

SPERM DISCHARGE AND RETENTION AFTER ARTIFICIAL INSEMINATION OF DAIRY CATTLE: A PRELIMINARY REPORT

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Story in Brief

Seven dairy cows were artificially inseminated to determine sperm numbers in the vagina, cervix, uterus, and oviducts 12 h after insemination. Numbers of sperm discharged in cervical mucus during the first 12 h after insemination were also determined. The cows had been inseminated with a dose of good quality semen containing 75×10^6 sperm. After slaughter, sperm remaining in the various tract regions were flushed out with sodium citrate for quantification with a hemacytometer. Of the inseminated sperm, 42.1% were discharged in the cervical mucus. At 12 h post insemination, the percentage of sperm numbers in the inseminate located in the vagina, cervix, uterus and oviducts were 7.83, .473, .319, and .039, respectively.

(Key Words: Female Tract, Sperm Transport)

Introduction

Optimum fertility is a substantial component of the reproductive efficiency needed to maintain a 12-13 month calving interval in dairy cattle. If services per conception are reduced from 2.0 to 1.5, it would be worth 20 million dollars to U.S. dairymen (Gerrits et al., 1979). Although 60% of artificially inseminated cows conceive under optimum conditions, 13% do not because of fertilization failure. A factor that can affect fertilization failure is sperm transport through the female tract to the site of fertilization. When fertilization failure occurs, inadequate sperm transport has been indicated by the absence of sperm in the zona pellucida of the unfertilized oocytes as well as at the site of fertilization near the time of ovulation (see review by Hawk, 1983). Recently Mitchell et al. (1985) reported that a large percentage of artificial inseminated sperm were discharged from the cow during the first several hours after insemination. They had inseminated 20-50 fold the normal number of sperm in a dose of semen of which a high proportion were abnormal. Thus, there appears to be real potential for increasing the sperm numbers in the oviduct by reducing the tremendous retrograde discharge of sperm. However, it is not clear at this time if substantial discharge occurs to the much lower numbers of high quality sperm normally used for artificial insemination. Thus, our objective was to determine the disposition of the sperm numbers of a normal dose of bull semen 12 h after insemination.

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Materials and Methods

Seven dairy cows at least 60 days postpartum with normal reproductive history and a normal reproductive tract (determined by palpation) were used. Both prostaglandin synchronized and natural estruses were used. Cows were inseminated in the uterine body 12 ± 4 h (range) after the onset of estrus with good quality semen containing $75 \times 10^6 \pm 10 \times 10^6$ sperm. The same experienced inseminator was used throughout the experiment. After insemination, a latex funnel was attached to the vulva with an industrial adhesive. This facilitated mucus collection without fecal contamination during the subsequent 12 h.

The cow was slaughtered 12 h after insemination to recover the reproductive tract. Just prior to slaughter, the vagina was flushed with 1 l of 2.9% sodium citrate. After stunning, the vulva was closed with cyanoacrylic adhesive and surgical staples. Through a mid-ventral incision, hemostats were placed at the infundibulum and 1/2 cm posterior to the utero-tubal junction (UTJ). Also, a ligature was located at the uterine body and the urethra. This isolated the tract regions of interest and prevented urine contamination of the vagina.

The oviducts with the UTJ were removed and flushed with 30 ml of 2.9% sodium citrate each. The vagina was flushed again with 1/3 l of sodium citrate and separated from the cervix. The exposed vaginal surface was covered with 0.5 N NaOH for 1/2 h to dissolve any remaining sperm containing mucus. The cervix was flushed with 0.15 l of sodium citrate and each uterine horn was flushed with .25 l. The uterine body was also flushed with an additional 0.1 l. Then, the cervix and uterine surfaces were exposed and covered with 0.5 N NaOH to dissolve sperm containing mucus. The mucus in each sample was liquified by increasing the volume of the recovered flush media by 50% with 1 N NaOH. Just prior to centrifugation, the recovered flush from each tract region was neutralized with 1 N HCL. Successive centrifugation steps at $3000 \times g$ for 20 min were used to concentrate sperm into volumes depending on potential numbers for a tract region. Sperm concentration was determined with a hemacytometer.

Results

Of the sperm inseminated, 58.6% were recovered from the seven cows in this study (Table 1). The largest proportion of inseminated sperm was recovered in the mucus discharged during the first 12 h after insemination. Another 6.5% could be recovered by lavaging the vagina increasing the total percentage of sperm recovered prior to slaughter to 56.9%. Of the inseminated sperm not discharged via cervical mucus, 7.8% were recovered from the vagina. Although the mean of the sperm recovered prior to and after slaughter from the vagina was 5.7×10^6 , the standard deviation was 4.8×10^6 . One cow accounted for this deviation as 15.2×10^6 sperm were recovered from the vagina prior to and after slaughter. There was a decrease in the percentage of inseminated sperm recovered from tract regions anterior to the vagina. The cervix, uterus and oviducts contained 0.47, 0.32 and 0.037% of inseminated sperm, respectively. Although the mean of the cervix recovery was 346,818 sperm, the standard deviation was 697,922. One cow accounted for this deviation as 2.56% of the inseminate was recovered from her cervix.

Table 1. Inseminated sperm numbers and percentage sperm in the inseminate recovered 12 hours post insemination from the female reproductive tract and discharged cervical mucus.

Tract region	Sperm recovered		Percentage of sperm in the inseminate	
	Mean	S.D.	Mean	S.D.
Vagina:				
Discharged Mucus	37,138,011	11,456,560	50.28	13.37
Flushed prior to slaughter	867,168	1,475,425	1.198	2.09
Total sperm recovered before slaughter	42,026,631	10,181,120	56.94	13.31
Total sperm recovered from vagina (not discharged)	5,703,054	4,802,171	7.83	6.72
Cervix	346,818	697,922	.473	.948
Uterine	231,063	193,544	.319	.277
Oviduct	29,542	18,982	.039	.025
Total sperm recovered	43,261,558	10,128,369	58.58	13.37
Sperm inseminated	73,697,142	5,237,753		

Discussion

Sperm are normally deposited during artificial insemination in the uterine body. However, from these data it is evident that a great majority of these sperm move retrograde into the vagina and are even discharged from the cow during the first several hours after insemination. Since cervical mucus contains the majority of inseminated sperm, it appears that such mucus circulates into the uterus, picking up the sperm at the site of semen deposition, and carrying them out. If the discharge of the sperm containing mucus could be decreased, then more sperm might remain in the uterus and be transported to the oviduct. This could enhance the chance of fertilization in those cows that have a deficiency of sperm at the site of fertilization. As fertilization failure affects a substantial portion of cows that don't conceive, efforts to improve sperm retention in the female tract should decrease services per conception and thus, improve reproductive efficiency.

Literature Cited

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