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# Early Pregnancy in the Ewe: Effects of Oestradiol and Progesterone on Uterine Metabolism and on Embryo Survival

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*Abstract*

The hormonal regulation of embryo development during early pregnancy in the ewe has been examined. Ovariectomized ewes received injections of oestradiol ($E_2$) and progesterone (P) according to schedules designed to simulate endogenous ovarian secretion during the luteal phase of the previous oestrous cycle (*priming P*), around the time of oestrus (*oestrous* $E_2$) and during early pregnancy (*maintenance P*, *maintenance* $E_2$). Embryos were transferred to the ewes on the 4th day after induced oestrus, and ewes were killed at 6 or 13 days after transfer to assess embryo development. Cytosol concentrations of oestradiol and progesterone receptors and RNA and protein metabolism in the endometrium and amounts of protein in uterine flushings were examined on the day of embryo transfer and 6 days after transfer.

Twenty one of 27 ewes which received *maintenance P*, *oestrous* $E_2$ and *priming P* carried normal embryos at the time of killing. Omitting *maintenance* $E_2$ had no effect on the proportion of ewes in which embryos developed normally. When either *priming P* or *oestrous* $E_2$ was omitted embryos ceased to develop normally within 1–2 days of the time of transfer. Omitting *oestrous* $E_2$ reduced the amount of protein in the uterine lumen and the RNA : DNA ratio, rate of synthesis of protein and amounts of oestradiol and progesterone receptors in the endometrium at the time of embryo transfer. Omitting *priming P* caused a small decrease in the concentration of progesterone receptor but no other significant changes in the endometrium or uterine flushings at the time of transfer. It is suggested that *oestrous* $E_2$ controls embryo development by regulating endometrial sensitivity to the progesterone of pregnancy. The mechanism by which *priming P* affects embryo development remains unknown.

## Introduction

In the ewe ovariectomized shortly after mating pregnancy may be maintained by the administration of progesterone alone (Foote *et al.* 1957; Moore and Rowson 1959; Bindon 1971; Cumming *et al.* 1974; Trounson and Moore 1974). The genital tract of the intact ewe is under the influence of progesterone secreted during the luteal phase of the oestrous cycle preceding mating, and of oestrogen secreted immediately before and during the oestrus at which mating occurs. In addition, further surges of ovarian oestrogen secretion occur during early pregnancy, the first and best characterized surge being that on days 3–4 after oestrus (Cox *et al.* 1971; Mattner and Braden 1972). In chronically ovariectomized animals pregnancy may be induced and maintained by the transfer of embryos to ewes receiving progesterone alone, but only if the ewes have previously received hormone injections which simulate ovarian luteal-phase progesterone and oestrous oestrogen secretion in the intact ewe. The priming progesterone and oestrous oestradiol treatments regulate several uterine metabolic activities some of which may determine the uterine environment

in which embryo development occurs after transfer (Miller and Moore 1976; Murphy *et al.* 1977). In these studies the genital tracts o fthe ewes were examined for embryos 21 days after the time of embryo transfer. Hence it could not be determined how soon after transfer that embryo development failed in ewes which did not receive the priming progesterone or oestrous oestradiol treatments. Also, it seemed possible that injections of oestradiol given to simulate ovarian oestrogen secretion during early pregnancy in intact ewes (maintenance oestradiol) might in some way protect the embryo in those ovariectomized ewes which had not received the priming progesterone or oestrous oestradiol treatment. Accordingly in the present study we have examined the effects of priming progesterone, oestrous oestradiol and maintenance oestradiol treatments on the survival and development of embryos during the first 13 days after transfer. In order to gain some understanding of the mechanism of these hormone effects, we have also examined RNA and protein metabolism and oestradiol and progesterone receptor concentrations in the uterus, and amounts of protein in uterine luminal flushings.

**Table 1. Steroid hormone treatment regimes**

| Group | No. of ewes | Treatment regime | | | | |
|---|---|---|---|---|---|---|
| | | *Base* $E_2$ | *Priming P* | *Oestrous* $E_2$ | *Maintenance P* | *Maintenance* $E_2$ |
| 1 | 17 | + | + | + | + | + |
| 2 | 16 | + | + | + | + | − |
| 3 | 15 | + | + | − | + | + |
| 4 | 16 | + | + | − | + | − |
| 5 | 16 | + | − | + | + | + |
| 6 | 15 | + | − | + | + | − |
| 7 | 16 | + | − | − | + | + |
| 8 | 15 | + | − | − | + | − |

## Materials and Methods

*Animals and Hormone Treatment Regimes*

Mature parous Merino ewes were used. They had been bilaterally ovariectomized at least 2 months prior to the commencement of the experiment. A total of 126 ewes was treated according to the factorial design shown in Table 1. At the start of the experiment (day 0) ewes in group 1 received a single injection of 25 μg oestradiol—*base* $E_2$. They were then given 5 mg progesterone twice daily (at about 0800 and 1800 h) from day 3 to day 14—*priming P*. During days 15 and 16 ewes received a total of 35 μg oestradiol given as five injections over a period of 32 h—*oestrous* $E_2$. Progesterone treatment recommenced on day 18 and continued to day 33—*maintenance P*. On days 20 and 21 a total of 36 μg oestradiol was given by four injections over a period of 36 h—*maintenance* $E_2$, as follows:

| Day of experiment | Dose oestradiol (μg) |
|---|---|
| 20 0600 h | 6 |
| 20 1800 h | 12 |
| 21 0600 h | 12 |
| 21 1800 h | 6 |

The *oestrous* $E_2$ and *maintenance P* dose regimes have been fully described; and the *maintenance P* regime used in this study was the low dose *maintenance P* regime previously employed (Miller and Moore 1976). In the remaining groups of ewes (groups 2–8, Table 1) one or more of *priming P*, *oestrous* $E_2$ or *maintenance* $E_2$ was omitted. From day 15 to day 18 all ewes were run with vasectomized rams equipped with marking crayons. Almost all ewes in groups 1 and 2 and the majority in groups 5 and 6 were marked between 2400 h on day 16 and 0800 h on day 17.

Three ewes from each group were killed at about 0800 h on day 21 for studies of uterine metabolism (4 days after oestrus in ewes which received *oestrous* $E_2$, and about 2 h after the third oestradiol injection in ewes which received *maintenance* $E_2$). Embryos collected from donor ewes 4 days after mating were transferred to the uteri of the remaining 102 ewes on day 21. Embryos were of 8–20 cells and within each group about one-half of the ewes each received two embryos and the remainder received a single embryo. Six ewes from each group were killed on day 27, for the recovery of embryos and for metabolic studies. The remaining ewes were killed on day 34 for the recovery of embryos.

*Recovery of Embryos and Luminal Protein*

Genital tracts from ewes killed on days 21 and 27 were dissected and packed in crushed ice promptly after slaughter. The chilled uteri were flushed with 20 ml 0·15 M NaCl. The solution was injected into the lumen near the utero–tubal junction of one uterine horn and collected through a polythene cannula inserted into the lumen at the utero–tubal junction of the opposite horn. The flushings were maintained at 5°C and embryos present in the flushing were removed and examined as fresh specimens and after staining with 1% orcein. Depending on their stage of development, embryos were classed as normal or abnormal. Normal embryos were hatched late blastocysts showing marked expansion (Green and Winters 1945), whilst abnormal embryos showed limited or no development beyond that observed at the time of transfer (see Figs 1 and 2).

Following the removal of embryos the flushings were centrifuged at 12 000 *g* for 30 min at 5°C. After decanting, the volume of the supernatants was adjusted to 20·0 ml with 0·15 M NaCl and aliquots (200 $\mu$l) were taken to determine protein (Hartree 1972).

Uteri collected from ewes killed on day 34 were not flushed. They were carefully opened and any embryos present were removed, examined and classed as normal or abnormal. Those classed as normal were at a stage of development similar to that found in intact ewes at the same stage of pregnancy (Green and Winters 1945), whilst abnormal embryos were either grossly retarded or resorbing.

*RNA and Protein Metabolism in the Endometrium and Oviduct*

Slices of endometrium and sections of isthmic oviduct were prepared from the chilled genital tracts promptly after slaughter, and *in vitro* rates of synthesis of protein and RNA : DNA ratios in each tissue were determined as previously described (Miller 1976), except for the following two modifications. To determine rates of protein synthesis each incubation was carried out in the presence of 1·0 $\mu$Ci L-[4,5-$^3$H]leucine (l Ci/mmol, Radiochemical Centre, Amersham, U.K.) instead of L-[methyl-$^3$H]methionine; and for each ewe RNA : DNA ratios and rates of synthesis of protein were determined in the same tissue samples, so that the results for protein synthesis could be expressed per milligram of tissue DNA. Duplicate determinations were carried out for each ewe and three endometrial slices or pieces of oviduct were used in each duplicate. Results are expressed as mean tissue RNA : DNA ratios and mean $^3$H dpm (protein) per microgram of DNA.

*Total [$^3$H]Oestradiol and [$^3$H]Progesterone Cytosol Receptor Estimation*

Tissues for receptor estimation were frozen at −70°C and stored at −30°C until assayed. Levels of oestradiol and progesterone receptor in whole uterus cytosols, in terms of picomoles of steroid bound per milligram of tissue DNA, were measured by agar gel electrophoresis at low temperature as previously described (Miller *et al.* 1977), with the following modification. Non-specific binding was estimated by the inclusion of incubations containing a 100-fold excess of unlabelled steroid with the labelled compounds. This binding did not change with treatment and thus although its inclusion changed the estimate of receptor concentration the pattern of change with treatment was not affected.

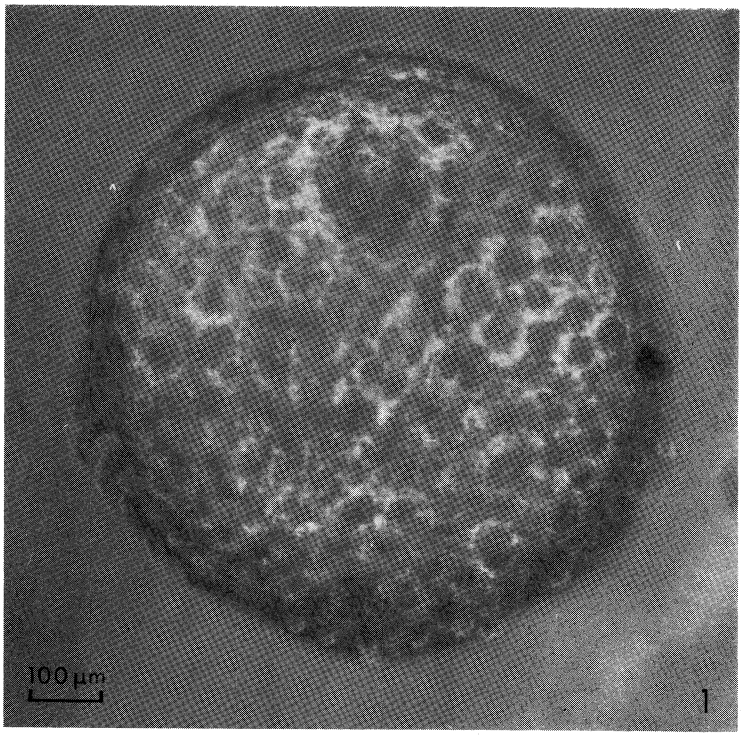
*Statistical Procedures*

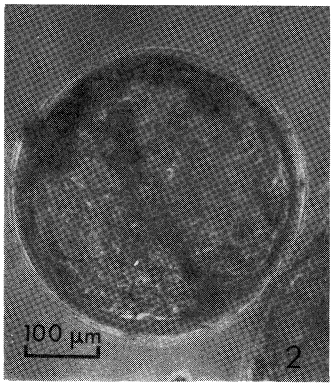
Standard tests of $\chi^2$ corrected for continuity (Snedecor 1956) and analyses of variance were used.

## Results

Embryos were recovered from 23 of 48 ewes on day 27 and from 27 of 54 on day 34 of the experiment (Table 2). However, a number of the embryos was classed as abnormal and on both days only 13 of the ewes killed had one or more normal

embryos. There was no effect of number of embryos transferred on either the proportion of ewes with embryos (normal or abnormal) or with normal embryos.




**Figs 1 and 2.** Embryos recovered on day 27 of the experiment. **1**, Normal hatched and expanded blastocyst. **2**, Retarted embryo; limited expansion and not hatched.

Overall, *maintenance* $E_2$ had no effect upon the proportion of ewes with normal embryos and within treatments the proportions of ewes with normal embryos at days 27 and 34 were remarkably similar. Omission of either *oestrous* $E_2$ (groups 3 and 4 *v.* 1 and 2; 0 of 25 *v.* 21 of 27; $\chi^2 = 29{\cdot}47$; $P < 0{\cdot}001$) or *priming P* (groups 5 and 6 *v.* 1 and 2; 4 of 25 *v.* 21 of 27; $\chi^2 = 19{\cdot}66$; $P < 0{\cdot}001$) markedly reduced the proportion of ewes with normal embryos. Where both *priming P* and *oestrous* $E_2$ were omitted there was a suggestion in ewes killed on day 27 that the further omission of *maintenance* $E_2$ increased the survival of embryos (group 7 *v.* 8; 0 of 6 *v.* 5 of 6;

$\chi^2 = 5{\cdot}49$; $P < 0{\cdot}05$). However, the majority of embryos recovered on day 27 from these ewes were grossly retarded and a similar difference was not observed on day 34.

**Table 2. Proportion of ovariectomized ewes with embryos**

*P.P, Priming P; O.$E_2$, oestrous $E_2$; M.$E_2$, maintenance $E_2$*

| Group | Treatment regime | | | Proportion of ewes | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | With normal or abnormal embryos | | | With normal embryos | | |
| | *P.P* | *O.$E_2$* | *M.$E_2$* | Day 27[A] | Day 34 | Total | Day 27 | Day 34 | Total |
| 1 | + | + | + | 5/6 | 7/8 | 12/14 | 5/6 | 6/8 | 11/14 |
| 2 | + | + | − | 6/6 | 6/7 | 12/13 | 5/6 | 5/7 | 10/13 |
| 3 | + | − | + | 1/6 | 2/6 | 3/12 | 0/6 | 0/6 | 0/12 |
| 4 | + | − | − | 1/6 | 2/7 | 3/13 | 0/6 | 0/7 | 0/13 |
| 5 | − | + | + | 2/6 | 4/7 | 6/13 | 1/6 | 1/7 | 2/13 |
| 6 | − | + | − | 3/6 | 5/6 | 8/12 | 1/6 | 1/6 | 2/12 |
| 7 | − | − | + | 0/6 | 0/7 | 0/13 | 0/6 | 0/7 | 0/13 |
| 8 | − | − | − | 5/6 | 1/6 | 6/12 | 1/6 | 0/6 | 1/12 |

[A] Days 27 and 34 of the experiment correspond to 10 and 17 days respectively after oestrus in ewes that received *oestrous $E_2$*.

The effects of the treatment regimes on uterine wet weight, amounts of luminal protein and endometrial RNA and protein metabolism are shown in Table 3. In ewes which received *priming P*, *oestrous $E_2$* and *maintenance $E_2$* (group 1) uterine weight and the amount of luminal protein were very similar on days 21 and 27, but

**Table 3. Effects of oestradiol and progesterone on endometrial RNA and protein metabolism**

*P.P, Priming P; O.$E_2$, oestrous $E_2$; M. $E_2$, maintenance $E_2$*

| Group | Treatment regime | | | Uterine weight (g) | | Luminal protein (mg) | | RNA : DNA | | Protein synthesis (dpm/μg DNA) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *P.P* | *O.$E_2$* | *M.$E_2$* | | | | | | | | |
| | Day of experiment: | | | 21 | 27 | 21 | 27 | 21 | 27 | 21 | 27 |
| | Days after induced oestrus[A]: | | | 4 | 10 | 4 | 10 | 4 | 10 | 4 | 10 |
| 1 | + | + | + | 42·6 | 41·4 | 5·04 | 5·35 | 0·618 | 0·521 | 71·3 | 31·9 |
| 2 | + | + | − | 31·0 | 34·5 | 2·84 | 3·90 | 0·493 | 0·481 | 45·2 | 29·5 |
| 3 | + | − | + | 39·4 | 35·4 | 3·07 | 2·58 | 0·498 | 0·514 | 65·3 | 33·0 |
| 4 | + | − | − | 24·6 | 26·8 | 1·59 | 3·34 | 0·366 | 0·423 | 23·4 | 26·8 |
| 5 | − | + | + | 35·2 | 46·6 | 4·03 | 3·87 | 0·668 | 0·555 | 85·4 | 39·8 |
| 6 | − | + | − | 25·5 | 34·6 | 2·09 | 3·97 | 0·535 | 0·473 | 51·2 | 32·6 |
| 7 | − | − | + | 25·2 | 32·1 | 2·34 | 4·30 | 0·468 | 0·523 | 76·6 | 38·3 |
| 8 | − | − | − | 15·2 | 22·6 | 1·89 | 3·65 | 0·322 | 0·427 | 24·4 | 24·9 |

[A] In ewes that received *oestrous $E_2$*.

in the endometrium the RNA:DNA ratio and particularly the rate of synthesis of protein decreased between these two times. When *priming P* was omitted (groups 5–8 *v.* 1–4) there was a decrease in uterine weight on day 21 ($P < 0{\cdot}001$) but not on day 27, and the rate of synthesis of protein, expressed per microgram of DNA, appeared to increase slightly on days 21 and 27, though the increase was significant only at the later time ($P < 0{\cdot}05$). There were no significant changes in luminal protein or RNA:DNA ratios on either day.

When *oestrous* $E_2$ was omitted (groups 3, 4, 7 and 8 *v.* 1, 2, 5 and 6) uterine weight diminished on days 21 ($P < 0{\cdot}01$) and 27 ($P < 0{\cdot}001$), and the amount of luminal protein was greatly reduced on day 21 ($P < 0{\cdot}001$) but not on day 27. The RNA:DNA ratio decreased markedly on day 21 ($P < 0{\cdot}001$) and to a lesser extent on day 27 ($P < 0{\cdot}01$), and the rate of synthesis of protein decreased on day 21 only ($P < 0{\cdot}01$).

The omission of *maintenance* $E_2$ (groups 2, 4, 6 and 8 *v.* 1, 3, 5 and 7) reduced the weight of the uterus on both days 21 and 27 ($P < 0{\cdot}001$), and luminal protein on day 21 ($P < 0{\cdot}001$) but not on day 27. The decreases in RNA:DNA ratios and rates of synthesis of protein on both days 21 and 27 were highly significant ($P<0{\cdot}001$). In each of the analyses of variance of these data almost all of the variation due to treatment regimes was attributable to primary effects of *priming P*, *oestrous* $E_2$ or *maintenace* $E_2$, and there were in most cases no significant interactions between these components of the treatment regimes.

**Table 4. Effects of oestradiol and progesterone on oestradiol and progesterone cytosol receptor concentrations in the uterus**

Values are expressed as mean pmol of steroid bound per mg of tissue DNA. *P.P, Priming P; O.*$E_2$, *oestrous* $E_2$; *M.*$E_2$, *maintenance* $E_2$

| Group | Treatment regime | | | Cytosol receptor | | | |
|---|---|---|---|---|---|---|---|
| | *P.P* | *O.*$E_2$ | *M.*$E_2$ | Oestradiol | | Progesterone | |
| | Day of experiment: | | | 21 | 27 | 21 | 27 |
| | Days after induced oestrus[A]: | | | 4 | 10 | 4 | 10 |
| 1 | + | + | + | 4·72 | 4·74 | 2·60 | 0·46 |
| 2 | + | + | − | 5·36 | 4·66 | 2·02 | 0·37 |
| 3 | + | − | + | 3·49 | 4·97 | 1·12 | 0·36 |
| 4 | + | − | − | 3·82 | 4·66 | 0·76 | 0·38 |
| 5 | − | + | + | 5·42 | 4·75 | 1·46 | 0·54 |
| 6 | − | + | − | 5·43 | 5·09 | 1·86 | 0·42 |
| 7 | − | − | + | 3·54 | 4·63 | 0·83 | 0·24 |
| 8 | − | − | − | 2·71 | 4·01 | 0·40 | 0·39 |

[A] In ewes that received *oestrous* $E_2$.

The effects of the treatment regimes on oestradiol and progesterone cytosol receptor concentrations in whole uterus are shown in Table 4. In ewes which received *priming P*, *oestrous* $E_2$ and *maintenance* $E_2$ (group 1), the concentration of oestradiol receptor was very similar on days 21 and 27, whereas that of the progesterone receptor decreased between these two times. In the case of the oestrogen receptor the omission of *oestrous* $E_2$ decreased receptor concentration on day 21 ($P < 0{\cdot}05$) but not on day 27, whilst omitting *priming P* or *maintenance* $E_2$ had no significant effect. The concentration of progesterone receptor on day 21 was decreased by omitting *oestrous* $E_2$ ($P < 0{\cdot}001$) and to a lesser extent by omitting *priming P* ($P < 0{\cdot}01$). On day 27 the effects of omitting *oestrous* $E_2$ or *priming P* on the progesterone receptor were greatly reduced, and the omission of *maintenance* $E_2$ had no significant effect at either time. There were no significant interactions between *priming P*, *oestrous* $E_2$ and *maintenance* $E_2$ on the cytosol concentrations of either type of receptor.

The oviducts were examined to determine RNA:DNA ratios and rates of synthesis of protein on day 21 only (Table 5). The omission of *oestrous* $E_2$ or *maintenance* $E_2$

decreased considerably the RNA:DNA ratio and protein synthesis ($P < 0{\cdot}001$), but omitting *priming P* had no effect.

## Discussion

From the several metabolic activities measured it appears that the uteri from ewes in each of the eight treatment groups were all rather similar by day 27 (i.e. by 10 days after oestrus in ewes which received *oestrous* $E_2$). In view of this and the finding that within groups the survival and development of embryos on days 27 and 34 was similar, it seems likely that subsequent survival and development of embryos was determined during a period close to the time of embryo transfer. The results for recoveries of normal embryos are in close agreement with our earlier data (Miller and Moore 1976) and they suggest that in the chronically ovariectomized ewe prior hormonal stimulation of the uterus by *priming P* and *oestrous* $E_2$ is required in order to establish at around the time of transfer a uterine environment suitable for the survival and development of embryos. Also, it appears from the present study that these as yet undefined components of endometrial metabolism which regulate embryo development in early pregnancy were not influenced by *maintenance* $E_2$ even though it markedly affected general uterine metabolism. *Maintenance* $E_2$ in no way compensated for deficiencies in the uterus resulting from the omission of the *priming P* or *oestrous* $E_2$ treatments.

**Table 5. Effects of oestradiol and progesterone on oviducal metabolism**

*P.P, Priming P; O.*$E_2$*, oestrous* $E_2$*; M.*$E_2$*, maintenance* $E_2$

| Group | Treatment regime | | | RNA : DNA Day 21[A] | Protein synthesis (dpm/μg DNA) Day 21 |
|---|---|---|---|---|---|
| | *P.P* | *O.*$E_2$ | *M.*$E_2$ | | |
| 1 | + | + | + | 0·960 | 116·5 |
| 2 | + | + | − | 0·741 | 68·4 |
| 3 | + | − | + | 0·796 | 110·3 |
| 4 | + | − | − | 0·534 | 42·8 |
| 5 | − | + | + | 1·073 | 144·0 |
| 6 | − | + | − | 0·788 | 77·5 |
| 7 | − | − | + | 0·668 | 94·9 |
| 8 | − | − | − | 0·479 | 41·6 |

[A] Four days after oestrus in ewes that received *oestrous* $E_2$.

A principal aim of this and the previous studies (Miller and Moore 1976; Murphy *et al.* 1977) was to determine possible mechanisms whereby *priming P* and *oestrous* $E_2$ might influence subsequent survival and development of embryos. Although *oestrous* $E_2$ increased protein synthesis and RNA:DNA ratios in the endometrium at the time of embryo transfer, these changes *per se* appear not to influence embryo survival, as *maintenance* $E_2$ also increased protein synthesis and mean cell content of RNA but had no effect on survival. However, qualitative differences in endometrial responses to *oestrous* $E_2$ and *maintenance* $E_2$ may be important. Any such differences would not have been revealed in the present study, where only total protein and RNA fractions were examined. Similarly, embryo survival appears unrelated to the total amount of protein in the uterine lumen, as both *oestrous* $E_2$ and *maintennace* $E_2$

increased total protein in uterine flushings at the time of embryo transfer. Perhaps the concentration of protein in the luminal fluid at the time of flushing, which we were unable to measure, regulates embryo development, as has been suggested in the rat (Surani 1975). In further studies (B. G. Miller, unpublished data) the composition of protein from uterine flushings was examined by fractionation techniques employing Sephadex G200 and polyacrylamide gels. The results suggested that almost all of the luminal protein was derived from plasma, and there was no evidence in ewes receiving any of the treatment regimes employed in the present experiment of progesterone-dependant secretion of low-molecular-weight uterine proteins, such as has been demonstrated in the rabbit and pig (Krishnan and Daniel 1967; Murray *et al.* 1972). Further, there appears to be no consistent effects of different hormone treatment regimes on the proportions of various plasma proteins which were recovered in uterine flushings.

The finding that oestradiol cytosol receptor concentrations in whole uterus were very similar on days 21 and 27 was surprising, as it was anticipated that *maintenance* $E_2$ would stimulate the synthesis of oestradiol receptor on days 20 and 21 (Jensen *et al.* 1969). Perhaps on day 21 increased synthesis of the receptor protein may have been hidden by the simultaneous loss of cytosol receptor to the nucleus (Jensen *et al.* 1968). Since oestrogens are apparently not required during early pregnancy for the normal development of sheep embryos it is difficult to envisage a biological role for the oestradiol receptor protein at the time of embryo transfer. On the other hand the amount of progesterone receptor protein in endometrial cytosol at around the time of embryo transfer may regulate the uterine response to the progesterone of pregnancy (*maintenance P* in this study) and hence embryo survival. The present results agree well with the earlier finding (Murphy *et al.* 1977) that *oestrous* $E_2$ greatly increased the progesterone receptor concentration in the uterus at around the time of embryo transfer. Regrettably, we have not so far measured any metabolic activity in the endometrium which is specifically regulated by progesterone. Apart from alkaline phosphatase (Murdoch and White 1968*a*, 1968*b*), we know of no enzyme whose activity in ewe endometrium is controlled specifically by progesterone. Nevertheless we tentatively suggest that in this model system the requirement for *oestrous* $E_2$ is explained by its regulation of cytosol progesterone receptor concentration in the endometrium at around the time of embryo transfer.

In contrast to *oestrous* $E_2$, *priming P* had no significant effect in the previous study (Murphy *et al.* 1977), and only a minor effect in the present experiment on the concentration of progesterone receptor during early pregnancy. Since *priming P* had no significant effect on endometrial RNA:DNA ratios or rates of synthesis of protein or on the amount of protein in uterine flushings at the time of embryo transfer, the mechanism by which *priming P* regulated embryo development in this study remains unknown. Of the various uterine parameters studied only uterine weight was markedly increased by *priming P*. Perhaps the 'priming' effect of *oestrous* $E_2$ at around oestrus is dependent on prior progesterone stimulation of the uterus, but this seems unlikely since there was little evidence of interactions between progesterone and oestradiol in this or early studies in the ovariectomized ewe (Miller 1976; Miller and Moore 1976; Murphy *et al.* 1977). In the present study the concentrations of both hormone receptors were lower than those seen in previous studies with intact or ovariectomized animals (Miller *et al.* 1977; Murphy *et al.* 1977). The reasons for this are not known.

In the intact ewe the first luteal-phase surge of oestrogen secretion on days 3–4 of the oestrous cycle (Cox *et al.* 1971) has little effect on uterine blood flow and capillary permeability (Brown 1973) or on endometrial metabolism and cytosol hormone receptor concentrations (Miller *et al.* 1977). Thus the considerable effects of *maintenance* $E_2$ on some aspects of endometrial metabolism in the present study may appear unphysiological. We cannot explain these differences between the uterine responses in intact and ovariectomized ewes. Perhaps the dose of *maintenance* $E_2$ chosen was too high, or the use of intermittent intramuscular injections of oestradiol and progesterone rather than continuous infusions of these hormones accounts for the differences.

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