Oestrogen concentrations in systemic plasma of pregnant pygmy goats*

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Summary. Concentrations of oestradiol-17β, oestradiol-17α, and oestrone in systemic plasma of pregnant pygmy goats (Capra hircus) remained low until about Day 60 and then rose to maximum values at Days 120–140 (parturition Day 140–145). Oestradiol-17α was the predominant oestrogen. All 3 oestrogens at Days 100–130 were higher in females carrying 3 fetuses than in those carrying only one, but at Days 70–90 only oestrone values were higher. It is suggested that the feto-placental unit is the source of oestrogens during gestation in goats.

Introduction

The pygmy goat is frequently used for physiological and pharmacological studies in pregnancy, and the reasons have been discussed elsewhere (Metcalf, Hoversland, Erickson, Rogers & Clary, 1968; Hoversland, Dhindsa & Metcalfe, 1971). Various workers have reported oestrogen concentrations in goat plasma in late pregnancy and at parturition (Thorburn, Nicol, Bassett, Shutt & Cox, 1972; Currie, Wong, Cox & Thorburn, 1973; Umo, Fitzpatrick & Ward, 1976; Flint, Kingston, Robinson & Thorburn, 1978). Although Challis & Linzell (1971) described total unconjugated oestrogens at various stages of pregnancy in the goat, little is known about individual oestrogen concentrations at these stages. In an effort to obtain more basic knowledge about fluctuations in oestrogens during pregnancy in this species, we have measured the quantities of oestrone, oestradiol-17β, and oestradiol-17α circulating in systemic plasma.

Materials and Methods

Six pygmy goats (Capra hircus) used in this study were mated at a spontaneous oestrus or at oestrus induced by Cronolone (9-fluoro-11β,17-dihydroxyprogesterone-17, acetate) treatment (Dhindsa, Hoversland & Metcalfe, 1971). Blood samples (4 ml) were taken from the jugular veins of unanaesthetized animals into heparinized syringes at about 10-day intervals during pregnancy. Blood samples were centrifuged immediately, and the plasma was removed and frozen at −16°C until steroid analyses were performed. Concentrations of oestrone, oestradiol-17β and oestradiol-17α were measured by radioimmunoassay. The steroids (free and conjugated to plasma proteins) were extracted with ether from 250 μl goat plasma, and were

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separated on Sephadex LH-20 columns in the solvent system hexane : benzene : methanol (62:20:13 by vol.). The dimensions of the columns and the fractions analysed have been reported elsewhere (Resko, Ploem & Stadelman, 1975). The specificity of the antisera and estimates of assay reliability are presented in Table 1.

Table 1. Specificity and reliability of oestrogen measurements with radioimmunoassay after chromatography on Sephadex LH-20

<table>
<thead>
<tr>
<th>Steroid tested</th>
<th>% Cross-reactivity*</th>
<th>Eluant volume (ml)</th>
<th>% Recovery</th>
<th>Water blank (pg/ml)</th>
<th>Within-assay coefficient of variation§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestrone</td>
<td>100.00</td>
<td>0.09</td>
<td>7-9</td>
<td>83.9 ± 2.0</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
<td>(9)</td>
</tr>
<tr>
<td>Oestradiol-17α</td>
<td>124.30</td>
<td>0.06</td>
<td>10-15</td>
<td>3.7 ± 1.6</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(10)</td>
<td></td>
<td></td>
<td>(10)</td>
</tr>
<tr>
<td>Oestradiol-17β</td>
<td>389.60</td>
<td>100.00</td>
<td>11-15</td>
<td>79.97 ± 1.83</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
<td>(10)</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>16.20</td>
<td>0.09</td>
<td>&gt;20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for the number of samples indicated in parentheses. The sensitivity of these assays is at least 5 pg/tube since measurements of 5 pg quantities are significantly different from steroid-free serum blanks (P < 0.05).

* Little or no cross-reactivity (with 500 pg oestrone or oestradiol) was found when 20 neutral steroids (androgens, progestagens and corticoids) were tested with both antisera.

† Antiserum obtained from a sheep immunized with oestradiol-17β-succinyl–bovine serum albumin (BSA). The percentage was calculated with 500 pg oestrone as the reference (100%).

‡ Antiserum obtained from a rabbit immunized with 6-keto-oestradiol-17β coupled to BSA. The percentage was calculated with 500 pg oestradiol as the reference (100%).

§ All samples were analysed in the same assay.

*c Recoveries of oestradiol-17β used for oestradiol-17α.

We used Antiserum A to measure oestrone and oestradiol-17α and Antiserum B to quantify oestradiol-17β. Approximately 6% of the oestradiol-17α may be removed and quantified as oestrone when oestrone is isolated on Sephadex LH-20 columns. Since oestradiol-17α and -17β do not separate on our chromatography system (see Table 1), we differentiated the isomers by collecting the oestradiol fraction from the column, dividing it into two parts and assaying each part with Antiserum A or B. Oestradiol-17α does not cross-react with Antiserum B used to quantify oestradiol-17β and our estimates of oestradiol-17β are therefore reliable. The oestradiol-17α measurements, however, may have been elevated slightly by the small quantities of oestradiol-17β in the serum samples since Antiserum A also cross-reacts with oestradiol-17β. However, the concentrations of oestradiol-17β were so small relative to those of oestradiol-17α that they did not significantly affect the measurements. This fact became evident when different aliquots of the oestradiol-17α extracted from plasma of several goats were assayed and compared with a standard solution of oestradiol-17α: different volumes of material extracted from goat plasma gave results parallel to those obtained with different concentrations of oestradiol-17α in our radioimmunoassay. Oestradiol-17α and oestradiol-17β standards are not parallel to each other under these test conditions.

Statistical comparisons were made by means of Student's t tests. The influence of the number of fetuses on oestrogen concentrations in the maternal circulation was estimated by the Mann–Whitney U test.

Results

Oestrone

The concentrations of oestrone in the peripheral plasma of 6 pregnant goats are shown in
Text-fig. 1a. From Days 10 to 30 of gestation oestrone levels remained low. Beginning on Day 40, oestrone levels rose significantly ($t = 3.5111$, 11 d.f., $P < 0.01$) and reached their highest value, $450 \pm 60$ (s.e.m.) ng/ml, on Day 120 of pregnancy. Oestrone levels then declined slightly but not significantly.

Text-fig. 1. Concentrations of (a) oestrone (●) and oestradiol-17β (O) and (b) oestradiol-17α in the systemic plasma of pregnant pygmy goats. Values are mean ± s.e.m. for 6 animals in (a) and the numbers indicated in (b). P = range for parturition.

Oestradiol-17β

Concentrations were quite low from Day 10 to Day 60 of gestation (Text-fig. 1a) but then rose steadily to reach the highest value, $32 \pm 9$ (s.e.m.) pg/ml, on Day 140, and then declined before parturition. The first significant elevation in oestradiol-17β levels above the basal level occurred on Day 60 ($t = 2.8985$, 13 d.f., $P < 0.02$).
**Oestradiol-17α**

Values were low from Days 10 to 60 of gestation (Text-fig. 1b) but then increased steadily and reached a peak value of $1.5 \pm 0.3$ (s.e.m.) ng/ml on Day 130. There was a decline to $1.1 \pm 0.2$ (s.e.m.) ng/ml plasma on Day 140, but this was not significant because of the large variation between samples.

**Fetal number and steroid concentrations**

To determine whether the number of fetuses affected steroid concentrations in the maternal blood, the data relating to oestrone and oestradiol were divided into two groups, one containing values for Days 70–90 and the other values for Days 100–130 of gestation (Table 2).

<table>
<thead>
<tr>
<th>Gestational age (days)</th>
<th>No. of fetuses</th>
<th>No. of pregnant females</th>
<th>Oestrogen conc. (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oestrone</td>
</tr>
<tr>
<td>70–90</td>
<td>1</td>
<td>3</td>
<td>$140 \pm 20$ (10)</td>
</tr>
<tr>
<td>70–90</td>
<td>2 or 3</td>
<td>3</td>
<td>$320 \pm 80$* (9)</td>
</tr>
<tr>
<td>100–130</td>
<td>1</td>
<td>3</td>
<td>$290 \pm 20$ (12)</td>
</tr>
<tr>
<td>100–130</td>
<td>3</td>
<td>2</td>
<td>$480 \pm 40$* (12)</td>
</tr>
</tbody>
</table>

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

Values differing significantly from those for goats carrying 1 fetus: *$P < 0.05$; **$P < 0.005$ (Mann–Whitney U test).

Between Days 70 and 90 the oestrone levels in goats carrying single fetuses were significantly lower ($P < 0.05$) than those in goats carrying 2 or 3 fetuses ($140 \pm 20$ versus $320 \pm 80$ ng/ml). Similarly, goats carrying single fetuses between Days 100 and 130 of gestation had significantly lower ($P < 0.002$) levels of oestrone than did goats carrying triplets ($290 \pm 20$ versus $480 \pm 40$ ng/ml).

Between Days 70 and 90 of gestation, concentrations of oestradiol-17β and oestradiol-17α in the peripheral plasma of pregnant goats were not significantly different ($P > 0.05$) in relation to the number of fetuses being carried. However, the levels of both isomers between Days 100 and 130 of gestation were significantly lower ($P < 0.05$) in goats carrying single fetuses than those carrying triplets.

**Discussion**

This study demonstrates changes in systemic concentrations of three physiological oestrogens throughout pregnancy in the pygmy goat, a breed of goat that is becoming popular for biological research.

Oestradiol-17β is produced in small quantities throughout pregnancy. The quantities of oestrone were much greater, and oestradiol-17α was produced in the largest amounts. Our data agree with those reported by other investigators who used other methods of oestrogen measurement (Thorburn et al., 1972). We did not observe an elevation in oestrogen before parturition. Our data, however, were not collected close enough to the time of parturition to establish this point with any degree of certainty. The fact that levels of all three oestrogens were
significantly higher in the plasma of pregnant animals with multiple fetuses than in the plasma of animals with only one fetus suggests a fetal–placental source of oestrogen in this species. This same conclusion was drawn by Thorburn et al. (1972) who compared oestrone and oestradiol-17α concentrations in uterine venous and arterial plasma and after infusion of fetuses with synthetic ACTH.

The function of oestradiol-17α in goats as the major oestrogen secreted during pregnancy is not understood. In fact, little is known about the biological activity of this oestrogen in the goat. Although oestradiol-17α is thought to have weak oestrogenic activity, Huggins & Jensen (1955) stimulated uterine growth with large amounts of this compound. Toft & Gorski (1966) demonstrated partial binding of oestradiol-17α to the cytoplasmic oestrogen receptor compared to oestradiol-17β. However, these studies were performed in rats not in goats. Perhaps oestradiol-17α is more active in goats than other species. Alternatively, the large amounts secreted during pregnancy may represent a detoxification mechanism of the pregnant female to high levels of oestrogen secretion.

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References


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