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DISTRIBUTION OF LIVE AND DEAD SPERMATOZOA IN THE GENITAL TRACT OF GILTS AT DIFFERENT TIMES AFTER INSEMINATION*

By

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VIRING, SVEN: *Distribution of live and dead spermatozoa in the genital tract of gilts at different times after insemination.* Acta vet. scand. 1980, *21*, 587—597. — Twenty-four gilts were inseminated pairwise with live or dead spermatozoa from the same ejaculate. The insemination dose was 100 ml undiluted semen containing, on average, 19×10^9 spermatozoa. The gilts were slaughtered 1, 2, 6 and 12 h after insemination. The numbers of spermatozoa were counted in the uterus, uterotubal junction and in four equally long segments of the oviduct, called I—IV, with a haemocytometer. IV was adjacent to the uterotubal junction. The numbers of spermatozoa recovered in the uterus diminished significantly during the first 12 h after insemination. From gilts inseminated with live spermatozoa more spermatozoa were recovered in the uterotubal junction than from gilts inseminated with dead spermatozoa. Two h after insemination spermatozoa were recovered in all oviducts. Significantly more live than dead spermatozoa were recovered in Segments III and IV of the oviduct, regardless of time. In gilts inseminated with live spermatozoa the sperm count in Segment I varied significantly with time, being higest 2 h after insemination. At 6 and 12 h there were no distinct differences in the distribution of live spermatozoa between the various oviduct segments. The numbers of spermatozoa recovered in the oviduct were at these times apparently related to the sperm depots in the uterotubal junction.

live spermatozoa; dead spermatozoa; genital tract; gilt; insemination.

At natural or artificial insemination boar semen is deposited directly into the uterus. The majority of the spermatozoa disappear rapidly from the uterus and a small percentage survive in the uterotubal junction for several hours after intra-uterine de-

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position (*du Mesnil du Buisson & Dauzier* 1955, *Rigby* 1964, *Viring et al.* 1980). The spermatozoa reach the upper part of the porcine oviduct within 15 min after insemination (*First et al.* 1968, *Baker & Degen* 1972).

Several studies have been made to elucidate whether boar spermatozoa are passively transported into the oviduct by way of the uterotubal junction. For this purpose immotile spermatozoa have been inseminated in oestrous sows and gilts. *First et al.* inseminated sows with live boar spermatozoa or spermatozoa from the same ejaculate that had been killed. Each insemination dose consisted of $35—40 \times 10^9$ spermatozoa extended in a buffer. Thirty min after insemination the spermatozoa were recovered from oviducts of sows inseminated with both kinds of spermatozoa. No significant difference in the numbers of spermatozoa recovered from the oviducts of the two groups of animals were found. *Schefels et al.* (1971) inseminated gilts with 20×10^9 killed or live spermatozoa suspended in a buffer solution. Twenty min after insemination no spermatozoa were found in oviducts from gilts inseminated with dead spermatozoa. It was concluded that no dead spermatozoa are transported into the oviduct. *Baker & Degen* inseminated live spermatozoa, dead spermatozoa or a mixture containing equal numbers of live and dead spermatozoa in oestrous gilts. The spermatozoa were in all cases suspended in a buffer. The females were killed 8 h after insemination. Significantly more spermatozoa were recovered from the oviducts of gilts inseminated with live spermatozoa. Similar results were obtained by *Baker & Degen* in a second experiment when using gilts cannulated left oviduct and right uterine horn. The gilts were inseminated under anaesthesia with equal numbers of live-stained and dead spermatozoa. Dead spermatozoa were collected together with live spermatozoa from the oviductal cannulas within 15 min after insemination. The transport of dead spermatozoa was, however, less efficient than that of live spermatozoa. There are thus different opinions as to whether transport of dead spermatozoa into the oviduct takes place or not and, if dead spermatozoa enter the oviduct, how efficient the transport is.

The purposes of the present investigation were to study

— the distribution of live and dead spermatozoa within the genital tract of gilts up to 12 h after insemination,
— the efficiency of transport of dead spermatozoa into the oviduct.

MATERIAL AND METHODS

Altogether 24 healthy crossbred gilts (Swedish Landrace × Swedish Yorkshire breed) were used in this experiment. The gilts were purchased directly from breeders at six months of age and were housed at the Department of Obstetrics and Gynaecology. They were checked for oestrus daily by experienced personnel in the presence of a vasectomized boar. During pro-oestrus and oestrus the gilts were checked for oestrus twice a day.

The gilts used in the insemination experiment were in their second or third oestrus. They were inseminated 12—24 h after the start of oestrus with semen from one of two boars. The two boars, one a Swedish Landrace and the other a Swedish Yorkshire, were housed at the Department for this experiment. They had satisfactory sperm production and a normal semen picture (according to *Holst* 1949 and *Bane* 1961) and documented good fertility.

The gilts were inseminated on split sample model, Gilt 1 with newly collected semen from one of the two boars. The semen was collected within 15 min before the insemination, and at collection time the gel portion was separated by straining through gauze. Gilt 2 was inseminated with killed spermatozoa from the same ejaculate as Gilt 1. The gilts were thus inseminated pairwise with equal numbers of spermatozoa, one of the pair with dead spermatozoa. The spermatozoa were killed by placing the insemination dose in liquid nitrogen for at least 10 min. In some cases the killed spermatozoa were kept in liquid nitrogen for up to one week, waiting for the gilt to come in heat. The frozen semen was thawed immediately before insemination and was at body temperature when inseminated. The sperm motility was investigated and was zero in all cases. Each gilt was inseminated with 100 ml undiluted semen via an insemination catheter ad modum *Melrose & O'Hagan* (1961). The sperm count per insemination dose varied between 6.4 and 31.2×10^9 (mean $19.1 \pm s$ 6.1). The mean numbers of inseminated spermatozoa were 25.0, 19.0, 14.6 and 17.7×10^9, respectively, in gilts slaughtered at 1, 2, 6 and 12 h after insemination.

The gilts were slaughtered at 1, 2, 6 and 12 h after insemination. Six gilts were slaughtered at each of these times, three of which having been inseminated with live and three with dead spermatozoa. The genital tracts were removed immediately after slaughter. Uterus and oviducts were dissected from their liga-

ments. Clamps were placed in positions, dividing each uterine horn into three parts, the length of the anterior part of the horn being 5 cm and the other two being of equal length. Uterotubal junction was taken separately. This part comprised 1 cm of the tip of the uterine horn and 1 cm of the most posterior part of the oviduct. The oviducts were divided into four segments of equal length, called I—IV, counted from the infundibulum towards the uterotubal junction. Each region was flushed with a measured volume of isotonic saline (0.9 % NaCl). The oviduct segments were flushed with 1 ml, the uterobal junction with 2 ml and the uterine segments with 10 ml saline. The flushings from the oviducts were centrifuged, about 5 min at 2700 $\times$ g, to concentrate the spermatozoa before counting. A Wifug angle centrifuge for eight tubes was utilized (Chemico, AB Vinkelcentrifug, Stockholm, Sweden). Eight to nine tenths of the supernatant were pipetted off. Resuspension was done by agitation, after which the volume was measured. The flushings from the uterotubal junction and uterine sections were as a rule counted without concentration. The sperm concentration was counted in a Bürker haemocytometer chamber. As a rule the spermatozoa were counted in 144 squares, each of volume 1/250 µl. In the case of very high sperm concentrations only 25 squares were counted. The total number of spermatozoa per segment was calculated. The numbers of spermatozoa recovered in the individual uterine segments are not recorded separately. The counts in the right and left sides of the uterotubal junction and of the oviduct segments were combined.

Statistics

The single sperm counts in the different parts were transformed to $\log_{10}$ for statistical analysis in order to stabilize the variance, as the standard deviation in the original scale varies directly as the means (*Snedecor & Cochran* 1973, p. 329 et seq.). When no sperm were found in the flushings from a segment of the tract, the log of the half of the lowest number of cells that would have been detected in the portion of the sample that was actually scanned was used. The transformed data were analyzed by completely randomized two-factor analysis of variance for live and dead spermatozoa at different time intervals. One-way completely randomized analysis of variance separately for live and for dead spermatozoa was also performed. In order to test the

hypothesis that the $\log_{10}$ of the number of spermatozoa decreased linearly with time within the uterine part, regression analysis was performed separately for live and dead spermatozoa (*Snedecor & Cochran*).

The degrees of significance are expressed as follows:

$P > 0.05$	not significant	NS
$0.05 > P > 0.01$	almost significant	*
$0.01 > P > 0.001$	significant	**
$P < 0.001$	highly significant	***

RESULTS

The means and log means of spermatozoa recovered from the genital tract are presented in Tables 1 and 2.

Uterus. As seen in Table 2, the number of spermatozoa recovered in the uterus diminished very greatly during the first 12 h after insemination (P=0.0006). The reduction was statistically significant in respect of the number of live spermatozoa (P=0.004), but not of dead (P=0.06, Table 1). Linear regression

Table 1. Number of spermatozoa ($\times 10^3$) recovered from the genital tract of gilts 1, 2, 6 and 12 h after insemination.

Time of recovery and insemination group	Segment of the genital tract: oviduct I	oviduct II	oviduct III	oviduct IV	UTJ	uterus
Alive spermatozoa						
1 h	< 0.1[a]	< 0.1	19.5	277	1 900	1 890 000
2 h	116	96	40	33	9 170	1 415 000
6 h	0.3	0.1	0.3	2.6	538	3 780
12 h	1.5	3.3	2.3	4.5	1 072	1 137
Dead spermatozoa						
1 h	1.7	2.3	< 0.1	106	14 200	3 129 000
2 h	3.5	3.8	0.7	0.4	16	214 000
6 h	2.0	0.1	0.1	< 0.1	9	80 100
12 h	< 0.1	7.6	0.2	< 0.1	7	285
Statistical comparisons[b] within segment and in insemination groups (one-way analysis of variance)						
Alive spermatozoa, time	**	NS	NS	NS	NS	**
Dead spermatozoa, time	NS	NS	NS	NS	NS	NS

[a] Each value is the mean of three gilts.

[b] Statistical analyses were made on logarithmic numbers (Table 3).

Table 2. Mean logarithmic ($\times 10^3$) ± s of spermatozoa recovered from the genital tract of gilts 1, 2, 6 and 12 h after insemination.

Time of recovery and insemination group	Segment of the genital tract					
	oviduct					
	I	II	III	IV	UTJ	uterus
1 h alive spermatozoa	—1.0 ± 0.0[a]	—1.0 ± 0.0	0.51 ± 1.38	0.87 ± 1.97	3.21 ± 0.35	5.99 ± 0.61
dead spermatozoa	—0.43 ± 0.98	—0.39 ± 1.07	—1.0 ± 0.0	0.17 ± 2.02	2.35 ± 2.31	5.81 ± 1.50
2 h alive spermatozoa	1.5 ± 0.94	0.44 ± 1.8	1.0 ± 0.96	1.10 ± 0.81	3.31 ± 1.31	5.30 ± 1.38
dead spermatozoa	0.14 ± 0.99	0.18 ± 0.84	—0.46 ± 0.69	—0.55 ± 0.54	0.81 ± 0.82	4.80 ± 0.89
6 h alive spermatozoa	—0.67 ± 0.58	—0.8 ± 0.35	—0.67 ± 0.58	0.32 ± 0.37	1.89 ± 1.66	3.52 ± 0.29
dead spermatozoa	—0.41 ± 1.03	—0.8 ± 0.35	—0.84 ± 0.28	—1.0 ± 0.0	0.90 ± 0.17	3.74 ± 2.12
12 h alive spermatozoa	—0.003 ± 0.49	0.47 ± 0.27	0.25 ± 0.43	0.47 ± 0.47	2.76 ± 0.59	3.03 ± 0.20
dead spermatozoa	—1.0 ± 0.0	—0.21 ± 1.36	—0.72 ± 0.49	—1.0 ± 0.0	0.58 ± 0.63	2.11 ± 0.82
Statistical comparisons within segment (two-factor analysis of variance)						
Time (T)	*	NS	NS	NS	NS	***
Sperm (S)	NS	NS	**	**	**	NS
T × S	NS	NS	NS	NS	NS	NS

[a] Each value is logarithmic mean for three gilts.

analysis, however, showed a significant reduction both for live (P=0.001) and for dead spermatozoa (P=0.008).

Uterotubal junction (UTJ). The number of spermatozoa recovered in the uterotubal junction of gilts inseminated with live and dead spermatozoa, respectively, did not vary significantly with the time factor during the first 12 h after insemination (Table 1). The mean number of spermatozoa recovered, on the other hand, was significantly higher in gilts inseminated with live compared with dead spermatozoa (P=0.004, Table 2). The high mean number of dead spermatozoa 1 h after insemination derived from one animal.

Oviducts. Two h after insemination spermatozoa were recovered in all oviducts (Table 3). Six and 12 h after insemination spermatozoa were recovered in five of six and six of six oviducts, respectively, after insemination with live spermatozoa. After insemination with dead spermatozoa, on the other hand, spermatozoa were recovered only in one of six and three of six oviducts, respectively, at the same times.

Table 3. Recovery of spermatozoa from oviducts of gilts inseminated with live spermatozoa or with dead spermatozoa at different times after insemination.

Time after insemination	Gilts with spermatozoa in oviducts		Oviducts with spermatozoa	
(h)	live	dead	live	dead
1	2/3	2/3	2/6	3/6
2	3/3	3/3	6/6	6/6
6	3/3	1/3	5/6	1/6
12	3/3	2/3	6/6	3/6

Statistical analysis of the number of spermatozoa recovered in the oviductal segments showed, regardless of time, significantly more live than dead spermatozoa in Segments III and IV (P=0.003 and P=0.009, respectively, Table 2). The very high mean numbers of spermatozoa in Segment IV 1 h after insemination were caused by one animal in each insemination group. The sperm count in gilts inseminated with live spermatozoa varied significantly with time in Segment I, being highest 2 h after insemination (P=0.01, Table 2).

One h after insemination larger mean numbers of live spermatozoa were recovered in the lower oviductal segments (III and IV) than in the upper (I and II). Two h after insemination of live spermatozoa the largest mean numbers of spermatozoa were recovered in the two upper segments (I and II). From one animal inseminated with live spermatozoa and slaughtered 2 h later relatively larger numbers of spermatozoa were recovered in all oviductal segments than from the other animals. At 6 and 12 h there were no distinct differences in distribution of live spermatozoa between the various oviductal segments. In gilts inseminated with dead spermatozoa there were no differences in numbers of spermatozoa recovered in respect of time or between the various oviductal segments.

DISCUSSION

Of the 19×10^9 spermatozoa, live or dead inseminated, on average, per individual animal only 10—15 % were recovered 1 h later. The rapid disappearance of the majority of the spermatozoa from the genital tract is in conformity with earlier findings (*du Mesnil du Buisson & Dauzier* 1955, *Rigby* 1964, *Lovell & Getty* 1968). The rate of disappearance from the uterus was approximately the same during the 12 h after insemination for live and dead spermatozoa.

In the uterotubal junction spermatozoa normally survive for a long time after the intra-uterine deposition in swine (*du Mesnil du Buisson & Dauzier, Rigby, Viring et al.* 1980). In the present study the sperm count in the uterotubal junction remained relatively unchanged up to 12 h after insemination with live spermatozoa. In the gilts inseminated with dead spermatozoa, on the other hand, only a small number were recovered in the uterotubal junction 2 h later. The dead spermatozoa thus remained in the uterotubal junction for a shorter period than the live ones. A similar conclusion was drawn by *Pursel et al.* (1978). They demonstrated that frozen-thawed boar spermatozoa remained in the uterotubal junction to a smaller extent than fresh spermatozoa.

From Table 3 it is evident that dead as well as live spermatozoa were present in the oviducts at 1 and 2 h after insemination. These results conform with the findings of *First et al.* (1968) and *Baker & Degen* (1972), namely that dead spermatozoa are transported through the uterotubal junction into the oviduct.

The transport of spermatozoa into the oviduct is thus of a passive nature. Radioactively labelled molecules of different sizes deposited in the uterus also pass into the oviducts of pigs (*Einarsson et al.* 1980). It is therefore not probable that any selection takes place in the uterotubal junction. On the other hand the uterobal junction, together with the isthmus, appears to limit the number of spermatozoa passing into the oviduct and thus to diminish the risk of polyspery (*Hunter & Léglise* 1971).

Baker & Degen proposed, in contrast to *First et al,* that the transport of dead spermatozoa into the oviduct is less efficient than that of live spermatozoa. It is impossible to decide definitely how the matter stands on the basis of the present results. It appears clear from Table 1, however, that, whether live or dead, a smaller number of spermatozoa in the uterotubal junction results automatically in a smaller number in the oviduct on the same side. From Tables 1 and 2 it is seen that the mean number of live spermatozoa was larger in the lower oviduct segments (III and IV) than in the upper 1 h after insemination, while the reverse applied 2 h after insemination. These results reflect the rapid transport of spermatozoa in the oviduct that takes place before the sperm depot in the uterotubal junction has been established. At 6 and 12 h, when the depot has been established (cf. *Rigby*), on the other hand, no distinct differences in sperm distribution between the various oviduct segments are observable.

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SAMMANFATTNING

Spermiedistributionen i den tubulära könsapparaten efter insemination av levande och döda spermier hos gylta.

Tjugofyra gyltor inseminerades parvis med levande eller avdödade spermier härrörande från samma ejakulat. Inseminationsdosen var 100 ml ospädd sperma innehållande i medeltal 19 miljarder spermier. Gyltorna slaktades 1, 2, 6 respektive 12 timmar efter inseminationen. Spermieantalet räknades i uterus, "uterotubal junction" och i fyra lika långa avsnitt av äggledaren benämnda I—IV med hjälp av haemocytometer. Avsnitt IV var beläget närmast "uterotubal junction". Antalet återfunna spermier i livmodern minskade signifikant under de första 12 timmarna efter inseminationen. Hos gyltor som inseminerats med levande spermier återfanns fler spermier i "uterotubal junction" än hos gyltor som inseminerats med avdödade spermier. Två timmar efter inseminationen återfanns spermier i samtliga äggledare. Signifikant fler levande än avdödade spermier återfanns i äggledaravsnitten III och IV oberoende av tid. Hos gyltor som inseminerats med levande spermier varierade antalet spermier i avsnitt I statistiskt signifikant

med tiden och uppvisade det högsta antalet 2 timmar efter inseminationen. Vid 6 och 12 timmar förelåg inga påvisbara skillnader i fördelningen av levande spermier mellan de olika äggledaravsnitten. Antalet återfunna spermier i äggledarna var vid dessa tidpunkter till synes relaterade till spermiedepåerna i "uterotubal junction".

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