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Luteinizing Hormone and Androgens in the Bovine Fetus after Gonadotropin-Releasing Hormone[1] (38899)

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Anterior pituitaries from 180- and 260-day-old bovine fetuses synthesize luteinizing hormone (LH) *in vitro* (1). Furthermore, LH increased in serum of the ovine fetus after administration of purified porcine gonadotropin-releasing factor (2), and hypothalamic extracts or synthetic gonadotropin-releasing hormone (GnRH) caused release of LH and FSH from human fetal pituitaries *in vitro* (3, 4). Additionally, fetal testes are capable of synthesizing androgens from labeled acetate or progesterone (5–7). However, no reports relate the release of gonadotropins with androgen secretion in the bovine fetus. Therefore, the present study was designed to determine release of LH and the consequent synthesis of androgens in the bovine fetus after injection of GnRH. To further define the endocrine function of the bovine fetal testis, testosterone and androstenedione synthesis after LH stimulation *in vitro* was investigated.

Materials and Methods. Gestational age of the fetus was determined from the date of artificial insemination in 10 Holstein heifers. On day 120 of pregnancy each heifer was anesthetized with halothane and placed in lateral recumbency. Using routine laparotomy procedures, the uterine horn containing the fetus was exteriorized through a laparotomy, and a "uterine window" (8) was made to expose the fetal membranes and vessels. In this procedure, a 3- to 5-cm incision was made through an avascular intracaruncular area in the wall of the uterus. A small artery was freed from fetal membranes by blunt dissection; a polyethylene cannula (PE60)[3] was inserted 15 to 20 cm into the artery toward the fetus and secured with the end of the cannula in the umbilical artery. The placental artery was selected for cannulation to prevent direct contact with the fetus when collecting blood samples during the experiment. Also, fetal arterial blood could be sampled before passing through the placentomes.

Six female and four male fetuses were given GnRH[4] (10 μg in 0.5 ml of sodium citrate) rapidly through the arterial cannulae, allowing the GnRH to circulate through the placentome before reaching the fetal pituitary. Blood was collected from each fetus at 0, 15, 30, 60, 90, and 180 min after injection of GnRH into the fetus. Jugular vein blood was collected from dams coincidentally with fetal samples. After collection of each blood sample from fetus and dam, an equal volume of sodium citrate solution was replaced through the cannulae. Serum was stored at −20° until assayed for LH, testosterone, and androstenedione by specific radioimmunoassay (9, 10).

At the end of the experimental period each fetus was removed and survival during the experiment was verified by detection of a heartbeat. Fetal pituitaries were removed, weighed, and stored at −20° for LH assay.

In the second experiment, testicular parenchyma from 10 fetuses which averaged 146 days with a range from 62 to 280 days of gestation age as estimated by crown–rump length (11) were obtained at a local abattoir and cut into 1-mm explants within 30 min after collection. Explants (20–40 mg) were

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[4] Gonadotropin-releasing hormone was supplied by Dr. R. Rippel, Abbott Laboratories, North Chicago, IL.

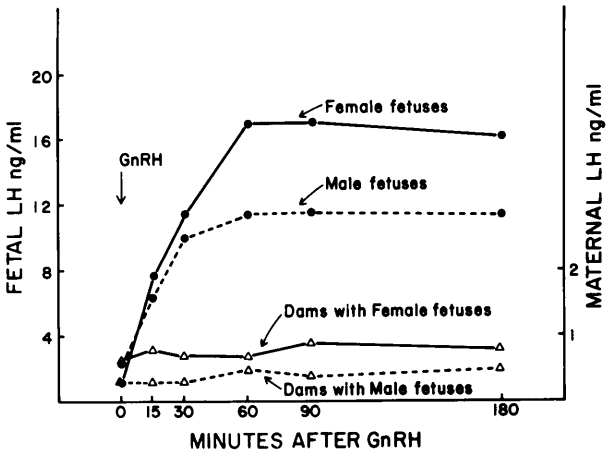

FIG. 1. Serum LH response in four male and six female fetuses and their dams. Synthetic gonadotropin releasing hormone (10 μg) was injected into the fetus.

placed into each of three 25-ml Erlenmeyer flasks containing 5 ml of Tissue Culture Medium 199 (Difco Laboratories, Detroit, MI) buffered with sodium bicarbonate. The contents of one flask were homogenized and immediately frozen. The tissue in the remaining flasks, one flask with and one flask without 500 ng NIH-LH-B8[5]/ml of medium, were incubated for 2 hr with gentle shaking in a 37° waterbath, and under an atmosphere of 95% O_2 and 5% CO_2. After the incubation, testicular explants were homogenized in the incubation medium and stored at −20° until assayed for testosterone and androstenedione (10). Net *in vitro* synthesis was calculated by subtracting the androgen concentration of the nonincubated tissues from those obtained for testicular explants after incubation. The differences in androgen concentrations between flasks with LH and those incubated without LH were attributed to LH stimulation.

In the first experiment, data were analyzed by the split-plot method (12) to account for repetitive measurements on individual fetuses. Selected contrasts were compared using multivariate T analysis (12). In the second experiment significant differences between treatments were determined by paired *t* analysis (13).

[5] NIH-LH-B8 supplied by the Endocrinology Study Section of the National Institutes of Health, Bethesda, MD.

Results. Serum LH (Fig. 1) for male fetuses averaged 2.3 ± 0.3 ng/ml prior to injection of GnRH, increased ($P < 0.01$) to 6.3 ± 0.8 at 15 min and peaked at 60 min (11.0 ± 1.0 ng/ml). Comparable values for the females were 1.0 ± 0.3, 7.7 ± 1.2, and 17 ± 3 ng/ml at 0, 15, and 60 min, respectively ($P < 0.01$). In both female and male fetuses, LH concentration was unchanged from 60 to 180 min after injection of GnRH. Over the entire period, the increase in serum LH was greater ($P < 0.07$) in female than in male fetuses.

In contrast to fetal LH, maternal serum LH did not change after GnRH treatment of the fetuses (Fig. 1). However, heifers with female fetuses averaged slightly greater serum LH concentrations than heifers with male fetuses throughout the experimental period.

Neither fetal pituitary weight (av 3.7 ± 0.3 mg) nor LH content (7.5 ± 2.1 μg) differed significantly between male and female fetuses. This failure to detect a difference in pituitary LH due to fetal sex is in agreement with our previous report (1).

Initially serum testosterone was higher ($P < 0.05$) in male fetuses than in female fetuses (averaging 630 ± 60 and 370 ± 60 pg/ml, respectively, Table I). Testosterone increased ($P < 0.05$) 2-fold in males during the 3-hr experimental period after injection of GnRH, but there was no change in

TABLE I. FETAL SERUM TESTOSTERONE AND ANDROSTENEDIONE AFTER INTRA-ARTERIAL GNRH[a]

Interval after GnRH (min)	Testosterone (pg/ml)		Androstenedione (pg/ml)	
	Male	Female	Male	Female
0	630 ± 60	370 ±60	740 ±220	840 ±200
15	620 ± 40	350 ±60	830 ±140	840 ±230
30	620 ± 50	340 ±60	780 ±200	620 ±130
60	930 ±150	340 ±60	900 ±280	870 ±230
90	980 ±170	380 ±70	860 ±200	480 ± 50
180	1260 ±310	370 ±70	810 ±200	370 ± 60

[a] Mean ± standard error, n = four (males) or six (females).

testosterone in females; this differential sex response caused a significant sex × time interaction ($P < 0.05$). The first significant increase in serum testosterone was at 60 min after GnRH and continued to increase until 180 min after GnRH when the last sample was collected. Androstenedione in serum from male and female fetuses was comparable.

After injection of GnRH into the male fetuses, a 2-fold increase ($P < 0.07$) in testosterone was observed in dams with male fetuses but not dams with female fetuses, causing a significant ($P < 0.05$) sex × time interaction (Table II). Maternal androstenedione did not change after injection of GnRH into fetuses. Although average testosterone (408 pg/ml vs 322 pg/ml) and androstenedione (460 pg/ml vs 315 pg/ml) appeared to be greater for dams with male than female fetuses, these differences were not significant.

Fetal testicular explants were incubated *in vitro* to determine the effect of LH on androgen synthesis. In the nonincubated testicular explants the concentrations of testosterone and androstenedione averaged 0.7 ± 0.4 and 1.7 ± 0.5 ng/mg, respectively. During a 2-hr incubation fetal testicular explants synthesized 0.6 ± 0.2 ng/mg of testosterone; however, LH significantly ($P < 0.05$) increased testosterone synthesis averaging 2.7 ± 0.8 ng/mg for testicular explants with added LH (Table III). Similarly, synthesis of androstenedione was significantly increased ($P < 0.05$) when LH was added to the incubation media.

Discussion. We have demonstrated that synthetic GnRH stimulates release of LH in the bovine fetus as early as 120 days of gestation. Others demonstrated that purified porcine gonadotropin-releasing hormone stimulates release of LH in the ovine fetus (2), and hypothalamic extracts or synthetic GnRH increased LH release by human fetal pituitaries *in vitro* (3, 4). Our observation of

TABLE II. MATERNAL SERA TESTOSTERONE AND ANDROSTENEDIONE AFTER GNRH TREATMENT OF MALE OR FEMALE FETUSES.[a]

Interval after GnRH (min)	Testosterone (pg/ml)		Androstenedione (pg/ml)	
	Male	Female	Male	Female
0	240 ± 80	280 ±80	350 ± 60	300 ±80
15	333 ±100	340 ±90	490 ± 40	300 ±40
30	400 ± 90	300 ±70	440 ± 40	310 ±60
60	490 ±100	320 ±90	520 ±100	333 ±60
90	510 ± 80	333 ±90	500 ±120	300 ±40
180	480 ±100	360 ±80	460 ±110	350 ±50

[a] Mean ± standard error, n = four (males) or six (females).

TABLE III. NET SYNTHESIS OF TESTOSTERONE AND ANDROSTENEDIONE BY BOVINE FETAL TESTES *IN VITRO*[a]

Androgen	LH added *in vitro*	
	0	2.5 μg
ng/mg	(ng/mg)	(ng/mg)
Testosterone	0.6 ± 0.2	2.7 ± 0.8
Androstenedione	0.3 ± 0.0	2.6 ± 0.6

[a] Mean ± standard error (n = 10) for fetuses at 146 ± 23 days of age. *In vitro* incubation of 1 mm^3 testicular explants were in TCM 199 for 2 hr at 37°.

higher basal LH in male fetuses is in contrast to the results in our previous study (14). However, LH release caused by GnRH in male fetuses was less than in female fetuses, consistent with negative feedback of fetal androgens on the fetal pituitary (14). Furthermore, serum LH in males was significantly lower than in females at 60 min after GnRH, coincidental with the first clear rise in serum testosterone concentrations for males.

The LH response observed after injection of GnRH into the bovine fetus is more sustained than those reported for adult males or females (15) which may suggest a slower clearance of LH or GnRH in the bovine fetus. But Foster *et al.* (2) found that the half-life of LH in the ewe was comparable to that in the fetus. If the half-life of LH in the bovine fetus is comparable to that of the adult, then GnRH stimulated LH release in the fetus for at least 3 hr because there was no evidence of declining LH at 3 hr after GnRH. The LH response in the present study resembled that seen after a brief exposure of adult bovine pituitary to GnRH in an *in vitro* superfusion system (15). Both studies suggest that the apparent biological effect of GnRH at the pituitary may be distinctly longer than the half-life of GnRH in the peripheral blood; approximately 4–7 min in adult human (16) and rats (17).

The absence of elevated concentrations of LH in maternal serum after GnRH was injected in the fetal circulation supports previous results (2) that LH was not transferred across the placenta. Additionally, GnRH may not cross the fetoplacental unit in sufficient quantities to cause maternal LH release. In our study, GnRH would first pass through the placentome before reaching the fetus. If the GnRH crossed quickly and quantitatively through the placentome to the dam, it would probably have caused increased serum LH, because 5 μg GnRH injected into yearling heifers caused a significant release of LH (15).

The results of this study suggest that testicular function can be regulated by LH secretion in the bovine fetus. Both the increased testosterone in the male fetuses and the testicular explants in response to LH support this view. Jost (18) concluded that pituitary gonadotropins were necessary for androgen secretion by the fetal testes and subsequent development of the male reproductive tract; however, the hormones were not quantified in his study. The source of higher testosterone in male fetuses is probably the testis, since testosterone did not increase in female fetuses after GnRH induced-LH release. Additionally, castration of the male monkey fetus reduced plasma testosterone relative to intact fetuses of the same age (19).

In contrast to previous studies in cows (10) and monkeys (20), we found no significant differences between testosterone and androstenedione concentrations of dams with male or female fetuses. In our study average androgen concentrations tended to be higher in dams with male fetuses relative to dams with female fetuses. This, together with the increased testosterone in dams coincidental with increased testosterone of male fetuses, suggest androgen leakage from fetal to maternal compartments.

Luteinizing hormone stimulated synthesis of both testosterone and androstenedione by bovine fetal testes during *in vitro* incubation. Failure to detect a significant increase in androstenedione *in vivo* may be due to epimerization of androstenedione to 17α-testosterone in the peripheral circulation (21). These enzymes required for conversion of androstenedione may not be present in a static *in vitro* system.

Summary. After an intra-arterial injection of 10 μg GnRH into four bovine male fetuses, serum LH increased ($P < 0.01$) 2-fold by 15 min and plateaued at approximately 11 ng/ml from 60 to 180 min. After GnRH in six female fetuses, LH increased ($P < 0.01$) 7-fold by 15 min and plateaued at approximately 17 ng/ml from 60 to 180 min. Maternal serum LH was not significantly influenced ($P > 0.05$) by sex of fetus or administration of GnRH to the fetus. After GnRH in male fetuses, serum testosterone increased ($P < 0.05$) and remained 2-fold greater than basal levels through 180 min. In contrast, serum testosterone from female fetuses averaged 370 $\pm$ 60 pg/ml at injection and was unchanged after GnRH.

Androgen synthesis was significantly increased during *in vitro* incubation of fetal testicular explants by the addition of LH. We conclude that GnRH causes release of LH from the bovine fetal pituitary as early as 120 days of age and that the testes respond with testosterone secretion after LH stimulation.

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