Summary

Jugular blood was collected from 10 Holstein heifers twice weekly from 26 to 6 days before parturition, twice daily from 6 days before to 5 days after parturition and on day 9 after parturition. Progestins were extracted from blood serum with trimethyl pentane, glucocorticoids were extracted with dichloromethane, and each was quantified by competitive protein binding assay. Estradiol and estrone were extracted with ether, isolated by column chromatography and determined by radioimmunoassays. Progesterone remained high until -2 days (7.6±9 ng/ml), fell to 3.0±7 ng/ml at -1 day and to 0.6±1 ng/ml at parturition, and remained near this low level for 9 days postpartum. Glucocorticoids remained low until -1 day (6.4±9 ng/ml), increased dramatically to 10.3±1.5 ng/ml at -0.5 day, to 16.7±3.5 ng/ml at parturition, and then fell to 5.1±0.8 at 0.5 day after parturition and remained near that basal level for 9 days postpartum. Estradiol increased linearly from 32±6 pg/ml at -26 days to 150±24 pg/ml at -5 days, and jumped to 295±53 pg/ml at -2 and -1.5 days. Then estradiol fell to 52±11 pg/ml at 1 day postpartum and averaged 14±10 pg/ml until day 9 postpartum. Changes in serum estrone generally followed those for estradiol, but estrone was about eight-fold higher.

Thus, a 10-fold increase in blood serum estrogens during the month before parturition was the first major change in steroid hormones in advance of parturition. Estradiol, estrone and progesterone all declined precipitously during the final 2 days of pregnancy, while glucocorticoid increased to a peak at parturition.

Introduction

Notwithstanding theories advanced to explain parturition and initiation of lactation (Csapo, 1956), the associated endocrine events have not been described completely. While others have quantified serum progesterone (Short, 1958; Donaldson, Basset and Thorburn, 1970; Stabenfeldt, Osburn and Ewing, 1970), serum glucocorticoids (Adams and Wagner, 1970) and urinary estrogens (Hunter et al., 1970) near the time of parturition in cattle, we found no report of both estradiol and estrone in blood from cows near parturition and to our knowledge no one has reported all three classes of steroids in the same animals. Our objective in the present research was to quantify progestins, glucocorticoids, estradiol and estrone in blood serum from heifers from 26 days prepartum to 9 days postpartum to determine the sequence of changes in steroids.

Materials and Methods

Blood (40 ml) was collected via jugular venipuncture from each of 10 pregnant Holstein heifers twice weekly beginning at about 26 days prepartum and continuing until about 6 days prepartum, twice daily (8:00 am and 5:00 pm) from 6 days before to 5 days after parturition and once on the 9th day postpartum. Blood was placed in centrifuge tubes containing 31.7 mg oxalic acid and centrifuged to precipitate blood cells. Then the blood plasma was transferred to tubes containing 27.8 mg CaCl₂ to initiate clotting. After 2 days at 4°C, the blood plasma was transferred to tubes containing 27.8 mg CaCl₂ to initiate clotting. After 2 days at 4°C, the blood was centrifuged to remove fibrin clots and serum was stored at -20°C until assayed for steroids.

Total Progestin and Glucocorticoid Assays. Approximately 2,000 dpm each of ³H-progesterone³ (specific activity=50 c/mM) and ³H-cortisol² (specific activity=45.3 c/mM) in 10 μl ethanol were added to 1.0 ml serum and progestins were extracted twice with 5 ml freshly redistilled trimethyl pentane by mixing on a vortex mixer at maximum speed for

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2 Department of Dairy Science.
3 Obtained from New England Nuclear, Boston, Mass.
2 minutes. Subsequently, glucocorticoids were extracted from the same aliquants of serum with dichloromethane and assayed as described previously from our laboratory (Smith, Convey and Edgerton, 1972). The combined trimethyl pentane extract was evaporated under nitrogen to approximately 3 ml and radioactivity in 0.5 ml was determined in a liquid scintillation spectrometer (Nuclear Chicago Model Mark I) to estimate procedural losses of progesterone.

Aliquants (0.5 and 1.0 ml) of the trimethyl pentane extract were assayed for total progestin by competitive protein binding procedures modified from the method described by Murphy (1967). Dog plasma (Colorado Serum Co., Denver, Colorado) was the source of binding protein. Endogenous steroids were adsorbed from 2.5% dog plasma in sterile water (Cutter Laboratories Inc., Berkeley, California) by stirring for 30 min. with 8 g Florisil (30–60 mesh, Matheson, Coleman and Bell, Cinn., Ohio) per 100 ml. Each batch of dog plasma was further diluted to 0.67% and approximately 8,000 dpm 3H-corticosterone (specific activity~45.3 c/mM) per milliliter was added as the competitor in the progestin assay. After the solvent was evaporated from tubes containing standard progesterone or unknown steroid, 1.5 ml diluted dog plasma was added and the mixture was vortex-mixed for 15 sec., then incubated at 4°C for 18 to 24 hours. Thereafter, 80 mg of Florisil was added and the contents of each tube were immediately vortex-mixed for 45 seconds. After the Florisil settled (about 30 sec.), 0.5 ml of supernatant fluid was transferred to a scintillation vial with 10 ml of Bray's scintillation fluid (Bray, 1960). Radioactivity was quantified in the liquid scintillation spectrometer and progestin was quantified by linear interpolation between progesterone standards (0, 0.1, 0.5, 1.0, 1.5, 2.0, 5.0 and 10.0 ng). Except the scintillation vials, all glassware was rinsed with 5% trimethylchlorosilane (Sargent Welch Scientific Co., Detroit, Mich.) in toluene and dried prior to use.

A preliminary experiment revealed 75% of the tracer progesterone and 3% of tracer cortisol and corticosterone were extracted with trimethyl pentane; 92% of tracer cortisol, 69% of corticosterone and 10% of the original progesterone were extracted with dichloromethane after trimethyl pentane. Thus, the progestin fraction was relatively free of cortisol or corticosterone, but the glucocorticoid fraction probably contained about 10% of the total progesterone. Since in our experience progesterone is only 10% as potent as cortisol in the glucocorticoid assay, endogenous progesterone should not have affected glucocorticoid assays. However, contamination in glucocorticoid assays with the progesterone tracer had the effect of increasing the glucocorticoid recovery values and reducing the calculated mass by approximately 10%.

To further validate the assay for progestins, we assayed 14 samples of serum (taken throughout the estrous cycle) after extraction with trimethyl pentane and after isolation of progesterone from LH-20 columns as described previously (Swanson, Hafs and Morrow, 1971). The correlation between determinations on the trimethyl pentane extract and determinations in fractions from the LH-20 Sephadex column was 0.85. The average progestin determined in trimethyl pentane extracts was slightly but not significantly less than average progesterone determined in fractions from column chromatography.

**Estradiol and Estrone.** Estradiol and estrone were estimated by radioimmunoassays (Wettemann et al., 1972). Briefly, the procedure involved extraction of 5 ml of serum twice with 2 volumes of anhydrous diethyl ether. Both estrogens were then isolated by elution from columns of Sephadex LH-20 (Pharmacia Fine Chemicals Co.) with chloroform:ethanol (24:1). Aliquots of the column fractions were counted in a liquid scintillation spectrometer to locate the estrogens and estimate procedural losses of tracers. Estradiol and estrone were then quantified by radioimmunoassay using an estradiol-17β antiserum. 4 Specificity of this antiserum was described by Tillson et al. (1970). The assay involved competition between 3H-estradiol and endogenous hormone for the antibody; the bound and unbound fractions were separated with dextran coated charcoal. Unknown estradiol and estrone were calculated by interpolation between standard estradiol or estrone, respectively.

**Results**

Average gestation length for the ten heifers in this study was 281.5 days with a range of 275 to 291 days. Serum glucocorticoids averaged 5.0 ng/ml from 26 days to 1 day before parturition, and increased (P<.01) to 10.3 ng/ml approximately 12 hr. before parturition

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4 Antibody SLC-4X was generously supplied by Dr. S. A. Tillson and associates of the Worcester Foundation for Experimental Biology, Shrewsbury, Mass.
and to 16.7 ng/ml at parturition (figure 1). By 12 hr. postpartum, serum glucocorticoids declined to 5.1 ng/ml; lower (P<.01) than the mean at parturition and at 12 hr. prep- partum but not different (P>.05) from the average during the previous 26-day prep- partum period. Orthogonal contrasts revealed that serum glucocorticoids increased (P<.01) again between 12 and 36 hr. after parturition. Inspection of the data in figure 1 revealed greater variation in serum glucocorticoids during the first 9 days postpartum than during the prepartum period. However, the average level of serum glucocorticoids after parturition (5.6 ng/ml) was not different (P>.05) from that before parturition (5.0 ng/ml). In samples collected at 8:00 am and 5:00 pm from days 5 to 1 prepartum, serum glucocorticoids in the morning averaged 5.2±.3 ng/ml (n=49), not significantly different (P>.05) from that in the afternoon (average 5.0±.5 ng/ml, n=50).

Serum progestins averaged 10.1 ng/ml from 26 to 9 days prepartum (figure 2), signifi- cantly greater (P<.01) than the comparable average for the remaining prepartum days; the average declined gradually from day 15 to day 3 prepartum, but not significantly (P>.05). At 2.5 days prepartum, serum progestins averaged 8.0 ng/ml; then declined precipi- tously (P<.01) to 0.6 ng/ml at parturition. During the early postpartum period serum progestin concentration remained low, averaging 0.6 ng/ml from day 0 to day 9.

Estradiol and estrone averaged 32 and 218 pg/ml, respectively, in serum at 26 days prior to parturition, and both nearly doubled (P<.05) by day 19 prepartum (figures 3 and 4). These increases continued to peaks at about day 2 prepartum when estradiol and estrone averaged 293 and 2,256 pg/ml, respectively. Thereafter, serum estrogens declined rapidly. Orthogonal contrasts revealed significant (P<.02) reductions in estrone and estradiol from 2.5, 2.0 and 1.5 days prepartum to days 1.0, 0.5 and parturition. Estradiol and estrone
averaged 180 and 726 pg/ml at parturition; they decreased (P<.01) further to 52 and 115 pg/ml, respectively, at 1.0 day postpartum and to averages of 28 and 14 pg/ml, respectively, on days 3, 5 and 9, significantly lower (P<.05) than on 1 day postpartum.

Discussion

Increased glucocorticoids in serum of heifers at parturition agrees with results for cows reported by Adams and Wagner (1970). However, in contrast to the sharp peak at parturition we report, they observed that glucocorticoids increased markedly to day 4 prepartum, plateaued through day 2 postpartum and declined gradually thereafter. This difference in changes in glucocorticoids at parturition may reflect differences in parity. Not all workers, however, have reported increased serum glucocorticoids at parturition. Brush (1958) observed no statistical change in plasma glucocorticoids between the last 5 days prepartum and parturition, although serum glucocorticoids were elevated near parturition in some cows. The increased glucocorticoids we observed prior to parturition may be related with initiation of parturition and lactation, as Adams (1969) and Adams and Wagner (1969) successfully induced parturition in cattle, sheep and rabbits with exogenous glucocorticoids during the final stages of pregnancy. Similarly, exogenous glucocorticoids initiate lactation in heifers (Tucker and Meites, 1965) and rats (Talwalker, Nicoll and Meites, 1961). Lochwood, Turkington and Topper (1966) reported increased milk proteins in pregnant mouse mammary tissue incubated in vitro with hydrocortisone. Alternatively, possibly serum glucocorticoids increase near parturition due to neural stimuli associated with discomfort of labor. In cattle, Gillette and Holm (1963) reported increased abdominal and uterine contraction for 2 days prepartum, coincidental with increased glucocorticoids reported here.

The precipitous decline in serum progesterins beginning at 48 hr. prepartum (figure 1) confirms previous reports (Short, 1958; Pope, Gupta and Munro, 1969; Stabenfeldt et al., 1970). Without exception, serum progesterins of each heifer declined precipitously beginning 72 to 48 hr. prepartum. Others (Short, 1958; Stabenfeldt et al., 1970) reported a gradual decrease in serum progesterone during the last 2 weeks of gestation, preceding the marked progesterone decline immediately pre-partum, but they provided no statistical verification of the initial gradual decline. Erb et al. (1968) reported a nonsignificant 25% decrease in serum progesterone during the last 2 weeks of gestation before the precipitous drop. The similar decrease we observed from 15 to 5 days prepartum (figure 1) was not significant, and it was followed by a nonsignificant increase at 3 days prepartum. In any event, the gradual changes in serum progesterone until day 3 before parturition are small relative to the precipitous decline during the 3 days before parturition.

Serum estradiol at 26 days before parturition (32±6 pg/ml) was nearly five-fold that reported earlier (Wettemann et al., 1972) for heifers during the first trimester of pregnancy. In general, the prepartum increases we found in blood serum estrogens might have been predicted from previous data on urinary estrogens. For example, Hisaw and Meyer (1929) found estrogens in ether extracts of urine during late pregnancy but none within 24 hr. after parturition, and Hunter et al. (1970) observed gradual increases in urinary estradiol-17a and estrone as parturition approached and marked declines immediately postpartum in cows. Mellin, Erb and Ester-green (1966) reported that excretion of estrone and estradiol-17a increased during the 40 hr. before calving, and declined after 16 hr. postpartum. In the light of the present investigation, perhaps high urinary excretion of estrogens continues beyond the point when serum estrone and estradiol begin to decrease at approximately 1 to 2 days prepartum.

In contrast to urinary estrogens, there is little information on serum estrogens during late pregnancy as reported here. Pope, Jones and Waynforth (1965), who quantified blood estrogens by bioassay, reported 1 to 10 ng/liter from cows in estrus and 7 ng/ml in blood from cows 7 to 9 months pregnant. Holm and Galligan (1966), using a fluorometric assay, reported a marked increase in both plasma estrone and estradiol during the last month of pregnancy and a sharp decline during the postpartum period of normal cows; Robinson et al. (1970) reported similar observations for blood estrone. When cows were bled daily and estrone measured fluorometrically, Robinson, Anistassiadis and Common (1971) found that the decline in blood estrone began a few days before parturition. Henricks et al. (1972) used a radioimmunoassay similar to ours, except they reported total immunoreactive estrogens around parturition; total estrogen
increased from 510 pg/ml 14 days prior to parturition to a peak of 2660 pg/ml at parturition. But total estrogens did not decline before parturition as we report for estradiol and estrone individually. The difference may be due to other immunoreactive estrogens not measured by our procedure. Inspection of our data revealed that both estradiol and estrone fell dramatically during the 2 days immediately prepartum. Although their values were about three-fold higher than ours, Robinson et al. (1971) also demonstrated marked reductions in blood estrone in each of seven cows during the final 2 days of gestation.

By 3 to 9 days postpartum, estradiol returned to values near those at 26 days prepartum (figure 3). In contrast, estrone at 3 to 9 days postpartum was only about 10% of that at 26 days prepartum. Whether this reduced estrone to estradiol ratio reflects a qualitative change in estrogen metabolism remains to be tested.

Data presented here are in agreement with the "progesterone block" theory of initiation of parturition (Csapo, 1956). The first major change in serum steroid hormones is a 10-fold increase in estrogens during the month before parturition, to a peak at about 2 days before parturition. Blood estrogens reach basal postpartum values within 3 days after parturition. Progesterone remains high until 3 days before parturition and falls principally during the last 2 days of pregnancy to values at parturition typical of estrus. The parturient rise in glucocorticoids occurs shortly after the fall in progesterone. Glucocorticoids peak at parturition and return to basal values within 12 hours. The large increase in estrogen probably increases contractility of the uterus during the final weeks of gestation (Csapo, 1956), and the precipitous decrease in progestins during the 48 hr. prepartum leaves the uterus under estrogen dominance at a time when coordinated uterine contractions begin in cattle.

Removal of the corpus luteum (McDonald, McNutt and Nichols, 1953) and administration of exogenous estrogen (Hill and Piersen, 1958) or corticoid (Adams, 1969) each terminate pregnancy in cattle; these observations are consistent with changes we observed in endogenous hormones at parturition. However, these facts do not prove that each of these changes in the maternal system normally initiate parturition. Increased glucocorticoids may be a response to the stress of parturition rather than an initiator of parturition. This view is supported by the recent observations that parturition induced by glucocorticoids 1) can be inhibited by progesterone administration (Jochle et al., 1972) and 2) is accompanied by a decline in progesterone (Schams et al., 1972) not unlike the decline reported here. If increased maternal serum glucocorticoids normally initiate parturition, the increase probably should occur prior to the decline in progesterone. Since glucocorticoids increased following the progesterone decline, we suggest that increased maternal glucocorticoids do not normally initiate parturition. Our data do not exclude a role for fetal glucocorticoids in initiating parturition as has been hypothesized for sheep (Liggins, Kennedy and Holm, 1967), and the increased maternal serum glucocorticoids may be related to initiation of lactation (Tucker and Meites, 1965). Reports of others (Holm and Gal- ligan, 1966; Henricks et al., 1972) that serum estrogen does not decline prior to parturition suggested a passive role for estrogens in parturition; estrogen declined only after parturition. But our observation that estradiol and estrone decline prepartum presents the possibility that altered estrogen metabolism may actively participate in the onset of parturition. Alternatively, the prepartum decline in serum estrogens may reflect incipient death of the placenta. Additional experimentation will be necessary to clarify the interaction of estrogen and progesterone prior to parturition, and a complete understanding of parturition and lactogenesis will require monitoring fetal endocrine events simultaneously.

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