0.01$), but synthesis due to addition of $PGF_2\alpha$ was not statistically significant ($P > 0.05$). Estrone synthesis was increased from 6 to 9 ng/g by addition of LH and from 6 to 10 ng/g by $PGF_2\alpha$, but neither increase was significant ($P > 0.05$). The effects of LH and PGF on *in vitro* synthesis of progesterone by bovine follicular tissues are also shown in Fig. 1. There was no increase in progesterone synthesis due to incubation alone, addition of LH, or addition of $PGF_2\alpha$ ($P > 0.05$).

These results show that bovine follicles at the 16th or 17th day of the estrous cycle contain appreciable testosterone (12.1 ng/g), estradiol (4.4 ng/g), and progesterone (623 ng/g). Both LH and $PGF_2\alpha$ stimulated

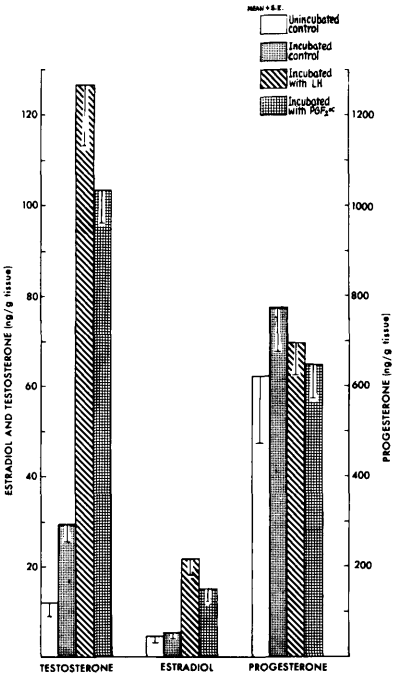

FIG. 1. Mean testosterone, estradiol 17β, and progesterone biosynthesis by bovine follicular tissue incubated alone and with added $PGF_2\alpha$ and LH. Vertical lines represent standard errors of the means.

the synthesis of relatively large amounts of testosterone and estradiol synthesis was stimulated by LH. Increased plasma estradiol levels noted in previous experiments following intrauterine administration of $PGF_2\alpha$ (11) and intraovarian administration of arachidonic acid (6), suggest that $PGF_2\alpha$ stimulates estrogen production by the follicle, even though the stimulated estradiol synthesis in this experiment was not statistically significant. The failure of LH or $PGF_2\alpha$ to stimulate *in vitro* progesterone synthesis may have reflected rapid conversion of progesterone into testosterone.

The high level of testosterone found and its stimulation by either LH or $PGF_2\alpha$ are in keeping with the high peripheral plasma testosterone levels found in the cow before the onset of estrus (1). Lindsay and Robinson (13) and Shemesh *et al.* (14) suggested the possible estrus-inducing activity of testosterone and other androgens. However, it is not clear whether testosterone per se, plays a role in the complete manifestation of estrus,

or whether it serves only as a precursor for estrogen. Recent evidence obtained for androgen-insensitive mice suggests that androgens may be needed for normal ovarian function (15). Rao (16) reported that testosterone and dihydrotestosterone enhanced [^{3}H]PGE_1 binding to bovine corpus luteum cell membranes, but estradiol-17β and estrone inhibited binding.

The precursors of the testosterone synthesized in response to LH and $PGF_2\alpha$ remain questionable. It is possible that the precursor is progesterone, since progesterone values did not increase concomitantly with the testosterone increase. On the other hand, one would expect a significant rise in progesterone biosynthesis, as demonstrated by Stoklosowa and Nalbandov (17) and others in rat follicular tissue cultures. It is possible that the Δ^4 pathway for testosterone synthesis through progesterone was activated, but one cannot rule out the possibility that the Δ^5 pathway for testosterone synthesis exists in the bovine follicle. Existence of the Δ^5 pathway could also explain the lack of progesterone stimulation by LH and $PGF_2\alpha$. YoungLai (18) suggested that Δ^5 androstene-3β, 17-diol may be a logical precursor in rabbit follicles. Stoklosowa and Nalbandov (17) reported that 10 ng of LH was able to cause maximal progesterone synthesis in rat follicular tissue cultures, while 10 times that amount of LH was required for maximal estrogen synthesis. The higher level did not increase the rate of progesterone synthesis. Thus, the high level of LH we used in the present study might explain the data obtained.

LH can terminate estrogen secretion and promote progestin secretion in the estrous rat (19). Thus, the high levels of LH used could have inhibited the aromatizing system. In the rat, the aromatizing system is located in the granulosa cells and is specifically stimulated by FSH (20). Moor *et al.* (21) indicated that whole sheep ovarian follicles less than 4.5 mm in diameter secreted considerable estrogen during the first 5 days of culture, but produced only small quantities of progesterone. Most follicles explanted at estrus produced only small amounts of estrogen *in vitro*, but synthetized large amounts of progesterone. *In vitro* studies indicate that follicles may contribute to preovulatory progestin secretion in the rat (17), and *in vivo* studies suggest that the same is true for the rabbit (22) and hamster (23).

Summary. Ovarian follicles isolated from bovine ovaries at the 16th and 17th days of the estrous cycle were minced, pooled, and incubated in Krebs–Ringer bicarbonate buffer at 37° for 2 hr. Samples were analyzed for testosterone, estrogens, and progesterone by radioimmunoassays. Incubation alone failed to cause an increase in testosterone (12.1 vs 29.3 ng/g of tissue; $P > 0.05$). Additions of 5 μg/ml of bovine LH or prostaglandin $F_2\alpha$ caused increases in testosterone synthesis to 125.0 ($P < 0.01$) and 103 ng/g ($P < 0.01$). Estradiol synthesis was also stimulated by LH ($P < 0.01$). However, stimulation due to addition of $PGF_2\alpha$ (5.1 to 15 ng/g) was not significant ($P > 0.05$). $PGF_2\alpha$ also caused a slight but nonsignificant increase in estrone secretion. Incubation failed to increase the progesterone concentration (623 vs 774 ng/g of tissue; $P > 0.05$). There was no increase in progesterone synthesis due to addition of either LH or $PGF_2\alpha$ ($P > 0.05$). The results show that bovine follicles produce much more testosterone than estrogen and that both LH and $PGF_2\alpha$ significantly stimulate testosterone synthesis in bovine follicular tissue without increasing net progesterone biosynthesis.

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