OVUM TRANSPORT IN THE EWE

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Abstract

Ovum transport was examined using 60 mature Border Leicester × Merino ewes, the time of ovulation being determined by endoscopy. By 6 hr after ovulation, all ova had reached the middle segment of the ampulla and some had reached the ampulla–isthmus junction (AIJ). At 8 hr, most ova were located at the AIJ. By 60 hr after ovulation half of the ova had passed into the isthmus; at this time all fertilized ova were at the 8-cell stage.

In a second experiment effects of progestagen synchronization and oestrogen treatment (red clover diet or 30 μg oestradiol-17β at 30 hr after ovulation) on the distribution of ova 60 hr after ovulation were studied. The synchronized ewes differed significantly ($P < 0{\cdot}044$) from the controls, with 75% of the ova being found in the isthmus and the remainder in the uterus. In the oestrogen-treated ewes a significant difference was not attained but there appeared to be a greater dispersion of ova, with 52% in the ampulla, 40% in the isthmus, and 8% in the uterus. The results suggest that disturbance of normal ovum transport in the oviduct may result from progestagen synchronization and perhaps from the ingestion of phyto-oestrogens.

I. Introduction

In most mammals the transport of ova through the oviduct requires approximately 3 days (Andersen 1927). The normal pattern of progress is a rapid passage through the ampulla to the ampulla–isthmus junction (AIJ), where the ovum is delayed; this is followed by a fairly rapid passage through the isthmus into the uterus (Blandau 1969). A similar pattern has been reported for the ewe (Kelley 1937; Lang 1965, 1969; Wintenberger–Torres 1967).

Disturbance of ovum transport by exogenous ovarian hormones has been reported in mice (Humphrey 1968), rabbits (Greenwald 1963; Chang 1966), pigs (Day and Polge 1968), cattle (Onuma, Hahn, and Foote 1970), and sheep (Shelton and Moore 1967). Both oestrogens and progestagens have been reported to produce either delay or acceleration depending on time of administration and dose level. Little is known of their effects on ovum transport in the ewe. It has been shown that endogenous levels of progesterone in peripheral blood (Thorburn, Bassett, and Smith 1969) are rising rapidly at 3 days after ovulation, at the time when the AIJ block to ovum progression disappears. Furthermore, levels of oestradiol-17β in ovarian vein blood (Cox, Mattner, and Thorburn 1971) have reached their peak and are beginning to fall at this time. It is possible that the balance between these two ovarian hormones is important in regulating ovum transport in the ewe (Braden *et al.* 1971). The present paper reports observations on ovum transport in ewes subjected to current methods of oestrus synchronization or to an intake of oestrogenic pasture.

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II. Materials and Methods

(a) *Experiment 1*

(i) *Experimental Animals and Mating Procedure*

Sixty mature, cyclic Border Leicester × Merino ewes and two vasectomized rams were placed in yards where continuous observation in times of activity could be maintained. When ewes were detected in oestrus they were immediately removed from the flock and placed in a yard isolated from rams. Duration of oestrus was determined by teasing at intervals of 4 hr for the first 24 hr of oestrus, and more frequently thereafter. Each ewe was permitted two matings with entire rams; usually at 8 and 20 hr after the onset of oestrus. Ewes were group-fed, receiving lucerne hay *ad libitum* and 0·25 lb oats per ewe daily throughout the experiment.

(ii) *Detection of Ovulation*

The time of ovulation was determined by direct observations of the ovary using an endoscope (Roberts 1968) under local anaesthesia (2% Lignocaine). Observations were made at intervals of < 2 hr beginning just after the cessation of oestrus until ovulation was detected. Care was taken to avoid manipulation of the oviduct, as preliminary studies indicated that this impaired ovum passage into the ampulla.

(iii) *Recovery of Ova*

Ewes were laparotomized under general anaesthesia (Pentobarbitone sodium) at a predetermined time following ovulation. Eight to twelve ewes were examined at 6, 8, 36, and 60 hr after ovulation and one ewe at 0·5 hr. The reproductive tract was exposed and the oviducts and surrounding mesosalpinx sprayed with 2% Lignocaine (without adrenaline) to stop motility of the oviduct. Following salpingectomy each oviduct was lightly compressed between two sheets of plain glass on the stage of a dissecting microscope and the AIJ clearly identified. The ampulla was then divided into three equal segments and the isthmus into two equal segments, numbered 1–5 starting from the cranial portion of the ampulla. Each segment was then flushed with 1–3 ml of 0·9% saline for the recovery of ova.

(b) *Experiment 2*

(i) *Experimental Animals and General Procedure*

Eight weeks after experiment 1, another 96 ewes from the same flock were divided into six treatment groups and placed in separate yards along with two harnessed vasectomized rams for each treatment group. Mating procedure and detection of ovulation were similar to that in experiment 1. All ewes were laparotomized at 60 hr after ovulation and a 5-cm section of the uterus removed along with the oviducts. The division and numbering of the oviducts was similar to that in experiment 1.

(ii) *Treatments*

Group I—control ewes were fed 800 g of pelleted lucerne–oats mixture per ewe daily.

Group II—fed as in group I but each ewe received 30 μg oestradiol-17β as a single intramuscular (i/m) dose in oil administered 30 hr after ovulation, i.e. 30 hr prior to laparotomy.

Group III—fed phyto-oestrogen (2·4 g formononetin per ewe daily) in the form of 800 g pelleted red clover (*Trifolium pratense*) per ewe daily, commencing at day 3 of the oestrous cycle and continuing till laparotomy one cycle later.

Group IV—fed as in group I, but each ewe received 20 μg oestradiol benzoate (ODB) in oil i/m daily, commencing at day 3 of the oestrous cycle and continuing till cessation of experiment.

Group V—fed as in group I but each ewe received 10 mg progesterone in oil i/m daily for 12 days, with laparotomy 60 hr after the subsequent ovulation.

Group VI—fed as in group I; a pessary containing 60 mg 6-methyl-17-acetoxy-progesterone (MAP) was inserted into the vagina of each ewe for 12 days, with laparotomy 60 hr after the subsequent ovulation.

III. Results

(a) *Experiment 1*

(i) *Oestrus and Ovulation*

Length of oestrus in the ewes was $24 \cdot 8 \pm 0 \cdot 2$ hr ($\pm$ S.E.). Ovulation occurred $27 \cdot 4 \pm 0 \cdot 3$ hr after the onset of oestrus (i.e. $2 \cdot 6$ hr after cessation of oestrus). In all ewes, ovulation occurred after cessation of oestrus.

(ii) *Site of Ovum Recovery*

The distribution of ova recovered from oviductal segments is shown in Table 1. At 6 hr after ovulation the majority of ova were located in the second segment with 25% in the third segment. At 8 hr after ovulation 70% of the ova were in the third segment. The majority of ova were still in the third segment at 36 hr but at 60 hr half had passed the AIJ and were found in either the fourth or fifth segments.

Table 1

Location of ova in the oviduct of the ewe in the first 3 days following ovulation

The ampulla and isthmus were divided into equal segments and numbered from the cranial end of the ampulla. Results are for one ewe at 0·5 hr and for 8–12 ewes at other times

Part of oviduct	Segment No.	No. of ova recovered at following times after ovulation (hr)				
		0·5	6	8	36	60
Ampulla	1	0	0	0	0	0
	2	1	9	3	1	1
	3	0	3	7	6	4
Isthmus	4	0	0	0	1	4
	5	0	0	0	0	1
Total recovered		1	12	10	8	10

The recovery rate of ova was 83% overall, and did not differ significantly between times. The average length of the oviducts was $13 \cdot 6$ cm, the ampulla $9 \cdot 2$ cm, and the isthmus $4 \cdot 4$ cm.

(iii) *Segmentation Data*

Only 1-cell ova were recovered up to 8 hr after ovulation. At 36 hr, fertilized ova were at the 4-cell (5 ova) and 6-cell (3 ova) stages and at 60 hr all fertilized ova were at the 8-cell stage. The fertilization rate of ova recovered at $\geqslant 36$ hr was 77%.

(*b*) *Experiment 2*

Although each treatment group initially consisted of 16 ewes, data are provided only for those ewes where accurate observations were obtained on the duration of oestrus and the time of ovulation.

(i) *Oestrus and Ovulation*

Duration of oestrus and time to ovulation from onset of oestrus are presented in Table 2. In group IV (20 μg ODB daily), the duration of oestrus in all ewes was

TABLE 2

OESTRUS, OVULATION, AND FERTILIZATION DATA FROM EWES IN EXPERIMENT 2

Treatment group*	No. of ewes†	Duration of oestrus (hr) ± S.E.	Time to ovulation (hr) ± S.E.	No. of ewes‡	No. of ova fertilized
I	12	26·5±0·5	29·6±0·8	12	14 (87·5%)
II	8	26·0±0·2	27·9±0·5	7	8 (72·7%)
III	13	27·0±0·2	29·2±0·5	10	8 (53·3%)
IV	16	>30	§	0	0
V	11	24·9±0·4	26·8±0·5	11	6 (50·0%)
VI	6	24·9±0·4	26·7±0·6	6	5 (62·5%)

* For details of treatments, see Section II(*b*)(ii).
† Number included in oestrus and ovulation data.
‡ Number included in fertilization data.
§ Failed to ovulate.

>30 hr. Ovulation had not occurred within 3 days after the anticipated time of ovulation. Ovulation rates for groups I and III were 1·60 and 1·66 ova per ewe respectively.

TABLE 3

LOCATION OF OVA IN THE OVIDUCT OF EWES 60 HR AFTER OVULATION (EXPT. 2)

Part of oviduct	Segment No.	Treatment group No.*				
		I	II	III	V	VI
Ampulla	1	0	0	0	0	0
	2	0	2	1	0	0
	3	4	3	7	0	0
Isthmus	4	7	5	5	8	5
	5	3	0	0	2	0
Uterus	—	0	1	1	2	3

* For details of treatments, see Section II(*b*)(ii).

(ii) *Site of Ovum Recovery*

Ovum transport was affected by the various treatments (Table 3). Comparing the number of ova that had passed the AIJ with those remaining in the ampulla, the

progestagen-treated ewes differed significantly from the controls ($P<0\cdot044$ by Fisher's exact method in 2×2 contingency tables). In these ewes all ova had passed the AIJ with some already in the uterus. On the other hand, the two oestrogen treatments were not significantly different from the controls ($P<0\cdot28$) but had the effect of retaining some ova in the mid-portion of the ampulla and at the AIJ, whilst the remainder had passed through into the isthmus and a few into the first part of the uterus.

The semen of the entire rams was not tested at any stage and the experiment was not specifically designed to provide fertilization rates. However, phyto-oestrogen ingestion appears to have reduced the number of ova fertilized ($87\cdot5\%$ *v.* $53\cdot3\%$, Table 2; $P<0\cdot044$, by one-tailed test).

IV. Discussion

The experiments provide precise information on the rate of progress of ova through the oviducts of crossbred ewes, and suggest that treatment with oestrogen or progesterone may interfere with normal progression.

The ovum recovery rate in experiment 1 did not differ significantly between the various times after ovulation (80, 76, 88, and 90%), suggesting that ova were not being lost to the uterus, though some ova could have been lost during the process of sectioning the oviduct. Furthermore, the passage of the ovum into the ampulla at ovulation does not appear to have been disturbed by endoscopy, as the recovery rate was not significantly higher in a group of 15 ewes not subjected to endoscopy but salpingectomized 60 hr after the estimated time of ovulation.

Delay of ova at the AIJ, following a period of rapid transport along the ampulla, has been noted in other species (Humphrey 1968). This delay may be associated with the necessity for maturation of the ovum to the 8-cell stage, as entry into the uterus prior to this stage is detrimental to embryo survival (Moore and Shelton 1964). The hormonal mechanisms responsible for this delay are not understood but probably involve the balance of circulating progesterone and oestrogen levels (Braden *et al.* 1971).

The mean time at which ova were found to pass into the isthmus (about 60 hr) is at variance with the reports of Lang (1965, 1969), who suggested that the mean time when ova passed into the isthmus was 49 hr after ovulation. Lang assumed that ovulation occurred at the end of oestrus, but his teasing procedure and the segmentation data presented would suggest that ovulation occurred some hours prior to cessation of oestrus (Parsons, Hunter, and Rayner 1967). If his times were corrected then 14, 29, and 50% of ova had passed into the isthmus by 44, 52, and 60 hr respectively. The data of Winterberger-Torres (1967) at the critical period (50–64 hr) are limited to four ova recovered from two ewes at 49–51 hr; three were in the ampulla and one in the isthmus. The time at which 50% of ova pass into the isthmus would thus appear to be between 52 and 60 hr post-ovulation.

In the progestagen-treated ewes, no ova were found in the ampulla 60 hr after ovulation; the majority were in the first part of the isthmus and some had passed into the uterus. It should be noted that no progestagen had been administered to these ewes for at least 5 days previously. Comparing the distribution of ova in the

oviduct of progestagen-treated ewes with that in the control ewes ($P < 0 \cdot 044$), the ova appear to have passed into the isthmus at an earlier time in the former group. This may not have been much earlier, for most of the ova from progestagen-treated ewes were located in the first part of the isthmus.

Synchronization of oestrus by progestagen in ewes generally results in reduced fertility, which is largely attributable to depressed fertilization rates caused by interference with sperm transport (Robinson 1968). The effect of progestagen synchronization on ovum transport has not been critically studied. Shelton and Moore (1967) reported that the percentage of ova recovered from the oviduct was normal when ewes were synchronized by injections (i/m) of progesterone in peanut oil, but was depressed when ewes were synchronized either with progesterone dissolved in propylene glycol or with a synthetic progestagen given in either vehicle. However, the time of recovery of ova in relation to ovulation was not accurately defined, being 54–78 hr after first observed oestrus. In the present study some ova from progestagen-synchronized (but not from control) ewes were already in the uterus 60 hr post-ovulation. It is possible that the premature entry into the uterus of a proportion (25%?) of ova in synchronized ewes makes a contribution, albeit small, to the reduced fertility of such ewes. The survival of ova placed in the uterus of ewes asynchronously is reduced (Moore and Shelton 1964).

Oestradiol-17β, administered as a single i/m injection 30 hr post-ovulation (when the ova should have been at the AIJ), produced an effect on ovum transport similar to that of phyto-oestrogens fed daily until laparotomy. There was retention of some ova in the ampulla with acceleration of others into the uterus. This increased dispersion, though not significantly different from the control ewes, has also been reported in mice receiving oestradiol after ovulation (Humphrey 1968).

The data from the clover-fed ewes, though limited, suggests that consumption of phyto-oestrogens during the mating period lowers the fertilization rate and also disturbs normal ovum transport. Ovulation rate was not reduced but daily formononetin intake was considerably lower than has been reported in grazing sheep.

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