Concentrations of spermatozoa in the vagina of heifers after deposition of semen in the uterine horns, uterine body or cervix*

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Summary. In Exp. I, virgin Holstein heifers (N = 18) were induced into oestrus with PGF-2α. Animals which stood to be mounted were paired for insemination ~8 h later with 56·1 × 10⁶ spermatozoa from a single bull. Semen was deposited in the uterine body of one female. Each matched female was inseminated by deposition of one-half of the inseminate into the right uterine horn and one-half into the left uterine horn ~7·0 cm anterior to the internal cervical os. In Exp. II, additional heifers (N = 18) were induced into oestrus and inseminated by deposition into the uterine horns or cervix (2·0 cm anterior to the external cervical os). A 1·0 ml aspirate of vaginal mucus was collected at hourly intervals for 8 h after insemination. Concentration of spermatozoa was determined by haemocytometry. In Exp. I, cumulative percentage spermatozoa recovered in an 8 h collection period were similar (P > 0·10) for insemination into the uterine horns (17·9 ± 2·9%) and uterine body (18·5 ± 4·5%). In Exp. II, cumulative % sperm recovery from the vagina was greater (P < 0·10) for cervical deposition (59·1 ± 14·1%) than for that into the uterine horns (30·9 ± 7·8%). In Exp. II, the insemination treatment × hour of sample interaction was significant (P < 0·08). Recovery of spermatozoa from the vagina was greatest (P < 0·05) within 3 h after cervical insemination (31·4 ± 9·9% compared to 9·4 ± 2·5% for uterine horn deposition). Percentage recovery of spermatozoa from the remaining hourly collections were similar (P > 0·10). These results suggest that retrograde movement of spermatozoa from the uterus to the vagina was similar after insemination into the uterine horns or uterine body and 2-fold greater after cervical deposition.

Keywords: cattle; insemination site; sperm loss

Introduction

In spite of the success of artificial insemination in cattle, little research has been conducted to determine the fate of spermatozoa in the female reproductive tract after deposition of semen. Until recently, it was assumed that spermatozoa moved predominantly in an anterior direction when deposited within the uterus. Mitchell et al. (1985) and Nelson et al. (1987) provided evidence that a large proportion (~60%) of the inseminate was discharged from the reproductive tract within 12 h after insemination.

Peters et al. (1984) and Swain et al. (1986) reported that both professional and herdsman-inseminators are inconsistent in placing the insemination syringe tip within the uterine body of the bovine uterus. In both studies, >20% of the insemination attempts were characterized by having the insemination syringe tip in the cervix rather than the uterine body. These cervical insemination errors could further increase the loss of spermatozoa.

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Cornual deposition offers a logical alternative to conventional uterine body insemination: deposition into the uterine horns does not appear to impair fertility (Hawk & Tanabe, 1986; Pallares et al., 1986) and in some cases improved conception (Zavos et al., 1985; Senger et al., 1988). The hypothesis being considered here was that cornual insemination increases conception by decreasing retrograde movement of spermatozoa from the uterus into the vagina. Our objective was to evaluate retrograde movement of spermatozoa following cornual, uterine body and cervical insemination.

Materials and Methods

Experiment I. Oestrus was induced in 18 virgin Holstein heifers (366 kg ± 4.9), with an i.m. injection of 35 mg prostaglandin F-2a (dinoprost tromethamine: The Upjohn Company, Kalamazoo, MI, 49001, USA). Observations for oestrus were conducted for 30 min at 8-h intervals beginning 48 h after injection and continuing for 104 h after injection. Standing to be mounted was the criterion for oestrus. Heifers observed in standing oestrus were paired, then placed in individual stanchions 7·6 ± 1·3 h later for insemination and were provided with food and water. Individuals within each pair were assigned randomly to receive cornual or uterine body insemination. Cornual insemination was accomplished by depositing one-half the inseminate in the left uterine horn and one-half into the right horn (Senger et al., 1988). Deposition of one-half of the semen within a straw in each uterine horn was approximately 7 cm anterior to the internal cervical os. Uterine body insemination was accomplished by depositing the semen in a single straw immediately anterior to the internal cervical os. All inseminations were conducted by a single technician.

Semen used in Exp. I was pooled from two consecutively collected ejaculates from a mature Holstein bull. Collection, extension, packaging in 0.5-ml French straws and freezing was conducted by Landmark Genetics, Watertown, WI, USA. Final concentration of spermatozoa was 112·2 × 10⁹/ml (56·1 × 10⁹ spermatozoa per straw). Immediately after thawing, motility was 37·5% and percentage of intact acrosomes was 78·2. At 4 h after thawing and incubation at 35°C for 4 h, motility was 22·5% and percentage of intact acrosomes was 62·7.

A 1-ml aspirate of mucus was collected from the vagina every hour, for an 8-h period beginning immediately after insemination (0 h). An insemination pipette was marked to designate a 1-ml volume. The pipette was inserted into the vagina, moved toward the fornix vagina and gentle suction applied using a syringe coupled to the pipette with surgical tubing. The 1-ml aspirate was discharged into a 15-ml plastic graduated test tube. The volume was recorded. Samples of mucus were fixed by the addition of 1 ml 0·3% glutaraldehyde. Dissolution of mucus in the sample and removal of spermatozoa adhering to the collection pipette were accomplished by flushing the pipette with 8 ml 0·5 N NaOH. The samples were sealed and shaken vigorously to dissolve the mucus.

Animals were observed continually throughout the 8-h period so that any mucus spontaneously discharged from the vagina could be collected. Volume of the discharged mucus was recorded and a 1-ml representative sample was aspirated and processed as previously described. Following the 8-h collection period all samples were taken to the laboratory for further processing.

Samples (1 ml mucus + 1 ml 0·3% glutaraldehyde + 8 ml 0·5 N NaOH) were vortexed for 3 min and transferred from the graduated test tubes to 15-ml Corex tubes for centrifugation. A 2-ml rinse of the graduated test tube was done with 0·5 N NaOH to remove adherent spermatozoa. Samples were again vortexed for 3 min and centrifuged at 12 000 g for 1 h. Then 10 ml of the supernatant were removed, leaving the pelleted sample in a 2-ml volume. The pellet was resuspended in 0·5 N NaOH twice to remove cellular debris and facilitate uniform sperm dispersion. Then 11 ml of the supernatant were removed to leave a final sample volume of 1 ml. Samples were sealed and refrigerated until spermatozoa were counted. All samples were evaluated within 21 days after collection. If more or less than 1 ml mucus was aspirated, an appropriate mathematical adjustment was made when computing final sperm concentration to a 1 ml equivalent basis. This occurred in approximately 9% of the total aspirated mucous samples taken.

Sperm concentration was determined using haemocytometry. Four haemocytometer grids were evaluated per sample. Each sample was prepared and placed in an enclosed humidified culture dish for 5 min to allow the cellular contents to settle. Enumeration of spermatozoa present was accomplished using phase-contrast microscopy (×100). All samples were evaluated by a single observer. Samples were coded so that the observer was unaware of the treatment or sample hour being evaluated.

Experiment II. Additional Holstein heifers (N = 18) were induced into standing oestrus as previously described. Pairs of heifers exhibiting standing oestrus were inseminated 8·2 ± 0·2 h later, by deposition of semen into the uterine horns or cervix. Surgical adhesive tape was wrapped around the insemination sheath, 2 cm posterior to the sheath tip. Each revolution of tape was superimposed on the previous wrap until a barrier of tape 5 mm in height relative to the sheath surface was obtained. This physical barrier allowed seminal deposition 2 cm anterior to the external cervical os. Cornual insemination was accomplished as previously described. All inseminations were conducted by the same technician as in Exp. I. The semen used was from a different bull, but concentration and post-thaw quality of spermatozoa were similar to those in Exp. I. Collection of samples, processing and determination of sperm concentration followed the same protocol as Exp. I. Sample evaluations were conducted by a different individual.

Validation of technique. Oestrus was induced in 12 Holstein heifers by i.m. injection of 35 mg prostaglandin F-2a. Animals exhibiting standing oestrus were placed in stanchions 8 h later. Heifers were inseminated with 13·9 × 10⁶,
7.0 × 10⁶, 3.5 × 10⁶ or 1.7 × 10⁶ spermatozoa per 0.5 ml dose. Site of insemination was the fornix vagina. This region was chosen to simulate the initial site of sperm loss from the uterus into the vagina. At 15, 30 and 45 min after insemination, a 1-ml vaginal mucus aspirate sample was collected from the vaginal fornix and processed as previously described. Concentration of spermatozoa was determined by haemocytometry.

The numbers of spermatozoa (mean ± s.e.m.) recovered per ml across all collection periods were 0.11 ± 0.07 × 10⁶, 0.08 ± 0.03 × 10⁶, 0.02 ± 0.01 × 10⁶ and 0.02 ± 0.01 × 10⁶ respectively for the high to low numbers of spermatozoa inseminated. These results indicate that the sampling technique provides an accurate estimate of the change in sperm concentration within the vagina over time.

Statistical analyses. Statistical analyses were performed using the SAS, GLM procedure for analysis of variance (SAS, 1982). Main effects evaluated on cumulative percentage of spermatozoa recovered from 1-ml vaginal mucus aspirates obtained during sampling times of 0–2 h, 3–5 h, 6–8 h and 0–8 h were insemination treatment and sample hour. Time from standing oestrus to insemination was included in the model as a covariate. The interaction of insemination treatment × sample hour was also included in the model. To account for animal variation, insemination treatment was tested by the error term of animal nested within treatment.

Results

Experiment I

Percentage of recovered spermatozoa collected hourly from the vagina is presented in Fig. 1(a). While percentage of spermatozoa recovered was independent of site of insemination, large variation in spermatozoa collected from animals within each treatment group was noted by the magnitude of standard error of the mean values. Samples collected during the later periods tended to contain fewer spermatozoa.

Cumulative percentage of spermatozoa (Fig. 1b) recovered from the 1 ml vaginal mucus samples during the 8-h collection period was similar (P > 0.10) between insemination sites. A total of 17.9 ± 2.9% and 18.5 ± 4.5% of the spermatozoa inseminated were recovered in the 8-h period after cornual or uterine body deposition, respectively (Fig. 1b). Mucus discharged from the vagina was collected once from each of 2 animals in the uterine body treatment group, between the 3 h and 4 h collection period in both heifers. Inclusion of spermatozoa recovered in the discharged mucus with the aspirated samples accounted for 46.6% ± 1.4 and 83.1% ± 2.6 of the total spermatozoa inseminated in these 2 heifers. Cumulative percentage of the inseminate recovered from each 1-ml sample collected at 0–2 h, 3–5 h or 6–8 h did not differ (P > 0.10) between insemination treatments. Site of insemination × hour of sampling was not significant (P > 0.10). Time from detection of oestrus to insemination did not differ between treatments (uterine horns, 7.8 ± 0.3 h; uterine body = 7.5 ± 0.3 h).

Experiment II

During the first 3 h after insemination, a greater number of spermatozoa was recovered following cervical deposition (Fig. 2a). Site of seminal deposition did not influence the percentage of spermatozoa which were recovered 4 h or more after insemination.

Cumulative percentage of spermatozoa recovered after deposition was greater (P < 0.05) when semen was deposited in the cervix compared to deposition in the uterine horns (Fig. 2b). Overall, a greater proportion (P < 0.10) of spermatozoa inseminated was recovered from the 1-ml vaginal mucus samples following the 8-h collection in heifers inseminated at mid-cervix (59.1 ± 14.1%) when compared to those inseminated into the uterine horns (30.9 ± 7.9%) (Fig. 2b).

One animal in the uterine horn insemination group discharged mucus from the vagina between the 3 and 4 h collection period. These samples contained a negligible number of spermatozoa. There was an insemination treatment × h of sample interaction (P < 0.08). Time from detection of oestrus to insemination did not differ between treatments (uterine horns, 8.3 ± 0.1 h; cervix, 8.0 ± 0.1 h) or influence percentage sperm recovery (P > 0.2).
Fig. 1. Percentages of spermatozoa recovered (a) hourly from aspirated 1-ml vaginal mucous samples following cornual or uterine body deposition, and (b) cumulatively (56.1 × 10⁶ spermatozoa deposited). Values are mean ± s.e.m. for 9 samples per treatment.

Discussion

This study demonstrates that there is significant retrograde movement of spermatozoa after insemination of sperm concentrations near conventional artificial insemination doses. Previous studies used from 300 × 10⁶ to 2.0 × 10⁹ spermatozoa per inseminate to ensure that adequate numbers of spermatozoa were recovered from the reproductive tract for counting (Quinlivan & Robinson, 1969; Dobrowolski & Hafez, 1970; Suga & Higaki, 1971; Mitchell et al., 1985), but only a small percentage of spermatozoa inseminated were accounted for within the female reproductive tract. In the present study, the aspiration of 1-ml samples of mucus hourly did not constitute an attempt to account for all spermatozoa in the vagina. Our effort addressed the relative movement of spermatozoa into the vagina following deposition of semen in various anatomical locations. It could not be determined whether our findings represent a physiological phase of sperm transport or passive drainage from the uterus to the vagina.

Mitchell et al. (1985) recovered ~60-7% of spermatozoa inseminated in the mucus expelled from the vagina in mature dairy cows. In the current study, recovery of 20–60% of spermatozoa inseminated, by obtaining 1-ml aspirate samples of vaginal mucus (n = 9) also provides evidence that there is massive loss of spermatozoa from the uterus to the vagina. The greatest proportions of spermatozoa recovered from the vaginal samples were within the first 3 h after insemination.

The greatest mucus production in the reproductive tract, under the influence of oestrogen, is during oestrus when natural mating occurs (Mattner, 1973). Although heifers in this study were
continually observed for mucus discharged from the vagina, mucus was recovered once from each of 2 animals in Exp. I, both within the uterine body treatment group. Expulsion of the mucus occurred between the 3- and 4-h collection periods in both heifers. In Exp. II, only one animal discharged mucus, between the 3- and 4-h collection periods, and this contained negligible numbers of spermatozoa. Mitchell et al. (1985) inseminated cows 3 h after detection of oestrus. In that experiment, half of the total spermatozoa recovered from discharged mucus was within 4 h after insemination. Heifers used in the current study were inseminated about 8 h after detection of oestrus. Therefore, much of the mucus produced may have been discharged before insemination. Also, failure to expel mucus present in the vagina could have resulted from altered behaviour due to experimental conditions. No animal exhibited ventral recumbancy during the 8 h collection period and this is often when mucus discharge is observed under natural conditions.

Fig. 2. Percentages of spermatozoa recovered (a) hourly from aspirated 1-ml vaginal mucous samples following cornual or cervical deposition, and (b) cumulatively (56.1 × 10⁶ spermatozoa deposited). Values are mean ± s.e.m. for 9 samples.
Differences in the proportion of spermatozoa recovered following cornual insemination of heifers in Exp. I and Exp. II may be due to several factors. Semen used in each experiment was similar in concentration and quality of spermatozoa, but from different bulls. Differential retention of spermatozoa from different males in the bovine reproductive tract has been reported (Mitchell et al., 1985). Evaluation of samples for each experiment was conducted by a different individual using different sample dilution rates to facilitate counting of spermatozoa by haemocytometry. Maximum sample dilution was 6-fold in Exp. I compared to 56-fold in Exp. II. While greater dilution rates diminish the time required in counting samples, there is the potential for magnification of errors in the counting process.

Controversy pertaining to site of semen deposition exists. Significant improvements in fertility have been reported when cows were inseminated in each uterine horn as compared to the uterine body (Zavos et al., 1985; Senger et al., 1988). Other studies evaluating fertilization rate in single-ovulating and superovulating cows inseminated in one or both uterine horns indicated no difference from uterine body deposition (Hawk & Tanabe, 1986; Pallares et al., 1986). Larsson (1986) deposited semen deep in one uterine horn, killed the animals, and found comparable numbers of spermatozoa within each horn of the excised reproductive tract.

The lack of difference in retrograde loss of spermatozoa from the uterus to the vagina when using cornual or uterine body insemination may be due to correct identification of the cervix and the body of the uterus during semen deposition. This is supported by the 2-fold increase in spermatozoa recovered from mucus in the vagina following mid-cervical insemination compared to cornual deposition. The consequences of seminal deposition in the cervical lumen may be of such magnitude that inadequate numbers of viable spermatozoa are maintained in the reproductive tract, resulting in fertilization failure.

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References


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