THE UTERUS OF THE EWE

I. SECRETION FROM THE CANNULATED UTERUS

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[Manuscript received March 15, 1971]

Abstract

Three methods of cannulating the uterus of the ewe are described and the patterns of secretion collected from the cannulated uteri are presented.

The first procedure involved cannulation of the uterus near the uterotubal junction. When one horn was cannulated by this method and the cannulated horn ligated at the external bifurcation, no change in the length of the oestrous cycle or in the ovulation rate was observed. However, in some animals examined 60–80 days after cannulation, these treatments caused a slight increase in the height of the luminal and glandular epithelium of the uterus.

There were large variations between ewes in the volume of fluid collected from the cannulated horn. In most ewes, a peak in flow was obtained close to oestrus and an average of 3 or 4 ml of fluid was collected during each oestrous cycle. However, larger volumes than average were obtained during the first oestrous period following ligation of the uterine horn at the external bifurcation. There was a considerable difference in the volume of fluid collected from each horn of the same animal. In animals with both the uterine horn and the fallopian tube cannulated, the volume of fluid collected from the uterus was less than that from the fallopian tube. In anoestrous ewes, secretion from one uterine horn averaged 0·12 ml/day and showed little variation with time.

In the second procedure, the uterus was cannulated by transection anterior to the cervix and insertion of a cannula into the transected end. Short oestrous cycles were frequently observed in these ewes after cannulation. The volume of uterine fluid collected varied greatly between animals but in most cases peaks in volume occurred at each oestrus.

Uterine fluid was also collected from four multiparous ewes with a cannula inserted through the cervical canal. In these ewes, the oestrous cycles were generally shorter than normal and there were large variations between animals in the volume of fluid collected.

Overall, cannulation via the uterine horn resulted in the collection of less fluid than if either of the other two methods were used. In all cases, the variation between animals was greater than the variation between cycles within the same animal. The physiological significance of secretion rates from cannulated uteri is discussed.

I. Introduction

There can be little doubt that the fluid in the uterine lumen plays an important role in reproductive processes. Spermatozoa are in contact with this fluid, at least for a short time, during transport to the site of fertilization. During this time the fluid probably provides a substrate for spermatozoal metabolism (Wales and Restall 1966) and may play some part in capacitation (Austin 1951; Chang 1951).

Of equal importance is the role of the luminal fluid in the nutrition of the developing embryo. This is particularly so in the ewe, where firm attachment of the

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trophoblast to the uterine caruncles occurs as late as day 44 of pregnancy. Thus the sheep conceptus must rely mainly on the histotrophic type of nutrition for a considerable part of pregnancy, first in the fallopian tube and later in the uterus.

Much information is available on the secretion and chemistry of tubal fluid in the ewe, but information on the secretion of uterine fluid during the oestrous cycle is confined to two reports, giving data for a total of four ewes (Perkins *et al.* 1965; Iritani, Gomes, and Van Demark 1969).

The present experiments provide further data on the rate of secretion of uterine fluid in ewes and the influence of various methods of cannulating the uterus on this secretion.

II. Materials and Methods

(*a*) *General*

The experiments were carried out on a total of 71 Border Leicester × Merino Crossbred ewes during the breeding seasons of 1965–1970 inclusive. Ewes were housed indoors and all exhibited normal oestrous cycles prior to operation. In the preliminary experiments the animals were kept in small colony enclosures but in all later experiments cannulated sheep were isolated in single pens. Special care was exercised in handling equipment and in cleaning the animal pens to decrease the risk of infection. During the period of the experiment, ewes were checked daily with a vasectomized ram for signs of oestrus.

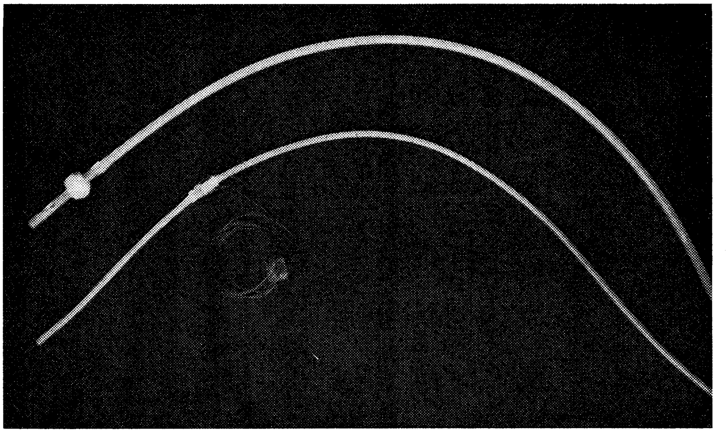

Fig. 1.—Silastic cannulae used in the present study. *Top*: That used for cannulation of the uterine horn and for cannulation of the body of the uterus at the uterocervical junction. *Bottom*: That used for cannulation through the cervical canal.

(*b*) *Surgical Procedures*

The reproductive tract was brought to the exterior through a midline abdominal incision anterior to the pubic symphysis. Three methods of cannulating the uterus were used.

(i) *Cannulation of the Uterine Horn*

The fallopian tube was ligated just anterior to the uterotubal junction and the uterus was transected 5–10 mm posterior to the uterotubal junction. A cannula of silicone rubber, 46 cm long and having a bore of 1·6 mm and a wall thickness of 0·8 mm (medical grade Silastic tubing, Dow Corning Co.) was inserted into the uterine lumen through the cut end of the uterus. Approximately 2 cm from the uterine end of the cannula, a collar of open-pore Silastic sponge was attached with Silastic cement (Dow Corning Co.). The 2 cm of cannula anterior to this collar entered the lumen of the uterus and was provided with two to three supplementary openings of 2 mm diameter to aid drainage. Details of the cannula are shown in Figure 1. The cannula was secured by suturing the collar of sponge to the cut edges of the uterus with interrupted sutures of 5/0 B.B. silk. The procedure was adopted as preliminary experiments involving 20 ewes indicated that when this cannula was used, fibroblasts invaded the open pores of the sponge collar and led to firmer attachment to the uterus than when other procedures were used.

(ii) *Cannulation at the Uterocervical Junction*

Longitudinal incisions on both the dorsal and ventral aspects of the uterus were made through the serosal layer in the vicinity of the uterocervical junction. The uterine vessels were then freed from the underlying tissue by careful dissection and the body of the uterus was transected at the cervical junction, leaving the uterine vessels intact. The cannula used in this procedure was the same as that used for cannulation of the uterine horn.

After insertion of the cannula, the uterine mucosa was sutured to the sponge with 3/0 catgut and the muscle layers sutured over the sponge pad to complete closure of the uterine opening. After the cervical opening was closed, the incisions in the serosal coat were closed with interrupted sutures of silk. The tubal junction of each horn was ligated and severed to prevent entry of tubal fluid into the uterus.

(iii) *Cannulation through the Cervical Canal*

A cannula of silicone rubber tubing with a bore of 1·0 mm and a wall thickness of 0·8 mm (Fig. 1) was inserted through the cervix. A midline laparotomy incision was made to expose the body of the uterus and a blunt probe was passed into its lumen through a puncture hole in the wall. The probe was then passed posteriorly through the cervical canal into the vagina. The cannula was attached to the probe and drawn into the uterus through the cervix until the sutures attached 6 cm from the uterine end of the cannula were positioned at the external cervical opening. The cannula was held in position by passing the free ends of the sutures through the lateral vaginal fornix and tying in the abdominal cavity. The portion of the cannula in the lumen of the uterus was provided with 4–5 holes to aid drainage of secretions. The external end of the cannula was attached to the collection apparatus on the flank of the animal well away from the perineal region.

(*c*) *Collection of Fluid*

Except in those animals cannulated through the cervical canal, all cannulae were brought to the surface through a small stab wound in the flank. The external end of the cannula was attached to a sterile bottle of approximately 7 ml volume, provided with an air vent. The collection bottle was protected in a rubber pouch attached to the flank of the animal with contact adhesive. The animals were inspected daily and the bottles replaced aseptically if secretion had collected. At the same time, the ewes were checked with a vasectomized ram for behavioural oestrus. On some occasions, circumstances precluded a daily collection and at such times, data for secretion rate were averaged over the 48-hr period of collection.

(*d*) *Histology*

Uterine tissue was fixed in Bouin's fluid, stained with haemotoxylin and eosin, and sections of 7 μm examined. The height of the glandular epithelium and the luminal epithelium of the uterus was measured in triplicate using an eye-piece micrometer.

(*e*) *Presentation of Data*

In the experiments where the cannula caused no change in the frequency of oestrus, mean patterns of secretion during each cycle following cannulation have been presented. These patterns were obtained by averaging the volume collected for all animals within a treatment group on the day of behavioural oestrus and on the 8 days before and after oestrus. Where the animals exhibited a varying frequency of behavioural oestrus after cannulation, it was not possible to present average values and data for individual ewes are given in the figures. All results were transformed to logarithms prior to variance analysis as this transformation greatly reduced the heterogeneity of the data.

III. Results

(a) *Cannulation of the Uterine Horn*

Cannulae were inserted into the uterine horn of a total of 45 ewes which were used in the following experiments.

(i) *Experiment 1: Effect of Cannulation on the Length of the Oestrous Cycle, Ovulation Rate, and Histology of the Uterus*

In 16 ewes, one uterine horn was cannulated and ligated at the external bifurcation. Half of these ewes were cannulated 0–8 days after oestrus while the other half were cannulated 9–16 days after oestrus. During cannulation, the time of last oestrus was verified and the ovulation rate ascertained by inspection of the ovaries.

Table 1

HEIGHT OF THE LUMINAL AND GLANDULAR EPITHELIUM OF EWES CANNULATED THROUGH ONE UTERINE HORN

Values are in arbitrary units and are means for three measurements in each animal

Stage at Slaughter	No. of Ewes	Height of Epithelium in Cannulated Horn		Height of Epithelium in Contralateral Horn	
		Lumen	Glands	Lumen	Glands
Cycling	3	7·9	6·9	7·3	7·0
Early anoestrus	6	6·9	6·8	6·2	6·4
Deep anoestrus	3	5·7	5·4	4·9	4·7

Summary of the Analysis of Variance

Source of Variation	Degrees of Freedom	Variance Ratios
Between animals:		
Between stages at slaughter		
Cycling *v.* deep anoestrus	1	6·57*
Early anoestrus *v.* rest	1	0·49
Between animals within stages (error 1)	9	*11·90*
Within animals:		
Effect of cannulation	1	2·86
Cannulation × lumen *v.* glands	1	0·17
Cannulation × animals interaction (error 2)	9	*3·33*
Lumen *v.* glands	1	3·20
Other interactions	24	0·92
Between measurements (error 3)	96	*0·49*

* $P < 0 \cdot 05$.

All ewes were checked daily for signs of oestrus and the ovaries of half of each group were inspected at laparotomy 1–2 days after the first oestrus post cannulation, to determine ovulation rate. The ovaries of all ewes were inspected by laparotomy after the second oestrus.

There were no significant effects of the treatments on the length of the oestrous cycle with an average of $17 \cdot 0 \pm 0 \cdot 6$ days for all ewes. This is not significantly different from the average of the same ewes determined prior to cannulation ($15 \cdot 2 \pm 0 \cdot 7$ days) nor is it different from the cycle length observed in eight of the ewes kept for a third oestrus after the second laparotomy ($17 \cdot 9 \pm 0 \cdot 9$ days). Ovulation rate in the eight ewes inspected at the first cycle after cannulation (1·125 ovulations/ewe) and in all ewes at the second cycle after cannulation (1·125 ovulations/ewe) was identical with the rate in these ewes prior to cannulation.

Twelve of the ewes were killed towards the end of the breeding season approximately 60–80 days after cannulation and were classified on ovarian morphology as cycling (3 ewes), early anoestrus (6 ewes), or in deep anoestrus (3 ewes). Tissue samples were taken from the uterine horn posterior to the insertion of the cannula and from the same position on the contralateral horn. Sections were examined histologically to determine the height of the luminal epithelium and of the glandular epithelium in the intercotyledonary areas. The results for those heights in arbitrary units are given in Table 1 together with the summary of the analysis of variance. There was a decrease in the height of the epithelium of the lumen and of the glands as the animals entered anoestrus. The slight mean increase in the thickness of the epithelium in cannulated ewes was not statistically significant.

(ii) *Experiment 2: Effect of Ligation of the Uterine Horn on the Secretion Pattern*

Sixteen ewes were cannulated in one uterine horn, eight ewes with a short (2 cm) intrauterine portion of cannula and the other eight with a long (5 cm) ended cannula. In the latter case, the openings in the cannula lay approximately half way along the length of the horn. Half of each group had the uterus ligated at the external bifurcation, the remaining ewes had no ligature on the uterus. Collections were made for three oestrous cycles in each ewe and the ewes were killed 5–8 days after the last cycle and the uteri inspected to check patency of the cannula, the absence of infection in the uterus, and ovarian morphology. The mean secretion pattern for each group of ewes is shown in Figure 2. The double peaks which occurred in the mean values for some cycles in Figure 2 were due to differences between ewes in the day of the cycle upon which a peak in the secretion of fluid occurred rather than to double peaks within individual ewes. The secretion data for the 12 days around oestrus were subjected to analyses of variance after logarithmic transformation. The 12 days of the cycle included in the analyses were divided into pre-oestrus (5 days), oestrus (day of and day following behavioural oestrus), and post-oestrus (5 days). The results of the analyses are given in Table 2. In unligated uteri, differences in the length of the intrauterine end of the cannula had no effect on the secretion rate. There were no differences between cycles and the mean volume of fluid collected from unligated uteri over an oestrous cycle was between 3 and 4 ml. In ewes with a ligated horn, there was no overall effect of length of the cannula on secretion rate but the results were variable as indicated by the significant interactions of this factor with the other factors studied. In these animals there was also a significant fall in the volume of secretion with each successive cycle; this fall being largely due to a decrease in the output at oestrus. In both groups of animals, more fluid was produced at oestrus than at other times.

The appearance of the fluids varied considerably from one collection to another and a number of collections showed some evidence of leucocyte invasion or were coloured by blood pigments. However, most collections were clear to straw-coloured. Of the 330 collections from the 16 ewes used in this experiment, 57% could be put into this

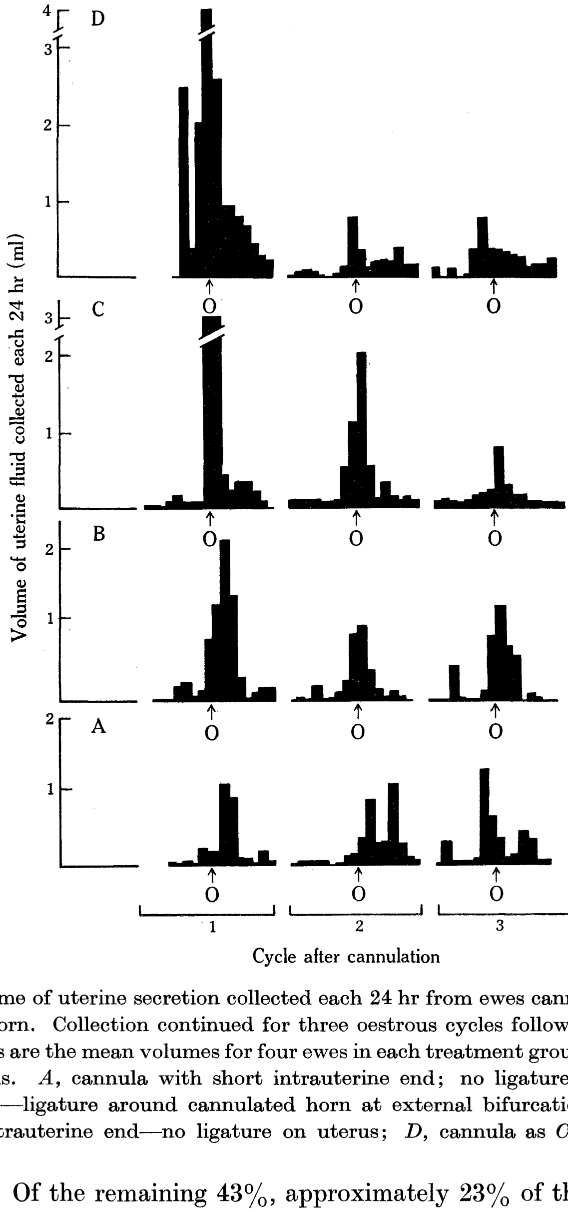

Fig. 2.—Volume of uterine secretion collected each 24 hr from ewes cannulated through one uterine horn. Collection continued for three oestrous cycles following cannulation and the values are the mean volumes for four ewes in each treatment group. O designates day of oestrus. *A*, cannula with short intrauterine end; no ligature on uterus; *B*, cannula as *A*—ligature around cannulated horn at external bifurcation; *C*, cannula with long intrauterine end—no ligature on uterus; *D*, cannula as *C*, ligature as *B*.

latter category. Of the remaining 43%, approximately 23% of the fluids showed a brown colour, presumably due to the breakdown of blood pigments. A further 10% were pink in colour and the remaining 10% were very red and blood-like. The fluids in the last category were collected from 9 of the 16 ewes. In five of these ewes, bloody fluids were collected at the commencement of the increased flow associated with the second or third oestrus. A further two ewes produced these fluids just prior to slaughter following the third oestrous period, while the final two ewes produced these fluids during the interoestrous period. There was no indication that the appearance of the fluid was related to the length of the intrauterine portion of the cannula or to the presence of a ligature on the uterine horn. In addition, the macroscopic appearance of the uterine mucosa in those ewes which gave blood-stained fluid was normal at post mortem and not different from that in the other ewes. However, in some of these ewes a small amount of blood-stained fluid was present on the mucosa of the cannulated side.

Table 2

SUMMARY OF THE ANALYSES OF VARIANCE FOR THE DATA OF FIGURE 2 FOLLOWING LOGARITHMIC TRANSFORMATION†

Source of Variation	Degrees of Freedom	Variance Ratios	
		Unligated Uterus	Ligated Uterus
Between animals:			
Effect of length of cannula (between methods)	1	1·25	0·67
Between animals within methods (error 1)	6	*379·48*	*135·09*
Collections within animals:			
Between cycles	2	0·18	7·73**
Between days of cycle			
Oestrus *v.* rest	1	18·45**	46·81**
Remainder	10	3·43**	0·78
Cycles × days of cycle (error 2)	22	*230·57*	*415·61*
Collections × method interactions	35	1·27	1·58*
Collections × animals interactions (error 3)	210	*140·34*	*179·17*

* $P < 0 \cdot 05$. ** $P < 0 \cdot 01$. † log transformation: $Y = 100 \log_{10} (X+1)$.

(iii) *Experiment 3: Cannulation of both Uterine Horns*

Five ewes were cannulated in both uterine horns and the secretions were collected over 44 days after cannulation. In three of the five ewes, the body of the uterus was ligated anterior to the cervix. As in the previous experiment, mean flow varied considerably between ewes, from 0·18 ml/day to 1·32 ml/day per horn, and there were considerable differences in the volume of fluid collected from the two horns of the same animal.

(iv) *Experiment 4: Cannulation of the Uterus and Fallopian Tube in the Same Ewe*

Secretions were collected from the fallopian tubes and uteri of three sheep. The fallopian tubes were cannulated through the fimbria as described by Restall (1966). The uterine horns were cannulated and no ligatures were applied to the uterus. Collection of fluid commenced 7 days after cannulation and continued for approximately 50 days and covered three oestrous cycles in each animal. The results are shown in the following tabulation in which each value represents the mean volume of fluid collected during each cycle:

	First Cycle	Second Cycle	Third Cycle
Tubal fluid collected (ml):	7·84	9·75	9·09
Uterine fluid collected (ml):	2·11	2·49	3·57

In all cases the volume of fluid collected from the fallopian tube was three times that from the uterus.

(v) *Experiment 5: Uterine Secretion in Anoestrous Ewes*

Five ewes were cannulated in one uterine horn at the end of the breeding season in order to observe the pattern of uterine secretion as the animals entered anoestrus. The uteri in all ewes were ligated at the external bifurcation. Four of the five ewes exhibited one oestrus before becoming anoestrus. The fifth continued to cycle and has not been included in the results which are presented in Figure 3. Intermittent

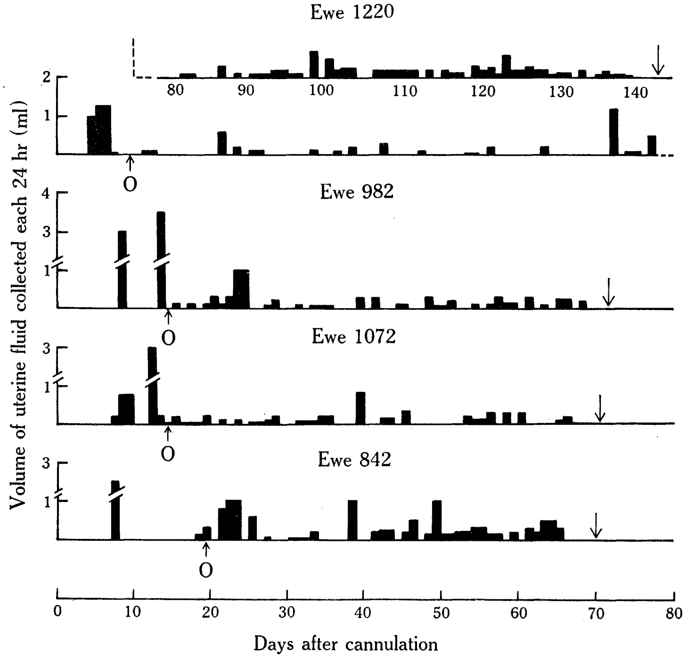

Fig. 3.—Daily collections of uterine fluid from ewes entering anoestrus. Each ewe was cannulated through one uterine horn and the cannulated horn ligated at the external bifurcation. Soon after cannulation, each ewe exhibited one oestrus (O) but subsequently, no cycles occurred. Arrows indicate days on which animals were killed.

secretion occurred in all ewes after the last oestrous cycle and the average collection of fluid during the whole of the anoestrous period was 0·12 ml/day (S.E. of difference between animals = 0·02 ml/day). In one ewe (ewe 1220) maintained for 130 days after the last oestrus, secretion continued at a rate of 0·1–0·2 ml/day until slaughter. At post mortem, the uteri in all animals appeared normal and ovarian morphology was typical of anoestrus.

(*b*) *Cannulation at the Uterocervical Junction*

The uteri of eight ewes were cannulated at the uterocervical junction. Collections were commenced 1–2 days after cannulation and continued for 24–27 days. In one ewe, there was a continuing problem with blockages in the cannula and the data on its secretion are not presented. The pattern of secretion from the remaining seven ewes is shown in Figure 4.

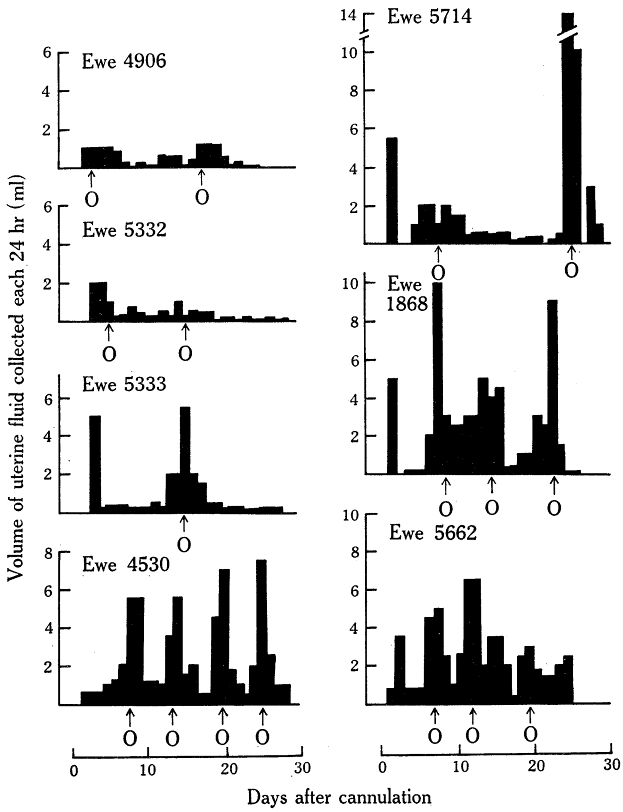

Fig. 4.—Pattern of secretion of uterine fluid collected from seven ewes whose uteri were cannulated at the uterocervical junction. O designates the days upon which behavioural oestrus occurred.

In all ewes there was a fluctuating pattern of fluid production with peaks related to the observed periods of oestrus. Both the volume of fluid and the frequency of oestrus varied greatly between animals. The average volume collected from individual ewes during the period of cannulation varied from 0·29 ml/day to 2·56 ml/day. In four ewes, the oestrous periods appeared to be separated by 14–16 days. In the remaining ewes, oestrus occurred at intervals of 6–9 days.

The appearance of the fluids collected from these seven ewes was similar to those collected from ewes cannulated through the uterine horn. Of the 118 collections made, 61% of the fluids were clear to light brown, 16% were pink, and the remaining 23% were brown to blood-like. Twenty-three of the 27 collections included in the last category were collected from ewes 5333, 4530, and 5662. In ewes 5333 and 4530, these collections were made within the first few days after cannulation while in ewe 5662, red fluids were collected at random intervals during the whole period of cannulation.

(c) *Cannulation through the Cervical Canal*

Cannulation of the uterus through the cervical canal was attempted in 18 ewes. However, considerable difficulty was experienced in threading the probe through the convolutions of the cervical canal and the procedure was unsuccessful in nulliparous and uniparous ewes. However, four successful cannulations were performed in multiparous ewes close to oestrus and the pattern of secretion from these ewes is shown in Figure 5.

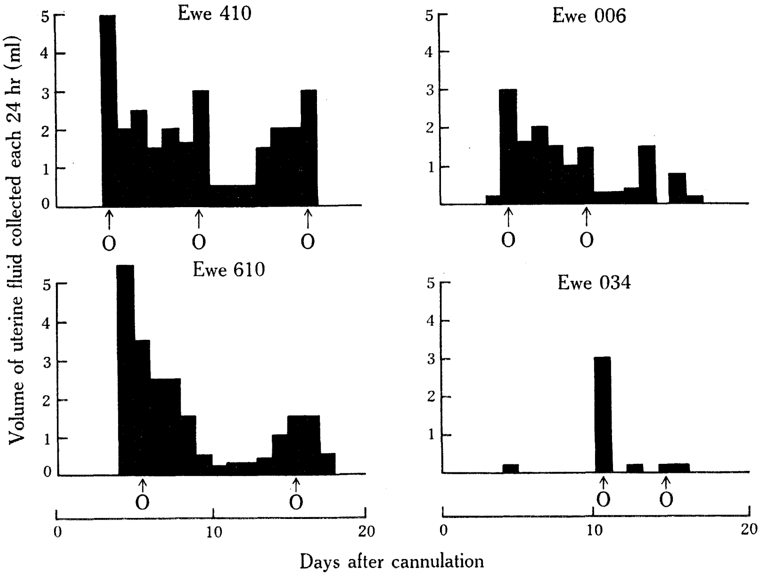

Fig. 5.—Pattern of secretion of uterine fluid collected from four ewes whose uteri were cannulated through the cervical canal. O designates the days upon which behavioural oestrus occurred.

Over the 14–20 days of collection, all ewes exhibited at least two periods of oestrus. In three of the four ewes, oestrus occurred at intervals of 4–6 days. In the fourth, the length of the oestrous cycle was normal. In general, each oestrus was accompanied by some increase in the volume of fluid collected from the cannula. As in the previous experiments, the volume of secretion collected varied considerably between ewes. The ewe with the lowest secretion rate produced an average of 0·3 ml/day, while all others averaged over 1·0 ml/day for the duration of the cannulation.

The fluids collected from these four ewes differed considerably in appearance from those collected using the other methods of cannulation. These fluids were cloudy to milky in appearance, apparently due to leucocyte invasion, and showed no evidence of coloration by blood pigments.

(*d*) *Comparison of Various Methods of Cannulation*

Although the pattern of secretion and the frequency of oestrus varied considerably with the method of cannulation, an attempt has been made to compare directly the various methods used to cannulate the uterus (Table 3). Comparisons were made using three parameters. The first parameter was the total volume of fluid collected during the elevated secretion associated with each period of behavioural oestrus. As the duration of elevated secretion at oestrus varied between animals, the data have also been compared on a daily secretion basis. Finally, the mean daily secretion over the whole period of cannulation for each animal was compared. Statistical analysis of the untransformed data indicated a high degree of heterogeneity between groups but this was almost eliminated by the use of the logarithmic transformation. The analysis of the data (Table 3) shows a larger variation between animals than between cycles

TABLE 3

SUMMARY OF THE SECRETION DATA FOR EWES CANNULATED BY VARIOUS METHODS

Site of Cannulation	Length of Intrauterine Portion of Cannula	Volume of Fluid per Oestrous Peak (ml)	Flow Rate at Oestrus (ml/day)	Average Daily Flow during Cannulation (ml/day)
Unligated horn	Short	2·9	0·66	0·21
Unligated horn	Long	3·7	1·13	0·25
Ligated horn	Short	3·8	0·91	0·29
Ligated horn	Long	6·1	1·12	0·43
Cervical junction	Short	8·6	3·59	1·57
Trans-cervical	Short	5·4	2·11	1·15

Analysis of Variance of the Data after Logarithmic Transformation†

Source of Variation	Degrees of Freedom	Variance Ratios		
		Fluid Volume per Oestrus	Flow Rate at Oestrus	Av. Daily Flow
Horn cannulation *v.* others	1	6·38**	16·12**	26·55**
Other treatment comparisons	4	1·11	0·72	0·76
Between-animal variance	21	*2755*‡	*2270*‡	*773*
Within-animal variance	47	*1110*	*1100*	—

** $P < 0{\cdot}01$.

† Transformations: fluid volume per oestrus, $y = 100 \log(10x+1)$; oestral flow and average flow, $y = 100 \log(100x+1)$.

‡ Used as error as significantly larger than within-animal variance ($P < 0{\cdot}05$).

within the same animal. Cannulation via the horn resulted in the collection of less fluid than if the other methods of collection were used. No other significant effects were found.

IV. Discussion

The present findings confirm earlier reports (Perkins *et al.* 1965; Iritani, Gomes, and Van Demark 1969) that the volume of secretion collected from the cannulated uterus of the ewe varies throughout the oestrous cycle. In most animals, a peak in the volume of fluid collected from the cannulated uterus occurred at or near oestrus and fell to low levels in dioestrus.

Although this pattern exists in cycling ewes, there is considerable variation between cycles and between animals in the volume of fluid collected. This raises the question as to the possible influence of the cannula on the secretion rates recorded from the cannulated uterus. Following healing after the initial surgical trauma, the end of the cannula lies in the uterine lumen in contact with the endometrium. In the ewe, as in other species, the presence of an intra-uterine foreign body interferes with the physiological function of the organ and causes infertility. Intra-uterine foreign bodies in the ewe have been shown to interfere with the development of the corpus luteum (Ginther, Pope, and Casida, 1965; Stormshak, Lehmann, and Hawk 1967), to modify uterine motility (Brinsfield and Hawk 1969), to inhibit sperm transport and promote spermicidal conditions (Hawk 1967), and in certain circumstances to modify cycle length (Moore and Nalbandov 1953; Nalbandov, Moore, and Norton 1955; Inskeep *et al.* 1962). Therefore, there would seem good reason to suggest that in the cannulated uterus, the volume of fluid collected may be influenced by local effects of the cannula on the uterus.

A number of observations in the present experiments would indicate the possibility of a direct effect of the cannula on the uterine mucosa. Although the endometrium in all cannulated horns appeared intact at post mortem, intermittent collection of blood-stained fluid from cannulated horns would point to some local irritation. This view is strengthened by the fact that in normal uteri collected from an abattoir, the film of blood-stained fluid, which was occasionally present on the surface of the endometrium of cannulated uteri, was never observed. In addition the finding that cannulation of the horn of the uterus caused a slight but variable increase in the height of the epithelium of the lumen and glands lends additional support to the suggestion of a local effect of the cannula on the endometrium.

Whatever the nature of local effects of the cannula on the volume of secretion, these effects would only appear to modify a pattern of secretion which is dependent on the hormonal status of the animal. In the anoestrous ewe, secretion is low and shows little variation with time or between animals. In cycling ewes, however, volume of secretion varies with the stage of oestrus and reaches a peak at or near oestrus and declines in the progestational stage of the cycle. Such a pattern would appear to be of benefit to spermatozoa by providing an ample fluid environment during passage to the site of fertilization. On the other hand, at the time the conceptus passes from the fallopian tube to the uterus, the volume of fluid would appear to be reduced, thus allowing closer apposition of the developing trophoblast with the endometrium.

The volumes of the collections recorded in the present experiments are substantially lower than those obtained by previous investigators (Perkins *et al.* 1965; Iritani, Gomes, and Van Demark 1969). Although some of these differences may be due to differences between animals, it would appear that the cannulae used in both the earlier reports were constructed of polyethylene tubing while those used in the present experiment were of silicone rubber. Silicone rubber tubing is more pliable and less likely to cause tissue irritation than polyethylene. Thus, if local irritation plays a role in the volume of secretion collected from the uterus, one may expect the differences noted between the results of the various studies.

Insertion of the cannula into the body of the uterus whether via the cervix or through the uterine wall had a variable effect upon the length of the oestrous cycles. Moore and Nalbandov (1953), Nalbandov, Moore, and Norton (1955), and Inskeep *et al.* (1962) noted a similar shortening in the length of the oestrous cycle in ewes with plastic beads sutured to the uterus. This effect appears to be due to a neural-mediated influence between days 3 and 8 of the oestrous cycle. The frequency and degree of shortening of the cycle found in the present cannulations of the body of the uterus are similar to those observed by Inskeep *et al.* (1962) and possibly a cannula in this position is exerting a similar influence to that of the plastic beads. On the other hand, cannulation of the uterine horn near the uterotubal junction causes no change in the cyclic pattern of oestrus, even when combined with ligation at the external bifurcation. It would therefore appear to be the method of choice for the collection of uterine secretions.

V. Acknowledgments

The authors are indebted to Professor C. W. Emmens for his interest and advice. The work was performed under a grant from the Australian Research Grants Committee.

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