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Oestradiol and Progesterone: Soluble Receptor Levels and Metabolism in the Uterus of the Ovariectomized Ewe

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Abstract

Ovariectomized ewes received injections designed to mimic to some extent oestradiol and progesterone secretion during early pregnancy (maintenance progesterone), during oestrus (oestrous oestradiol) and during the luteal phase of the previous cycle (priming progesterone). The animals were killed at times equivalent to 1, 4 or 7 days after oestrus in those animals which had received oestrous oestradiol. The level of soluble oestradiol and progesterone receptors in whole uterus, and [^{3}H]oestradiol and [^{3}H]progesterone metabolism by uterus minces were measured.

Oestradiol receptor level was highest on day 1 in those animals receiving oestrous oestradiol with no significant effect at any stage of the inclusion or omission of priming or maintenance progesterone. Progesterone receptor level was also high on day 1 in those animals receiving oestrous oestradiol with high levels maintained to day 4. Again, inclusion of priming or maintenance progesterone was without effect. In animals not receiving oestrous oestradiol the level of both receptors was uniformly low.

Metabolism of [^{3}H]oestradiol was low and not affected by treatment. [^{3}H]Progesterone metabolism, although more variable, was also low and not affected by treatment.

Introduction

It is known that progesterone will maintain early pregnancy in ewes ovariectomized a few days after mating (Foote *et al.* 1957; Moore and Rowson 1959; Bindon 1971; Cumming *et al.* 1974; Trounson and Moore 1974). It is not clear, however, just what influences progesterone secreted during the previous luteal phase and oestradiol secreted to induce oestrus have on this ability of progesterone to maintain early pregnancy. Using a model system in the ovariectomized animal, Miller and Moore (1976) have described the effects of priming progesterone administered prior to oestrus, oestradiol administered to induce oestrus and maintenance progesterone administered subsequent to oestrus, on protein and RNA metabolism in the endometrium and on the ability of the uterus to support transferred embryos. In this paper the levels of soluble oestradiol and progesterone receptors, and oestradiol and progesterone metabolism in uterine tissue in those same animals are described.

Methods

Forty-five parous mature Merino ewes which had been ovariectomized at least 2 months prior to the initiation of the treatments were used. The treatments and their injection and slaughter schedules have been described in detail (Miller and Moore 1976). Briefly, all animals received an initial injection of oestradiol and were then placed on one of five treatments. These treatments comprised the inclusion or omission of priming (pre-oestrous) progesterone, oestradiol to induce oestrus, and maintenance (post-oestrous) progesterone as summarized in Table 1. Within each treatment three

animals were killed at times equivalent to 1, 4 or 7 days after oestrus in those animals which had received the oestrous oestradiol.

Uterine tissue was removed quickly after slaughter and kept in crushed ice until frozen at −196°C for storage. Soluble oestradiol and progesterone receptor levels (expressed as pmol of steroid bound per mg of tissue DNA) were measured as previously described by Miller *et al.* (1977) except that 5 volumes rather than 3 volumes of homogenization medium were used. Incubation of [^{3}H]oestradiol and [^{3}H]progesterone with uterine minces was also as previously described (Miller *et al.* 1977) but only tissues from animals killed on days 4 and 7 were included.

Statistical significance of the results was tested by analysis of variance and Duncan's multiple range test (Steel and Torrie 1960).

Table 1. Summary of experimental design

Treatment code	Base oestradiol	Priming progesterone	Oestrous oestradiol	Maintenance progesterone
ABC	+	+	+	+
AB–	+	+	+	—
–BC	+	—	+	+
A–C	+	+	—	+
––C	+	—	—	+

Table 2. Soluble oestradiol and progesterone receptor levels in the uterus of the ewe

Values are expressed as pmol of steroid bound per mg of tissue DNA. Each value is the mean ± s.e. for three animals. For coding of treatments see Table 1

Treatment	Days after oestrus[A]		
	1	4	7
	(a) Oestradiol		
ABC	11·3±1·0	8·7±0·2	5·7±0·1
AB–	12·2±1·8	8·5±0·2	5·5±0·3
–BC	9·5±0·8	9·0±1·0	6·0±0·4
A–C	5·1±0·0	5·1±0·7	6·6±1·2
––C	4·7±0·6	5·3±0·5	5·4±0·2
	(b) Progesterone		
ABC	7·2±0·5	8·6±0·8	4·5±0·3
AB–	8·5±1·9	8·0±0·3	5·3±0·5
–BC	7·3±0·9	8·3±0·6	4·8±0·9
A–C	4·0±0·0	4·1±0·3	4·3±0·8
––C	3·4±0·5	3·8±0·7	4·1±0·5

[A] In those animals which received oestrous oestradiol.

Results

The level of oestradiol receptor is given in Table 2. Values were significantly higher in those groups which received oestrous oestradiol ($P < 0·001$), and within these groups the highest level was found one day after oestrus ($P < 0·001$) and this was not significantly influenced by the omission or inclusion of the priming and maintenance progesterone treatments. When oestrous oestradiol was omitted levels were uniformly low. By day 7 there were no significant differences between groups.

Results for the progesterone receptor are also given in Table 2. Values on days 1 and 4 were significantly higher in those groups which received oestrous oestradiol ($P < 0{\cdot}001$) and again this was not influenced by the inclusion or omission of the progesterone treatments. In contrast to results with the oestradiol receptor no difference was seen between days 1 and 4, suggesting a difference in the regulation of these two receptors. By day 7 progesterone receptor levels in the groups receiving oestrous oestradiol had fallen to those shown throughout by the groups not receiving oestrous oestradiol.

In vitro oestradiol metabolism was low in all groups with a mean ($\pm$s.e.) of $98{\cdot}6\pm0{\cdot}2\%$ of the recovered radioactivity associated with the incubated steroid. This low level of metabolism did not differ significantly with steroid treatment or time of killing. Progesterone metabolism, although more variable, also did not differ with treatment, $72{\cdot}6\pm2{\cdot}5\%$ of the recovered radioactivity being associated with progesterone.

Discussion

In laboratory animals the synthesis of soluble receptors for both oestradiol and progesterone is in part regulated by oestradiol [see Brenner and West (1975) for a review]. The results reported here for the ewe indicate that in this species also the oestradiol secreted to induce oestrus is a major regulator of these two receptor proteins. However, the more prolonged elevation of progesterone receptor levels suggests a difference in their regulation or their stability *in vivo*. Similar patterns have been seen in the intact ewe during the oestrous cycle (Miller *et al.* 1977). Progesterone administered to mimic secretion either during the luteal phase of the previous cycle or during the period of early pregnancy under study was without significant influence on the level of either receptor.

The support of a high proportion of normal embryos requires the inclusion of all three treatments studied here (Miller and Moore 1976). The present results indicate that the likely role of oestrous oestradiol is to maintain the sensitivity of the uterus to oestradiol and to progesterone by virtue of its ability to regulate their receptor levels. The role of progesterone secreted during the previous luteal phase is not clear either from the present study or from influences on endometrial metabolism (Miller and Moore 1976) and requires further investigation.

In laboratory animals the circulating levels of oestradiol also influence the uterine metabolism of oestradiol and progesterone *in vivo* and *in vitro* (Martin and Stone 1965; Pack and Brooks 1970; Armstrong and King 1971; Clark 1973; Saffron *et al.* 1974; Holtermann and Lisboa 1975). In view of this the generally low level of metabolism of these two steroids, particularly to less active compounds, and the lack of influence of steroid treatment, in the present study, is rather surprising. The low metabolism may serve to maintain the activity of these steroids in the uterus. A similar low metabolism was noted in tissues from intact animals (Miller *et al.* 1977).

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