INFLUENCE OF OESTROGEN AND PROGESTERONE ON FLOW OF FLUID FROM THE FALLOPIAN TUBE IN THE OVARIECTOMIZED EWE

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Summary. The volumes of Fallopian tube secretion which pass into the peritoneal cavity (ampullar flow) and uterus (isthmic flow) were recorded at 12-hr intervals in ovariectomized ewes, and after injection of 30 μ g, 90 μ g or 500 μ g oestradiol benzoate (ODB). Ovariectomized ewes were also treated sequentially with progesterone, oestrogen, and, in some animals, a further series of progesterone injections in an attempt to achieve the pattern of directional flow of tubal fluid found in the intact, cyclic ewe.

A decline in total daily secretion and fluid flow followed ovariectomy and was counteracted by the administration of ODB. Both ampullar and isthmic flows were markedly increased at all dose levels of ODB until the flows were comparable with, or exceeded, those which occurred during oestrus in the intact ewe. But the sequence of maximum ampullar flow and maximum isthmic flow was reversed by ODB, the difference being further accentuated by each increase in dose level. As isthmic flow was stimulated at all dose levels of ODB, an effective restriction of the tubo-uterine junction and/or isthmus was not apparent. Progesterone in combination with ODB did not effectively restrict the isthmic flow relative to the ampullar flow.

INTRODUCTION

Fluid secreted by the Fallopian tube in the ewe varies in quantity with the stage of the oestrous cycle (Black, Doby & Reisen, 1963; Perkins, Goode, Wilder & Henson, 1965; Restall, 1966) and is at a maximum about 24 hr after the onset of oestrus. Cannulation of both ends of the Fallopian tube and collection of the secretions (Bellve & McDonald, 1968) has shown that most of the fluid flowed through the ampulla orifice and presumably enters the peritoneal cavity in the intact animal. The flow of fluid through the tubo-uterine junction into the uterus is low for most of the cycle, but markedly increased 4 days after the onset of oestrus. The increase in flow through the isthmus coincides with the expected time at which ova enter the uterus.

The mechanism which determines the direction that secretions leave the Fallopian tube is probably under hormonal control. Some evidence in support

of this suggestion is shown by the prolonged duration of fluid distension in the ligated ampulla which results after administration of oestrogen to the mated rabbit (Black & Asdell, 1958, 1959), ewe (Edgar & Asdell, 1960) and cow (Black & Davis, 1962). The present study was therefore conducted to evaluate the effect of exogenous oestrogen, and of a combined progesterone-oestrogen treatment simulating the hormonal changes that occur during the oestrous cycle of the intact ewe, on the flow of tubal fluid in the ovariectomized ewe.

MATERIALS AND METHODS

Procedure

Twenty-one, 5- and 6-year-old Romney ewes, kept in individual pens, were used for three experiments during the anoestrous season (November 1966 to January 1967). Each animal was ovariectomized and one Fallopian tube doubly cannulated (Bellve & McDonald, 1968). One cannula was ligated into the ovarian end of the ampulla for the collection of fluid (ampullar flow) and the other was passed through the uterine wall and ligated at the utero-tubal junction (isthmic flow). Both cannulae were exteriorized and connected to separate collecting tubes attached to the animal's flank. The fluid in each collection tube was withdrawn twice daily (at 08.30 and 20.30 hours) and the amount measured. The total quantity of fluid secreted by the Fallopian tube was taken as the sum of ampullar flow and isthmic flow and the results expressed for 24 hr. The data from one ewe were excluded owing to failure of the isthmic cannulation within a few days of surgery.

Vaginal smears, taken daily from all ewes, were stained with haematoxylin and eosin and examined. The cellular appearance of smears was classified for response to oestrogen according to the method of Robinson & Moore (1956).

Experiment 1

Ten ewes were studied immediately after ovariectomy and daily collections of fluid made for 15 or 16 days. At the end of this period, two groups, each of four ewes, were treated with 30 and 90 μg of oestradiol benzoate (ODB) respectively. The ODB was dissolved in arachis oil (30 $\mu\text{g}/\text{ml}$) and injected intramuscularly at 09.00 hours (Day 0). The two remaining ewes were not treated with oestrogen and acted as controls. Collections of fluid were made from all ewes until Day 11.

Experiment 2

Seven days after the completion of the first experiment, four ewes (two from each of the oestrogen-treated groups) were injected (Day 0) with 500 μg ODB. Two ovariectomized ewes not injected with ODB acted as controls. Collections of fluid were made until Day 11.

Experiment 3

Ten ewes were fitted with cannulae in January 1967, and hormonal treatments started 12 days after surgery. Eight ewes were pre-treated with subcutaneous injections of 10 mg progesterone daily for 8 days (Day -8 to Day -1).

On Day 0 (the day after progesterone pre-treatment), a single intramuscular injection of 30 µg ODB was given to four ewes (Group A) and of 90 µg ODB to four ewes (Group B). Two ewes in each group received 5 mg progesterone on Day 4 and 10 mg daily from Day 5 to Day 10. Two ovariectomized ewes, not treated with progesterone or oestrogen, acted as controls. Animals were allocated to groups and sub-groups at random and fluid was collected from all ewes until Day 10.

To enable separation of treatment effects, the data on total fluid secreted and on ampullar and isthmus flow were considered during the following periods: (a) Day -8 to Day 0; (b) Day 1 to Day 4; (c) Day 5 to Day 10. In each analysis of variance, main effects of treatment were tested against the appropriate first order interaction.

RESULTS

EXPERIMENTS 1 AND 2

Effect of ovariectomy

Text-figure 1 shows the mean daily fluid secretion, ampullar flow and isthmus flow in eight ovariectomized ewes prior to injection with oestrogen (Experiment 1). Analysis of variance (Table 1) indicated that considerable

TABLE 1
SUMMARY OF ANALYSES OF VARIANCE† FOR FLUID SECRETED, AND FLUID FLOW, IN OVARIECTOMIZED AND OESTROGEN-TREATED OVARIECTOMIZED EWES (ML/24 HR)

Source	d.f.	Total fluid secretion M.S.	Ampullar flow M.S.	Isthmic flow M.S.
Ovariectomized				
Days	14	7.71**	7.59**	4.84**
Sheep	7	5.52**	5.77**	3.21**
Error	98	0.042	0.010	0.026
Treatments				
Between treatments	2	0.618	0.191	0.123
Between days	13	1.664	0.796*	0.336**
Treatment × days	26	1.795**	0.334**	0.108
Error	126	0.161	0.060	0.063

† Main effects of treatment tested against appropriate first-order interaction.

M.S. = Mean square.

* $P < 0.05$; ** $P < 0.01$.

variation existed between animals ($P < 0.01$) in daily fluid secretion, ampullar flow and isthmus flow; much of the variation was observed during the immediate post-operative period. This was particularly noticeable with isthmus flow where the volume recorded ranged from 0 to 1.07 ml/24 hr (mean 0.42 ± 0.16 ml) on the 1st day after ovariectomy and 0 to 0.81 ml/24 hr (mean 0.31 ± 0.11 ml) on the 2nd day.

Total fluid secretion and isthmus and ampullar flow rates declined significantly ($P < 0.01$) and reached relatively constant levels about 12 days after surgery. Isthmus flow in some animals was often spasmodic and 15 days after surgery only intermittent recordings were obtained.

TEXT-FIG. 1. Mean daily secretion of tubal fluid (●), and rate of flow through the ovarian (ampullar, Δ) and uterine (isthmus, ○) ends of Fallopian tubes in ewes ovariectomized on Day 0 ($n = 8$).

The two ovariectomized ewes which were used as control animals in Exps. 1 and 2 gave results for tubal fluid similar to those shown in Text-fig. 1. These two animals continued to secrete fluid at low levels with a very small isthmus component.

Effect of oestrogen

Data from the ewes in Exps. 1 and 2 are considered together as there was no evidence that previous treatment with ODB influenced the results from the four oestrogen-treated ewes in Exp. 2. The levels of tubal fluid immediately before injection in Exp. 2 were similar to those recorded in the same sheep before the first study and were similar to levels in the control ewes.

TABLE 2

DURATION OF RESPONSE AND TIME OF MAXIMUM FLUID SECRETION, AND FLUID FLOW, IN OESTROGEN-TREATED OVARIECTOMIZED EWES

Measurement	Dose level (μg)	Day of maximum recording (Mean $\pm$ S.E.)	Duration of response in days (Mean $\pm$ S.E.)
Total fluid secretion	30	3.2 $\pm$ 0.3	7.0 $\pm$ 0.6
	90	3.9 $\pm$ 0.6	9.8 $\pm$ 0.8
	500	5.0 $\pm$ 0.8	10.5 $\pm$ 0.8
Ampullar flow	30	3.4 $\pm$ 0.5	6.9 $\pm$ 0.6
	90	4.0 $\pm$ 0.7	9.0 $\pm$ 0.9
	500	5.3 $\pm$ 0.7	10.4 $\pm$ 0.8
Isthmic flow	30	2.1 $\pm$ 0.2	5.3 $\pm$ 0.4
	90	2.3 $\pm$ 0.2	5.9 $\pm$ 0.5
	500	2.3 $\pm$ 0.4	7.3 $\pm$ 0.7

TEXT-FIG. 2. Mean daily secretion of tubal fluid, ampullar flow and isthmic flow in ovariectomized ewes injected with either 30 μg ($\bullet$), 90 μg (Δ) or 500 μg ($\circ$) ODB ($n = 4$).

In all ewes, oestrogen markedly increased total fluid secretion and flow from both sections of the Fallopian tube (Text-fig. 2). There were no significant differences between doses of oestrogen in terms of mean daily secretion or ampullar and isthmic flows, although there was significant interaction between treatment and days mainly due to differences between treatments on Days 5 to 9 inclusive (Table 1).

The effect of treatment on tubal fluid was further examined by determining the time at which maximum daily fluid secretion and maximum fluid flows occurred and also the 'duration of response' which was regarded as the interval (days) from injection of ODB until recordings of fluid secretion and fluid flow returned to pre-treatment levels (Table 2). Both for daily fluid secretion and ampullar flow, the maximum response to treatment occurred later, and the duration of response was longer as the dose level of oestrogen increased, but the time of peak isthmic flow was not markedly altered by the dose level of oestrogen. In ewes given 30, 90 or 500 μg ODB, peak isthmic flow preceded that of ampullar flow by 1.3 ± 0.5 , 1.8 ± 0.5 and 3.0 ± 0.6 days, respectively. The duration of response for isthmic flow was considerably less than that for ampullar flow.

Examination of daily vaginal smears showed that increased quantities of vaginal mucus and a positive cellular response to oestrogen occurred in all ewes after injection with ODB.

EXPERIMENT 3

Preparatory progesterone

The ampullar and isthmic flows appeared to decrease during progesterone treatment, but on several days flows were too low to record (Text-fig. 3). Considerable day-to-day fluctuations in the direction of fluid flow occurred within individual ewes (Table 3).

Sequential oestrogen-progesterone

All ewes showed positive vaginal smear responses to ODB. Oestrogen significantly increased ($P < 0.01$) the output of fluid from both cannulae during Days 1 to 4 (Text-fig. 4; Table 3), but an effect of dose level was recorded only for isthmic flow. A peak flow of 0.25 ± 0.05 ml and 0.13 ± 0.03 ml/24 hr occurred after injection of 90 μg and 30 μg ODB respectively and in this period more fluid passed through the isthmus in the animals treated at the highest dose level. During the remainder of the experiment, the daily secretion and ampullar flow declined ($P < 0.01$) and progesterone given at this time further decreased ($P < 0.01$) the flow from both ends of the Fallopian tube. The effect of progesterone in reducing daily secretion and flow was also indicated by a reduction in the duration of response that followed the injection of oestrogen (Table 4).

In all treated ewes, peak isthmic flow preceded that of maximum ampullar flow by 1.1 ± 0.8 and 2.1 ± 0.8 days after 30 μg and 90 μg ODB respectively (Table 4). In the ewes injected with 30 μg ODB, the isthmus was only a minor outlet for the fluid secreted.

TEXT-FIG. 3. Mean daily secretion of tubal fluid (●) ampullar flow (Δ) and isthmic flow (○) in ovariectomized ewes injected with 10 mg progesterone daily from Day -8 to Day -1 (n = 8).

TABLE 3

SUMMARY OF ANALYSES OF VARIANCE† FOR FLUID SECRETED AND FLUID FLOW IN OVARIECTOMIZED EWES PRE-TREATED WITH PROGESTERONE AND GIVEN SEQUENTIAL OESTROGEN-PROGESTERONE TREATMENT (ML/24 HR)

	<i>d.f.</i>	<i>Total fluid secreted M.S.</i>	<i>Ampullar flow M.S.</i>	<i>Isthmic flow M.S.</i>
Pre-treated with progesterone				
Days	9	0.0043**	0.0020	0.0006
Sheep	7	0.0047**	0.0001	0.0008
Error	63	0.0013	0.0013	0.0004
Oestrogen				
Days	3	0.6413**	0.6651**	0.0329**
Treatments	1	0.0043	0.0351	0.0435**
Days × treatments	3	0.0431	0.0307	0.0009
Error	24	0.0144	0.0153	0.0041
Concluding progesterone				
Days	5	0.1570**	0.1357**	0.0011
Oestrogen	1	0.0253	0.0165	0.0011
Progesterone	1	0.2352**	0.1599**	0.0066**
Days × oestrogen	5	0.0029	0.0016	0.0007
Days × progesterone	5	0.0095	0.0086	0.0007
Progesterone × oestrogen	1	0.0032	0.0460	0.0004
Progesterone × oestrogen × days	5	0.0060	0.0009	0.0002
Error	24	0.0119	0.0121	0.0002

† Main effects of treatment tested against appropriate first order interaction.

M.S. = Mean square.

** $P < 0.01$.

TEXT-FIG. 4. Mean daily secretion of tubal fluid, ampullar flow and isthmic flow in ovariectomized ewes injected with progesterone from Day -8 to Day -1 (see Text-fig. 3), and with 30 μ g (●) or 90 μ g ODB (▲) on Day 0 ($n = 4$). Data for ewes treated with progesterone from Days 4 to 10 are shown with open symbols (○, △) corresponding to oestrogen-treated groups ($n = 2$).

TABLE 4

DURATION OF RESPONSE AND TIME OF MAXIMUM FLUID SECRETION, AND FLUID FLOW, IN OVARIECTOMIZED EWES PRE-TREATED WITH PROGESTERONE AND GIVEN SEQUENTIAL OESTROGEN-PROGESTERONE TREATMENT

Treatment	Day of maximum recording (Mean $\pm$ S.E.)			Duration of response (days) (Mean $\pm$ S.E.)		
	Total fluid secretion	Ampullar flow	Isthmic flow	Total fluid secretion	Ampullar flow	Isthmic flow
30 μ g ODB	3.9 $\pm$ 0.3	4.2 $\pm$ 0.2	2.3 $\pm$ 0.2	7.3 $\pm$ 0.9	7.0 $\pm$ 0.7	6.5 $\pm$ 0.4
30 μ g ODB + progesterone				6.7 $\pm$ 0.6	6.8 $\pm$ 0.6	3.8 $\pm$ 0.3
90 μ g ODB	4.4 $\pm$ 0.3	4.5 $\pm$ 0.3	2.4 $\pm$ 0.2	9.5 $\pm$ 0.9	9.3 $\pm$ 0.9	7.8 $\pm$ 0.5
90 μ g ODB + progesterone				7.5 $\pm$ 0.5	7.3 $\pm$ 0.6	6.8 $\pm$ 0.4

DISCUSSION

Fluid secretion

The decline in the daily volume of tubal fluid following ovariectomy confirmed previous reports on the rabbit (Bishop, 1956; Mastroianni, Beer, Shah & Clewe, 1961) and the ewe (Restall, 1966). In ovariectomized ewes, fluid secretion decreased to 0.05 to 0.09 ml/24 hr which is about one-third of the daily production in the anoestrous sheep (Bellve & McDonald, unpublished data).

Oestrogen increased the daily volume of fluid. A single injection of 30 μ g ODB raised tubal secretion to a level comparable to that occurring during normal oestrus in the intact ewe (Bellve & McDonald, 1968). Higher doses of ODB (90 and 500 μ g) did not induce further substantial increases in secretion, but elevated levels of fluid occurred over a longer period than after 30 μ g ODB.

Progesterone depressed the secretion of tubal fluid in both the untreated and oestrogen-primed ovariectomized ewe. In the ovariectomized, progesterone-treated ewe, the low levels of fluid secretion may be induced by oestrogen from extra-ovarian sources (Bulbrook & Greenwood, 1957).

Direction of fluid flow

Ampullar flow, after a single injection of 30 μ g or 90 μ g ODB, approximated that in the untreated animal in oestrus (Bellve & McDonald, 1968) while the response to 500 μ g ODB was only slightly greater. Isthmic flow, however, was substantially increased by the higher levels of oestrogen. It is clear that these oestrogen treatments did not lead to the sequence of fluid flow from both ends of the Fallopian tube that is found in the intact ewe in oestrus; the times of maximum flow from each source were completely reversed by oestrogen and further accentuated by each increase in dose level.

The stimulation of isthmic flow by ODB in the ovariectomized ewe suggests that a mechanism may exist in the cyclic animal which prevents a premature surge in isthmic flow during the oestrogen-dominant pre-ovulatory phase of the

cycle. Progesterone from a waning corpus luteum may facilitate the action of oestrogen in promoting an oedematous occlusion of the isthmus (Black & Asdell, 1959; Edgar & Asdell, 1960; Black & Davis, 1962) to restrict flow of fluid into the uterus. In Exp. 3, the effect of preparatory progesterone treatment on the secretion of fluid which followed the injection of ODB was not determined. The decreased output of fluid during the period of progesterone injections may have reduced the daily flow response to oestrogen. However, the sequential progesterone-ODB treatment did not delay the flow of fluid through the tubo-uterine junction. Extension of progesterone treatment to merge with the ODB injection might have suppressed isthmic flow. The fluid distension incurred by ligation at the ovarian end of the Fallopian tube in the cyclic ewe (Edgar & Asdell, 1960) does not frequently occur in the sheep induced to ovulate in the non-breeding season and therefore without endogenous progesterone.

Progesterone given after ODB significantly reduced both ampullar and isthmic flows. The latter result does not agree with the long-standing concept that rising levels of ovarian progesterone after ovulation allow the passage of ova (Hartman, 1939; Chang & Bedford, 1961) and tubal fluid (Black & Asdell, 1959) through the isthmus. The absence of isthmic flow in the progesterone-treated ovariectomized ewe is more indicative of retarded ovum transport as in the pseudopregnant rabbit (Austin, 1949) and ovariectomized rabbit treated with progesterone (Adams, 1958). In the rabbit given sequential oestrogen-progesterone treatment (Harper, 1965), the transport of artificial ova through the isthmus was also hindered, not enhanced, by the concluding phase of progesterone.

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