Role of the adrenals in the maintenance of pregnancy in cows*

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Summary. On Day 215 of gestation, cows were treated as controls (Group 1), bilaterally ovariectomized (Group 2), bilaterally adrenalectomized (Group 3) or bilaterally adrenalectomized and ovariectomized (Group 4). The mean ± s.e.m. lengths of gestation (days) were 278 ± 3 (N = 5), 270 ± 2 (N = 7), 277 ± 1 (N = 5) and 219 ± 1 (N = 3) in Groups 1, 2, 3 and 4 respectively. In Group 4, serum progesterone concentrations declined to undetectable levels within 24 h after surgery and all the cows aborted 3-6 days later. All the cows in Groups 1, 2 and 3, except 1 cow in Group 2, completed gestation and delivered live calves. Progesterone concentrations in Group 2 cows declined to approximately one-third those of cows in Groups 1 or 3. All 3 cows in Group 4 and 7/8 in Group 2 had a retained placenta. At 1 and 2 days after surgery there were no significant differences in plasma levels of oestradiol-17β, but by the third day this difference was significant (P < 0.01). Oestrogen levels were high before parturition in cows in Groups 1 and 3 but were not noticeably elevated in those in Groups 2 and 4. The abrupt termination of pregnancy and negligible concentrations of progesterone in adrenalectomized–ovariectomized cows indicate that the bovine adrenals contribute to progesterone production and are capable of maintaining pregnancy after ovariectomy at 215 days of gestation.

Introduction

Bilateral ovariectomy before 200 days of gestation in the cow terminates pregnancy (Tanabe, 1966; Estergreen, Frost, Gomes, Erb & Bullard, 1967). However, gestation is completed and live offspring are born when ovariectomy is performed around 200 days of gestation (Estergreen et al., 1967) or later (Chew, Erb, Fessler, Callahan & Malven, 1979a; Hoffmann, Wagner, Hixon & Bahr, 1979). The ability to maintain pregnancy following ovariectomy after 200 days of gestation suggests either that cows have an extraovarian source of progesterone at this time in gestation or they do not require progesterone for maintenance of pregnancy after 200 days of gestation. Results of several studies have suggested that the adrenal gland is a source of progesterone in the non-pregnant cow (Wiersma & Stott, 1969; Gwazdauskas, Thatcher & Wilcox, 1972; Wagner, Strohbehn & Harris, 1972). Balfour, Comline & Short (1957) found progesterone levels in the adrenal vein to be 10–100 times greater than concentrations in the corresponding adrenal artery in a cow at 240 days of pregnancy. The present study was conducted to test the hypothesis that the cow requires intact adrenal glands or ovaries for the maintenance of late pregnancy.

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Materials and Methods

Twenty-three multiparous Hereford × Angus or Angus cows were assigned randomly to four treatment groups in a 2 × 2 factorial design. The treatment groups were: (1) control, N = 5; (2) bilateral ovariectomy, N = 8; (3) bilateral adrenalectomy, N = 5; and (4) bilateral adrenalectomy and ovariectomy, N = 5. Three additional cows were assigned to Group 2 since the literature indicated that ovariectomy might cause abortion in some cows.

Daily blood sampling via jugular puncture began on Day 211 of gestation and continued until either the day of parturition or the day of placental shedding in cows with retained placentas. Placentas were considered retained if cows failed to shed their placentas within 24 h after parturition. Blood was centrifuged at 12,000 g for 10 min and the plasma was removed and stored at −20 °C for subsequent radioimmunoassay.

Blood plasma concentrations of progesterone were assayed by double-antibody radioimmunoassay using the antisera and procedures of Staigmiller, Short, Bellows & Carr (1979). The antiserum was raised in rabbits against progesterone-11β-bovine serum albumin. The radio label was an iodinated tyrosine methyl ester derivative of progesterone and the second antibody was raised in sheep against rabbit gamma globulin. The antiserum was shown to be specific by comparison with 10 steroids and by no change in activity before and after LH-20 chromatography using a bed volume of 3 ml and elution with a hexane : benzene : methanol solvent system. For the assays reported herein, samples were extracted at a dilution of 1:5 with hexane (v/v) after 2000 c.p.m. [3H]progesterone had been added to all tubes. The average recovery was 89%. Accuracy was shown by linearity and quantitative confirmation of known amounts of progesterone added to ovariectomized cow serum. The correlation coefficient, regression coefficient and y intercept of recovered values against added values were 0.98, 1.003 and 0.22, respectively. The interassay and intra-assay coefficients of variation were 12.3% and 15.0% for a sample with a mean concentration of 4.63 ng/ml. Assay sensitivity of at least 50 pg/ml was determined by defining the lower limit of sensitivity as twice the standard deviation of the blank.

Serum concentrations of oestradiol-17β were determined using the antiserum and procedures of England, Niswender & Midgley (1974) as modified by Schillo, Dierschke & Hauser (1982). Samples were corrected for a recovery of [3H]oestradiol at 74 ± 1.3%. The lower limit of detection (twice the s.d. of the blank) was 0.78 ± 0.24 pg/tube.

The within- and between-assay coefficients of variation were 9.6 and 18.2%, respectively. The assay was performed as previously published without chromatography to remove oestrone. The cross-reactivity of the antiserum with oestrone is 3%.

On Day 213 of gestation, the cows were isolated from the herd and food and water were withheld until surgery was performed. On Day 215, the cows were tranquilized with acepromazine (Ayerst Laboratories Incorporated, Rouses Point, New York), anaesthetized with sodium thiampylal (Surital: Parke-Davis, Detroit, Michigan) and maintained under general anaesthesia with halothane (Fluothane: Ayerst).

The adrenalectomy was performed by a retroperitoneal method (W. C. Wagner, personal communication). An incision, approximately 36 cm long, was made in the anterior lumbar–posterior thoracic area, 10 cm ventral and parallel to the vertebral column on the right side of the cow. A 10-cm section of ribs 12 and 13 was removed to facilitate the approach. Both adrenals were removed by blind dissection through this incision. Bilateral ovariectomy was through a 15-cm incision on the right side in the ventral area of the lumbar fossa. The ovaries were located and exteriorized when possible. The hilus of the ovary was ligated and the ovary excised. To control for the effect of surgery on the maintenance of pregnancy, control cows were anaesthetized and subjected to the same incision and rib removal procedures as the adrenalectomized cows, and both adrenal glands were palpated. All adrenalectomized cows were given daily i.m. injections of 50 mg cortisone acetate (Merck, Sharp, Dohme, Rahway, New Jersey) and 10 mg deoxycorticosterone acetate (Sigma Chemical Company, St Louis, Missouri) dissolved in saline (9 g NaCl/l).
began the day of surgery and were continued until 7 days before the cows were killed 18–60 days after parturition.

Two criteria (Thompson & Wagner, 1974) were used to determine whether a cow had been completely adrenalectomized: absence of adrenal tissue at necropsy, and exhibition of muscular fatigue following a 7-day interruption of hormone replacement therapy before necropsy. Two cows in Group 4 failed to meet both of these criteria and were, therefore, excluded from the experiment.

The results were assessed by analysis of variance using an SAS programme for unbalanced data sets with individual means compared by orthogonal contrasts.

Results

The mean lengths of gestation are shown in Table 1. Gestation was normal length for the cows in Groups 1 and 3, but significantly shorter ($P < 0.05$) for those in Group 2 and terminated abruptly about 4 days after operation in Group 4.

![Table 1. Effect of adrenalectomy and ovariectomy at Day 215 of gestation on length of gestation and retention of placenta in cows](image_url)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of cows</th>
<th>Mean ± s.e.m. length of gestation (days)</th>
<th>No. of cows with retained placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>5</td>
<td>278 ± 3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Ovariectomy</td>
<td>7†</td>
<td>270 ± 2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Adrenalectomy</td>
<td>5</td>
<td>277 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Adrenalectomy–ovariectomy</td>
<td>3</td>
<td>219 ± 1*</td>
<td>3</td>
</tr>
</tbody>
</table>

* Interaction between adrenalectomy and ovariectomy ($P < 0.05$).
† One cow aborted at Day 235 and was not included in the data.

Plasma concentrations of progesterone (Text-figs 1a & b) were not significantly different amongst the four groups of cows for 2 days before the surgery on Day 215. After surgery and on Day 216, maternal plasma concentrations of progesterone remained high and slightly elevated above pre-surgery concentrations for cows in Groups 1 and 3 (10–14 ng/ml) but declined significantly ($P < 0.01$) to 3.5–4 ng/ml in Group 2 and fell below a detectable level (<0.5 ng/ml) in Group 4 ($P < 0.01$). Control cows (Group 1) maintained progesterone concentrations of at least 4 ng/ml until the day before parturition. In Group 3 progesterone values paralleled those of control cows but mean values were consistently lower by 1–2 ng/ml. This difference was never significant ($P > 0.05$). In Group 2 plasma concentrations of progesterone were significantly lower than those in Groups 1 and 3 ($P < 0.01$) but higher by 2–4 ng/ml than those in Group 4.

Plasma concentrations of oestriadiol-17β fluctuated considerably before and after surgery and among cows. Statistical analysis revealed no significant differences among treatments in oestriadiol-17β concentrations before the first 2 days after surgery although they tended to be higher in Group-3 cows (Text-fig. 1c). By Day 218 this difference was significant ($P < 0.01$). Oestrogen levels were high before parturition in cows in Groups 1 and 3 but without appreciable elevation in those in Groups 2 and 4 in which pregnancy was terminated early ($P < 0.01$; Text-fig. 1d).

All but one of the ovariectomized cows (Group 2) and all of the cows in Group 4 had retained placentas. None of the cows in Groups 1 and 3 had a retained placenta (Table 1).
Text-fig. 1. Changes in mean ± s.e.m. serum concentrations of progesterone and oestradiol-17β in cows in Group 1 (sham-operated controls), Group 2 (bilaterally ovariectomized), Group 3 (bilaterally adrenalectomized) and Group 4 (bilaterally adrenalectomized and bilaterally ovariectomized). S = surgery; P = parturition.

Discussion

Ovariectomy at Day 215 of gestation did not immediately terminate pregnancy even though the maternal plasma concentration of progesterone declined to a low level. The ability of cows ovariectomized in late pregnancy to maintain a low level of progesterone in their blood was also observed by Chew et al. (1979a) and Hoffmann et al. (1979). This period when pregnancy is supported by an extraovarian source of progesterone coincides with the period when Tanabe, Hokanson & Griel (1968) found that no exogenous progesterone was needed to maintain pregnancy after ovariectomy. Around Day 270, the time of parturition of ovariectomized cows in the present study, Tanabe et al. (1968) found that exogenous progesterone was again needed to maintain pregnancy. This is at a time when concentrations of oestrogens are rapidly increasing (Chew et al., 1979a; see also review by First, 1979). The 2–4 ng progesterone/ml blood observed in this study or the 1–2.5 ng/ml observed by Chew et al. (1979a) may be adequate to maintain pregnancy in ovariectomized cows as long as oestrogens are relatively low but inadequate when rising levels of oestrogen create a large oestrogen to progesterone ratio. If this ratio is critical for the development of oxytocin receptors in cattle, as shown for rats and guinea-pigs (Alexandrova & Soloff, 1980a, b, c) and for the development of myometrial gap junctions as suggested by Garfield, Kannan & Daniel (1980a), Garfield, Merrett & Grover (1980b) and Garfield, Puri & Csapo (1982), then the
premature parturition of ovariectomized cows (Table 1; Estergreen et al., 1967; Chew et al., 1979a; Hoffmann et al., 1979) may be due to an earlier arrival of the critical ratio due to the absence of progesterone.

The source of the extraovarian progesterone has been considered by Hoffmann et al. (1979) to be body fat. Ovariectomy in that experiment was at 235–260 days of gestation and 2–3 days before abortion. It does not seem likely that residual progesterone would be maintained in body fat for the prolonged period from 215–218 days to approximately Day 270 as occurred in the present experiment and that of Chew et al. (1979a). The postulate that the extraovarian progesterone is from the maternal adrenals is supported by the present results because (1) pregnancy was terminated in the absence of the ovaries and adrenals but maintained when either the ovaries or adrenals were present, (2) 2–4 ng progesterone/ml were present in the blood of ovariectomized cows but there was none after ovariectomy plus adrenalectomy, and (3) there were slightly, though not significantly, lower plasma concentrations of progesterone in adrenalectomized cows than in sham-operated controls.

It is unlikely that the adrenalectomized–ovariectomized cows aborted because of the stress of surgery. The principal stress was adrenalectomy and the adrenalectomized cows as well as sham adrenalectomized–ovariectomized cows had gestations of normal duration.

This postulate is also supported by the observation of Balfour et al. (1957) that the progesterone concentration in blood in the adrenal vein at 240 days of gestation was 10–100 times greater than in the maternal artery, and by studies showing that the adrenal gland contributes to the total blood content of progesterone in the non-pregnant cow (Wiersma & Stott, 1969; Gwazdauskas et al., 1972; Wagner et al., 1972). Whether progesterone production by the adrenals of the pregnant cow is hormonally regulated is unknown. Wagner et al. (1972) found that ACTH administered to sexually mature ovariectomized heifers elevated plasma concentration of progesterone and concluded that the source of progesterone was probably the adrenal glands. However, Schmidt, Hoffmann & Rattenberger (1977) found that this extraovarian progesterone of ovariectomized pregnant cows was not suppressed by 5–20 mg flumethazone.

Placental production of progesterone was not determined in the present study. There is evidence that the fetus and placenta do not contribute to maternal plasma progesterone. A fetal contribution to maternal progesterone or oestrogen has not been found either by analysis of hormones in fetal and maternal blood (Hunter, Fairclough, Peterson & Welch, 1977) or by assay of maternal hormones after fetectomy (Hoffmann et al., 1979). The bovine placenta at this time does not contain detectable amounts of progesterone (Erb, Randel & Callahan, 1971; Peterson, Hunter, Welch & Fairclough, 1975). However, when incubated in vitro it can release progesterone into the medium, but the amounts are small compared to those produced by the placentas of other species (Ainsworth & Ryan, 1967). Evidence from the present study suggesting that the conceptus and not the maternal adrenals may be the extraovarian source of progesterone is the complete loss of progesterone from the blood of ovariectomized and control cows after parturition and expulsion of the conceptus.

The oestrogen assayed in these experiments was oestradiol-17β. It is recognized that the concentrations of oestrone are higher in late bovine pregnancy than oestradiol-17β. However, the changes in concentrations of the two oestrogens are approximately parallel immediately before parturition with oestrone rising earlier and remaining higher (Smith, Edgerton, Hafs & Convey, 1973; Chew et al., 1979a). The pre-partum levels of oestradiol-17β in control and adrenalectomized cows were similar to those reported by Edqvist, Ekman, Gustafsson & Johansson (1973), Smith et al. (1973) and Robertson (1974), and were significantly higher than those of the other cows (Text-fig. 1d). This difference occurred between both groups of ovariectomized cows terminated pregnancy (Days 270 and 219) before the normal completion of the rise in plasma oestradiol-17β. These results suggest that the pre-term rise in oestradiol-17β may result from mechanisms related to gestational age rather than mechanisms initiating parturition or that the placenta was incapable of initiating events leading to the oestrogen elevation before Day 266. The significantly higher
concentration of oestradiol-17β seen at Day 218 in plasma of adrenalectomized than in control cows was not maintained at later times and may only represent a difference of chance (Text-figs 1c & d).

The high frequency of retained placentas in ovariectomized cows (Table 1) is in agreement with results reported by Estergreen et al. (1967) and Chew et al. (1979a). Under the conditions of the present experiment, the cows with retained placentas (ovariectomized and ovariectomized–adrenalectomized groups) had lower peripartum levels of progesterone and oestradiol-17β than did cows without retained placentas. Low concentrations of oestrogen pre partum have been associated with retained placentas. Chew et al. (1979a) found lower than normal pre-partum concentrations of progesterone and oestrogens in cows with retained placentas caused by ovariectomy. They have also shown that retained placentas in ovary intact cows are associated with low plasma concentrations of oestradiol-17β 6 days pre partum (Chew et al., 1979b). Interpretation of this relationship as cause and effect may be misleading because the cows with retained placentas usually have a short gestation and deliver before completion of the normal late term elevation of oestradiol.

The results of the present experiment support the hypothesis that adrenal glands can produce sufficient progesterone to maintain pregnancy in cows after Day 215.

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References


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