Aust. J. Biol. Sci., 1976, **29**, 565–73

Effects of Progesterone and Oestradiol on RNA and Protein Metabolism in the Genital Tract and on Survival of Embryos in the Ovariectomized Ewe

B. G. Miller and N. W. Moore

Department of Animal Husbandry, University of Sydney, Camden, N.S.W. 2570.

Abstract

The hormonal regulation of metabolism in the genital tract and the development of embryos during early pregnancy in the ewe have been examined. Ovariectomized ewes received injections of maintenance progesterone, oestrous oestradiol and priming progesterone according to schedules designed to simulate endogenous ovarian secretion during early pregnancy, around the time of oestrus and during the luteal phase of the oestrous cycle immediately preceding oestrus.

The survival and development of embryos was dependent upon the dose of maintenance progesterone and the duration of treatment at the time of transfer, but changes in progesterone dose did not change endometrial protein or RNA metabolism on particular days. Both priming progesterone and oestrous oestradiol were required for normal embryo development. Priming progesterone and oestrous oestradiol each increased endometrial RNA/DNA ratios during early pregnancy. There were no interactions between priming progesterone and oestrous oestradiol, their effects being simply additive. Neither maintenance nor priming progesterone had any effect on protein and RNA metabolism in the oviduct. It is suggested that in the intact ewe oestrogen secreted at oestrus and progesterone secreted prior to oestrus play important roles in the establishment of a uterine environment suitable for the subsequent normal development of embryos.

Introduction

The survival and development of pre-implantation mammalian embryos is dependant upon the uterine environment, and in a number of species, particularly the rat and rabbit, it has been well demonstrated that the uterine environment can be markedly influenced by ovarian steroid hormones (for reviews see McLaren 1973; Psychoyos 1973).

In the ewe it has been shown in animals ovariectomized a few days after mating that progesterone will maintain early pregnancy (Foote *et al.* 1957; Moore and Rowson 1959; Bindon 1971; Cumming *et al.* 1974; Trounson and Moore 1974). However, prior to ovariectomy the ewe would have been under the influence of oestrogen secreted around the time of oestrus, and of progesterone secreted during the luteal phase of the oestrous cycle immediately preceding oestrus. Hence, these studies have been unable to suggest any role that these two phases of ovarian secretion might play in the subsequent development of embryos.

The present study was designed firstly to examine in some detail the progesterone requirements of the ewe during the first 3–4 weeks of pregnancy and to determine the

roles that progesterone secreted prior to oestrus and oestrogen secreted during pro-oestrus might play in the subsequent survival and development of embryos. Secondly, we have sought to relate embryo development to changes in the uterine environment, by also examining changes in RNA and protein metabolism within the genital tract. Portions of this study have been briefly described elsewhere (Moore 1975; Miller and Moore 1976; Moore and Miller 1976).

Table 1. Steroid hormone treatment schedules for experiments 1 and 2

Day of experiment	Time	Progesterone (mg)		Oestradiol (μg)	Operation or event	
0	a.m.	—		25		
3–14	a.m.	5				
	p.m.	5				
15	1600 h	—		3·5		
	2400 h	—		7·0		
16	0800 h	—		14·0		
	1600 h	—		7·0		
	2400 h	—		3·5	Oestrus	
17	a.m.	—			Oestrus	
	p.m.	—				
		Low	High		Experiment 1	Experiment 2
18	a.m.	0·50	1·00		Slaughter	Slaughter
	p.m.	0·625	1·25			
19	a.m.	0·75	1·50			
	p.m.	1·00	2·00		Slaughter and embryo transfer	
20	a.m.	1·25	2·50			
	p.m.	1·50	3·00			
21	a.m.	2·0	4·0		Slaughter and embryo transfer	Slaughter and embryo transfer
	p.m.	2·5	5·0			
22	a.m.	3·0	6·0			
	p.m.	4·0	8·0		Slaughter and embryo transfer	
23	a.m.	5·0	8·0			
	p.m.	6·0	8·0			
24	a.m.	8·0	8·0		Slaughter	Slaughter
	p.m.	8·0	8·0			
25	a.m.	8·0	8·0			
	p.m.	8·0	8·0			
26–41	a.m.	12·0	12·0			
42					Slaughter	Slaughter

Materials and Methods

Experimental Animals

Mature parous Merino ewes were used in the study and they had been bilaterally ovariectomized at least 2 months prior to the commencement of the experiments.

Experiment 1

A total of 128 ovariectomized ewes was treated as shown in Table 1. At the start of the experiment (day 0) all ewes 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 all ewes received a total of 35 μg oestradiol given as five injections over a period of 32 h—*oestrous* E_2. From day 15 to day 18 the ewes were run with vasectomized rams equipped with marking crayons, and all were marked between 2400 h on day 16 and 0800 h on day 17. Progesterone treatment recommenced on day 18 and continued to day 41—*maintenance P*. Two regimes were used and from day 18 to day 25 progesterone was administered twice daily, and thereafter once daily. Oestradiol and progesterone were dissolved in peanut oil and administered by intramuscular injection.

Twenty-seven of the 128 ewes were killed for studies of uterine metabolism; three at 0600 h on day 18 and three from each of the two *maintenance P* regimes at 1800 h on day 19, 0600 h on day 21, 1800 h on day 22 and 0600 h on day 24 (1, $2\frac{1}{2}$, 4, $5\frac{1}{2}$ and 7 days after oestrus). Embryos collected from donor ewes 4 days after mating were transferred to the uteri of the remaining 101 ewes at $2\frac{1}{2}$, 4 or $5\frac{1}{2}$ days after oestrus (Moore and Shelton 1964). Embryos were of 8–20 cells and each ewe received one embryo. On day 42 (25 days after oestrus) the ewes were killed to recover embryos.

Experiment 2

A total of 104 ovariectomized ewes was used and all received *base* E_2 on day 0. They were then placed under one of five regimes. The *priming P* and *oestrous* E_2 treatments used were the same as those used in experiment 1, but only the low dose *maintenance P* treatment of experiment 1 was used. In four of the five regimes one or more of *priming P*, *oestrous* E_2 or *maintenance P* was omitted (Table 2).

Table 2. Steroid hormone treatment regimes for experiment 2

Group	Treatment regime			
	Base E_2	*Priming P*	*Oestrous* E_2	*Maintenance P*
1	+	+	+	+
2	+	+	+	−
3	+	+	−	+
4	+	−	+	+
5	+	−	−	+

Forty-five of the ewes were killed for metabolic studies; three from each of the five treatment groups at 0800 h on each of days 18, 21 and 24 (1, 4 and 7 days after induced oestrus in ewes which received *oestrous* E_2). A single 4-day-old embryo was transferred to the uterus of each of the remaining 59 ewes on day 21, and these ewes were killed on day 42.

RNA and Protein Metabolism in the Endometrium and Oviduct

Ewes destined for metabolic studies did not receive *maintenance P* on the morning or evening on which they were killed. Genital tracts were dissected and packed in crushed ice promptly after slaughter by exsanguination. Uniform slices of endometrium were prepared, using a Stadie–Riggs microtome (A. H. Thomas Company, Philadelphia, U.S.A.). In experiment 2, but not in experiment 1, sections each about 1·0 cm long, were collected from the isthmic portions of the oviducts. Rates of synthesis of protein and tissue RNA/DNA ratios were determined as previously described (Miller 1976). 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/μg protein.

Statistical Procedures

Standard tests of χ^2 corrected for continuity (Snedecor 1956) and analyses of variance were used. Data on proportions of ewes with embryos at slaughter in experiment 1 were subjected to analyses of variance after angular transformation of the raw data.

Results

Experiment 1

A single embryo was recovered from 73 ewes, but only 45 embryos were classed as normal (Table 3). Embryos classed as normal were all at a stage of development similar to that found in intact ewes at the same stage of pregnancy (Green and Winters 1945). The remaining 28 embryos were either grossly retarded or showed advanced resorption and were considered to be non-viable. Retarded embryos were 3 or more days less advanced than those classed as normal. There was an effect of time of transfer ($P < 0{\cdot}01$; d.f. 1, ∞) on the proportion of ewes with normal embryos.

Table 3. Proportion of ovariectomized ewes with normal and abnormal embryos at slaughter in experiment 1

Time of transfer (days after induced oestrus)	*Maintenance P* regime				Total	
	Low		High			
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
$2\frac{1}{2}$	6/17	5/17	13/16	0/16	19/33	5/33
4	12/17	2/17	7/17	6/17	19/34	8/34
$5\frac{1}{2}$	5/17	8/17	2/17	7/17	7/34	15/34
Total	23/51	15/51	22/50	13/50	45/101	28/101

There was no main effect of progesterone regime, but there was a significant interaction between regime and time of transfer ($P < 0{\cdot}01$; d.f. 2, ∞). In ewes under the low progesterone regime maximum survival and development followed the synchronous transfer of 4-day-old embryos to recipients 4 days after oestrus, whereas in those under the high regime there was a linear effect of time of transfer ($P < 0{\cdot}01$; d.f. 1, ∞), with maximum survival in recipients which received an embryo $2\frac{1}{2}$ days after oestrus.

Table 4. Rate of synthesis of protein in the endometrium at different times after induced oestrus in experiment 1

Values expressed are means for three ewes

	Maintenance P regime	Days after induced oestrus				
		1	$2\frac{1}{2}$	4	$5\frac{1}{2}$	7
Rate of synthesis of protein (^{3}H dpm/μg protein)	Low	20·0	14·4	12·8	13·3	9·7
	High	20·0	14·5	12·5	11·5	9·6

Rates of synthesis of protein in the endometrium are shown in Table 4. The rate declined over the sampling period ($P < 0{\cdot}001$; d.f. 1, 18), but there was no significant difference between ewes under the two progesterone regimes in rates of synthesis on particular days after oestrus. Similarly, there was no effect on particular days of progesterone regime on endometrial RNA/DNA ratios.

Experiment 2

A single embryo was recovered from 21 of 59 ewes, but 10 embryos were either grossly retarded or resorbing and were classed as non-viable (Table 5). When *main-*

tenance P (group 2) or *priming P* and *oestrous* E_2 (group 5) were omitted from the treatment regime no ewe had an embryo, either normal or abnormal. Survival and normal development was greatest in ewes which received all components of the regime (group 1). Omission of *oestrous* E_2 markedly reduced the proportion of ewes with normal embryos (1/13 *v.* 8/13; $\chi^2 = 8{\cdot}01$; $P < 0{\cdot}01$), whilst when *priming P* was omitted there was a reduction, which approached significance, in the proportion of ewes with normal embryos (2/11 *v.* 8/13; $\chi^2 = 3{\cdot}00$; $0{\cdot}10 > P > 0{\cdot}05$).

Table 5. Proportion of ovariectomized ewes with normal and abnormal embryos at slaughter in experiment 2

Proportion of ewes	Groups, and treatments omitted					Total
	1 Nil	2 *Maintenance P*	3 *Oestrous* E_2	4 *Priming P*	5 *Priming P, oestrous* E_2	
With normal embryos	8/13	0/11	1/13	2/11	0/11	11/59
With abnormal embryos	1/13	0/11	6/13	3/11	0/11	10/59
Total	9/13	0/11	7/13	5/11	0/11	21/59

The effects of the various treatment regimes on uterine wet weight and on endometrial RNA and protein metabolism are shown in Table 6. When *maintenance P* was omitted (group 2 *v.* group 1; 4 and 7 days after oestrus) there were decreases in the rate of synthesis of protein ($P < 0{\cdot}05$; d.f. 1, 30) and in the RNA/DNA ratio ($P < 0{\cdot}01$, d.f. 1, 30), but there was no significant difference in uterine wet weights.

Table 6. Effects of priming progesterone and of oestrous oestradiol on RNA and protein metabolism in the endometrium in experiment 2

Values expressed are means for three ewes. *M. P, Maintenance P*; *O.* E_2, *oestrous* E_2; *P. P, priming P*

	Day of experiment	Days after induced oestrus[A]	Groups, and treatments omitted				
			1 Nil	2 *M.P*	3 *O.*E_2	4 *P.P*	5 *P.P, O.*E_2
Uterine wet weight (g)	18	1	41·5	43·8	25·3	36·3	18·8
	21	4	33·4	26·6	18·5	28·4	21·8
	24	7	27·5	24·2	26·3	34·3	21·1
Rate of synthesis of protein (^{3}H dpm/μg protein)	18	1	13·3	12·9	10·0	13·2	5·9
	21	4	13·1	10·3	7·5	10·6	9·8
	24	7	9·4	8·6	8·6	10·1	11·2
Tissue RNA/DNA ratio	18	1	0·913	0·865	0·585	0·742	0·410
	21	4	0·786	0·676	0·512	0·667	0·423
	24	7	0·649	0·530	0·558	0·723	0·544

[A] In ewes which received *oestrous* E_2.

In ewes which received *maintenance P* the omission of *oestrous* E_2 (groups 3 and 5 *v.* groups 1 and 4) markedly reduced uterine weight, rate of protein synthesis and the RNA/DNA ratio ($P < 0{\cdot}001$; d.f. 1, 30). By day 24 the differences in protein

synthesis had disappeared and those for uterine weight and RNA/DNA ratio were reduced. Omitting *priming P* in ewes which received *maintenance P* had no effect on uterine weight or on rate of protein synthesis, but did reduce RNA/DNA ratios on days 18 and 21 (groups 4 and 5 *v.* groups 1 and 3; $P < 0{\cdot}001$; d.f. 1, 30). There were no interactions between *oestrous* E_2 and *priming P*, their effects being simply additive.

Table 7. Effects of priming progesterone and of oestrous oestradiol on RNA and protein metabolism in the oviduct in experiment 2

Values expressed are means for three ewes, except in the case of group 4 on day 21 where only two ewes were available. *M. P, Maintenance P*; *O.* E_2, *oestrous* E_2; *P. P, priming P*

	Day of experiment	Days after induced oestrus[A]	Group, and treatments omitted				
			1 Nil	2 *M.P*	3 *O.*E_2	4 *P.P*	5 *P.P, O.*E_2
Rate of synthesis of protein (^{3}H dpm/μg protein)	18	1	10·4	10·2	7·0	10·4	5·9
	21	4	7·6	8·2	5·8	8·5	5·9
	24	7	7·4	7·2	6·3	7·6	6·7
Tissue RNA/DNA ratio	18	1	0·995	0·921	0·585	1·079	0·517
	21	4	0·769	0·734	0·544	0·798	0·584
	24	7	0·630	0·720	0·552	0·601	0·434

[A] In ewes which received *oestrous* E_2.

When the oviducts were examined (Table 7) the omission of *maintenance P* had no effect on the RNA/DNA ratio or rate of protein synthesis. The omission of *oestrous* E_2 in ewes which received *maintenance P* markedly reduced RNA/DNA ratios and the rates of protein synthesis ($P < 0{\cdot}001$; d.f. 1, 29). However, the omission of *priming P* had no effect on any of the metabolic parameters under study.

Discussion

In experiment 1 the progesterone dose regime employed after oestrus had a marked effect upon the survival and development of embryos. In the intact ewe, as in other mammalian species, survival and development of transferred embryos are dependent upon close synchronization between the post-ovulatory age of recipients and age of embryos (Moore and Shelton 1964). The low dose progesterone regime would appear to have satisfied these requirements. In animals under the high progesterone regime, however, the greatest survival followed transfer to uteri younger, in terms of time after oestrus, than the embryos transferred. Clearly, both dose and duration of progesterone stimulation were important in the development of a uterine environment suitable for survival and normal development. However, no difference in rates of synthesis of protein in the endometrium of ewes under the two regimes could be detected. If progesterone induces specific secretory proteins in the ewe (Menezo 1973) as in porcine (Murray *et al.* 1972) and rabbit (Krishnan and Daniel 1967) endometrium then the results suggest that the synthesis of any such proteins, which possibly regulate embryo development, would comprise only a small portion of the total protein synthesis occurring in the endometrium.

The results of experiment 2 suggest that in the intact ewe oestrogen secreted during

pro-oestrus and oestrus and progesterone secreted prior to oestrus have effects on the uterus which regulate the subsequent survival and normal development of embryos. Omission of *oestrous* E_2 markedly reduced the proportion of ewes with normal embryos, but the effect of *priming P* was not as apparent. However, in further studies involving larger numbers of animals a marked effect of omission of *priming P* has been demonstrated (B. G. Miller and N. W. Moore, unpublished data). In the ewe, the first ovulation of the breeding season is generally not accompanied by oestrus (Grant 1934), and Robinson (1955) has suggested that failure of oestrus is due to the lack of a phase of progesterone secretion prior to secretion of oestrogen. Similarly, oestrus usually does not accompany the first ovulation after parturition, and Hunter (1968) regards failure to exhibit oestrus and mate at the first ovulation as a source of reproductive wastage. However, results of the present study would suggest that even if mating and fertilization occurred early embryonic development would fail.

In ewes which received *maintenance P* the stimulatory effects of *priming P* and *oestrous* E_2 on RNA/DNA ratios in the endometrium followed the same pattern as did their effects on survival and normal development of embryos. We suggest that changes in RNA/DNA ratios reflect metabolic effects of *priming P* and *oestrous* E_2 on the potential of the endometrium for glandular secretion under the subsequent influence of *maintenance P*. Further studies are in progress to determine the effects of *priming P* and *oestrous* E_2 on the protein composition of the luminal fluid. There appears to be no published information on the roles of priming progesterone and oestrous oestrogen in early pregnancy in other species which have an oestrus cycle that includes an extended luteal phase. In the rat and mouse, oestrous oestrogen increases glandular epithelial and stromal cell mitotic activity in the endometrium during early pregnancy (Finn and Martin 1969), and is essential for maximal endometrial sensitivity to an oil decidual stimulus (Finn 1966; Finn and Martin 1972). However, oestrous oestrogen is not necessary in these species for the induction of the decidual reaction by trauma (Rothchild and Meyer 1942; Finn 1966), or for implantation and development of transferred embryos (Humphrey 1969). Clearly, major species differences exist in the pattern of steroid hormone secretion required for early pregnancy, and perhaps these differences are related to the duration of the oestrous cycle in different species. Species differences are further illustrated by the unexpected and consistent absence of any interactions between the effects of *oestrous* E_2 and of *priming P* on any of the parameters studied. In the rat and mouse major interactions between the effects of oestrogen and progesterone on uterine weight and on RNA synthesis have been clearly demonstrated (Miller and Emmens 1969; Bronson and Hamilton 1972; Trams *et al.* 1973). However, the absence of such interactions was consistent with the finding (Miller 1976) that the effects of the two hormones on RNA and protein synthesis in the endometrium of the ewe at pro-oestrus are simply additive.

The changes in oviducal metabolism were, presumably, unrelated to the development of embryos transferred to the uterus. However, they are of interest, as such changes may have important effects on early pregnancy in intact ewes (Brenner 1973; Hafez 1973; Hamner 1973). The inability of either priming or maintenance progesterone to influence total RNA or protein metabolism in the oviduct is surprising, but does not exclude the possibility that progesterone induces the synthesis of small amounts of specific proteins in the luminal epithelium of the oviduct, as has been suggested by Bronson and Hamilton (1972) and Miller (1976).

Acknowledgments

This work was aided by a grant from the Australian Research Grants Committee. The authors wish to thank Mr J. Ellsmore and Mr L. O'Dea for the care of the experimental animals and Mrs A. Budden and Miss S. Hocking for their valuable technical assistance.

References

Bindon, B. M. (1971). The role of progesterone in implantation in the sheep. *Aust. J. Biol. Sci.* **24**, 149–58.

Brenner, R. M. (1973). Hormonal regulation of oviducal epithelium. In 'The Regulation of Mammalian Reproduction'. (Eds S. J. Segal, R. Crozier, P. A. Corfman and P. G. Condliffe.) pp. 337–348. Charles C. Thomas: Illinois.)

Bronson, F. H., and Hamilton, T. H. (1972). A comparison of nucleic acid synthesis in the mouse oviduct and uterus: interactions between estradiol and progesterone. *Biol. Reprod.* **6**, 160–7.

Cumming, I. A., Baxter, R., and Lawson, R. A. S. (1974). Steroid hormone requirements for the maintenance of early pregnancy in sheep: a study using ovariectomised adrenalectomised ewes. *J. Reprod. Fertil.* **40**, 443–6.

Finn, C. A. (1966). Endocrine control of endometrial sensitivity during the induction of the decidual cell reaction in the mouse. *J. Endocrinol.* **36**, 239–48.

Finn, C. A., and Martin, L. (1969). The role of 'oestrous' oestrogen in the preparation of the endometrium for implantation. *Acta Endocrinol. (Copenhagen) Suppl.* **138**, 38.

Finn, C. A., and Martin, L. (1972). Endocrine control of the timing of endometrial sensitivity to a decidual stimulis. *Biol. Reprod.* **7**, 82–6.

Foote, W. D., Gooch, L. D., Pope, A. L., and Casida, L. E. (1957). The maintenance of early pregnancy in the ovariectomised ewe by injection of ovarian hormones. *J. Anim. Sci.* **16**, 986–9.

Grant, R. (1934). Studies on the physiology of reproduction in the ewe. *Trans. R. Soc. Edinburgh* **58**, 1–47.

Green, W. W., and Winters, L. M. (1945). Prenatal development of the sheep. Univ. Minn. Agr. Exp. Sta., Tech. Bull. No. 169.

Hafez, E. S. E. (1973). Endocrine control of the mammalian oviduct. In 'Handbook of Physiology'. Section 7, Vol. 2, Part 2. (Ed. R. O. Greep.) pp. 97–122. (American Physiological Society: Washington.)

Hamner, C. E. (1973). Oviducal fluid—composition and physiology. In 'Handbook of Physiology'. Section 7, Vol. 2, Part 2. (Ed. R. O. Greep.) pp. 141–151. (American Physiological Society: Washington.)

Humphrey, K. W. (1969). Induction of implantation of blastocysts transferred to ovariectomised mice. *J. Endocrinol.* **44**, 299–305.

Hunter, G. L. (1968). Increasing the frequency of pregnancy in sheep. II. Artificial control of rebreeding and problems of conception and maintenance of pregnancy during the post-partum period. *Anim. Breed. Abstr.* **36**, 533–53.

Krishnan, R. S., and Daniel, J. C. (1967). 'Blastokinin': inducer and regulator of blastocyst development in the rabbit uterus. *Science* **158**, 490–2.

McLaren, A. (1973). Endocrinology of implantation. *J. Reprod. Fertil. Suppl.* **18**, 159–66.

Menezo, M. Y. (1973). Etude électrophretique des protéines des sécretions uterines de brébis au debut de la gestation. *C. R. Acad. Sci. Ser. D* **277**, 337–40.

Miller, B. G. (1976). RNA and protein metabolism in the oviduct and endometrium of the ewe at pro-oestrus: regulation by oestradiol and progesterone. *J. Endocrinol.* **69**, 57–66.

Miller, B. G., and Emmens, C. W. (1969). The effects of oestradiol and progesterone on the incorporation of tritiated uridine into the genital tract of the mouse. *J. Endocrinol.* **43**, 427–36.

Miller, B. G., and Moore, N. W. (1976). Effects of progesterone and oestradiol on endometrial metabolism in the ovariectomised ewe. *J. Reprod. Fertil.* **46**, 535–6.

Moore, N. W. (1975). Progesterone requirements for the maintenance of pregnancy in the ovariectomised ewe. *J. Reprod. Fertil.* **43**, 386.

Moore, N. W., and Miller, B. G. (1976). Progesterone and oestrogen requirements for the survival of embryos in the ovariectomised ewe. *J. Reprod. Fertil.* **46**, 536–7.

Moore, N. W., and Rowson, L. E. A. (1959). Maintenance of pregnancy in ovariectomised ewes by means of progesterone. *Nature* (*London*) **184**, 1410.

Moore, N. W., and Shelton, J. N. (1964). Egg transfer in sheep. Effect of degree of synchronisation between donor and recipient, age of egg and site of transfer on the survival of transferred eggs. *J. Reprod. Fertil.* **7**, 145–52.

Murray, F. A., Bazer, F. W., Wallace, H. D., and Warnick, A. C. (1972). Quantitative and qualitative variation in the secretion of protein by the porcine uterus during the estrous cycle. *Biol. Reprod.* **7**, 314–20.

Psychoyos, A. (1973). Hormonal control of ovoimplantation. *Vitam. Horm.* (*N.Y.*) **31**, 201–56.

Robinson, T. J. (1955). Quantitative studies on the hormonal induction of oestrus in spayed ewes. *J. Endocrinol.* **12**, 163–73.

Rothchild, I., and Meyer, R. K. (1942). Studies of the pretrauma factors necessary for placentoma formation in the rat. *Physiol. Zool.* **15**, 216–23.

Snedecor, G. W. (1956). 'Statistical Methods'. 5th Edn. p. 217. (Iowa State University Press: Iowa.)

Trams, G., Brewitt, H., Möllmann, H., and Maass, H. (1973). Effect of progesterone on RNA and protein synthesis in the rat uterus. *Acta Endocrinol.* (*Copenhagen*) **73**, 740–50.

Trounson, A. O., and Moore, N. W. (1974). Effect of progesterone and oestrogen on the survival and development of fertilized ova in the ovariectomized ewe. *Aust. J. Biol. Sci.* **27**, 511–17.

Manuscript received 24 June 1976