Several factors affecting pattern and rate of sperm transport in the female reproductive tract have been indicated by recent research. The maximal number of spermatozoa in the oviduct coincides with the time of ovulation (El-Banna and Hafez, 1970). In cattle (Quinlan, Mare and Roux, 1932; Kirillov, 1937; Beschlebnov, 1938; Sergin et al., 1941; Mattner, 1963, 1968), goats (Mattner, 1966) and man (Cohen and Stein, 1951), the motile spermatozoa remain in the cervix longer than in other regions of the reproductive tract; these observations suggest that the cervix may function as a reservoir for spermatozoa. Copulation (Van Demark and Hays, 1952, 1954; Hays and Van Demark, 1953a, b; Mattner and Braden, 1963) or massage of the uterus during insemination causes the release of oxytocin, which increases the rate of sperm transport by stimulating the contraction of the uterus.

The present experiments were done to determine the distribution of spermatozoa in the bovine female tract at different times after insemination and to investigate the effect of copulation on the distribution of spermatozoa.

Materials and Methods

Eighteen Hereford heifers about 1.5 years of age and weighing 350 to 400 kg were used. Estrus was detected by use of a vasectomized bull. Semen, collected with an artificial vagina, was examined microscopically for motility and density, and only sperm with good initial motility were used. A regular hemocytometer chamber was used for sperm count. The heifers were inseminated, at the end of estrus, with a constant number of spermatozoa (2,000 x 10⁶). Semen was deposited with a speculum at the external os of the cervix.

The heifers were divided into four groups. Three groups of five animals each were slaughtered at 1, 8 and 24 hr. post-insemination. A fourth group of three heifers, mated by a sterile bull 10 to 15 min. after insemination, was slaughtered 1 hr. after copulation.

Recovery of Spermatozoa

Immediately after slaughter the reproductive tracts were removed, and the different parts separated by a method previously described (El-Banna and Hafez, 1970), except that the vagina was not separated into two parts. The oviducts and the uterotubal junctions were cleaned of surrounding tissues and their lumina were washed in a measured volume of flushing solution (97% distilled water, 2% formalin, 1% liquinox). The uterine horns were flushed and emptied into a plastic container. The cervix and vagina were cleaned of the remaining blood vessels, and their lumina were washed separately in glass containers. Mucus from these regions, collected with a glass syringe, was transferred to glass containers and dissolved in measured volumes of 0.5 N NaOH. Sperm counts in the flushings were counted microscopically (×430) as described by El-Banna and Hafez (1970), using Fuchs Rosenthal Ultra Plane Chambers.

Results and Discussion

All heifers slaughtered 8 or 24 hr. after insemination had already ovulated.

The total number of spermatozoa recovered from the whole tracts varied from 14 to 335 x 10⁶, that is, from 0.7% to 16.7% of the number inseminated (table 1, figure 1). The percentage of inseminated sperm recovered decreased from an average of 13.4% at 1 hr. to 0.9% at 24 hr. after insemination.

The number of sperm recovered from the vagina was maximal 1 hr. after insemination and decreased progressively during the following 24 hr., while in the uterus the number of spermatozoa recovered increased between 1 and 24 hours. From the oviducts, the maximal number of spermatozoa (2,000,000) was re-
TABLE 1. NUMBERS OF SPERMATOZOA RECOVERED IN THE CATTLE REPRODUCTIVE TRACT AT DIFFERENT INTERVALS AFTER INSEMINATION AND PERCENT OF TOTAL SPERM RECOVERED

<table>
<thead>
<tr>
<th>Time after insemination (hr)</th>
<th>Total no. of sperm recovered x 10^6</th>
<th>Percentage of the total no. of sperm inseminated</th>
<th>Mean no. of spermatozoa x 10^6 (±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vagina</td>
</tr>
<tr>
<td>1</td>
<td>268.6</td>
<td>13.4</td>
<td>207.4</td>
</tr>
<tr>
<td></td>
<td>±6.3</td>
<td>±13.0</td>
<td>±0.2</td>
</tr>
<tr>
<td>8</td>
<td>76.0</td>
<td>3.8</td>
<td>51.0</td>
</tr>
<tr>
<td></td>
<td>±2.2</td>
<td>±3.1</td>
<td>±0.6</td>
</tr>
<tr>
<td>24</td>
<td>17.8</td>
<td>0.9</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>±0.8</td>
<td>±0.9</td>
<td>±0.1</td>
</tr>
<tr>
<td>1, with copulation</td>
<td>77.4</td>
<td>3.9</td>
<td>67.2</td>
</tr>
<tr>
<td></td>
<td>±3.4</td>
<td>±1.8</td>
<td>±0.03</td>
</tr>
</tbody>
</table>

* Both left and right sides are included.

The number of spermatozoa in the reproductive tract increases gradually and then declines. More spermatozoa were recovered from the vagina (51 to 86%) or cervix (13 to 26%) than from the uterus, uterotubal junctions, or oviducts. The percent of sperm recovered from the cervix was the same at different times following insemination. Spermatozoa may be moving through the cervix according to a constant rate and/or the cervix may serve as a regulator of movement.

Eventually, the spermatozoa are lost from the reproductive tract by phagocytosis due to an intensive leukocytic invasion (Quinlan et al., 1932; Howe and Black, 1963; Bedford, 1965; Howe, 1967) and by passage from the oviducts into the peritonial cavity (Mattner, 1963). Various reports indicate that the muscular activity of the uterus and oviducts facilitates sperm transport. Magnitude of contraction of uterine muscles is increased by oxytocin.

![Figure 1. Sperm distribution in the reproductive tract of cattle at different hours after insemination as a percentage of the total number of sperm recovered (logarithmic scale).](image-url)
Vagina  Cervix  Uterus  UTJ  Oviduct

without copulation  with copulation

Figure 2. Sperm distribution in the reproductive tract of cattle 1 hr. after artificial insemination as a percentage of the total number of sperm recovered (logarithmic scale).

(Hays and Van Demark, 1953b). The release of this substance is triggered by stimulation of the receptors in the vagina (De Backere and Peeters, 1960; Van Demark and Hays 1952, 1954), vulva or cervix during coitus. Stress at the time of coitus inhibits the initial rapid sperm transport in sheep (Mattner, 1963) and cattle (Van Demark and Hays, 1952), although the long-term distribution of spermatozoa is not affected. The percentage of sperm recovered from the uterotubal junctions and oviducts of the heifers mated after insemination and slaughtered 1 hr. later was higher than in the group of unmated heifers slaughtered at the same time. The total numbers of sperm recovered from these animals were markedly lower than in the unmated heifers (table 1, figure 2). Sperm loss in mated animals was caused by the expulsion of a large volume of mucus from the vagina after copulation. The number of sperm recovered in the oviduct was decreased as a result of copulation, even though percent of total recovery was higher.

Summary

The distribution of spermatozoa in the regions of the reproductive tracts of heifers was studied 1, 8 and 24 hr. after insemination. Comparison was made of the distribution of spermatozoa with and without mating, using animals slaughtered the same length of time after insemination (1 hr.).

The numbers of spermatozoa recovered from the vagina and cervix decreased progressively following insemination. Sperm numbers recovered from the uterotubal junction and the oviducts reached a maximum 8 hr. after insemination. Data suggest that copulation decreased the concentration of spermatozoa in the uterotubal junction and oviduct due to expulsion of spermatozoa by mucus. Copulation may increase rate of transport of spermatozoa that are present.

Literature Cited

El-Banna, A. A. and E. S. E. Hafez. 1970. Sperm


