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A NON-SURGICAL TECHNIQUE FOR THE COLLECTION OF UTERINE FLUID FROM THE MARE¹

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SUMMARY

A non-surgical, transcervical technique was developed for collection of uterine secretions from unanesthetized horse mares. Uterine flushings were collected on days 4, 8, 12, 14, 16, 18 and 20 postovulation of the estrous cycle. The uterus was flushed with sterile .33 M saline, which was left in the uterus for a minimum of 5 minutes. During this time, the uterus was massaged per rectum to insure adequate mixing of luminal contents and saline. Fluid in the uterus was aspirated into sterile syringes and stored on ice until centrifuged. Centrifugation was carried out at $12,062 \times g$ for 20 min in a refrigerated centrifuge. Flushings were filtered through a .45 μ filter to remove bacteria and/or cellular debris. After the volume of each uterine flushing was measured, it was stored at -20 C until analyzed for protein. Volume of flushing medium collected from uteri (overall mean 106.3% fluid recovered) was not affected by day of the estrous cycle. Total amount of protein obtained in uterine flushings was affected ($P < .025$) by day of the estrous cycle with maximal amounts of recoverable protein in the mid-to-late luteal phase.

(Key Words: Uterine Secretion, Nonsurgical Collection Technique, Protein, Mare.)

INTRODUCTION

Oviductal and uterine fluids provide a biochemical environment in which fertilized ova develop. Uterine secretions, called histotrophe, are products of the maternal endometrium. In most mammals, histotrophe is most important during early embryonic development, but in sows and mares may provide nutriment throughout gestation (Amoroso, 1956). Uterine

fluids have been collected from many species, including ovine (Perkins *et al.*, 1965; Iritani *et al.*, 1969), bovine (Olds and VanDemark, 1957a,b; Heap, 1962, Fahning *et al.*, 1966) and porcine (Murray *et al.*, 1972; Squire *et al.*, 1972), but due to difficulties involved in obtaining uterine fluids, results have been limited by recovery techniques used. Collection of uterine fluid by ligation of the uterine horn results in a condition (hydro-uterus) which may mask cyclic secretory patterns (Hombberger *et al.*, 1957). Additionally, it was demonstrated that ligation of the uterine horns of rabbits caused a change in the chemical composition by comparison with fluid taken by a surgical procedure (Heap, 1962).

Procedures for non-surgical collection of uterine fluids have been reported for cows (Heap, 1962; Fahning *et al.*, 1966). In the procedure of Heap (1962), an endotracheal tube was passed transcervically into the uterus. Isotonic saline was infused into the uterus, then recovered by massaging the uterine horns per rectum. Using this procedure, the percent flushing medium recovered was only 58%. Fahning *et al.* (1966), using a uterine infusion pipette, were able to collect uterine fluids from cows by passing the pipette through the cervix with the aid of a local anesthetic. With this collection technique, the collection apparatus was frequently occluded by clotted blood and cellular material, especially early in an estrous cycle.

The purpose of this report is to describe a non-surgical, transcervical technique for the collection of uterine fluid from the unanesthetized mare, and to describe changes in protein content of uterine fluids throughout an estrous cycle.

MATERIAL AND METHODS

Experimental Animals. Uterine flushings were collected from 16 normally cycling mares of Quarter Horse or Thoroughbred breeding. Days of collection were 4, 8, 12, 14, 16, 18 and

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20 post-ovulation. Estrus was detected daily by teasing with an active, vigorous stallion. Ovulation was detected by daily rectal palpation of the ovaries. Rectal palpation was performed by the same person each day to eliminate variation among individuals. Throughout the experiment 42 uterine flushings were collected. Mares were assigned to the day of collection in ascending order (day 4, 8, 12, 14, 16, 18 or 20) within a replicate as they became available (ovulated). Several of the mares were used for collection of more than one uterine flushing in succeeding estrous cycles but at least one estrous cycle was permitted to intervene between two collections.

Animal Preparation. Mares were restrained in a metal frame palpation chute. Tails were banded and the external genital area washed thoroughly with a disinfectant². Tranquilization of the mares was not necessary at any time during the procedure.

Design of Catheter. The design of the catheter was based on a modification of the apparatus described by Fahning *et al.* (1966) to collect uterine flushings from cows. The modified catheter consisted of a 26 to 30 French (8 to 10 mm) Foley catheter with an inflatable 30 cc cuff. This catheter served as an outside casing. Inside the casing was a 16 gauge stainless steel inner catheter. Attached to the anterior end of the inner catheter was a Silastic tip approximately 13 cm in length, with several side holes. The function of this tip was to aid in collection of uterine fluid and to reduce endometrial trauma. This catheter was sterilized before use.

Sample Collection. The sterile catheter was placed transcervically into the uterus by manipulation through the cervical canal. Once in the uterus, the inflatable cuff was positioned just anterior to the internal os of the cervix. The cuff was inflated with approximately 60 cc of air. A cervical block which prevented the flushing medium from leaking back into the vagina was thus established, as shown in figure 1.

Eighty milliliters of sterile .33M NaCl were infused through the inner catheter into the uterine lumen. The uterus was massaged per rectum, for a period of 5 min to insure adequate mixing of the luminal contents and saline. After 5 min, fluid containing uterine secretions was slowly withdrawn from the uterus.

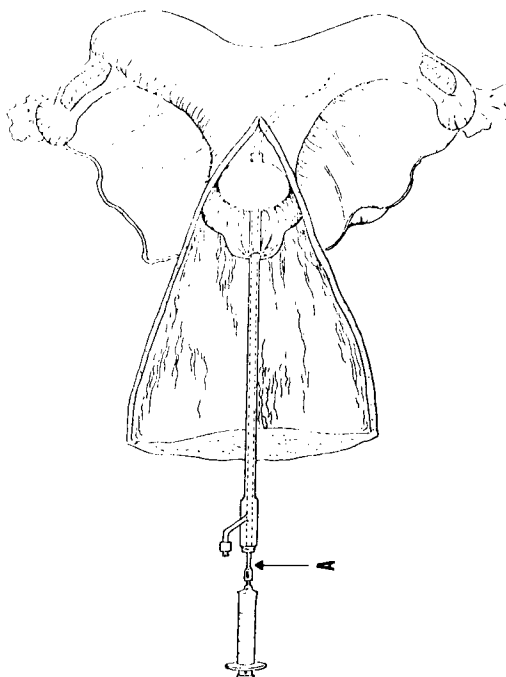


Figure 1. Diagrammatic representation of uterine secretion collection device in position in the uterus. The inner catheter (A) is depicted in the extended position.

Uterine flushings were aliquoted into sterile vials, and placed in an ice bath. A 1 ml sample of flushing was saved for bacteriological evaluation. After the last flushing had been aspirated, 50 ml of an anti-microbial agent³ were infused into the uterus. The catheter was removed and the mare returned to the herd. Mares subjected to the collection procedure were observed daily for indications of post-collection complications, such as uterine or vaginal discharge.

Volume of uterine flushings was measured in a graduate cylinder preliminary to centrifugation in a refrigerated centrifuge (4 C) for 20 minutes at $12,062 \times g$. The supernatant was then filtered through a $.45 \mu$ filter, to remove bacteria and/or cellular debris. The filtrate was then transferred into sterile vials and stored at -20 C for subsequent analysis of protein concentrations. Protein concentration of the uterine flushings was determined by the method of Lowry *et al.* (1951).

Statistical Analysis. Percent flushing medium recovered and total protein were analyzed by the method of least squares (Harvey, 1960). The model included mares, (15 df) days, (6 df) and residual variation (20 df).

² Nolvasan — Fort Dodge.

³ Furacin-(nitrofurazone) Eaton Veterinary Laboratories.

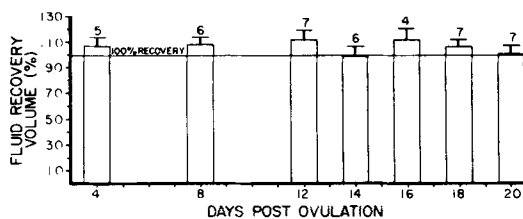


Figure 2. Least squares means histogram of recoverable fluid volume of mare uterine flushings (%). Numbers above bars indicate the number of animals per observation group (total = 42) and lines above bars represent the standard errors of the least squares means.

RESULTS

The average volume of flushing medium recovered was 85 ml (106.3%, $n=42$) and was not significantly affected by day of the estrous cycle (figure 2). There was an increase ($P < .025$) in total uterine protein recovered during the late luteal phase of an estrous cycle, followed by a rapid decline on days 18 and 20. Protein content of mare uterine flushings is expressed on a concentration (mg/ml) and total basis (mg/flushing) in table 1.

To determine if any uterine fluids were retained after the collection procedure, uterine flushings were collected from four additional mares which were sacrificed immediately post-collection. Two of the mares were in the early luteal stage of an estrous cycle (day 8) and two were in the late luteal stage (day 15 and 16). Each uterus was removed and clamped at the cervix and oviductal ends. A longitudinal sec-

tion was made in both the horns and body of the uterus. Residual fluid was collected by syringe. In each case, the residual volume was less than 1.5 milliliters.

No uterine infection was observed following any collections and evaluation of the uterine flushings for pathogenic bacteria indicated none present. Collection of uterine flushings did not have a negative effect upon mare fertility. Several of the mares were bred at subsequent estrous cycles and became pregnant. In a later experiment (M. T. Zavy, Fuller W. Bazer and Dan C. Sharp, *unpublished observations*), uterine flushings were collected from 35 pony mares using this non-surgical technique. These mares were bred at subsequent estrous cycles with a 75.0% conception rate. Arthur (1975) and Neely *et al.* (1975) observed that intrauterine infusion of 500 or 250 milliliters of saline after day 4 of a cycle, and with no attempt to contain the saline within the uterus beyond 2 min, resulted in a decreased interovulatory interval. In this experiment, a short (11 days) interovulatory interval was observed in one mare only (collected on day 4) and the mean interovulatory interval from all mares providing uterine flushings on days 4, 8, 12, 14 and 16 was 22.6 days. In a subsequent experiment in which uterine secretions were collected in a similar manner from 32 mares on day 4, but in addition deep uterine cultures and endometrial biopsies were taken, the interovulatory interval was reduced (13.83 days) in six mares (M. T. Zavy and D. C. Sharp, *unpublished results*). Therefore, collection of uterine

TABLE 1. PROTEIN CONTENT OF MARE UTERINE FLUSHINGS^a

Days	No. of observations	Protein concentration ^b (mg/ml)	Total protein ^c (mg)
4	5	.46 ± .28	39.5 ± 22.9
8	6	.75 ± .23	64.0 ± 19.1
12	7	1.10 ± .24	105.0 ± 19.6
14	6	1.11 ± .26	97.4 ± 21.5
16	4	1.30 ± .28	120.2 ± 23.3
18	7	.93 ± .22	77.8 ± 18.2
20	7	.64 ± .22	48.6 ± 17.9
	42		

^aLeast squares mean ± standard errors.

^bProtein concentration (overall mean ± SEM = .90 ± .09; coefficient of variation = 75.4%).

^cTotal protein (overall mean ± SEM = 78.9 ± 7.5; coefficient of variation = 79.2%).

secretions with this method may not, by itself, be sufficiently stimulatory to elicit corpus luteum regression.

Several mares providing uterine secretions on day 18 and 20, however, had interovulatory intervals greater than anticipated. Four of fourteen mares had interovulatory intervals of 40 days or greater. Quantification of actual intervals in these latter mares would not have been valid since three mares were treated with prostaglandin $F_{2\alpha}$ to regress their corpora lutea. Whether these prolonged diestrous periods were a result of the collection technique or were chance occurrence cannot be determined from these data.

Discussion

The non-surgical, transcervical method of recovering uterine fluid from mares appears to eliminate several problems noted by previous researchers, e.g., low recovery volume (Heap, 1962) and blood contamination of flushing (Fahning *et al.*, 1966). Recovery volumes in this study were greater than those reported in previous studies using different non-surgical techniques for collection of uterine fluids from different species. Contamination of flushing by mucus and blood was avoided, with one exception. The collection from this mare was excluded from protein analysis.

Changes in protein content of mare uterine flushings during the estrous cycle are similar to those reported for the gilt by Murray *et al.* (1972), (Figure 3). In both species, there is a distinct peak in the late luteal phase of the cycle. The luteal phase of the cycle, when the uterine endometrium is under strong progesterone influence, is classically described as the preparatory stage for pregnancy. During this time, the endometrium becomes more differentiated and the uterine glands become more secretory.

In mares, after conception has occurred, the embryo lies free within the uterine lumen for nearly 40 days before implantation is initiated (Allen *et al.*, 1973). During this time, the embryo presumably relies on uterine secretions for part, if not all, of its metabolic needs. Virtually nothing is known about the environment of the embryo *in utero* in the mare, although it is the site of physiological and biochemical events which are critical to reproduction.

The technique described herein may be used for collection of uterine secretions which may

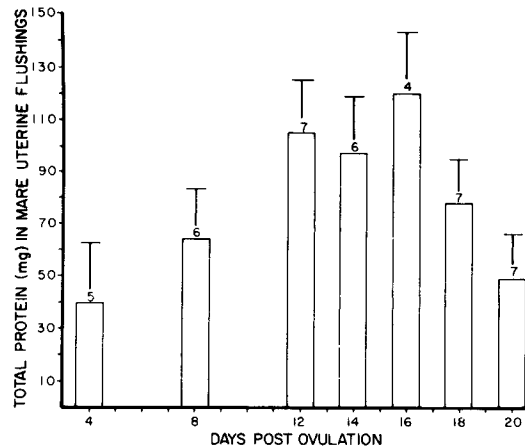


Figure 3. Least squares means histogram of total recoverable protein in mare uterine flushings. Numbers above bars indicate the number of animals per treatment day (total = 42), lines above bars represent standard errors of the least squares means.

then be characterized and evaluated as to their role in pre-implantation growth and development of the equine conceptus.

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