Fertilization and early embryonic development in androstenedione-immunized Merino ewes

M. P. Boland*, C. D. Nancarrow, J. D. Murray, R. J. Scaramuzzi, R. Sutton, R. M. Hoskinson and I. G. Hazelton

C.S.I.R.O. Division of Animal Production, P.O. Box 239, Blacktown, New South Wales 2148, Australia

Summary. Ewes were immunized against androstenedione (Fecundin) and assigned to be mated 14 days (179 ewes Group C) or 25 days (174 ewes Group B) after a booster immunization with Fecundin. The anti-androstenedione titres at these times were 6790 and 3240 respectively ($P < 0.01$). The remaining 169 ewes were untreated controls (Group A). Ewes were mated to entire rams (12 rams to 180 ewes) at their second oestrus after synchronization of oestrus. Immunization against androstenedione caused a shortening of the time from sponge removal to mating (Day 0) and a decrease in the percentage of ewes mated by the rams. Also, ovulation rate was increased after immunization ($P < 0.01$), being 1.42, 2.16 and 1.93 for Groups A, C and B respectively.

Egg recovery rates on Day 2 were lower in immunized ewes and there was some indication that fertilization rates were lowered. On Day 13 after mating a higher proportion of blastocysts was recovered from ewes in Group A than from those in Groups B and C. Immunization resulted in lower fertilization rates and smaller blastocysts with lower mitotic indexes ($P < 0.01$). At Days 24–32 of pregnancy fetal weight was lower in the immunized ewes. At all sampling stages, the proportion of ewes pregnant (fertility) was lowered in immunized ewes.

The results of the present study show that significant reproductive wastage occurs in androstenedione-immunized Merino ewes, with lower rates of embryo recovery and delayed embryonic development being found in comparison to controls. Despite increases in ovulation rate of 36–52% in Fecundin-treated ewes, the number of fetuses obtained per ewe mated decreased from 1.00 (control, Group A) to 0.89 (Group B) and to 0.69 (Group C). Higher titres of androstenedione antibody were associated with higher reproductive wastage.

Introduction

Normal fertilization rates for sheep exceed 80%, but fertility of mated ewes is usually lower (Kelly, 1984). This loss of potential lambs, which is of considerable economic importance, has been ascribed primarily to early embryonic mortality. Several attempts have been made to define the extent of the losses which occur at various stages between fertilization and parturition and Edey (1969) concluded that 20–30% of embryos are lost, generally during the first 3–4 weeks of pregnancy. Other studies have reported the incidence of reproductive wastage after a variety of nutritional and other treatments; collectively they show great variability in the extent of embryonic mortality (Edey, 1976; Kelly & Allison, 1976; White, Rizzoli & Cumming, 1981; Parr, Cumming & Clarke, 1982; Lunstra & Christenson, 1982). However, it is generally accepted that the proportion of embryonic wastage increases with ovulation rate (Kelly, 1984).

* Present address: University College, Dublin, Department of Agriculture, Lyons, Newcastle, P.O., Co. Dublin, Ireland.

An immunization procedure in which ewes are actively immunized against androstenedione (Fecundin: Glaxo Animal Health Ltd, Boronia, Victoria, Australia) has become available to increase twinning rate in sheep. Early results indicate that although the ovulation rate can be increased by about 0.4–0.6 eggs per ewe, lambing rates are only increased by 0.15–0.30 lambs per ewe (Scaramuzzi, Geldard, Beels, Hoskinson & Cox, 1983). Therefore, the present experiment was designed to measure and partition the reproductive wastage occurring between oestrus and Day 32 of pregnancy in Fecundin-treated and control Merino ewes. This experiment was conducted with 3 replicates; results from the first have already been reported (Boland *et al.*, 1984). The occurrence of chromosomal abnormalities in the Day 2 embryos (Murray *et al.*, 1985), the Day 13–14 blastocysts and the Day 24–32 fetuses (Murray *et al.*, 1986) has been reported separately.

Materials and Methods

Animals and immunization procedures. The 522 mature Merino ewes were allocated to 3 treatment groups as follows: (A) untreated controls; (B) immunized with Fecundin with an estimated interval of 25 days from booster injection until mating; (C) immunized with Fecundin with an estimated interval of 14 days from booster injection until mating. Immunization with Fecundin consisted of a primary injection, followed 4 weeks later by a booster injection, each of 2 ml injected subcutaneously behind the ear in the anterior third of the neck. The work was carried out as 3 replicates, 3 weeks and 1 week apart, during the breeding season in March–May, 1984. Twenty-four entire and 18 vasectomized rams were used for mating and detection of oestrus.

Antibody titre measurement. Antibody titres were determined in plasma samples collected 7 days after booster immunization and around the time of mating (14 or 25 days after boosting) and stored at -20°C until assayed (Boland *et al.*, 1985). Radiolabelled androstenedione was used as the ligand and titres were expressed as mean reciprocal dilutions which bound 50% of the added ligand.

Experimental procedure. Flock management and mating was facilitated by using synchronization procedures such that ewes were mated at the second oestrus after removal of progestagen-containing intravaginal sponges (Repromap: Upjohn Pty Ltd, Rydalmere, New South Wales, Australia) (Table 1). At sponge withdrawal the flock was placed with vasectomized rams (1 ram to 10 ewes) which were replaced 14 days later by fertile rams fitted with mating harnesses and crayons (Sire-Sine, Hortico Aust. Pty Ltd, New South Wales, Australia). Two relays of 12 rams per 180 ewes were alternated twice daily at the morning and evening checks for oestrus. Mated ewes were removed at 12-h intervals.

Embryo recovery. Embryo recovery was attempted on Day 2, Days 13–14 or Days 24–32 after mating. Embryos were recovered surgically on Day 2 between 09:00 and 11:00 h or between 19:00 and 21:00 h, the time chosen being that closest to 48 h after the detection of oestrus. The oviduct was flushed with 5 ml Na/K phosphate-buffered saline (pH 7.0; 0.01 M) containing 5% (v/v) heat-treated sheep serum. On Days 13–14 animals were killed and each uterine horn was flushed with 15 ml phosphate-buffered saline. Remaining ewes not returning to service were killed between Days 24 and 32 (106 of 122 ewes between Days 25 and 29) and the fetuses were dissected out from the tract and freed from membranes. At Days 13–14 the length of the expanded blastocyst was measured while fetuses collected at Days 24–32 were weighed. Additional data collected included the number of corpora lutea and the number and development of eggs, embryos and fetuses. As all embryos and fetuses were processed for chromosome analysis (Murray *et al.*, 1985, 1986) mitotic indexes (percentage of mitotic figures per total number cells counted) were calculated for 10 embryos collected 13 days after mating from each treatment group based on 350–1150 cells/embryo. All ewes allocated to the 24–32-day collection group were examined endoscopically before Day 17 so that ovulation rates of non-pregnant ewes could be determined.

Table 1. Experimental protocol

	Group A (untreated controls)	Group B (immunized 25 days)	Group C (immunized 14 days)
Day 1	—	Primary immunization	—
Day 11	—	—	Primary immunization
Day 20	Intravaginal sponges inserted	Intravaginal sponges inserted	Intravaginal sponges inserted
Day 28	—	Booster immunization	—
Day 34	Sponges removed and vasectomized rams introduced	Sponges removed and vasectomized rams introduced	Sponges removed and vasectomized rams introduced
Day 35	—	Blood sample	—
Day 39	—	—	Booster immunization
Day 46	—	—	Blood sample
Day 48	Placed with fertile rams	Placed with fertile rams	Placed with fertile rams
Day 53–54	—	Blood sample	Blood sample

Statistical analyses. Egg recovery rates and fertilization rates were subjected to χ^2 analysis while ovulation rates were compared using an analysis of variance. The distribution of embryo lengths at Day 13 and weights at Day 29 were not normal so these data were ranked and analysed by a non-parametric Wilcoxon–Rank sum procedure. Embryo weights at Day 25 and mitotic indexes were compared using Student's *t* test. Antibody titres were normalized by $\log_{10}$ transformation and the means were compared by Student's *t* test.

Results

Oestrous response

There were no differences between the 3 replicates within treatment groups and the data have been pooled. Oestrus was recorded from 14 days after removal of sponges and the intervals from sponge withdrawal until this oestrus are presented in Table 2. The interval was shorter in Group C than in the other two groups. The distribution of this oestrus, presumed to be the second after sponge withdrawal, is illustrated in Fig. 1 which shows that 25% of Group C ewes were mated within 17 days of sponge withdrawal, compared with 1.4% of those in Group B and 1.3% of those in Group A. A lower proportion of the ewes in Group C were mated in the 10-day period after mixing with entire rams than in either of the other two groups.

Ovarian response

The ovulation rate of ewes in Groups B and C (immunized) was increased over that of controls Group A with that of Group C ewes being higher than that of Group B. Mean androstenedione antibody titres of 6790 ($n = 20$) and 6490 ($n = 60$) for ewes in Groups C and B sampled on Days 14 and 7 after boosting respectively were not different. These titres were significantly higher than that of 3240 for 20 ewes subsampled from Group B 26 days after booster injection.

Table 2. Effect of androstenedione immunization on characteristics of oestrus and ovulation in Merino ewes

	Group A (controls)	Group B (immunized 25 days)	Group C (immunized 14 days)
No. of ewes	169	174	179
% Marked	95 ^a	96 ^a	84 ^b
Days from sponge removal to second oestrus	19.2 ± 0.1 ^a	19.5 ± 0.1 ^a	17.2 ± 0.1 ^b
Ovulation rate	1.42 ± 0.04 ^c	1.93 ± 0.05 ^d	2.16 ± 0.06 ^e
Antibody titre*	—	3240 ± 940 ^f	6790 ± 1830 ^g

Values are mean ± s.e.m.

* Mean reciprocal antiandrostenedione antibody titre.

Different superscripts within a row indicate significant differences (a,b $P < 0.05$; c,d,e $P < 0.05$; f,g $P < 0.01$).

Fig. 1. Frequency distribution of mating times in untreated and androstenedione-immunized Merino ewes. The arrow indicates the lower limit of normal oestrous cycles for mating at the second oestrus after sponge withdrawal and the numbers indicate the percentage of ewes with abnormal, short cycles in each treatment group.

Embryo recovery

The recovery rate of ova on Day 2 was affected by treatment, with all groups being significantly different. The lowest recovery rate was recorded for Group C (Table 3). Fertilization rates for eggs recovered from all 3 groups were not significantly different. However, the fertility for control ewes (84%) was greater than that for ewes in Group C (63%). The recovery rate of eggs and blastocysts at Day 13 was lower in Group C (56%) than in Group A (77%) (Table 4). The proportion of eggs

Table 3. Effect of androstenedione immunization on recovery and fertilization rates of eggs 48 h after mating in Merino ewes

	Group A (controls)	Group B (immunized 25 days)	Group C (immunized 14 days)
No. of ewes mated	57	60	60
No. of corpora lutea (ovulation rate)	84 (1.47)	108 (1.80)	129 (2.15)
No. of eggs recovered (%) [*]	75 (89) ^a	81 (75) ^b	78 (60) ^c
No. of eggs fertilized (%) [†]	65 (87)	61 (75)	61 (78)
No. of embryos per 100 corpora lutea	77 ^a	56 ^b	47 ^b
No. of ewes with fertilized eggs (%)	48 (84) ^d	42 (70) ^{e,f}	38 (63) ^f

^{*} Percentage of total number of corpora lutea.

[†] Percentage of eggs recovered.

Different superscripts within a row indicate significant differences (a,b,c $P < 0.05$; d,e,f $P < 0.01$).

Table 4. Effect of androstenedione immunization on fertilization and blastocyst recovery at Day 13 after mating in Merino ewes

	Group A (controls)	Group B (immunized 25 days)	Group C (immunized 14 days)
No. of ewes mated	54	54	51
No. of corpora lutea (ovulation rate)	71 (1.31)	92 (1.70)	117 (2.29)
No. of eggs plus blastocysts recovered (%) [*]	55 (77) ^a	77 (84) ^a	66 (56) ^b
% of eggs showing development	95 ^a	78 ^b	79 ^b
No. of blastocysts recovered (%) [*]	52 (73) ^a	60 (65) ^a	52 (44) ^b
No. of normal blastocysts recovered (%) [†]	51 (98) ^c	54 (90) ^c	42 (81) ^d
No. of normal blastocysts per 100 corpora lutea	72 ^e	59 ^{e,f}	36 ^f
No. of ewes with hatched blastocysts (%)	41 (76) ^a	33 (61) ^{a,b}	29 (57) ^b

^{*} Percentage of total number of corpora lutea.

[†] Percentage of total blastocysts recovered. Abnormal blastocysts were those that were significantly retarded, e.g. collapsed and containing heteropycnotic nuclei or still enclosed within the zona pellucida.

Different superscripts within a row indicate significant differences (a,b $P < 0.05$; c,d $P < 0.01$; e,f $P < 0.005$).

which had cleaved and developed to some further stage was lower for both immunized groups (B & C) than for the controls (A). There was also a lower proportion of morphologically normal blastocysts, for this age, in Group C ewes at Day 13 as well as the lower proportion of total blastocysts recovered. The proportion of mated ewes that yielded morphologically normal blastocysts was

Table 5. Effect of androstenedione immunization on pregnancy rates at Days 24–32 after mating in Merino ewes

	Group A (controls)	Group B (immunized 25 days)	Group C (immunized 14 days)
No. of ewes mated	50	53	39
No. of corpora lutea (ovulation rate)	74 (1.48)	88 (1.66)	68 (1.74)
No. of ewes returning to service (%)	8 (16) ^a	19 (36) ^b	20 (51) ^b
No. of fetuses recovered (%) [*]	52 (70) ^c	50 (57) ^{c,d}	28 (41) ^d
No. of normal fetuses recovered (%) [†]	50 (96)	47 (94)	27 (96)
No. of normal fetuses per 100 corpora lutea	68 ^a	53 ^{a,b}	40 ^b
No. of ewes with normal fetuses (%)	38 (76) ^e	31 (58) ^{e,f}	18 (46) ^f

^{*} Percentage of total number of corpora lutea.

[†] Percentage of total fetuses recovered.

Different superscripts within a row indicate significant differences (a,b $P < 0.05$; c,d $P < 0.001$; e,f $P < 0.01$).

Table 6. Effect of androstenedione immunization on the development of fetuses at three stages of pregnancy in Merino ewes

Stage of pregnancy	Group A (controls)	Group B (immunized 25 days)	Group C (immunized 14 days)
Day 13 (length, mm)	8.52 ± 1.25 ^a (43)	5.80 ± 1.26 ^b (52)	3.54 ± 1.02 ^c (24)
Singles	8.04 ± 1.59 ^a (25)	4.48 ± 1.61 ^b (14)	—
Twins	8.87 ± 1.97 ^a (18)	6.58 ± 1.91 ^b (32)	—
Day 25 (wt, g)	0.211 ± 0.016 ^d (14)	0.198 ± 0.011 ^d (14)	0.167 ± 0.010 ^e (10)
Day 29 (wt, g)	0.567 ± 0.020 ^a (16)	0.437 ± 0.017 ^b (16)	—

Values are mean ± s.e.m. for the no. of fetuses in parentheses.

Different superscripts within a row indicate significant differences (a,b $P < 0.001$; a,c $P < 0.001$; b,c $P < 0.03$; d,e $P < 0.05$).

lower in Group C than in the control group (76%). During the 4 weeks after mating, more ewes from the immunized groups returned to service compared to controls (Table 5). At 24–32 days after mating, more Group A (control) ewes were pregnant (76%) than were Group C ewes (46%) (Table 5), but there was no significant difference in pregnancy rate between Group B and Groups C or A. Recovery rates at this stage were also decreased from 70 to 41% of the recorded ovulations. More than 90% of all fetuses recovered at this stage appeared to be morphologically normal.

Embryo development

The development of the embryos over the sampling period is shown in Table 6. At Day 13 blastocysts from control ewes were longer than those from Group C or Group B while the blasto-

cysts from Group B were longer than those from Group C. Within Groups A and B there were no significant differences in size between singleton and twin blastocysts. By Day 25 there was no difference in fetal weight between control (14 fetuses) and Group B (14 fetuses) ewes, but the values were greater than for Group C (10 fetuses). At Day 29, 16 fetuses from Group A were heavier than 16 fetuses from Group B. Too few fetuses were available from Group C at this stage or from other sampling days between Day 24 and 32 for meaningful comparisons.

The means $\pm$ s.e.m. for the mitotic indexes of 10 Day-13 blastocysts were 12.40 ± 1.41 , 5.87 ± 0.62 and 5.63 ± 0.89 for Groups A, C, and B respectively, the last 2 values being significantly lower than that for Group A.

Discussion

The treatments with Fecundin in which different times elapsed between booster immunization and the mating period with entire rams (Groups B and C) enabled the effects of significantly different concentrations of antibody on reproductive characteristics to be assessed. Between 7 and 14 days after the booster injection, the mean titre was unchanged and had declined significantly by Day 25. This resulted in effects on several of the reproductive measures taken, both between treated and control groups of ewes and, in some cases, between the 2 treated groups.

The altered oestrous response in Group C may be due to the antibody titres, higher at that stage compared to those in Group B, rendering some ewes temporarily anoestrous. The higher titres may also lead to the shortening of the oestrous cycle as judged by the interval from sponge withdrawal to mating at the second oestrus. However, we do not know whether the time of immunization relative to the stage of the cycle was important in producing these differences; the ewes in Group C received the booster injection 5 days after sponge withdrawal while those in Group B received it before sponge withdrawal.

Immunization of Merino ewes against androstenedione increased the ovulation rate. Similar increases in other breeds of ewes have resulted in increased lambing percentages (Geldard, Scaramuzzi & Wilkins, 1984). The present study indicates that immunization of the Merino ewe with Fecundin may lead to problems with egg pick-up by the fimbria and embryonic growth. Decreases in egg recovery and fertilization rates have been reported after progestagen-PMSG treatment (Lunstra & Christenson, 1982) but ewes in the present experiment were mated at a subsequent oestrus. Recovery rates for control ewes are comparable to data in the literature (Mattner & Braden, 1967; Lunstra & Christenson, 1982) but the consistently lower recovery rates over 3 sampling periods in immunized ewes, especially in Group C, suggest 3 possibilities—failure to release ova, failure of fimbrial collection, or a subsequent loss of eggs from the oviduct.

There were some indications in this study that fertilization may be impeded in Fecundin-treated ewes although these results remain equivocal. Fertilization rates for these ewes recorded at Day 2, which is the only time that satisfactory collections of non-fertilized or non-developed eggs could be made, were not significantly lower than for control ewes ($P \geq 0.05$). However, the proportions of single-cell eggs collected at Day 13 were higher from both Fecundin-treated groups than from the control group. The lower recovery rate suggests that significant reproductive wastage results from immunization. The number of apparently normal embryos observed at Days 2, 13 and 24–32 were 77, 72 and 68% for Group A; 56, 59 and 53% for Group B; and 47, 36 and 40% for Group C ($P < 0.001$ (Day 2); $P < 0.001$ (Day 13); $P < 0.004$ (Days 24–32) of corpora lutea (Tables 3, 4 & 5). Therefore, despite higher ovulation rates in immunized ewes, reproductive wastage has resulted in significantly reduced fertility associated with pregnancy rates of 58 and 46% in these ewes compared to 76% in controls at Day 24–32 (Table 5). Most of these losses were present at Day 2 after mating and are therefore mainly attributable to a failure to pick up eggs.

The percentages of abnormal blastocysts encountered at Day 13 were 2, 10 and 19 for Groups A, B and C and at Days 24–32 these estimates were 4, 6 and 4% respectively. Only in the group with

higher mean concentrations of circulating androstenedione antibody was there significantly higher abnormality at Days 13 than in controls, and this difference was eliminated by Days 24–32. However, a constant proportion of genetically abnormal embryos (11%) was recovered from all groups at Day 2 in this experiment (Murray *et al.*, 1985) and these were mostly lost by all 3 groups before Day 13 of gestation (Murray *et al.*, 1986). At Days 13, 25 and 29 there were significant decreases in blastocyst length and fetal weight and mitotic indexes of embryos in Groups B and C at Day 13 were also lower. This evidence suggests that Fecundin treatment has caused, directly or indirectly, some major metabolic changes within the population of embryos which results in retardation of development and wastage of a significant proportion of the embryos by Day 24–32 of gestation. Estimates of embryonic mortality in the literature vary from 0 to 33% (see review by Edey, 1969). The total losses (% of corpora lutea) found in this experiment estimated from normal fetuses at Days 24–32 were 32, 47 and 60%, and so it would appear that Fecundin treatment in Merinos does increase reproductive wastage beyond the norm. This could be partly associated with the higher ovulation rates, as it has been suggested that the probability of embryo survival is higher for ewes ovulating one rather than two eggs (White *et al.*, 1981; Kelly, 1984). Nevertheless, there is no evidence to suggest that high ovulation rates are associated with low embryo recovery rates in other than Fecundin-treated ewes and consequently this new source of reproductive wastage may be specific for this means of increasing ovulation rate.

The causes of retardation in embryo development in the immunized ewes cannot be identified. Retardation is unlikely to result from the high ovulation rates in the immunized animals. In fact, when measured (Table 6) twin blastocysts were larger than single blastocysts. Although short-term undernutrition has been shown to affect embryo growth rate (Parr *et al.*, 1982), it is not a factor in this experiment. The lower rates of cell division, as measured by the mitotic indexes, confirm these observations (Table 6). We conclude that retardation has been caused by the altered steroid environment induced by androstenedione immunity.

Fecundin treatment increases lambing percentages by increasing ovulation rate and consequently litter size (no. of embryos/no. of ewes pregnant). Fertility, as measured by percentage of ewes pregnant, is not improved by Fecundin treatment in cross-bred sheep (Scaramuzzi *et al.*, 1983; Geldard *et al.*, 1984). Improved lambing percentages therefore are normally a result of increased litter size and unchanged fertility. The present experiment clearly indicates that in some situations the fertility of Fecundin-treated Merino ewes can even be lowered and this serves to offset the gains in litter size, leading to the reduced lambing responses occasionally reported from field tests (Scaramuzzi *et al.*, 1983). The variability of responses seen with Fecundin usage in Merinos therefore appears to be due to a depression in fertility associated with a loss of ova, a possible decrease in fertilization rates and wastage of early embryos during the first 3 weeks of pregnancy. These effects may be more pronounced at higher levels of circulating androstenedione antibody.

The present results were obtained with synchronized ewes given a single service opportunity. In commercial applications of Fecundin, when individual ewes may have 2 or 3 service opportunities, reproductive performance could be expected to be superior to that observed in this study.

We thank Mr J. Fenn, Mr H. Thompson and Mr J. Knobbs for assistance with animal care and Mrs A. Dafter, N. Hamilton, J. Marshall, B. Harrison and R. Welch for technical assistance. M.P.B. was the recipient of a Reserve Bank of Australia Fellowship.

References

Boland, M.P., Murray, J.M., Hoskinson, R.M., Hazelton, I.G., Sutton, R. & Nancarrow, C.D. (1985) Ovarian response to PMSG treatment in ewes immunized against oestradiol-17β. <i>Aust. J. biol. Sci.</i> 38, 339–345.	Boland, M.P., Murray, J.D., Scaramuzzi, R.J., Moran, C., Sutton, R., Hoskinson, R.M., Hazelton, I. & Nancarrow, C.D. (1984) Reproductive wastage and chromosomal abnormalities in early embryos from

- androstenedione-immune and control Merino ewes. In *Reproduction in Sheep*, pp. 137–139. Eds D. R. Lindsay & D.T. Pearce. Australian Academy of Science, Canberra.
- Edey, T.N. (1969) Prenatal mortality in sheep: a review. *Anim. Breed. Abstr.* **37**, 173–190.
- Edey, T.N. (1976) Embryo mortality. In *Sheep Breeding*, pp. 400–410. Eds G. J. Tomes, D. E. Robertson & R. J. Lightfoot. Western Australian Institute of Technology, Perth.
- Geldard, H., Scaramuzzi, R.J. & Wilkins, J.F. (1984) Immunization against polyandroalbumin leads to increases in lambing and tailing percentages. *N.Z. vet. J.* **32**, 2–5.
- Kelly, R.W. (1984) Fertilization failure and embryonic wastage. In *Reproduction in Sheep*, pp. 127–133. Eds D. R. Lindsay & D. T. Pearce. Australian Academy of Science, Canberra.
- Kelly, R.W. & Allison, A.J. (1976) Returns to service, embryonic mortality and lambing performance of ewes with one and two ovulations. In *Sheep Breeding*, pp. 418–423. Eds G. J. Tomes, D. E. Robertson & R. J. Lightfoot. Western Australian Institute of Technology, Perth.
- Lunstra, D.D. & Christenson, R.K. (1982) Fertilization and embryonic survival in ewes synchronized with exogenous hormones during the anoestrous and estrous seasons. *J. Anim. Sci.* **53**, 458–466.
- Mattner, P.E. & Braden, A.W.H. (1967) Studies in flock mating of sheep. II. Fertilization and pre-natal mortality. *Aust. J. exp. Agric. Anim. Husb.* **7**, 110–116.
- Murray, J.D., Boland, M.P., Moran, C., Sutton, R., Nancarrow, C.D., Scaramuzzi, R.J. & Hoskinson, R.M. (1985) Occurrence of haploid and haploid/diploid mosaic embryos in untreated and androstenedione-immune Australian Merino sheep. *J. Reprod. Fert.* **74**, 551–555.
- Murray, J.D., Moran, C., Boland, M.P., Nancarrow, C.D., Sutton, R., Hoskinson, R.M. & Scaramuzzi, R.J. (1986) Polyploid cells in blastocysts and early fetuses from Australian Merino sheep. *J. Reprod. Fert.* **78**, 439–446.
- Parr, R.A., Cumming, I.A. & Clarke, I.J. (1982). Effects of maternal nutrition and plasma progesterone concentrations on survival and growth of the sheep embryo in early gestation. *J. agric. Sci., Camb.* **98**, 39–46.
- Scaramuzzi, R.J., Geldard, H., Beels, C.M., Hoskinson, R.M. & Cox, R.I. (1983) Increasing lambing percentages through immunization against steroid hormones. *Wool Technol. Sheep Breed.* **31**, 87–97.
- White, D.H., Rizzoli, D.J. & Cumming, I.A. (1981) Embryo survival in relation to number and site of ovulation in the ewe. *Aust. J. exp. Agric. Anim. Husb.* **21**, 32–38.

Received 4 February 1986