Role of the Central Nervous System in the Regulation of Pregnancy, Parturition and Lactation in Beef Heifers

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Summary

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 on (SOC, *n* = 9) during midgestation. The main finding was that endogenous PRL and GH maintained progesterone secretion in HST heifers similar to that in SOC throughout pregnancy. Serum PRL averaged 37 vs. 187 and GH 2 vs. 4 ng/ml in HST 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 and GH secretion. The results suggest that PRL, GH, and possibly placental lactogen are luteotropic during pregnancy in cattle.

Introduction

Progesterone secretion is crucial for maintaining pregnancy to parturition in mammalian species. 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 hours, whereas ovarian removal at 139–268 days results in fewer delivered living calves and 100% retained fetal membranes. Calving difficulties, including uterine inertia and partial cervical dilation, are common. The corpus luteum in the ovary is the major source of progesterone during pregnancy in cattle.

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. A luteal microsomal 32-kilodalton phosphoprotein, a PRL receptor associated protein (PRAP), is expressed in the corpus luteum of the rat, mouse, hamster, cow, pig, and human. Coexpression of PRL-receptor long (PRL-R_L) and short (PRL-R_S) forms is elevated during pregnancy, with large luteal cells expressing the bulk of PRL-R. Bovine 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 of the molecule found in rodents and sheep.

In cattle, our evidence indicates that the corpus luteum develops and secretes progesterone after HST early (day 2) in the estrous cycle. 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.

This study focuses on the role of PRL, GH, and luteinizing hormone (LH) in corpus luteum function during pregnancy in HST beef heifers.

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 previously described. Briefly, anesthesia was induced by intravenously-injected thiopental sodium (11 mg/kg body wt) for intubation with an inflatable endotracheal catheter. The heifers were maintained on a closed-circuit system of halothane (2–4%) and oxygen (800–1800 ml/min) and suspended in ventral recumbency by canvas belts. An animal head restrainer, 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 intervention. Cortisone acetate (100 mg) was intramuscularly-injected before surgery was begun, and 20% mannitol was intravenously-infused for 20 minutes 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 hours. 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 control (SOC, *n* = 9) included all surgical procedures except transection of the stalk. After recovery, all heifers were maintained under pasture conditions.

Anterior vena cava blood was withdrawn every fourth day beginning on day 100 of pregnancy and continuing through day 330 from breeding. Blood was cooled on ice, allowed to clot at 15°C, and then centrifuged at 5°C for 20 min at 1500 x *g*. Serum was stored frozen (-20°C) for hormone assays.

Hormone Radioimmunoassay (RIA)

Progesterone RIA was identical to that described previously with the exception of the extraction procedures. Serum aliquots (200 µl) of each unknown, in duplicate, were added to 2 tubes without tracer and 1 tube containing dried tracer (5000 cpm; [1, 2, 6, 7-N-^{3}H-progesterone]; 97.0 Ci/mmol) to determine extraction efficiency. Two milliliters of benzene-hexane (1:2) were added to all tubes. Each tube was shaken vigorously for 30 seconds, and then was placed on dry ice to freeze the aqueous phase. The organic phase of the extracts containing ^{3}H-progesterone was decanted into scintillation vials, whereas the extracts from the remaining two aliquots of each unknown were decanted into assay tubes and dried for subsequent RIA. Preliminary experiments revealed little variance in procedural losses (94.6±0.9% extraction efficiency). Assay sensitivity was 50 pg/tube. Inter-assay and intra-assay coefficients of variation were 11.7% (n = 28) and 8.5% (n = 6), respectively.

The estradiol-17ß (E_2-17ß) and estrone (E_1) RIA was a modification of procedure to allow a more sensitive determination of estradiol in ovine and bovine serum. Three thousand dpm [2, 4, 6, 7-^{3}-H] estradiol-17ß (114 Ci/mmol) was added to 2 ml serum to facilitate the determination of procedural losses. The samples were extracted twice with 3 volumes of double-distilled benzene, and the final benzene extract was washed twice with 0.1 vol deionized water. Following each extraction, an aqueous-organic solvent phase separation was achieved by centrifugation at 500 x g for 10 minutes, and the organic solvent removed by aspiration. Assay sensitivity was about 2 pg. Intra-assay coefficients of variation for E_1 and 17ßE_2 were 3.0% and 2.9%, respectively. Inter-assay coefficients of variation for E_1 and 17ßE_2 were 7.1% and 9.5%, respectively.

LH was measured in 100-µl to 300-µl aliquots of serum, in duplicate, by using highly purified bovine LH (bLH) for labeling with ^{125}I (IMS 30) and for standards (36 pg–20 ng). Assay sensitivity was 0.2 ng/ml. Intra-assay and inter-assay coefficients of variation were 8.2% and 11.2%, respectively.

PRL was measured in 20-µl to 100-µl aliquots of serum, in duplicate, by using highly purified ovine PRL (oPRL) for labeling with ^{125}I and purified bovine PRL (bPRL) for standards (40 pg–20 ng). Assay sensitivity was 0.28 ng/ml. Intra-assay and inter-assay 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 ^{125}I by the chloramine T method, highly purified bGH for standards (0.125-2 ng), and incubation at 4°C for 72 hours by procedures similar to those previously described. Assay sensitivity was 0.125 ng/tube. Intra-assay and inter-assay coefficients of variation were 3.5% and 11.2%, 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 intramuscular 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-hour period, and the cow was milked twice (0800 and 1600 hours) during that period. Aliquots of milk (n = 116) from these animals were analyzed for fat, protein, lactose, and total solids by absorption of infrared light, with 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 at 6 µm and stained with performic acid-Alcian blue-periodic acid-Schiff-orange G, whereas other sections were stained with hematoxylin and eosin.

Statistical Analysis

Experimental units in this study were the individual heifers, each assigned to treatments at random. Least-squares analyses were based on a weighted average of sample variance for experimental and control groups. 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. Data are presented as geometric mean ± S.E.M., and statistical significance was concluded when $P < 0.05$.

Results

Pregnancy and Parturition after HST

Six of 7 HST heifers delivered living calves (Table 1). There was no evidence of onset of labor in 4 HST heifers, and parturition was induced in these animals by intramuscular injection of dexamethasone, followed approximately 30 hours later with an intravenous injection of oxytocin (Tables 1 and 2). Delivery required no assistance in 8 of 9 control animals, but cesarean section was necessary in 1 SOC heifer.

Lactation and Postpartum Estrus after HST

Lactation was maintained in both HST and SOC animals (Table 1).

None of the HST heifers exhibited a postpartum estrus during periods exceeding 300 days (Table 2). SOC heifers returned to estrus within 2 months of 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). By 100 days after birth, body weight and growth rate of calves from HST heifers was 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. Milk production in the first week postpartum was less ($P < 0.001$) in HST than in SOC females in the first week postpartum (Fig. 1); paired comparisons indicated reduced ($P < 0.001$) milk secretion in HST compared with SOC throughout the 30 wk lactation ($t = 8.87$, *d.f.* = 58). There was no significant difference in milk composition for percentage fat, protein, α-lactose, and total solids in HST and SOC (Table 3).

Ovarian Function, and progesterone, E_1, 17bE_2, 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 of parturition; and in 4 of 9 SOC, estrus, ovulation, and corpus luteum formation occurred by 45 days postpartum. Corpus luteum diameter (range of 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 ($P > 0.05$) similar to SOC from day 100 through parturition (Fig. 2a). Near onset of parturition, progesterone concentration decreased in both HST and SOC, and during lactation it remained at basal to nondetectable levels to day 330 from breeding (Fig. 2a). Duration of pregnancy was similar ($P > 0.05$) in HST (280–293 days) and SOC (281–295 days) animals.

A coincident steady increase in E_1 and 17ßE_2 concentration occurred in both groups of HST and SOC heifers from day 100 to peak values at parturition, followed by an abrupt decrease to <10 pg/ml during early lactation (Figs. 2b and c). The results indicate that E_1 and 17ßE_2 are primarily of placental origin during the later 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.

GH and LH Secretion

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

LH decreased ($P < 0.01$) abruptly after HST to undetectable levels throughout the remainder of gestation. In SOC, LH was maintained at only 0.3–0.9 ng/ml, a level greater ($P < 0.01$) than that in HST heifers. LH remained consistently low, frequently at undetectable levels, and a complete absence of episodic secretion throughout 24 hours during late pregnancy existed in 2 SOC heifers.

At death, the pituitary gland weight of HST heifers was 35% ($P < 0.01$) that of the SOC heifer (1.19 ± 0.10 vs. 3.20 ± 0.19 g). The pituitary gland expressed as g/100 kg body wt was 0.19 ± 0.02 in HST compared to 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 in SOC. Pituitary and thyroid histology in these HST and SOC heifers was similar to that observed after long-term growth in HST and SOC calves.

Discussion

The main finding in this study was that progesterone secretion in HST beef heifers was maintained at a level similar to that seen in SOC throughout pregnancy. Although PRL secretion in cattle is tonically inhibited by the hypothalamus and remains significantly greater in the first 14 days after HST than in SOC, circulating PRL concentration gradually drifts lower but remains seasonally regulated. A similar transient increase in PRL secretion occurs soon after hypothalamo-pituitary disconnection of ewes during the anestrus and the breeding season. 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 concentration peaking in summer and reaching a nadir in winter. In this study, serum PRL was sevenfold greater (37 ng/ml) in HST beef heifers during pregnancy compared with prepubertal HST beef heifer calves, whereas LH decreased abruptly to undetectable levels after HST at midgestation.

Dopamine may be involved in tonic regulation of PRL secretion in rats, pigs, sheep, cattle, and monkies, based on elevated circulating PRL concentration after HST and acute stimulatory effects of haloperidol and *a*-methyl-*p*-tyrosine on PRL secretion. The maintenance of progesterone secretion by aging corpora lutea with daily PRL treatment in hysterectomized-hypophysectomized animals provides further evidence for PRL's luteotropic action.

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. 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. Bovine PL may act through this putative

unique receptor (R), through the PRL-R and(or) through a heterodimer of the PRL-R and GH-R.

Bovine PRL receptors (bPRL-R) in the bovine corpus luteum, mammary gland, and liver have been measured. There also is evidence for bovine GH receptor (bGH-R) and bPRL-R transcripts in bovine extraembryonic membranes and in the glandular uterine endometrium, but much lower levels of both receptor mRNAs are found in the caruncles. Bovine corpus luteum and endometrium have a unique mRNA that hybridizes with a cDNA for bGH-R. The giant cells of 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. Thus, the biological activity of these related hormones depends not only on receptor distribution and affinity of hormone for the receptor but also on transmission of signal in response to binding.

The second finding in this study was that pregnancy continued and parturition and lactation occurred, in beef heifers HST at mid-gestation (138–201 days), with calf delivery occurring at the same time as 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 the 30 weeks of lactation and postpartum estrus was obliterated compared with SOC. Studies in dairy cows have demonstrated that bPL treatment increases milk production, suggesting that bPL may have GH-like galactopoietic actions. 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. Although circulating concentrations of estrogen, progesterone, bGH, bPRL, and possibly bPL were adequate to stimulate mammogenesis during the later half of pregnancy in HST heifers in the present study, the markedly reduced milk production during lactation suggests that decreased circulating concentrations of PRL and GH were the primary limiting factors compared with SOC heifers.

HST heifers remained anovulatory by blocking GnRH secretion to pituitary gonadotropes, whereas postpartum estrus and ovulatory cycles resumed within 2 months in SOC animals. Although ovarian follicles abruptly regressed in HST heifers, the corpus luteum was maintained similarly to that seen in SOC throughout pregnancy. Postpartum, corpus luteum regression was abrupt and ovarian follicular growth was arrested for at least 300 days in HST compared to SOC heifers.

Implications

The results from this study show that endogenous PRL, GH, and possibly bPL secretion maintained corpus luteum function and progesterone secretion in HST beef heifers 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 and GH, and presumably decreased cortisol release around parturition and lactation compared to 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.

Reference

Anderson, L. L., D. L. Hard, L. S. Carpenter, E. K. Awotwi, M. A. Diekman, A. H. Trenkle and S.-J. Cho. 1999. Pregnancy, parturition, and lactation in hypophyseal stalk-transected beef heifers. *J Endocrinol* **163**:463-475.

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

	Heifer no.	Day of surgery	Duration of pregnancy (days)	Type of delivery	Lactation during 30 weeks	Calf			
						Sex	Body weight (kg) Birth	100 days of age	Gain (kg/day)
HST	40	201	289	induced†	+	♀	34.02	82.29	0.48
	70	200	280	unassisted	+	♀	24.95	66.53	0.42
	74	161	293	induced†	+	♂	41.75	117.29	0.76
	71	161	284	induced†	–	♂	34.02'		
	33	149	283	unassisted	+	♂	34.02	97.19	0.63
	73	143	–*		–	♂			
	66	138	287	induced†	+	♂	31.75	75.05	0.43
SOC	44	205	284	unassisted	+	♀	36.29	143.10	1.07
	47	201	286	unassisted	+	♀	36.29	131.65	0.95
	38	200	285	unassisted	+	♂	34.02	145.26	1.11
	36	162	287	unassisted	+	♂	29.48	93.09	0.64
	148	160	286	unassisted	+	♀	41.73	152.57	1.11
	75	159	295	cesarean	–	♂	42.63	84.77	0.42
	44A	145	288	unassisted	+	♀	38.55	108.54	0.70
	149	141	281	unassisted	+	♀	35.38	113.71	0.78
	45	140	283	unassisted	+	♀	29.48	115.79	0.86

*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 intramuscularly followed by oxytocin intravenously at time of delivery.

'Calf died within 5 minutes after delivery.

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

	Pregnancy duration (days)	No. of heifers Requiring induction of delivery	Lactating 30 weeks	Exhibiting postpartum estrus	Days to postpartum estrus	No. of calves born alive	Calf Body weight (kg) Birth	100 days of age	Gain (kg/day)
HST	286 ± 1.9	4 of 6	5 of 6	0 of 7[§]	>300	6	33 ± 2.2	88 ± 8.9[b]	0.54 ± 0.06[a]
SOC	286 ± 1.3	1[†] of 9	8 of 9	9 of 9	67 ± 14	9	36 ± 1.5	121 ± 7.9	0.85 ± 0.08

[§] Pregnancy failed after surgery in one heifer.

[†] Cesarean.

[a] $P < 0.025$.

[b] $P < 0.001$.

Table 3. Serum LH concentration before surgery, and after SOC and HST at midgestation, parturition, and early lactation in beef heifers. Values are means ± S.E.M.

Reproductive State	Day	LH (ng/ml) Presurgery (n = 12)	SOC (n = 6)	HST (n = 6)
Pregnancy	-40	0.27 ± 0.01		
	-32	0.28 ± 0.01		
	-24	0.32 ± 0.04		
	-16	0.29 ± 0.01		
	-8	0.33 ± 0.06		
	0	0.63 ± 0.32		
	8		0.37 ± 0.01	ND[a]
	16		0.31 ± 0.02	ND
	24		0.28 ± 0.01	ND
	32		0.44 ± 0.14	ND
	40		0.31 ± 0.03	ND
	48		0.37 ± 0.01	ND
	56		0.36 ± 0.06	ND
	64		0.29 ± 0.01	ND
	72		0.30 ± 0.01	ND
	80		0.29 ± 0.01	ND
	88		0.31 ± 0.04	ND
	96		0.29 ± 0.01	ND
	104		0.28 ± 0.01	ND
	112		0.27 ± 0.01	ND
Parturition	120		0.28 ± 0.01	ND
Lactation	128		0.30 ± 0.02	ND
	136		0.39 ± 0.09	ND
	144		1.48 ± 0.60	ND

[a]Not detectable (<0.2 ng/ml).

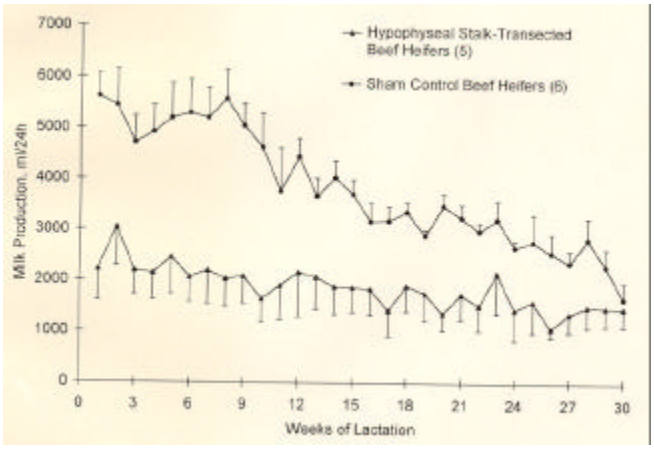

Figure 1. Milk production in HST (?) and SOC (?) beef heifers. Calves suckled their dams throughout this 30-week period. Dams were isolated from their calves once each week for 24 hours and were milked twice (0800 and 1600 hours) during that period. The number of heifers is indicated in parentheses. Values are means ± S.E.M.

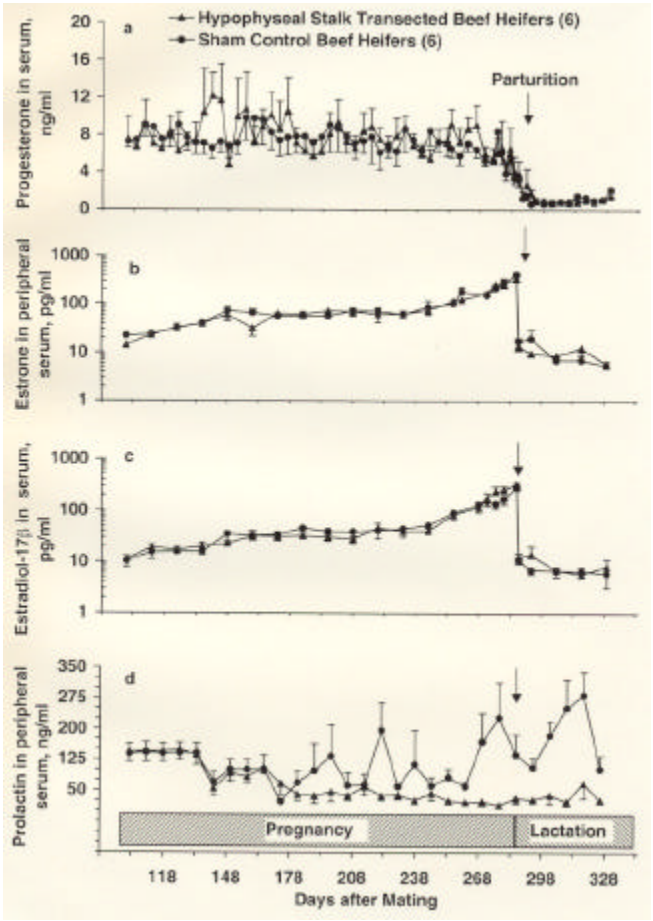

Figure 2. Concentration of progesterone (a), estrone (b), estradiol-17b (c), and prolactin (d) in peripheral serum at 4-day intervals in HST (?) and SOC (?) beef heifers during pregnancy, parturition, and lactation (day 0 = estrus). Three SOC heifers died within 3 days after surgery; postmortem examination revealed bleeding from the severed internal carotid in one animal, and accumulation of blood in the cranial sinus from vasculature of the dura mater and calvarium in two animals. The number of heifers is indicated in parentheses. Values are means ± S.E.M.