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Effect of Progesterone and Oestrogen on the Survival and Development of Fertilized Ova in the Ovariectomized Ewe

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Abstract

Two experiments were carried out to investigate the importance of progesterone and oestrogen for the survival and development of fertilized ova in ovariectomized ewes. In experiment 1 ewes were treated with progesterone (P) alone or progesterone and 17β-oestradiol (P+E2) following ovariectomy on the second (day 2) or sixth day (day 6) after mating to vasectomized rams. Fertilized ova were transferred to the ewes either immediately after ovariectomy or on day 6 after ovariectomy on day 2. In experiment 2 ewes that had been given an equine anterior pituitary extract to induce multiple ovulation were ovariectomized on day 2 after mating to fertile rams, and then treated with P or P+E2. On day 18 the uteri of all ewes were flushed to recover embryos. Treatment with P commenced immediately after ovariectomy and E2 was given from day $2\frac{1}{2}$ to day 5, to ewes ovariectomized on day 2. Ovarian vein blood was collected just prior to ovariectomy and was assayed for its progesterone and oestrogen contents.

In both experiments oestradiol had no effect upon the proportion of ewes with normal embryos. In experiment 1 delay of transfer until day 6 after ovariectomy on day 2 increased the proportion of ewes with normal embryos [28 of 43 (65%) *v.* 16 of 46 (35%)]. Transfers on day 6 after ovariectomy on the same day tended to be less successful than transfers on day 6 after ovariectomy on day 2 [11 of 23 (48%) *v.* 28 of 43 (65%)]. In all ewes in experiment 2, and in experiment 1 where ovariectomies and transfers were carried out on the same day, survival and normal development of ova occurred only in ewes in which the ovarian vein progesterone levels at ovariectomy, expressed as progesterone per corpus luteum, fell within precise limits. There was no such relationship in ewes ovariectomized on day 2 and given progesterone prior to transfer on day 6.

It would appear that the secretion of oestradiol during the luteal phase of the oestrous cycle in the ewe has little effect on the survival and development of ova. However, progesterone appears to have an important role. Further, it would appear that as early as the second day after oestrus the activity of the corpus luteum, as assessed by its progesterone secretion, may well influence the success or failure of pregnancy.

Introduction

Studies on the steroid hormone requirements for the maintenance of pregnancy in the ovariectomized ewe have shown that progesterone is essential. Studies in which ewes were either ovariectomized $3\frac{1}{2}$–4 days after mating (Foote *et. al.* 1957; Bindon 1971) or were ovariectomized and received fertilized ova 3–4 days after mating to vasectomized males (Moore and Rowson 1959) have shown that 10–20 mg/day of progesterone will maintain pregnancy in up to 70–80% of ewes. Daily doses of 4 mg progesterone appear to be insufficient to maintain pregnancy (Bindon 1971). Oestrogen given daily together with progesterone failed to have a beneficial effect and in fact oestradiol benzoate at a daily dose of 5 μg (Moore and Rowson 1959) or oestradiol daily at 10–20 ng/5 lb body weight (Foote *et al.* 1957) appeared to depress the proportion of ewes in which pregnancy was maintained.

Failure to implicate oestradiol in the early pregnancy in the ewe is somewhat surprising, particularly as it has been demonstrated that oestrogens are secreted during the luteal phase of the cycle. A marked rise in the plasma oestradiol levels 3–4 days after oestrus has been well documented (Cox *et al.* 1971; Mattner and Braden 1972). Holst *et al.* (1972) have shown that the rise is associated with ovarian follicular development and Braden *et al.* (1971) and Holst and Braden (1972) suggested that the secretion of oestrogen during the immediate post-oestrus period may be of considerable importance in the transport of embryos from the oviduct to the uterus and in the preparation of the uterus for the developing embryo. Therefore, it seemed desirable to investigate in some detail the survival and development of embryos in ovariectomized ewes given progesterone alone or in combination with oestradiol. Oestradiol was given in a series of graded doses from day $2\frac{1}{2}$ to day 5 and progesterone was given in a series of graded doses until day 7 and thereafter at a level of 12 mg daily.

Materials and Methods

The investigation involved two experiments in which mature parous Merino ewes were treated with progesterone alone or progesterone together with 17β-oestradiol following ovariectomy carried out within the first 6 days after oestrus.

Experiment 1

Fertilized ova were collected, 2 (day 2) or 6 days (day 6) after oestrus, from donor ewes in which multiple ovulation had been induced with an equine anterior pituitary extract. Ova were transferred, at a rate of two per animal, to each of 112 recipient ewes. The recipients were ovariectomized on either day 2 or day 6 and transfers were carried out either immediately after ovariectomy or on day 6 following ovariectomy on day 2. Prior to transfer the donor and recipient ewes had been run with entire and vasectomized rams respectively, and were inspected for oestrus twice daily at 0600 and 1800 h. Ova were transferred to only those recipients that were first observed in oestrus within 12 h of their respective donors. Ova collected on day 2 were of two and four cells and they were transferred to the oviducts. Day-6 ova were morulae and early blastocysts and were transferred to the uterine horns.

Immediately prior to ovariectomy 5–10 ml of blood was obtained from the ovarian vein of each ovary of 65 of the 112 recipients. The two samples from individual ewes were pooled, centrifuged at 4°C and the plasmas were then stored at −20°C until assayed for their progesterone and oestrogen contents. Plasma progesterone determinations were performed on duplicate 0·5-ml aliquots of plasma using the competitive protein binding method described by Neill *et al.* (1967) and Johansson (1969). The protein binding solution (CBG) was prepared from selected batches of dog plasma and Florisil was used to separate bound and free steroid. Standard plasma (4 ng progesterone/ml) was prepared by evaporating a known quantity of progesterone in purified ethanol to dryness under nitrogen and redissolving it in ovariectomized ewe plasma at 45°C. The standard curve was obtained from duplicate 0·5-ml aliquots of standard and ovariectomized ewe plasma. Serial dilutions of the unknown plasma samples were made in triple-distilled water and assay values accepted when the results on two dilutions were within the range of the standard curve and duplicates differed by less than 15%. Following the recommendation of Clark and Gurpide (1972) values obtained were not corrected for extraction losses.

Plasma 17β-oestradiol was determined by the solid-phase radioimmunoassay reported by Abraham (1969). Determinations were performed in triplicate on 0·5-ml aliquots of plasma and the values obtained were corrected for extraction losses. The values obtained would probably be more correctly termed total immunoreactive oestrogens although 17β-oestradiol is, proportionally, the most significant oestrogen present in sheep plasma (Moore *et al.* 1969; Cox *et al.* 1971; Shutt and Cox 1973). After ovariectomy, in those ewes in which ovarian vein blood was collected the corpora lutea were dissected free of extraneous ovarian tissue and then weighed.

Immediately following ovariectomy the ewes were treated with either progesterone alone or progesterone together with 17β-oestradiol, according to the schedule shown in Table 1. Up until the

seventh day after oestrus treatments were carried out at 12-h intervals, and thereafter treatments were once daily. The treatment schedule used was an attempt to simulate the pattern of progesterone and oestradiol levels within ovarian vein plasma as described by Moore *et al.* (1969) and Cox *et al.* (1971).

Table 1. Schedule of steroid hormone treatment after ovariectomy

Steroid	Days after oestrus: 2	2½	3	3½	4	4½	5	5½	6	6½	7–18
Progesterone (mg)	0·6	1·2	1·8	2·4	3·0	3·6	4·2	4·8	5·4	6·0	12[A]
17β-Oestradiol (μg)	—	2	4	6	6	4	2	—	—	—	—

[A] Once daily.

Eighteen days after oestrus the uteri of recipient ewes were flushed *in situ* to recover embryos. A ligature was placed around the cervix and a glass cannula of 4 mm internal diameter was inserted into the lumen of the uterus at the extremity of one uterine horn. Sterile normal saline (10–20 ml) was injected into the tip of the other horn and the saline and contents of the uterus were gently expressed from the uterus through the cannula into collecting dishes. Embryos and membranes were examined under a dissecting microscope and classified as normal, retarded or resorbing according to the criteria of size and morphology described by Bryden *et al.* (1972).

Experiment 2

Thirty ewes that had been treated with an equine anterior pituitary extract to induce multiple ovulation were ovariectomized 2 days after mating to entire rams of proven fertility. Following ovariectomy they were treated with progesterone or progesterone and 17β-oestradiol. The procedures used for the collection and assay of ovarian vein blood, the steroid hormone treatments after ovariectomy, and the recovery of embryos were the same as those employed in experiment 1.

Table 2. Survival of ova transferred to ovariectomized ewes (experiment 1)

Ewes were treated with progesterone alone (P) or progesterone and 17β-oestradiol (P+E2) according to the schedule given in Table 1. Embryos were recovered 18 days after oestrus

Days after oestrus: Ovariectomy	Transfer	No. of ewes	Treatment	Number of ewes that yielded: Normal embryos	Abnormal embryos[A]	No embryos
2	2	23	P alone	8	2	13
2	2	23	P+E2	8	5	10
Total		46		16	7	23
2	6	21	P alone	13	4	4
2	6	22	P+E2	15	4	3
Total		43		28	8	7
6	6	23	P alone	11	6	5
Grand total		112		55	20	37

[A] Number of ewes with only retarded or resorbing embryos.

Results

Experiment 1

(i) *Survival of transferred ova*

Embryos were recovered from 75 of the 112 recipients (Table 2). In all, 55 ewes had one (22 ewes) or two (23 ewes) normal embryos while 15 ewes had one (nine ewes) or

two (six ewes) retarded embryos and the remaining five ewes had resorbing embryos. In 3 of the 55 ewes that had normal embryos, one normal embryo was recovered together with a retarded embryo.

In ewes ovariectomized on day 2 treatment with oestradiol from day $2\frac{1}{2}$ to day 5 had no effect upon the number of ewes with normal embryos and this occurred irrespective of whether ova were transferred on day 2 or day 6. In ewes ovariectomized on day 2 the delay of transfer until day 6 increased the proportion of ewes with normal embryos [28 of 43 (65%) *v.* 16 of 46 (35%); $P < 0{\cdot}01$] and following transfer on day 6 there was a suggestion that the proportion of ewes with normal embryos on day 18 was greater for ewes ovariectomized on day 2 than for those ovariectomized on day 6 [28 of 43 (65%) *v.* 11 of 23 (48%)]. Neither treatment nor day of ovariectomy and transfer had any effect upon the number of ewes with abnormal embryos. Asynchronous transfers of ± 12 h appeared to have no effect on the survival of embryos. Of 17 ewes that were first observed in oestrus 12 h before or after their respective donors, normal embryos were recovered from 10, retarded or resorbing embryos from three and no embryo from four ewes.

Table 3. Ovarian vein plasma levels of progesterone and oestrogen and weight of corpora lutea at ovariectomy (experiment 1)

Days after oestrus: Ovariectomy	Days after oestrus: Transfer	Type of embryo recovered	No. of ewes	Range of concentrations:[A] Progesterone (ng/ml)[B]	Range of concentrations:[A] Oestrogen (pg/ml)	Weight of corpora lutea (mg)[C]
2	2	Normal	6	61–135 (102)	7–56 (20)	111 ± 24
2	2	Abnormal	5	32–43 (36)	5–33 (18)	81 ± 5
2	2	None	10	3–59 (29)	5–77 (23)	93 ± 13
2	2	None	4	154–602 (440)	10–141 (72)	129 ± 40
2	6	Normal	20	6–373 (67)	5–65 (17)	85 ± 7
2	6	Abnormal	3	6–64 (21)	5–43 (20)	70 ± 19
2	6	None	3	20–82 (59)	10–13 (12)	114 ± 4
6	6	Normal	5	445–675 (515)	7–110 (39)	413 ± 49
6	6	Abnormal	4	787–1165 (1018)	5–38 (20)	399 ± 39
6	6	None	3	907–1203 (1072)	12–17 (14)	369 ± 74

[A] Mean values in parentheses. [B] Per corpus luteum. [C] Mean wet weight ± S.E.

(ii) *Ovarian vein progesterone and oestrogen*

In ewes in which ovarian vein blood was collected prior to ovariectomy on day 2 or day 6, the plasma concentrations of progesterone and oestrogen showed marked variations, but there was no apparent indication of any relationship between levels of progesterone or oestrogen and subsequent survival and development of transferred ova. However, a number of ewes had more than one corpus luteum and when progesterone levels were divided by the number of corpora lutea present, a distinct relationship became apparent, but the relationship was observed only in those ewes in which transfers and ovariectomies were carried out on the same day (Table 3). In animals that were ovariectomized on day 2 all six with normal embryos had ovarian vein plasma levels of progesterone, expressed as progesterone per corpus luteum, of between 61 and 135 ng/ml plasma. Progesterone levels at ovariectomy in all 19 ewes that had abnormal or no embryos fell outside this range. Within recipients that were

ovariectomized on day 6 and received ova on the same day, a similar but somewhat less precise relationship between progesterone levels and survival and development of ova was observed. With the exception of two animals, one of which had a normal embryo and a progesterone level of 1024 ng/ml and the other a resorbing embryo and a level of 512 ng/ml, progesterone levels in ewes with normal embryos were less than in ewes with abnormal or no embryos. In ewes that received ova on day 6 following ovariectomy on day 2, similar relationships between progesterone levels on day 2 and subsequent survival and development of transferred ova could not be demonstrated. In all recipients in which ovarian vein blood was collected, the mean weight of individual corpora lutea tended to follow progesterone levels but the correlation coefficient was relatively low (day-2 ovariectomies: $r = 0{\cdot}389$, $P < 0{\cdot}01$; day-6 ovariectomies: $r = 0{\cdot}561$, $P < 0{\cdot}05$).

There was no relationship between ovarian vein oestrogen levels at ovariectomy and the subsequent survival and development of transferred ova (Table 3).

Experiment 2

Of the 30 ewes ovariectomized on day 2 following induction of multiple ovulation with an equine anterior pituitary extract, normal embryos were recovered on day 18 from 6 of 15 that were treated with progesterone alone (16 embryos) and from 3 of 15 given progesterone and oestradiol (five embryos, one retarded). No ewe had only abnormal embryos.

The ovarian vein plasma progesterone concentrations per corpus luteum in eight ewes that were bled at ovariectomy and had one or more normal embryos at day 18 fell within the range 52–146 ng/ml, with a mean of 92 ng/ml, and the numbers of corpora lutea ranged between 2 and 14. Progesterone concentrations in 14 ewes that were bled and had no embryos ranged between 23 and 352 ng/ml per corpus luteum, and in only four of these ewes were progesterone concentrations within the range of 52–146 ng/ml observed in the ewes that had normal embryos.

Oestrogen levels fell within the range 2·8–40·4 pg/ml plasma (mean 12·1) but there was no suggestion of any relationship between oestrogen levels at ovariectomy and the subsequent state of any animals at the time of embryo recovery.

Discussion

The failure to detect any beneficial effect upon the survival and development of fertilized ova of oestradiol given with progesterone from day $2\frac{1}{2}$ to day 5 would tend to exclude the post-oestrus rise in ovarian oestrogen secretion, on the third to fourth day after oestrus, as an important factor in the survival and development of sheep ova. Holst and Braden (1972) reported that the post-ovulatory rise in ovarian oestrogen secretion occurred when ova were passing through the ampulla–isthmus junction of the oviduct and they suggested that oestrogen delayed passage through the junction and prevented the premature entry of ova into the uterus, a situation known to have a deleterious effect upon subsequent survival of ova (Averill and Rowson 1958; Moore and Shelton 1964). However, in the present study treatment with oestradiol following transfer of ova to the oviducts on day 2 did not result in any increase in the proportion of ova that continued to develop normally.

In ewes that received ova on day 6 following ovariectomy on day 2, progesterone levels in ovarian vein blood collected at the time of ovariectomy were not related to

survival and normal development of ova. Further, a larger proportion of these ewes had normal embryos than ewes that received ova on day 2. This may simply indicate an increase in the survival rate of older ova (Moore and Shelton 1964). However, there was a tendency for increased survival of day-6 ova transferred to ewes ovariectomized on day 2 when compared to ewes ovariectomized on day 6. It would appear that progesterone treatment given between day 2 and day 6 may maintain and perhaps even restore a suitable state for embryo development within recipients.

It would appear that progesterone has a major role in the determination of embryo development in the ewe but in the present study it was not possible to determine how the effects are brought about. In this connection it would appear important to examine uterine changes in the relation to ovarian progesterone secretion in entire ewes and in relation to dose and duration of progesterone treatment given to ovariectomized ewes.

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