Placental production of progesterone in ovariectomized goats treated with a synthetic progestagen to maintain pregnancy

E. L. Sheldrick, A. P. Ricketts* and A. P. F. Flint


Summary. Pregnant goats were ovariectomized or lutectomized and treated with medroxyprogesterone acetate. The circulating concentration of progesterone, which was reduced by 85% after ovariectomy or lutectomy, increased almost 3-fold between 120 and 140 days gestation, suggesting increased placental secretion. Measurement of veno-arterial differences across the uterus confirmed that an intrauterine organ was secreting progesterone at this time. Progesterone levels decreased (by 38%) in intact animals treated with medroxyprogesterone acetate alone. Concentrations of total unconjugated oestrogens were not altered by ovariectomy or lutectomy or by medroxyprogesterone acetate alone, but were related to the birth weight of the kids. Chronic treatment with medroxyprogesterone acetate reduced milk yield during lactation by up to 64%.

Introduction

The placenta of the goat produces insufficient progesterone to maintain pregnancy at any stage of gestation; ovariectomy during pregnancy invariably causes abortion (Drummond-Robinson & Asdell, 1926; Meites, Webster, Young, Thorp & Hatch, 1951; Cowie, Daniel, Prichard & Tindal, 1963). However, the uptake of progesterone by the gravid uterus decreases after approximately 110 days gestation (Thorburn & Schneider, 1972), and the placenta contains 3β-hydroxysteroid dehydrogenase after Day 116 (Wiener, 1976; Flint, Kingston, Robinson & Thorburn, 1978). Therefore it seems possible that the placenta may produce small amounts of progesterone during late pregnancy.

To determine the placental contribution to circulating progesterone concentrations in late pregnancy we have measured plasma progesterone levels in ovariectomized goats in which pregnancy was maintained with a synthetic progestagen, medroxyprogesterone acetate (MPA). Data are also presented on the effects of MPA on plasma oestrogen levels and milk yields.

Materials and Methods

Animals. Sixteen Saanen goats of the Institute’s herd were used; with 2 exceptions they were studied in their first pregnancies. For measurement of peripheral progesterone concentrations the ovaries (1 animal) or corpora lutea (3 animals) were removed after mid-line ventral laparotomy, using aseptic surgical techniques, between 71 and 96 days gestation. Anaesthesia was induced with intravenous pentobarbitone sodium and maintained after endotracheal intubation with

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halothane in oxygen in a closed-circuit system. When necessary ovarian vessels exposed after excision of corpora lutea (luteectomy) were ligated with chromic catgut sutures. For collection of uterine venous and arterial blood, a further 3 goats were ovariectomized by identical techniques, between 125 and 129 days gestation. Polyvinyl catheters (i.d. 1.5 mm, o.d. 2.1 mm; NT3-SH90: Portex Ltd, Hythe, Kent) were inserted in the uterine vein draining the pregnant uterine horn and in a femoral artery; the uterine venous catheter was exteriorized through the flank, and both catheters were located dorsally.

Medroxyprogesterone acetate (MPA; Depo-Provera or Promone-E: Upjohn Ltd, Crawley, Sussex) was administered intramuscularly, 6 mg once per week, starting at least 3 days before surgery, and treatment was ended at delivery; 5 intact animals were also treated with MPA (identical doses), starting on Days 65–79 of gestation, and 4 intact, untreated pregnant goats acted as controls. Blood samples (20 ml) were taken once per week during pregnancy and more frequently towards term by jugular venepuncture into heparinized syringes and immediately centrifuged to obtain plasma, which was stored frozen. Milk yields from each gland were measured separately, for both daily milkings, and the average daily yields were calculated for each week of lactation. Udder volumes were determined at weekly intervals during the last third of pregnancy by the water displacement method of Linzell (1966) without milking or oxytocin treatment.

**Measurement of progesterone and oestrogens.** Concentrations of progesterone and total unconjugated oestrogens (oestriadiol-17β plus oestradiol-17α plus oestrone) were measured in plasma by radioimmunoassays described elsewhere (Flint, Anderson, Patten & Turnbull, 1974; Sheldrick, Mitchell & Flint, 1980). These assays have previously been validated for plasma from goats (Flint et al., 1978); for the progesterone and oestrogen assays the respective values were 84 and 91% for mean extraction recovery, 8.5 and 8.6% for intra-assay coefficient of variation, and 5.4 and 4.0% for the inter-assay coefficient of variation. The sensitivities were 0.016 ng progesterone/ml and 5.6 pg total oestrogens/ml.

For measurement of progesterone in plasma from ovariectomized or luteectomized goats it was necessary to purify the extracts before assay, and this was achieved by reverse-phase chromatography on columns of celite (Abraham, Hopper, Tulchinsky, Swerdloff & Odell, 1971). The need for a purification step was demonstrated by assaying these samples with and without purification. For intact animals, samples after chromatography gave 91 ± 6.7% (mean ± s.e.m., n = 10) of the concentration before chromatography; for ovariectomized/luteectomized animals the corresponding value was 57 ± 13·4% (n = 5). The blank value in the progesterone assay, determined in an ovariectomized animal post partum, was 34 pg/ml.

Radioimmunoassay cannot be regarded as providing unequivocal identification of a compound because of the limited specificity of antisera, and therefore possible cross-reactants in the progesterone assay were considered. The major known cross-reactants with the antibody used are 11-deoxycorticosterone, 5α-pregnanedione and pregnenolone, which interact at 4·0%, 2·0%, and 1·5% respectively. To assess the possible contribution of these compounds, an extract of plasma from an ovariectomized, MPA-treated goat on Day 135 was chromatographed on celite before being subjected to thin layer chromatography. Assay of consecutive bands from the thin-layer plate showed no cross-reacting material co-chromatographing with 11-deoxycorticosterone, pregnenolone or 5α-pregnanedione (Text-fig. 1). An important metabolite of progesterone in vitro, which is known to circulate at high concentrations in late gestation, is 5β-pregnane-3α,20α-diol (Flint et al., 1978; E. L. Sheldrick, unpublished observations); however, this compound is separated from progesterone by celite chromatography (as is 11-deoxycorticosterone). The cross-reaction of MPA in the progesterone assay is below 0·04%. Thus as far as can be judged by these means the radioimmunoassay carried out on extracts purified by celite chromatography is specific for progesterone.

**Measurement of medroxyprogesterone acetate.** Medroxyprogesterone acetate was measured by radioimmunoassay, using an antibody raised in goats by Cornette, Kirton & Duncan (1971).
Placental progesterone in the goat

Plasma (1 ml samples in duplicate) was extracted with 5 ml peroxide-free diethyl ether and the ether extracts were dried under a stream of N₂. Antibody (0.25 ml of a 1:6000 dilution) and tracer (0.25 ml containing approximately 50 000 d.p.m. [³H]medroxyprogesterone acetate; sp. act. 56.8 Ci/mmol; New England Nuclear, Dreieich, W. Germany) were added to each tube, in a buffer consisting of 50 mM-sodium phosphate, pH 7.5, 0.2% (w/v) bovine γ-globulin, 0.9% (w/v) sodium chloride and 0.1% (w/v) sodium azide. After mixing, the tubes were incubated overnight at 4°C. To separate free and bound steroid, 0.5 ml of a solution of polyethylene glycol-6000 (30% w/v in water) was added, and after vortex mixing for 1 min, γ-globulins were sedimented by centrifugation (1000 g for 30 min). Supernatants were removed and discarded, and pellets resuspended in 1 ml water before adding to 5 ml toluene-based scintillation fluid. Samples were counted after shaking and standing for 8 h. Extraction recovery was 78.6 ± 2.6% (mean ± s.e.m., n = 5) and values were corrected for losses at this stage. The sensitivity of the assay (determined from 2 × s.d. about the zero point) was 0.18 ng, and the blank value (plasma from untreated pregnant goats) was 0.12 ng/ml (n = 9). Intra- and inter-assay coefficients of variation were 11.4% (n = 12) and 9.0% (n = 5) respectively. The antibody showed negligible cross-reaction with all the naturally occurring steroids tested: cross-reactions with deoxycorticosterone, corticosterone, progesterone, 17α-hydroxyprogesterone, 5β-pregnane-3α,20α-diol, oestradiol-17α, oestradiol-17β and oestrone were below 0.01% (confirming the data of Cornette et al., 1971).

For the assay of MPA in milk, samples of colostrum or whole milk (0.1 ml) were diluted with 0.4 ml water and extracted with 5 ml diethyl ether. The extracts were dried under N₂, dissolved in 1 ml 70% cold aqueous methanol and stored overnight at -15°C. After centrifugation (1000 g for 30 min), supernatants were decanted into glass tubes and dried under N₂. The subsequent assay procedure was as for plasma (see above); each sample was assayed in triplicate and corrected for extraction losses on the basis of recovery of [³H]MPA added to the same sample and processed in parallel. Mean extraction recoveries were 44% for colostrum, and 61% for milk. Blank values (which were subtracted from the results) were 0.9 ng/ml for colostrum and 0.7 ng/ml for milk.
Results

Effect of MPA on gestation length and fetal viability

Median gestation length in the herd under study is 150 days (mean 149.7 days), with 90% of animals giving birth between 146 and 154 days of gestation (Peaker, 1978). In the group of animals studied here, the 4 untreated goats delivered spontaneously after 146, 150, 151 and 151 days of gestation. Since pregnancy can be maintained beyond term with progesterone treatment (Meites et al., 1951; Rawlings & Ward, 1973), it was considered advisable to deliver kids by Caesarean section in animals receiving MPA, and this was done in all but one goat, which kidded spontaneously at Day 151. In the remaining 8 animals, Caesarean section was performed on Days 149 (2 animals), 150 (1), 151 (1), 154 (3) and 155 (1). All but one of the kids were delivered alive; the dead kid, which was one of twins and born to an animal in the intact MPA-treated group, was estimated to have died in utero during the last 2 days of pregnancy.

Birth weight of kids was not affected by MPA treatment or ovariectomy/lutectomy. Mean (± s.e.m.) birth weights (kg) of singleton kids from untreated, MPA-treated and ovariectomized/lutectomized MPA-treated animals were 3.33 ± 0.46 (N = 3); 3.00 and 2.75 (2); 3.94 and 3.70 (2), respectively. Comparable values for animals with multiple pregnancies were 2.00 and 2.40 (2); 3.04 ± 0.18 (7); and 3.00 ± 0.12 (4).

Maternal progesterone and oestrogen levels after ovariectomy or lutectomy

Circulating progesterone and total unconjugated oestrogen concentrations are shown in Table 1 and Text-figs 2 and 3. Ovariectomy or lutectomy reduced progesterone values at 110–120 and 135–145 days of gestation (P < 0.001 by Student’s t test at both times). The level was also reduced, relative to that of untreated control animals, by MPA treatment alone (P < 0.001 at both stages tested). In ovariectomized/lutectomized animals, the progesterone concentration increased from 0.46 ± 0.04 ng/ml (mean ± s.e.m., n = 18 samples) between 100 and 129 days to 1.09 ± 0.06 ng/ml (n = 11; P < 0.001) between 136 and 148 days, and then dropped before term.

Table 1. Maternal peripheral plasma concentrations of progesterone and total unconjugated oestrogens, birth weight of kids and milk yields during the first week of lactation in intact untreated (Group 1), intact MPA-treated (Group 2) and ovariectomized/lutectomized, MPA-treated goats (Group 3)

<table>
<thead>
<tr>
<th>Hormone conc. (ng/ml)</th>
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<tbody>
<tr>
<td>No. of goats</td>
</tr>
<tr>
<td>Group</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for the number of observations in parentheses.

To determine whether the gravid uterus was the source of the progesterone in peripheral plasma, catheters were placed in 3 ovariectomized animals at Days 125–129 of gestation. In a total of 13 pairs of uterine venous and femoral arterial plasma samples obtained from these goats between 128 and 141 days of gestation, the mean ± s.e.m. veno-arterial difference in progesterone concentration was 0.57 ± 0.18 ng/ml (Table 2).

Neither ovariectomy/lutectomy nor MPA treatment had any effect on circulating oestrogen concentrations, which appeared to be related only to the total birth weight of kids (Table 1).
Text-fig. 2. Maternal peripheral plasma progesterone concentrations during the last third of pregnancy before parturition in 4 ovariectomized/luteectomized MPA-treated goats. The number of samples assayed is given at each point. Values are means for samples obtained between each of the times indicated on the abscissa; vertical bars indicate s.e.m.

Text-fig. 3. Variation of mean total unconjugated oestrogen concentrations measured in maternal peripheral plasma of goats between 112 and 142 days gestation with total weight of fetuses carried determined at birth. Regression analysis gives $y = 3.85 \log_{10} x - 0.648$; correlation coefficient, $r = 0.8595; P < 0.001$. ○, control untreated animals; ●, intact MPA-treated animals; △, ovariectomized/luteectomized animals.

Text-fig. 3). The oestrogen level increased during the last week of pregnancy in all treatment groups; the mean concentrations (± s.e.m.) at 136–147 and 148–153 days were 1.56 ± 0.16 (n = 35 samples) and 2.09 ± 0.22 ng/ml (n = 16; P < 0.02) respectively.

**Circulating concentrations of medroxyprogesterone acetate**

MPA was measured in maternal plasma throughout the treatment period and in a limited number of samples obtained during lactation (Text-fig. 4). Concentrations increased during the first 30 days of treatment before reaching a plateau. Treatment was stopped at parturition, and the MPA level dropped during lactation; in 5 plasma samples obtained between 40 and 60 days after delivery it was 12 ± 4% (s.e.m.) of that at term. MPA subsequently became undetectable in
blood between 100 and 170 days post partum, and shortly thereafter (at the start of the next breeding season) the goats returned to oestrus. The interval between delivery and the subsequent return to oestrus was not affected by treatment with MPA. MPA was not measured in fetal or neonatal blood, and therefore it is not known whether it crosses the placenta from the mother. Puberty and oestrous behaviour occurred normally in female kids born to MPA-treated goats.

### Table 2. Progesterone concentrations in uterine venous and femoral arterial plasma of ovariectomized MPA-treated goats during late pregnancy

<table>
<thead>
<tr>
<th>Goat</th>
<th>Gestation age (days)</th>
<th>Progesterone conc. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uterine vein</td>
</tr>
<tr>
<td>567</td>
<td>134–141</td>
<td>3.46 ± 0.60 (6)</td>
</tr>
<tr>
<td>G29</td>
<td>128</td>
<td>1.12 ± 0.12 (3)</td>
</tr>
<tr>
<td>G30</td>
<td>131</td>
<td>0.87 ± 0.13 (4)</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for the no. of observations in parentheses. For all differences between venous and arterial concentrations, $P < 0.01$ (Wilcoxon signed rank test for paired samples).

Milk production during the first week of lactation was reduced by 64% ($P < 0.001$), irrespective of whether MPA administration was accompanied by ovariectomy/lutectomy. Although MPA treatment was stopped at parturition, milk yield continued to be adversely affected throughout.
**Placental progesterone in the goat**

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Weeks of lactation

**Text-fig. 5.** Effect of treatment with medroxyprogesterone acetate during pregnancy in the goat on milk yield during the subsequent lactation. Values are mean (± s.e.m.) daily yields calculated for each week of milking; yield from each gland was determined separately at 2 milkings per day. Total no. of values = 6552. Untreated animals (○), intact and ovariectomized/lutectomized, MPA-treated animals (●).

lactation. Measurement of udder volume between Days 110 and term showed that MPA had no consistent inhibitory effect on mammary growth.

Concentrations of MPA in milk were higher than in plasma at the start of lactation; the concentration in samples obtained 5 min before inducing anaesthesia for Caesarean section ranged from 2.1 to 9.4 ng/ml (mean ± s.e.m. 4.61 ± 1.63 ng/ml for 4 animals). However, MPA disappeared at least as rapidly from milk as from plasma after delivery and the end of treatment; in 2 animals in which plasma and milk levels were measured simultaneously it had dropped by 87% within 20–27 days of the start of lactation compared with a decline of 75% in plasma.

**Discussion**

Measurement of circulating progesterone concentrations in ovariectomized or lutectomized goats showed that the level increased between 130 and 142 days and subsequently dropped before term. Veno-arterial differences across the uterus suggested that the placenta was secreting progesterone between Days 128 and 141. This confirms circumstantial evidence described in the ‘Introduction’. The mechanism underlying increased placental progesterone production in late pregnancy is not known; it may be related to the mechanism controlling the increase in progesterone secretion by the placenta in the sheep at about 50–70 days gestation, which is itself poorly understood (Thorburn, Challis & Robinson, 1977; Ricketts & Flint, 1980). The fact that ovariectomy or lutectomy has no effect on circulating oestrogen levels, which are influenced primarily by fetal weight, is consistent with the proposal that the ovary is not a major source of oestrogens during pregnancy in the goat (see Currie & Thorburn, 1977). An alternative source
of oestrogens, and one which would not be expected to be affected by ovariectomy or lutectomy, is the mammary gland, and this has been shown to produce a large proportion of the oestrogen circulating near term (Maule Walker & Pealker, 1978).

The fall in the circulating concentration of progesterone during the last week of pregnancy in intact goats reflects luteal regression (Currie & Thorburn, 1977). The cause of the decrease in progesterone before term in ovariectomized or lutectomized animals treated with MPA (Text-fig. 2) is less certain. One possibility is that it results from the activation or induction of placental enzymes which metabolize C₂₁ steroids to oestrogens; an increase in placental 17α-hydroxylase activity in response to raised fetal glucocorticoid concentrations has been implicated in the mechanisms leading to the onset of labour in goats (Flint et al., 1978), and a similar series of events is thought to occur, and to cause a decrease in placental progesterone secretion, in sheep before parturition (see Flint & Ricketts, 1979).

Progesterone levels in intact goats receiving MPA were consistently reduced relative to those in untreated animals. This effect, which was not accounted for by any differences in the incidence of multiple pregnancies in this group, suggests that MPA reduces luteal progesterone secretion. This may be brought about through inhibition of LH release; the corpus luteum in LH-dependent in late pregnancy in the goat (Buttle, 1978), and LH levels are reduced in sheep by chronic progestagen treatment (see Karsch, Legan, Ryan & Foster, 1978). Some inhibition of LH release might be expected if the gestational effect of circulating MPA was greater than that normally exerted in pregnancy by progesterone. The potency of MPA has been quoted as 30–40 times greater than that of progesterone in the rat and guinea-pig (Miyake & Pincus, 1958; Illingworth & Deanesly, 1972); if this is also true for the goat, then the circulating MPA concentration (which reached 0·6–0·8 ng/ml; Text-fig. 4) is equivalent to a concentration of progesterone of 18–36 ng/ml, which is above the normal range (Table 1).

MPA has been shown to inhibit 3β-hydroxysteroid dehydrogenase in human corpus luteum and rat testis (Johansson, 1971; Satyaswaroop & Gurpide, 1978; Shinada, Yokota & Igarashi, 1978) and it may therefore have a direct effect in reducing progesterone secretion by the placenta. If this did occur it might lead to underestimation of the placental contribution to circulating progesterone levels. However, MPA treatment had no effect on oestrogen concentrations, and any inhibitory effect on steroid synthesis in the placenta is likely, therefore, to be small; this may reflect the low peripheral concentrations of MPA in vivo, compared to those shown to be inhibitory in vitro.

Treatment with MPA during pregnancy reduced milk yield during the subsequent lactation. Although this effect was observed in animals which underwent Caesarean section, it did not appear to be caused by surgical delivery of the kids; lactational yields were also low in the one intact MPA-treated animal which delivered spontaneously. This effect was not unexpected; MPA used as a long-term contraceptive interferes with lactation in some women (Parveen, Chowdhury & Chowdhury, 1977), and a reduction in circulating progesterone concentration is an important factor controlling lactogenesis in goats (Davis et al., 1979) and sheep (Hartmann, Trevethan & Shelton, 1973). The MPA preparations used in the present study (Depo-Provera & Promone-E) are long-acting, microcrystalline formulations released over a long period in primates (Cornette et al., 1971; Kirton & Cornette, 1974; Shrimanker, Saxena & Fotherby, 1978), and MPA was found in the circulation of the goats 1–2 months after the end of treatment. Nonetheless, the level did drop dramatically during early lactation, and it is possible that the decreasing levels of MPA and the rising milk yield in treated animals are causally related. The mechanism whereby MPA reduces milk yield is uncertain. Since progesterone reduces oxytocin release in response to vaginal distension in goats (Roberts, 1975) MPA may reduce oxytocin secretion in response to milking, thereby preventing normal let down of milk; however, administration of oxytocin during milking did not increase milk yield in MPA-treated animals (E. L. Sheldrick, unpublished observations). An alternative mechanism is that MPA treatment reduces the mammary prolactin receptor concentration, as does progesterone in the rat (Djiane...
& Durand, 1977). The possibility that MPA reduces the circulating prolactin concentration seems less likely, in view of the increased release of prolactin in response to TRH which occurs in sheep treated chronically with progesterone (Wright, Jenkin, Heap & Walters, 1978).

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References


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