Pregnancy, parturition, and lactation in hypophyseal stalk-transected beef heifers

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Abstract

Progesterone secretion is crucial for maintaining pregnancy to parturition in mammalian species, and in cattle the corpus luteum is the primary source of this hormone. This study determined the roles of prolactin (PRL), growth hormone (GH) and luteinizing hormone (LH) in the luteotropic process in beef heifers hypophyseal stalk-transected (HST, n = 7) or sham operated (sham operated controls, SOC, n = 9) during midgestation. The main finding was that endogenous PRL and GH maintained progesterone secretion in HST heifers in a similar manner to that in SOC throughout pregnancy. Serum PRL averaged 37 vs 187 and GH 2 vs 4 ng/ml in HST heifers compared with SOC, whereas LH abruptly decreased to undetectable levels after HST compared with a modest 0.4 ng/ml in SOC heifers. The second finding was that parturition and lactation occurred in HST heifers with calf delivery induced to occur at the same time as SOC. Milk production in HST animals was severely limited, and postpartum estrus obliterated compared with SOC. The suckling stimulus sustained milk ejection in HST heifers in spite of diminished PRL, GH, thyroid stimulating hormone, thyroxine and tri-iodothyronine secretion. The results suggest that PRL, GH and possibly placental lactogen are luteotropic during pregnancy in cattle.

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Introduction

Progesterone secretion is crucial for maintaining pregnancy to parturition in mammalian species (Rothchild 1965, 1981, Bazer & First 1983). Ovarian production of progesterone is required for at least 200 days of the approximate 280-day gestation in cattle; ovariectomy at 48–117 days causes abortion within 96 h whereas ovarian removal at 139–268 days results in fewer delivered living calves and 100% retained fetal membranes (Tanabe 1966, Estergreen et al. 1967, Chew et al. 1979). Calving difficulties, including uterine inertia and partial cervical dilation are common. In sheep ovariectomized at day 50, pregnancy is maintained to term (day 150), whereas in goats, another ruminant species, ovariectomy causes abortion (Rothchild 1965, Bazer & First 1983). The corpus luteum is the major source of progesterone during pregnancy in cattle; ovariectomy abruptly decreases blood plasma progesterone to <1 ng/ml (Chew et al. 1979). Progesterone, essential for maintaining pregnancy in ovariectomized cows, may be as low as 1.0–1.5 ng/ml through day 251 (Chew et al. 1979). After day 256, a modest increase in progesterone to 2.5 ng/ml in ovariectomized cows indicates an extraovarian source probably from the maternal adrenal glands and placenta, as well as from fetal gonads and adrenal glands.

Prolactin (PRL) and luteinizing hormone (LH) play pivotal roles in the luteotropic process, and progesterone produced by the corpus luteum might function as a universal luteotropic hormone by controlling its own production through an autocrine mechanism (Rothchild 1991a, Rothchild 1996). The role of pituitary PRL in corpus luteum function of the pregnant rat is well established by not only maintaining progesterone secretion but also stimulating, in synergy with estradiol, protein synthesis and luteal cell hypertrophy (Rothchild 1981, Gibori 1993). In contrast, the role of LH is transient in rat corpus luteum survival during the switch from dependency on adrenohypophyseal PRL to PRL and PRL-like hormones of decidual and placental origin. A luteal microsomal 32 kDa phosphoprotein, a PRL receptor associated protein (PRAP), is expressed in the corpus luteum of the rat, mouse, hamster, cow, pig, and human (McLean et al. 1990, Parmer et al. 1992, Duan et al. 1997). Coexpression of PRL-receptor long (PRL-Rl) and short (PRL-Rs) forms is elevated during pregnancy with large...
luteal cells expressing the bulk of PRL-R (Kelly et al.
tissues contain two transcripts for the PRL-R, a short form
that includes an additional 39 bases at a position identical
to the deviation from the long form found in rodents and
sheep (Schuler et al. 1997). Differences in size between
the bovine PRL-R and receptors in other species may
relate partly to glycosylation and post-translational modifi-
cations that alter ligand affinity. Although bovine placental
lactogen (bPL) can activate transcription through the
extracellular domain of the homologous bPRL-R, bPRL
exhibits greater binding affinity with bPRL-R than does
bPL (Tchelet et al. 1995).

In sheep, luteal receptors for LH, but not the circulating
concentration of LH, correlate with serum progesterone
concentration throughout the estrous cycle (Niswender
et al. 1985). In contrast, PRL injected for prolonged
periods maintained corpora lutea in hypophysectomized-
hysterectomized ewes and in hypophyseal stalk-transected
(HST)-hysterectomized ewes by maintaining endogenous
PRL plasma concentration, whereas LH was undetectable
(Denamur et al. 1966, Kann & Denamur 1973). In cattle,
our evidence indicates that the corpus luteum develops
and secretes progesterone after HST early (day 2) in the
estrous cycle (Anderson et al. 1969). Progesterone peaks
at day 12 and decreases to a low level by day 20, but
these HST beef heifers remain anovulatory thereafter.
Progesterone secretion continues beyond 48 days in
HST-hysterectomized heifers (Anderson 1968, Anderson
et al. 1969).

This study focuses on the role of PRL, growth hormone
(GH) and LH in corpus luteum function during pregnancy
in HST beef heifers. The results show that pregnancy can
be maintained to normal term in HST cattle in which
endogenous PRL, GH and possibly bPRL secretion was
maintained while LH was undetectable.

Materials and Methods

Animals and surgery

Crossbred (Hereford × Aberdeen Angus) heifers 15–
30 months old and weighing 240–410 kg were bred by
artificial insemination. The day of breeding was designated
day 0.

The heifers were hypophyseal stalk-transected (HST,
n=7) during midgestation by a supraorbital approach that
we have described previously (Anderson & Oxenreider
1967). Briefly, anesthesia was induced by i.v.-injected
thiopental sodium (11 mg/kg body weight, Abbott
Laboratories, North Chicago, IL, USA) for intubation
with an inflatable endotracheal catheter. The heifers were
maintained on a closed-circuit system of halothane (2–4%,
Ayerst Laboratories, Rouses Point, NY, USA) and oxygen
(800–1800 ml/min) and suspended in ventral recumbency
by canvas belts. An animal head restrainer (Anderson &
Girard 1985), attached to the front of a cattle squeeze
chute, permitted the head to be raised, lowered, tilted and
turned to the desired position for neurosurgical interven-
tion. Cortisone acetate (100 mg, Merck & Co., Inc., West
Point, PA, USA) was i.m.-injected before surgery was
begun, and 20% mannitol (Abbott) was i.v.-infused for
20 min immediately preceding the opening of the dura
mater and the lifting of the left cerebral hemisphere
to expose the hypophyseal stalk. Surgical intervention
required 5–6 h. After the hypophyseal stalk was severed
by dissection with spherical-tipped platinum probes, a nylon
disc (9.5 mm diameter and 0.45 mm thickness) was
inserted between the severed ends of the tubular stalk
to prevent vascular regeneration. Sham operation (sham
operation controls, SOC, n=9) included all surgical
procedures except transection of the stalk. After recovery,
all heifers were maintained under pasture conditions.

Heifers and day of pregnancy for HST were as follows:
heifer 66, day 138; heifer 73, day 143; heifer 33, day 149;
heifers 71 and 74, day 161; heifer 70, day 200; heifer 40,
day 201. Sham operations were performed on days 140,
145, 159, and 242. Anterior vena cava blood was with-
drawn every fourth day beginning on day 100 of preg-
nancy and continuing through day 330. Blood was
collected from two SOC heifers at 20-min intervals
throughout 24 h on days 273 and 278 of pregnancy. Blood
was cooled on ice, allowed to clot at 15 °C, and then
centrifuged at 5 °C for 20 min at 1500 × g. Serum was
stored frozen (−20 °C) for hormone assays.

Hormone RIA

Progesterone RIA was identical to that described by
Anderson et al. (1979) with the exception of the extraction
procedures. Serum aliquots (200 µl) of each unknown, in
duplicate, were added to two tubes without tracer and
one tube containing dried tracer (5000 c.p.m.; 3H-
progesterone; 97-0 Ci/mmol; New England Nuclear-
Dupont, Boston, MA, USA) to determine extraction
efficiency. Two milliliters benzene-hexane (1:2) were
added to all tubes and each tube shaken vigorously for 30 s,
then placed on dry ice to freeze the aqueous phase. The
organic phase of the extracts containing progesterone was
decanted into scintillation vials whereas the extracts from
the remaining two aliquots of each unknown were de-
canted into assay tubes and dried for subsequent RIA.
Preliminary experiments revealed little variance in pro-
cedural losses (94-6 ± 0.9% extraction efficiency); thus,
the mass of progesterone determined in each unknown
was corrected for average loss of tracer. Specificity of
progesterone antibody (GDN 337) used in this investiga-
tion has been described previously (Niswender 1973).
Assay sensitivity was 50 pg/tube. The interassay and
intra-assay variabilities for progesterone were determined
from replicates of a peripheral serum pool from mid-
gestation SOC heifers.
Interassay and intra-assay coefficients of variation were 11.7% (n=28) and 8.5% (n=6) respectively.

The estradiol-17β (17β E₂) and estrone (E₁) RIA was a modification of the procedure of Wu and Lundy (1971) to allow a more sensitive determination of estradiol in ovine and bovine serum. Three thousand d.p.m. ³H-estradiol-17β (114 Ci/mmol, New England Nuclear-DuPont) was added to 2 ml serum to facilitate the determination of procedural losses. The samples were extracted twice with 3 vol double-distilled benzene, and the final benzene extract washed twice with 0.1 vol deionized water to remove any water-soluble contaminants. Following each extraction, an aqueous–organic solvent phase separation was achieved by centrifugation at 500 × g for 10 min and the organic solvent removed by aspiration. Column chromatography and RIA of the extracts was exactly as described by Wu and Lundy (1971). Validation of this procedure with serum from ewes and cows has been published previously (Koligean & Stormshak 1977).

Assay sensitivity, defined as E₁ or 17βE₂ standard that yielded 95% of radioactive counts in buffer control tubes, was about 2 pg. Intra-assay coefficients of variation for E₁ and 17βE₂ were 3.0 and 2.9% respectively. Interassay coefficients of variation for E₁ and 17βE₂ were 7.1 and 9.5% respectively.

LH was measured in 100- to 300-µl aliquots of serum, in duplicate, by using highly purified bovine LH (bLH, NIH, Bethesda, MD, USA) for labeling with ¹²⁵I (IMS 30, Amersham Corp., Arlington Heights, IL, USA) and for standards (36 pg to 20 ng) similar to procedures described previously (Niswender et al. 1969). After dilution of ovine LH antiserum (GDN #15, 1:40 000) with 1:400 normal rabbit serum (NRS) (Antibodies Inc., Davis, CA, USA), 200 µl were added to each assay tube containing the serum unknown and PBS; the samples were incubated for 24 h at 4 °C, and ¹²⁵I-bLH was added and incubated for 24 h at 4 °C. Two hundred microliters of a 1:45 dilution of anti-rabbit γ-globulin (#130330 produced in goat; Cappel, Organon Teknika Corp., West Chester, PA, USA) were added and incubated for 72 h at 4 °C; the assay tubes were centrifuged, liquid was decanted, and the radioactivity of the precipitate was determined in a γ scintillation spectrometer. Assay sensitivity, defined as the amount of hormone that yielded 90% of optimum binding, was 0.28 ng/ml. Intra-assay and interassay coefficients of variation were 4.9 and 9.4% respectively.

GH was measured in 100-µl aliquots of serum in duplicate using highly purified bGH (USDA-bGH-I-1, 3.2 IU/mg) for labeling with ¹²⁵I (Amersham Corp.) by the Chloramine T method, highly purified bGH (Dr C H Li, University of California, Berkeley, CA, USA) for standards (0–125–2 ng), and incubation at 4 °C for 72 h by procedures similar to those we described previously (Trenkle 1976). Assay sensitivity was 0.125 ng/tube. Intra-assay and interassay coefficients of variation were 3.5% and 11.2% respectively.

Thyrotropin (TSH) was measured in 200-µl aliquots of serum, in duplicate, using highly purified bovine TSH (bTSH, 30–40 IU/mg, Dr J G Pierce, University of California, San Francisco, CA, USA) for labeling with ¹²⁵I (IMS 30, Amersham Corp.) by the Chloramine T method, purified bTSH (21 IU/mg, NIH) for standards (0–150–50 ng), and incubation at 4 °C for 72 h by procedures similar to those described previously (Borger & Davis 1974). Ovine TSH antiserum (anti-oTSH, Dr S L Davis, Oregon State University, USA) was diluted with 1:400 NRS and then preabsorbed with follicle-stimulating hormone (FSH) (NIH-FSH-B1) and LH (NIH-LH-B8) to remove non-specific antibodies that react with these gonadotropins. PBS-1% BSA (pH 7. to 500 µl) and anti-oTSH (1:80 000, 200 µl) were added, and the assay tube was incubated for 24 h. Then 100 µl ¹²⁵I-bTSH were added, incubated for 24 h followed by the addition of anti-rabbit γ-globulin produced in goats (#130330, Cappel, Organon Teknika), and incubated for a further 72 h. To each assay tube 2-5 ml PBS (pH 7) were added, centrifuged, the supernatant decanted, and the precipitate counted for 2 min in a γ scintillation counter. Assay sensitivity ranged from 0.3–1.0 ng/tube. Intra-assay and interassay coefficients of variation were 6.4% and 9.8% respectively.

Thyroxine (T4) was measured using T₄¹²³I immunoassay procedures described by Chopra et al. (1971a). Serum samples of 20 µl in duplicate were assayed with T₄ standards ranging from 2–32 µg/dl. Assay sensitivity was 2.5 ng/ml. Intra-assay and interassay coefficients of variation were 3.6% and 8.2% respectively.

Tri-iodothyronine (T3) was measured by using T₃¹²³I immunoassay procedures described by Chopra et al. (1971b). Serum samples of 100 or 150 µl in duplicate...
were assayed with T3 standards ranging from 25 to 800 ng/dl. Assay sensitivity was 4.5 ng/dl. Intra-assay and interassay coefficients of variation were 2.5% and 6.0% respectively.

**Parturition**

HST and SOC heifers were closely monitored near the time of expected parturition (day 280 in this herd). With onset of labor, manual assistance was given when required. In animals showing no signs of spontaneous delivery, parturition was induced by i.m. injection of dexamethasone and subsequent oxytocin treatment to ensure safe delivery of a calf, or by cesarean section.

**Lactation and milk composition**

Calves were allowed to suckle their dams throughout 30 weeks. Milk production by HST and SOC heifers was determined at weekly intervals. Calves were separated from their dams for a 24-h period, and the cow was milked twice (0800 and 1600 h) during that period. Aliquots of milk (n=116) from these animals were analyzed for fat, protein, lactose, and total solids by absorption of infra red light, and the constituents expressed as percentage composition of whole milk.

**Histology**

Postmortem examination of each animal confirmed the completeness of stalk transection. The nylon disc was in the proper location and had prevented vascular regeneration of the stalk in each heifer. Pituitary glands from HST and SOC heifers were cut transversely and fixed in Susa’s solution for histological evaluation. Coronal sections of the glands were cut at 6 µm and stained with performic acid-Alcian blue-periodic acid-Schiff-orange G by the method of Heath (1965), whereas other sections were stained with hematoxylin and eosin. The thyroid gland was transected from the middle of the left lobe and fixed for 4 h in Bouin’s fluid (35 °C); picric acid was removed by several changes of 70% ethanol containing saturated lithium carbonate. The tissues were stored in 70% ethanol, dehydrated, embedded, and cut at 7 µm. One set of sections was stained with hematoxylin and eosin, and another with Mallory triple stain.

**Statistical analysis**

Experimental units in this study were the individual heifers, each assigned to treatments at random. Least-squares analyses were based upon a weighted average of sample variance for experimental and control groups. Paired comparisons of treatment means and analyses of covariance were used where appropriate (Snedecor & Cochran 1980). Hormone data were analyzed by a split-plot analysis using a one-way analysis of variance, and Student’s t-tests for continuous variables were used for comparisons between groups (SAS 1997). Data are presented as geometric means ± s.e.m. and statistical significance was concluded when P<0.05.

**Results**

**Pregnancy and parturition after HST**

Six of seven heifers HST during midgestation delivered living calves (Table 1). Three of the heifers in the control group died within 3 days after surgery and were excluded from the study; pregnancies were maintained to term in 9 of 9 remaining SOC heifers. There was no evidence of onset of labor in 4 HST heifers, and parturition was induced in these animals by i.m. injection of dexamethasone, followed approximately 30 h later with an i.v. injection of oxytocin (Tables 1 and 2). Delivery required no assistance in 8 of 9 controls, but cesarean section was necessary in one SOC heifer.

**Lactation and postpartum estrus after HST**

Lactation was maintained in both HST and SOC (Table 1) animals. Calves were allowed to suckle their dams from birth through 7 months of age. The calf from HST heifer 71 died soon after delivery, and the mammary glands involuted. Although a living calf was delivered by cesarean section from one SOC heifer, she refused to allow the calf to suckle, and the mammary glands involuted. None of the HST heifers exhibited a postpartum estrus during periods exceeding 300 days (Table 2). SOC heifers returned to estrus within 2 months after parturition.

**Calf performance and milk production**

Birth weight of calves delivered from HST heifers was similar (P>0.05) to that produced by SOC (Tables 1 and 2) heifers. By 100 days after birth, body weight and growth rate of calves from HST heifers were less (P<0.001, P<0.025 respectively) than in calves from SOC. Limited neonatal growth of calves born to HST heifers resulted primarily from decreased milk production by the dams. In calves from HST dams, accelerated growth occurred after they began eating grain and roughage, and before puberty, these calves attained body weights similar to those of calves born to SOC. Milk production was less (P<0.001) in HST heifers compared with that in SOC in the first week postpartum (Fig. 1); paired comparisons indicated reduced (P<0.001) milk secretion in HST compared with SOC animals throughout the 30 week lactation (t=8.87, d.f.=58). There was a gradual decrease in milk secretion during the 30 weeks in SOC (Y=5688–122X) and HST heifers (Y=2418–37X) (Y=milk

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pregnancy failed.

were present in all stages of pregnancy. Ovarian follicular
ovarian follicles (Fig. 2a). Duration of pregnancy was similar (P<0.05) in HST (280–293 days) and SOC (281–295 days) heifers. In HST heifer 73, progesterone averaged 0.025 ng/ml (± S.E.M.) from day 100 to day 236; pregnancy failed. Dexamethasone i.m. followed by oxytocin i.v. at time of delivery. §Calf died within 5 min of delivery.

secretion; X=weeks). The regression coefficients of milk secretion rate differed (P<0.001, F=78), and adjusted means were markedly different (P<0.001, F=58) in HST and SOC animals. The decrease in milk secretion in HST heifers and SOC was correlated during 30 weeks of lactation (r=0.79, P<0.01). There was no significant difference in milk composition for percentage fat, protein, α-lactose, and total solids in HST and SOC heifers (Table 3).

Ovarian function, and progesterone, E₁, 17βE₂ and prolactin secretion

Ovarian follicles regressed soon after HST; only very small follicles (<4 mm in diameter) were detected by rectal palpation during pregnancy and postpartum. In SOC, ovarian follicles (>5 mm in diameter; range 5–18 mm) were present in all stages of pregnancy. Ovarian follicular growth occurred within 30 days after parturition and in 4 of 9 SOC estrus, ovulation, and corpus luteum formation occurred by 45 days postpartum. Corpus luteum diameter (range, 15–24 mm) remained similar during pregnancy in HST and SOC heifers. The secretory activity of the corpus luteum was maintained after HST as indicated by serum progesterone concentration which was similar (P>0.05) to SOC from day 100 through parturition (Fig. 2a). Near the onset of parturition, progesterone concentration decreased in both HST and SOC heifers, and during lactation it remained at basal to nondetectable levels until day 330 (Fig. 2a). Duration of pregnancy was similar (P>0.05) in HST (280–293 days) and SOC (281–295 days) heifers. In HST heifer 73, progesterone averaged 0.9 ng/ml from day 100 to day 236; pregnancy failed in this animal.

A coincident steady increase in E₁ and 17βE₂ concen-

Table 1 Pregnancy, parturition, lactation and calf development in individual HST and SOC beef heifers

<table>
<thead>
<tr>
<th>HST</th>
<th>SOC</th>
<th>Duration of pregnancy (days)</th>
<th>Type of delivery</th>
<th>Lactation during 30 weeks</th>
<th>Calf</th>
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<tbody>
<tr>
<td>40</td>
<td>44</td>
<td>201</td>
<td>289</td>
<td>Induced††</td>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>76</td>
<td>70</td>
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<td>280</td>
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<td>34.02</td>
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<td>74</td>
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<td>33</td>
<td>164</td>
<td>149</td>
<td>283</td>
<td>Unassisted</td>
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<tr>
<td>73</td>
<td>66</td>
<td>143</td>
<td>—§</td>
<td>—</td>
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</tr>
<tr>
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<td>287</td>
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<td>285</td>
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<td>140</td>
<td>283</td>
<td>Unassisted</td>
<td>29.48</td>
</tr>
</tbody>
</table>

*Progesterone concentration in peripheral blood serum from 35 bleedings at 4-day intervals averaged 0.9 ± 0.10 ng/ml (± S.E.M.) from day 100 to day 236; pregnancy failed. †Dexamethasone i.m. followed by oxytocin i.v. at time of delivery. §Calf died within 5 min of delivery.

Table 2 Pregnancy, parturition, lactation and calf development in HST and SOC beef heifers. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Pregnancy duration (days)</th>
<th>Number of heifers</th>
<th>Calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>HST 286 ± 1.9</td>
<td>4 of 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 of 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 of 7§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;300</td>
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</tr>
<tr>
<td></td>
<td>67 ± 14</td>
<td>6</td>
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<tr>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33 ± 2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>88 ± 8.9b</td>
<td>0.54 ± 0.06*</td>
</tr>
<tr>
<td></td>
<td>36 ± 1.5</td>
<td>121 ± 7.9</td>
</tr>
<tr>
<td></td>
<td>0.85 ± 0.08</td>
<td></td>
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</tbody>
</table>

§Pregnancy failed after surgery in one heifer. †Cesarean. *P<0.025, bP<0.001 vs SOC.
an abrupt decrease to <10 pg/ml during early lactation (Fig. 2b and c). The results indicate that E1 and 17βE2 are primarily of placental origin during the latter half of gestation in cattle. Furthermore, HST does not disrupt placental production of these estrogens.

PRL averaged 156 ng/ml before surgery, decreased ($P<0.01$) by 16 days after HST, and remained at 37 ng/ml throughout the latter half of pregnancy (Fig. 2d). In SOC, PRL concentration remained similar (187 ng/ml; $P>0.05$) during the last half of gestation to that seen before surgery. PRL concentration remained consistently lower ($P<0.01$) in HST than SOC heifers. During the first 40 days of lactation, PRL concentration averaged 37 ng/ml in HST heifers and was significantly less ($P<0.001$) than in SOC (Fig. 2d).

**TSH, T4, T3, GH and LH secretion**

Before surgery, TSH averaged 23 ng/ml (Fig. 3a). After HST, TSH decreased ($P<0.05$) and remained consistently lower than in SOC throughout the remainder of gestation and during early lactation. T4 averaged 95 ng/ml before surgery and abruptly decreased ($P<0.01$) soon after HST, whereas in SOC it remained unchanged (Fig. 3b).

Although T4 concentration in SOC gradually decreased during late gestation, during early lactation it was consistently higher than in HST heifers. T3 averaged 158 ng/dl before surgery and decreased ($P<0.01$) after HST compared with SOC (Fig. 3c). Although a transient increase in T3 occurred in HST heifers in late gestation, T3 levels decreased ($P<0.05$) during early lactation compared with levels in SOC heifers.

GH averaged 2–5 ng/ml before surgery, but it decreased ($P<0.05$) soon after HST and remained consistently lower than in SOC throughout the latter half of gestation (Fig. 3d). Likewise, GH concentration remained lower ($P<0.05$) in HST than in SOC heifers during early lactation.

LH decreased ($P<0.01$) abruptly after HST to undetectable levels throughout the remainder of gestation.
Hypothalamic regulation of pregnancy in cattle  ·  L L ANDERSON and others

Figure 3 Concentration of (a) TSH, (b) T4, (c) T3, and (d) GH in peripheral serum of beef heifers before (○) and after HST (●) and SOC (■) during pregnancy, parturition and lactation. The number of heifers is indicated in parentheses. Values are means ± s.e.m.

In SOC animals, LH was maintained at only 0.3–0.9 ng/ml, but this was greater (P < 0.01) than in HST heifers. LH remained consistently low and frequently was at undetectable levels; there was a complete absence of episodic secretion throughout 24 h during late pregnancy in two SOC heifers (Fig. 4). Although LH increased (P < 0.001) by the 3rd week of lactation in SOC, the modest amount (<0.39 ng/ml) was insufficient to induce ovulation at this early stage of lactation.

At death, the pituitary gland weight of HST heifers was 35% (P < 0.01) that of SOC animals (1.19 ± 0.10 vs 3.20 ± 0.19 g). Pituitary gland weight expressed as g/100 kg body weight was 0.19 ± 0.02 in HST compared with 0.55 ± 0.03 (P < 0.01) in SOC heifers. The severed ends of the hypophyseal stalk remained separated by the nylon disc in all HST heifers. The pituitary glands from HST heifers indicated persistence of PRL secretory cells in the same areas of the adenohypophysis as SOC (Fig. 5). In coronal sections of pituitary glands from HST and SOC heifers stained with performic acid–Alcian blue–periodic acid (PAS)–Schiff–orange G, acidophils, basophils, and chromophobes were present. Pituitary and thyroid histology in these HST and SOC heifers was similar to that observed after long-term growth in HST and SOC calves (Anderson et al. 1999).

Discussion

The main finding in this study was that progesterone secretion in HST beef heifers was maintained at a similar level to that seen in SOC throughout pregnancy. Ovarian production of progesterone is required to maintain pregnancy in cattle; ovariectomy causes an abrupt drop in circulating progesterone levels with abortion occurring in most cases (Tanabe 1966, Estergreen et al. 1967, Chew et al. 1979). Furthermore, the antiprogestin, RU 486, causes an abrupt decrease in progesterone and precisely induces premature parturition in cattle (Li et al. 1991b). Although PRL secretion in cattle is tonically inhibited by the hypotalamus and remains significantly greater during the first 14 days after HST than seen in SOC animals, circulating PRL concentration gradually drift lower but remains seasonally regulated (Benoit et al. 1989, Cho et al. 1998). A similar transient increase in PRL secretion occurs soon after hypothalamo-pituitary disconnection of ewes during anestrus and the breeding season (Thomas et al. 1986). HST beef calves had consistently lower serum PRL (5 ng/ml) compared with SOC (40 ng/ml), but both groups remained acutely sensitive to seasonal changes throughout the year with hormone concentrations peaking in summer and reaching a nadir in winter (Cho et al. 1998). Furthermore, haloperidol, a neuroleptic drug that blocks dopamine receptors on lactotropes in the adenohypophysis, and α-methyl-p-tyrosine, a drug that inhibits catecholamine synthesis in the hypothalamus by blocking activity of tyrosine hydroxylase, acutely increase PRL plasma concentration in cattle with greater peak release in summer than winter (Benoit et al. 1987). In this study, serum PRL was sevenfold greater (37 ng/ml) in HST beef heifers during pregnancy compared with prepubertal HST beef heifer calves (Cho et al. 1998), whereas LH decreased abruptly to undetectable levels after HST at midgestation. Thomas et al. (1986) also observed an abrupt decrease in both LH and FSH to undetectable levels within 2 days after hypothalamo-pituitary disconnection in sheep.

Dopamine may be involved in tonic regulation of PRL secretion in the rat, pig, sheep, cattle, and monkey based on elevated circulating PRL concentration after HST and acute stimulatory effects of haloperidol and α-methyl-p-tyrosine on PRL secretion (Kanematsu & Sawyer 1973, Kann & Denamur 1973, Diefenbach et al. 1976, Gibbs & Neill 1978, Langer et al. 1978, Spies et al. 1979, Anderson et al. 1982, Benoit et al. 1989, Cho et al. 1998). We were unable to show marked differences, however, in dopamine concentration of sequential samples of portal blood and peripheral blood collected during 4 h from Holstein heifers.
The maintenance of progesterone secretion by aging corpora lutea with daily PRL treatment in hysterectomized-hypophysectomized animals provides further evidence for PRL’s luteotrophic action (Rothchild 1981, Li et al. 1989). The placentae of rodent, human, primate and ruminant species secrete hormones structurally related to pituitary L.L. Anderson and others (1999) report that ovine PL is a non-glycosylated 22 kDa protein (Anthony et al. 1995b). Bovine PL (bPL), a glycosylated hormone produced by trophoblast binucleate cells only during pregnancy, and bovine GH from the anterior pituitary are members of the same gene family and have structural and functional similarities (Byatt et al. 1992a). Ovine PL and bovine PL can act through PRL-R and elicit PRL-like effects in ovine and bovine mammary gland and rat Nb2 lymphoma cells (Gertler et al. 1998). Bovine endometrium possibly contains unique PL receptors during pregnancy that differ from those of PRL and GH (Galosy et al. 1991). Bovine PL may act through this putative unique receptor, through the PRL-R and(or) through a heterodimer of the PRL-R and the GH receptor (GH-R) (J C Byatt, unpublished data). Unlike GH, interaction of PRL with its homologous receptors is transient but sufficient to activate signal transduction (Gertler et al. 1996). This may result from JAK2 kinase, a member of the Janus family of tyrosine kinases, which serves as an initial mediator for both receptors, and is already associated with lactogenic receptors before hormone binding-induced dimerization (Lebrun et al. 1994, Goupille et al. 1997). The existence of the homodimer is no longer required after the signal is initiated, and the activated tyrosine-phosphorylated JAK2 continues its enzymatic activity by docking and(or) phosphorylating down-stream proteins (Helman et al. 1998).

Bovine PRL receptors (bPRL-R) in bovine corpus luteum, mammary gland and liver have been measured (Saito & Saxena 1975, Poindexter et al. 1979, Scott et al. 1992). There is also evidence for bGH-R and bPRL-R transcripts in bovine extra-embryonic membranes and in the glandular uterine endometrium, but much lower levels of both receptor mRNAs in the caruncles (Kolle et al. 1997). Recombinant bPL (rbPL) treatment increased the size of bovine corpus luteum, increased concentrations of progesterone in plasma, and specifically bound to luteal membranes, whereas the responses of bovine corpus luteum to recombinant bovine GH (rbGH) were much less than to rbPL (Lucy et al. 1994a). Bovine corpus luteum and endometrium have a unique mRNA that hybridizes with a cDNA for bGH-R. The giant cells of

### Table 4

<table>
<thead>
<tr>
<th>Reproductive state</th>
<th>Day</th>
<th>LH (ng/ml)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Presurgery (n=12)</td>
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<tr>
<td>Pregnancy</td>
<td>40</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0.28 ± 0.01</td>
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<td></td>
<td>24</td>
<td>0.32 ± 0.04</td>
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<tr>
<td></td>
<td>16</td>
<td>0.29 ± 0.01</td>
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<td></td>
<td>8</td>
<td>0.33 ± 0.06</td>
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<tr>
<td></td>
<td>0</td>
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<td>16</td>
<td>0.31 ± 0.02</td>
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<td>24</td>
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<td></td>
<td>32</td>
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<td>56</td>
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<td>64</td>
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<td></td>
<td>72</td>
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<td>80</td>
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<td>88</td>
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<td></td>
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<tr>
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<td>112</td>
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<tr>
<td>Parturition</td>
<td>120</td>
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<tr>
<td>Lactation</td>
<td>128</td>
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<td>136</td>
<td>0.39 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>1.48 ± 0.60</td>
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ND, not detectable (<0.2 ng/ml).

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the bovine corpus luteum have been shown to be rich in GH-R message and to stain positively by immunohistochemistry for the presence of cell surface GH-R (Lucy et al. 1993, 1994a). The administration of rbGH increased both the size of the bovine corpus luteum and progesterone secretion; it also increased the progesterone response to gonadotropin-releasing hormone infusion in bGH-treated animals (Lucy et al. 1994b, Adriaens et al. 1995). Thus, the biological activity of these related hormones not only depends on receptor distribution and affinity of hormone for the receptor, but also on transmission of signal in response to binding (Scott et al. 1992, Anthony et al. 1995b). The affinity of bPL for its own putative receptor and receptors for PRL and GH indicate that this placental hormone may act via three receptor types (Lucy et al. 1994a). Differences in the primary structure of bPL compared with both bPRL and bGH present the possibility that bPL may participate in hormone–receptor dimers in a different manner to that of the pituitary hormones. Thus, the presence of a modified mRNA in corpus luteum may suggest that the bovine corpus luteum is a target for placental hormones related to bGH. Additionally, the placentae of ruminant ungulate species (cattle, sheep, goats, red deer, bison, moose, elk) produce pregnancy-associated glycoproteins, but these have no luteotropic action (Xie et al. 1991, Roberts et al. 1995, Xie et al. 1997, Garbayo et al. 1998).

The second finding in this study was that in beef heifers HST at midgestation (138–201 days), pregnancy continued, and parturition and lactation followed with calf delivery occurring at the same time as in SOC (286 days). Although HST heifers maintained pregnancy, hormonally induced parturition (dexamethasone and oxytocin) was required in most animals; milk production was severely limited during 30 weeks lactation, and postpartum estrus was obliterated compared with SOC. Ductal growth of the mammary gland is mostly regulated by ovarian and/or placental steroids, estrogen and progesterone (Cowie et al. 1966, Hart & Morant 1980). Mammogenesis in pregnant heifers was not affected by a dopamine antagonist, bromocriptine, administered throughout the last half of gestation, suggesting that a lactogen hormone of placental origin, bPL, can substitute for bPRL in mammary growth (Schams et al. 1984). Studies in dairy cows have demonstrated that bPL treatment increases milk production, suggesting that bPL may have GH-like galactopoietic actions (Byatt et al. 1992b, 1994). Long-term effects of bGH treatment in lactating cows suggest that bGH acts primarily by changing tissue responsiveness to homeostatic signals so that a greater proportion of nutrients is partitioned for increased milk yield (Adriaens et al. 1992). Although circulating concentrations of estrogen, progesterone, bGH, bPRL and possibly bPL were adequate to stimulate mammogenesis during the latter half of pregnancy in HST heifers in the present study, the markedly reduced milk production during lactation compared with SOC heifers suggests that decreased circulating concentrations of PRL and GH were the primary limiting factors.
Additionally, diminished TSH, T4 and T3 secretion probably contributed to the marked difference in milk production by HST heifers compared with SOC. The reduced lactational performance of HST heifers is also probably related to reduced mammary differentiation (lactogenesis) that is triggered during the periparturient period in large part by high levels of PRL (Akers et al. 1981a). Cattle have an absolute requirement for PRL to stimulate the onset of milk production, even though it is apparently not required for maintenance of lactation (Akers et al. 1981b). In HST heifers, cortisol release around parturition and thereafter, an important competence factor for lactogenesis, presumably was decreased and could at least partly explain the reduced milk production.

The suckling stimulus by calves sustained a milk ejection reflex in these HST heifers throughout lactation. We did not measure oxytocin in sera and thus cannot say whether hypothalamic oxytocin in HST cows was released in sufficient amounts for milk ejection in response to suckling. In the HST beef heifers, the prolonged period of suckling by the calves and sustained serum PRL and GH concentration probably maintained the consistent, yet modest, level of lactation. Growth rate of calves born to HST dams was significantly decreased compared with SOC primarily as a result of reduced milk production.

HST heifers remained anovulatory by blocking gonadotropin-releasing hormone (GnRH) secretion to pituitary gonadotropes, whereas postpartum estrus and ovulatory cycles resumed within 2 months in SOC animals. In a previous experiment, we found that circulating progesterone concentration was maintained for 43 days in a beef heifer HST at day 51; a living fetus was recovered at day 94 (Anderson et al. 1969). Although ovarian follicles abruptly regressed in HST heifers, the corpus luteum was maintained in a similar manner to that seen in SOC throughout pregnancy. Postpartum, corpus luteum regression was abrupt and ovarian follicular growth

**Figure 5** Histology of the adenohypophyses from two SOC and four HST beef heifers. Coronal sections (6 μm) are from the middle one-third of the anteromedial adenohypophysis. Acidophils with cytoplasm were dispersed in anteromedial regions of the gland in both groups of SOC and HST heifers. Acidophils are associated with somatotropes, lactotropes, and adrenocorticotropes. Basophils are associated with thyrotropes and gonadotropes. Chromophobes were evident throughout the adenohypophysis in SOC and HST beef heifers. Acidophils were characterized by positive staining reaction to orange G and were further differentiated by yellow (GH cell) and orange (PRL cell) granules in the cytoplasm. These representative histological sections indicate survival of adenohypophyseal cells in HST beef heifers, but the cytoplasm was less abundant and more intercellular space was evident in pituitary glands from HST compared with SOC heifers. Bar=50 μm. The number denotes the beef heifer ear tag identification.
arrested for at least 300 days in HST compared with SOC heifers.

The results from this study show that endogenous PRL, GH and possibly bPRL secretion maintained corpus luteum function and progesterone secretion in beef heifers HST at midgestation; LH decreased to undetectable levels. Furthermore, the HST heifers delivered live calves and sustained a modest lactation during 30 weeks of suckling by the calves. The decreased milk production in HST cows corresponded with significantly decreased serum concentrations of PRL, GH, TSH, T4 and T3, and presumably decreased cortisol release around parturition and lactation compared with that in SOC heifers. HST blocked GnRH-induced gonadotropin secretion with the cows remaining anovulatory for more than 300 days whereas estrous cycles had resumed in SOC animals by 2 months postpartum.

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