Bovine Fetal Pituitary Concentration and *In Vitro* Synthesis of Prolactin, GH, and LH[1] (36288)

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As early as 1929, Smith and Dortzbach (1) demonstrated gonadotropin and growth hormone (GH) activity in pituitaries obtained from fetal pigs, and prolactin activity was demonstrated in the fetal bovine pituitary (7, 8). More recently LH concentration in fetal pituitaries from man (2), sheep (3, 4), cattle (5), and swine (6) have been reported.

Meneghelli and Scapinelli (9), using immunofluorescent techniques, found that pituitaries from 100-day bovine fetuses contained GH acidophils which became more numerous with advancing fetal age. GH also increases with fetal age in pituitaries of rats (10) and sheep (11). In addition, GH, LH, and TSH are released from, and apparently synthesized by, human fetal pituitaries *in vitro* (12, 16). Evidence that fetal pituitaries are responsive to hypothalamic neurohumors was recently reported (4).

The objectives of this study were to quantify LH, GH, and prolactin in bovine fetal pituitaries at various ages and to evaluate the ability of the fetal pituitary to synthesize these hormones *in vitro*.

Materials and Methods. Thirty-four bovine fetuses were removed surgically at 90, 180, or 260 days of gestation. Pituitaries were removed after decapitation; and the posterior pituitary was discarded. Anterior pituitaries (AP) from 90-day fetuses were weighed and stored at −20° for hormone assay. Explants (2 or 3) for *in vitro* incubation were cut from 180- and 260-day AP; and the remaining AP tissue was weighed and stored at −20°. Combined weight of explants used for *in vitro* incubation averaged 2.7 mg. In all cases, incubation was initiated by 15 min after death of the pituitary donor.

Medium used for culture was TC 199 containing 0.224% $NaHCO_3$ and 0.064 mg/ml of penicillin-G-phosphate. Triangular stainless steel screen platforms (15 mm/side) overlayed with lens paper wicks supported the explants in disposable organ culture dishes (Falcon Plastics, Inc., Los Angeles, CA). One milliliter of medium placed in the center well just covered the top of the platform so that pituitary explants were exposed to the gaseous environment and yet received adequate nutrition from the culture medium. Explants were incubated for 72 hr at 37° under continuous gassing (300 ml/min) with 95% O_2 and 5% CO_2.

In preparation for hormone assay, pituitaries were minced on a wax block and homogenized by sonication (Sonifier Cell Disruptor, Model W185D, Heat Systems-Ultrasonics, Inc., Plainview, NY) in 2 ml of phosphate buffered saline (pH 7.0) at 4° at 60 to 80 W applied intermittently for a total of 3 min.

The double antibody radioimmunoassays for prolactin, LH, and GH were described (13–15). Pituitary homogenates and incubation media assayed at different dilutions formed curves parallel to the standard curves for GH, LH, and prolactin. The culture medium was tested in the assay procedure and did not affect the radioimmunoassays.

Hormone synthesis *in vitro* was calculated; postincubation pituitary hormone content plus media hormone content minus preincubation hormone content.

Results and Discussion. Average anterior

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TABLE I. Fetal Pituitary Concentration and *in Vitro* Synthesis of Luteinizing Hormone.[a]

Age (days)	Sex of fetus	No.	(ng/mg) Pituitary LH: Before incubation	Pituitary LH: After incubation	LH in media[b]	LH synthesis *in vitro* 72 hr[c]
90	Male	5	285 ± 93	—	—	—
	Female	8	347 ± 81	—	—	—
	Av		323 ± 59[d]	—	—	—
180	Male	5	413 ± 74	508 ± 197	561 ± 161	655 ± 169
	Female	5	535 ± 93	212 ± 85	534 ± 113	212 ± 144
	Av		474 ± 60	360 ± 113	548 ± 93	434 ± 128[e]
260	Male	6	609 ± 114	279 ± 78	603 ± 185	273 ± 230
	Female	5	446 ± 106	255 ± 61	332 ± 76	141 ± 153
	Av		535 ± 79	268 ± 49	480 ± 111	213 ± 138[e]

[a] Values are means ± standard errors.

[b] LH assayed in the culture medium was assumed to be released.

[c] LH synthesized *in vitro* was calculated as postincubation pituitary LH plus media LH minus preincubation pituitary LH.

[d] Less than comparable av at 180 and 260 days ($p < .01$).

[e] Greater than zero ($p < .05$).

pituitary LH (ng/mg) increased from 323 to 474, and to 535 at 90, 180, and 260 days of gestation, respectively ($p \cong .07$, Table I). Although pituitary LH concentration was 36% higher in males than in females at the end of the third trimester of pregnancy (260 days), differences in pituitary LH concentration due to sex of the fetus were not significant. Increasing pituitary LH with advancing fetal age agrees with data reported previously for swine (1, 6), sheep (3, 4), and cattle (5). Decreased LH release may account in part for increased pituitary LH in older fetuses because serum samples from these fetuses averaged 3.0, 1.3, and 0.8 ng/ml of LH at 90, 180, and 260 days, respectively (13). In agreement with results for fetal sheep (3, 4), our results also demonstrated greater pituitary LH concentration in males than females at 260 days pregnancy.

Average LH concentration of explants following *in vitro* culture was lower than for nonincubated pituitary tissue. Incubation media contained average LH concentrations of 548 and 480 ng/ml for 180- and 260-day fetuses, respectively (Table I). Pituitaries cultured *in vitro* synthesized significant ($p < .05$) quantities of LH, and net synthesis in 180-day pituitaries was greater than that in 260-day fetuses (434 vs 213 ng/ml). But differences in LH synthesis *in vitro* due to sex of pituitary donor were not significant. We believe this is the first demonstration of LH synthesis by bovine fetal pituitary tissues *in vitro*; it agrees with results reported for human fetal pituitary cell cultures (12).

Fetal pituitaries contained considerably more GH (Table II) than LH (Table I) or prolactin (Table III); 4.2, 8.9, and 18.1 μg/mg at 90, 180, and 260 days of gestation, respectively. The increases from 90 to 180 days and from 180 to 260 days were significant ($p < .01$). Pituitary GH for male and female fetuses were similar at 90 and at 180 days, but males averaged (12.1 μg/mg) less than females (25.3 μg/mg) at 260 days ($p < .05$). Increased pituitary GH content with advancing fetal age was previously reported for rats (10), sheep (11), and cattle (9). In contrast to LH, serum GH in the same fetuses (42, 65, and 103 ng/ml at 90, 180, and 260 days pregnancy, respectively) increased with fetal age (13). Correlations of crown–rump length with pituitary GH concentration

TABLE II. Fetal Pituitary Concentration and *in Vitro* Synthesis of Growth Hormone.[a]

Age (days)	Sex of fetus	No.	Pituitary GH (μg/mg) Before incubation	Pituitary GH (μg/mg) After incubation	GH in media[b] (μg/mg)	GH synthesis *in vitro* 72 hr[c] (μg/mg)
90	Male	5	4.1 ± 1.5	—	—	—
	Female	8	4.2 ± 0.8	—	—	—
	Av		4.2 ± 0.7[d]	—	—	—
180	Male	5	8.9 ± 1.9	2.1 ± 0.7	7.1 ± 1.6	1.2 ± 2.6
	Female	5	8.9 ± 1.2	2.8 ± 1.1	23.3 ± 18.6	17.1 ± 19.1
	Av		8.9 ± 1.1[e]	2.4 ± 0.6	15.2 ± 9.2	8.7 ± 9.5
260	Male	6	12.1 ± 4.3	2.7 ± 1.4	6.8 ± 2.6	0.7 ± 2.5
	Female	5	25.2 ± 6.5[f]	10.7 ± 2.9[f]	13.5 ± 3.7	2.8 ± 4.8
	Av		18.1 ± 4.1	6.0 ± 2.1[g]	8.9 ± 2.4	1.7 ± 2.5

[a] Values are means ± standard errors.

[b] GH assayed in the culture medium was assumed to be released.

[c] GH synthesized *in vitro* was calculated as postincubation pituitary GH plus media GH minus preincubation pituitary GH.

[d] Less than comparable av at 180 and 260 days ($p < .01$).

[e] Less than comparable av at 260 days ($p < .01$).

[f] Greater than comparable av for male ($p < .05$).

[g] Greater than comparable av at 180 days ($p < .05$).

($r = .60$) and with serum GH concentration ($r = .57$) were significant ($p < .01$). Therefore, both synthesis and release of GH by fetal pituitaries increased with advancing age, but the increase in rate of synthesis must have exceeded the increase in rate of release to account for increased pituitary stores of GH.

Following 72 hr culture *in vitro*, GH concentration in explants was lower ($p < .05$) than for nonincubated tissues (Table II). The culture media contained substantial quantities of GH, suggesting that fetal bovine pituitaries may be capable of synthesizing GH *in vitro* similar to human pituitary explants (12, 16). However, because of large variations between animals within age and sex, calculated values for net synthesis of GH *in vitro* did not differ from zero ($p < .05$).

Fetal pituitary prolactin (Table III) increased ($p < .01$) from 72 to 2508 ng/ml as fetal age increased from 90 to 260 days. Fetal serum prolactin also increased with fetal age (13) suggesting increased synthesis and release of prolactin as pregnancy advances. To our knowledge, this is the first report of fetal prolactin measured at three stages of pregnancy, although others (7, 8, 11) have demonstrated prolactin activity in fetal pituitaries.

Fetal sex did not influence ($p < .05$) prolactin concentration in nonincubated pituitary tissue, but pituitary explants from female fetuses synthesized three times more ($p \cong .07$) prolactin *in vitro* than explants from males. Whether this represents differential exposure to gonadal steroids or development of hypothalamic control mechanisms, or both, remains to be tested. Significant ($p < .05$) quantities of prolactin were synthesized *in vitro*; averaging 24 μg/mg of anterior pituitary tissue/72 hr incubation. Prolactin synthesis by human fetal pituitaries in cell culture has been reported (17).

Compared with prolactin, relatively low net synthesis of GH and LH *in vitro* may indicate that hypothalamic stimulation is required for GH and LH synthesis as early as 180 days gestation. As with mature animals, prolactin synthesis by fetal pituitaries appears to be enhanced by removal of hypothalamic influence as evidenced by marked

TABLE III. Fetal Pituitary Concentration and *in Vitro* Synthesis of Prolactin.[a]

Age (days)	Sex of fetus	No.	(ng/mg) Pituitary prolactin: Before incubation	Pituitary prolactin: After incubation	Prolactin in media[b]	Prolactin synthesis *in vitro* 72 hr[c]
90	Male	5	85 ± 41	—	—	—
	Female	8	63 ± 32	—	—	—
	Av		71 ± 24[d]	—	—	—
180	Male	5	903 ± 510	2.9 ± 1.7	8.4 ± 2.5[f]	10.4 ± 3.0[f]
	Female	5	1397 ± 318	1.2 ± 0.4	37.8 ± 24.7	37.5 ± 24.0
	Av		1150 ± 295[e]	2.0 ± 0.9	23.1 ± 13.0	24.0 ± 12.5[g]
260	Male	6	2593 ± 615	1.6 ± 0.7	13.0 ± 3.3[f]	12.1 ± 3.5[f]
	Female	5	2408 ± 325	3.7 ± 0.7	35.2 ± 10.2	36.5 ± 10.0
	Av		2508 ± 351	2.6 ± 0.6	23.1 ± 5.9	23.2 ± 6.2[g]

[a] Values are means ± standard errors.

[b] Prolactin assayed in the culture medium was assumed to be released.

[c] Prolactin synthesized *in vitro* was calculated as postincubation pituitary prolactin plus media prolactin minus preincubation pituitary prolactin.

[d] Less than comparable av at 180 and 260 days ($p < .01$).

[e] Less than comparable av at 260 days ($p < .01$).

[f] Less than comparable av for females ($p < .05$).

[g] Greater than zero ($p < .05$).

synthesis *in vitro*.

Summary. Luteinizing hormone, GH, and prolactin were quantified by radioimmunoassay in 34 fetal bovine pituitaries. Pituitary LH concentration increased with fetal age from 323 to 474, and to 535 ng/mg at 90, 180, and 260 days, respectively. Pituitaries cultured *in vitro* synthesized significant quantities of LH. Differences in LH synthesis due to sex or age of pituitary donor were not significant. Fetal pituitary GH increased during gestation (4.2, 8.9, and 18.1 μg/mg at 90, 180, and 260 days, respectively). GH for male and female fetuses was similar at 90 and 180 days, but pituitaries from males contained less GH (12.1 μg/mg) than females (25.3 μg/mg) at 260 days. Growth hormone was not synthesized *in vitro* in significant quantities. Fetal pituitaries contained increasing quantities of prolactin (72, 1150, and 2508 ng/mg for 90, 180, and 260 days, respectively) with advancing fetal age, and synthesized *in vitro* more prolactin than LH. Pituitaries from female fetuses synthesized three times more prolactin than males at 180 and 260 days.

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