Acta vet. scand. 1980, *21*, 598—606.

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INFLUENCE OF BOAR SEMINAL PLASMA ON THE DISTRIBUTION OF SPERMATOZOA IN THE GENITAL TRACT OF GILTS*

By

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VIRING, SVEN and STIG EINARSSON: *Influence of boar seminal plasma on the distribution of spermatozoa in the genital tract of gilts.* Acta vet. scand. 1980, *21*, 598—606. — The main purpose of the present study was to investigate whether boar seminal plasma affects the transport of spermatozoa in the genital tract of oestrous pigs or not, with special reference to the sperm transport into the oviducts. Altogether 17 gilts were used in three experiments.

Experiment I. In nine gilts one uterine horn was injected surgically with 10^{10} spermatozoa suspended in seminal plasma and the other uterine horn with 10^{10} spermatozoa suspended in TESNaK-glucose buffer solution. The sperm deposition was performed under general anaesthesia. The gilts were slaughtered 1—2 or 4—6 h after insemination. The genital tract was removed and the numbers of spermatozoa determined in oviducts and in uterine horns.

Experiment II. The insemination doses were prepared exactly as in Experiment I. Approx. 24 h before insemination polyvinylchloride cannulas were inserted into the uterine lumen of the horns, drawn via the midventral incision at linea alba subcutaneously to cutaneous incisions ventral to the vulva opening. One cannula was placed in each uterine horn. At standing heat the insemination doses were slowly injected through the cannulas. The gilts were slaughtered 1 h after insemination and the numbers of spermatozoa within the genital tract were counted.

Experiment III. In three gilts under general anaesthesia the uterine horns were ligated 10 cm from the uterotubal junction. The semen doses (containing 2×10^9 spermatozoa), prepared as in Experiment I, were deposited into the uterine horns anterior to the ligatures through a cannula. The gilts were slaughtered 1 h after insemination, and the numbers of spermatozoa within the oviducts and ligated part of the uterine horns were counted.

In all three experiments more spermatozoa were, on average, recovered in the oviducts connected to uterine horns inseminated with spermatozoa suspended in seminal plasma. In Experiments I and

* This work was supported by grants from the Swedish Council for Forestry and Agricultural Research and "Anslaget för främjande av ograduerade forskares verksamhet".

II this was the case for 10 of 14 gilts and in Experiment III for all the three gilts. It is therefore suggested that boar seminal plasma promotes sperm transport into the oviduct of oestrous pigs. The background mechanism for this is discussed.

seminal plasma; spermatozoa; genital tract; gilt.

In the pig, semen is deposited into the uterine lumen during natural or artificial insemination. The spermatozoa reach the upper parts of the female tract shortly after the intra-uterine deposition (e.g. *Burger* 1952, *First et al.* 1968, *Baker & Degen* 1972). The sperm motility cannot account for the rapid transport. Muscular contractions of the genital tract itself is considered to be mainly responsible for the rapid transport of spermatozoa (cf. *Blandau* 1969). Also radiolabelled substances of different molecular size reach the upper part of the oviduct within 5 min after intrauterine deposition (*Viring et al.* 1980). Only a very small proportion of the spermatozoa reach the site of fertilization in the oviducts. Several factors are considered to affect the number of spermatozoa reaching the fertilizing site.

Baker et al. (1968) suggested that some minimum volume of semen must be deposited to achieve optimum fertility, and the concentration of spermatozoa must also be maintained above some minimum value. It has been proposed that seminal plasma mediates rapid transport within the female genital tract of several mammals (e.g. *Ventura et al.* 1968 — guinea pig, *Ventura & Freund* 1972 — rat, *Leidl* 1968 — pig). The mode of action of boar seminal plasma within the genital tract is unknown. A possible role of seminal plasma might be to facilitate the transport of the spermatozoa into the oviduct. The constricted lumen of the isthmus during oestrus together with the oedematous condition of the longitudinal folds are thought to be of functional importance (*Hunter* 1975).

The purpose of the present experiments was to test whether boar seminal plasma affects the transport of spermatozoa within the genital tract of oestrous gilts, with special reference to the transport into the oviduct.

MATERIALS AND METHODS

Seventeen sexually mature crossbred (Swedish Landrace × Swedish Yorkshire) gilts were used in these experiments. Daily heat control was performed with a vasectomized boar and the

gilts were experimentally inseminated 12 h after the onset of their second or third heat.

Two boars (purebred Swedish Landrace and Swedish Yorkshire, respectively) of good fertility provided semen for the present study. The sperm-rich fraction of the ejaculate was collected. The gel portion of the semen was removed at the time of collection by straining through gauze.

The sperm motility was assessed and only semen containing spermatozoa exhibiting a lively forward movement ($\geq$ 70 %) was used. The sperm concentration was determined using an absorptiometer (Corning-EEL, Evans Electroselenium, Halsted, England).

Experiment I

For each of nine gilts the following insemination doses were prepared. Two semen samples from the same ejaculate, each containing 10^{10} spermatozoa, were measured and put into test tubes. The tubes were filled up to 15 ml with TESNaK-glucose buffer (*Crabo et al.* 1972) and centrifuged for 5 min plus time of retardation, at a maximum force of 2700 $\times$ g. A Wifug angle centrifuge for 8 tubes was utilized (Chemico, AB Vinkelcentrifug, Stockholm, Sweden). The supernatant was removed by aspiration and the spermatozoa were resuspended to a total volume of 5 ml with TESNaK-glucose buffer and with sperm-free seminal plasma, respectively. The seminal plasma was collected from a boar with testicular hypoplasia. To one sample of the pair was immediately added another 35 ml of TESNaK-glucose buffer and seminal plasma to the other. The sperm motility was checked and found to be about the same in pairs of samples ($\geq$ 70 motile spermatozoa). The time from collection of semen and to intrauterine deposition did not exceed 45 min.

The operation of the gilts was performed under general anaesthesia with a 5 % solution of thiopentone sodium (Pentothal, Abbott) injected into an ear vein. An incision was made at linea alba and the uterine horns were localized and exposed. Insemination was performed very slowly, directly through a cannula in the cervico-tubal direction, into the lumen of the uterine horns about 10 cm from the uterine body. Backflow of semen was prevented by manual pressure during the insemination. The sample with spermatozoa and seminal plasma was inseminated into one horn and the sample with spermatozoa and

TESNaK-glucose buffer into the other. After insemination the midventral incision was closed. The gilts were slaughtered 1—2 or 4—6 h after insemination. Immediately after slaughter the genital tract was removed. Oviducts, uterine horns and uterine body were exposed. Clamps were placed in various positions, dividing the oviducts into two equal parts and each uterine horn into three parts, the length of the anterior part being 5 cm and the other two being of equal length. Each region was flushed with a measured volume of isotonic saline. The washings from the oviducts were concentrated by centrifugation before counts were made. The sperm concentration was determined in a Bürker's haemocytometer chamber. The total numbers of spermatozoa in each segment were then calculated.

Experiment II

For each of five gilts insemination doses were prepared exactly as described in Experiment I, and with the same number of spermatozoa per insemination dose (10^{10}).

On the first day of heat the following surgical operation was made on each gilt. The operation was performed under general anaesthesia as in Experiment I. An incision was made at linea alba and the uterine horns were exposed. Polyvinylchloride cannulas (ERU®, Urethral Catheter, W. Rüsch, 7053 Rommelshausen bei Stuttgart, Germany) were inserted into the lumen of the uterine horns, to a length of about 10 cm, through a small incision, cut about 10 cm from the uterine body. The cannulas were fixed with ligatures to the uterine wall. The cannulas were placed in the cervico-tubal direction. The free ends of the cannulas were drawn via the midventral incision subcutaneously to cutaneous incisions situated 6—8 cm ventral to the vulva opening. The cannulas were fixed to the edges of the cutis and cut to a length of 5 cm. The cut-off ends of the cannulas were closed and kept close to the skin. The midventral incision was closed.

On the second day of heat (about 24 h from the onset of heat) the gilts were inseminated. The gilts were stimulated to standing position, a rubber catheter was placed in the cervix, and the two insemination doses were slowly injected by syringe through the cannulas into the uterine horns. After insemination the openings of the cannulas were closed. The insemination procedure took about 5 min. Spermatozoa suspended in seminal plasma were inseminated into the right uterine horn in one gilt

and into the left horn in the next gilt. The gilts were slaughtered about 1 h after insemination and the genital tracts were removed. After exposure, clamps were placed so as to divide the oviducts into two equal parts and the uterine horns separately. Flushing and counting of the spermatozoa were performed as in Experiment I.

Experiment III

For each of three gilts the following insemination doses were prepared. Two test tubes with semen from the same ejaculate, each containing 2×10^9 spermatozoa, were filled up to 10 ml with TESNaK-glucose buffer and treated in the same way as described in Experiment I. After removal of the supernatant the spermatozoa were resuspended to a total volume of 10 ml: in one of the tubes with TESNaK-glucose buffer and in the other with sperm-free boar seminal plasma.

The operation of the gilts was performed as in Experiment I. The uterine horns were ligated with double catgut ligatures (No. 2) 10 cm from the uterotubal junction. This ligation did not influence the uterine contraction pattern (unpublished observation). The semen was deposited into the uterine horns anterior to the ligatures through a cannula. Spermatozoa and seminal plasma were deposited into one horn and spermatozoa and buffer solution into the other. The gilts were slaughtered 1 h after insemination. After exposure of the uterine horns and the oviducts, clamps were placed so as to divide the oviducts into two equal parts and the anterior 10 cm of the uterine horns separately. Flushings and countings of spermatozoa were performed as in Experiment I.

In Experiments I and II the numbers of spermatozoa recovered from the three parts of each uterine horn were combined.

RESULTS

The results from Experiment I are presented in Table 1. As is seen, the total numbers of spermatozoa recovered from the uterine horns decreased with the time in both groups.

At 1—2 as well as 4—6 h after intra-uterine deposition a higher mean number of spermatozoa was recovered from the oviducts belonging to uterine horns inseminated with seminal

Table 1. Recovery of spermatozoa from the genital tract of gilts at different times after experimental insemination. Spermatozoa were suspended in seminal plasma and in TESNaK-glucose buffer (= buffer), respectively.

Segment of the genital tract	Mean number of spermatozoa recovered ($\times 10^3$) ± s			
	1—2 h		4—6 h	
	seminal plasma	buffer	seminal plasma	buffer
Uterine horn	804 000 ± 1 123 000	1 307 000 ± 2 633 000	171 000 ± 280 000	68 000 ± 92 000
Oviduct	283 ± 438	194 ± 371	481 ± 807	89 ± 100
posterior half	282 ± 438	194 ± 370	469 ± 815	81 ± 105
anterior half	0.5 ± 0.9	0.3 ± 0.4	11.7 ± 16.4	8.8 ± 12.9
Number of gilts	5		4	

plasma as medium. There was a great difference between animals concerning numbers of recovered spermatozoa at each time interval. In seven out of the nine gilts the highest oviductal number of spermatozoa was recovered from that side where seminal plasma was used as the deposition medium.

In the gilts in Experiment II the positions of the cannulas were found to be the same at slaughter as at operation. The mean numbers of spermatozoa from the different regions of the genital tract are presented in Table 2. A higher mean number of oviductal spermatozoa was recovered from the side inseminated with spermatozoa suspended in seminal plasma. In single gilts this was the case in three out of five gilts. In one gilt the difference was extremely great.

Table 2. Number of spermatozoa recovered from the genital tract of gilts inseminated with spermatozoa suspended in seminal plasma and TESNaK-glucose buffer (= buffer) via cannulas placed in the posterior part of the uterine horns.

Segment of the genital tract	Mean number of spermatozoa recovered ($\times 10^3$) ± s	
	seminal plasma	buffer
Uterine horn	2 532 000 ± 820 000	2 746 000 ± 1 368 000
Oviduct	748 ± 1 618	11.4 ± 21.6
posterior half	730 ± 1 593	11.2 ± 21.7
anterior half	18.8 ± 27.8	0.3 ± 0.4
Number of gilts	5	

The results from Experiment III are summarized in Table 3. In all three gilts more spermatozoa were recovered from the oviducts belonging to the ligated uterine horns inseminated with spermatozoa suspended in seminal plasma.

Table 3. Recovery of spermatozoa from the ligated anterior part of the uterine horns and from the oviducts 1 h after experimental insemination of spermatozoa suspended in seminal plasma and TESNaK-glucose buffer (= buffer) into the ligated parts.

Segment of the genital tract	Mean number of spermatozoa recovered ($\times 10^3$) ± s	
	seminal plasma	buffer
Anterior part of uterine horn (10 cm)	1 329 000 ± 963 000	2 249 000 ± 503 000
Oviduct	3 492 ± 2 648	550 ± 747
posterior half	3 472 ± 2 645	539 ± 729
anterior half	19.6 ± 10.7	10.5 ± 18.0
Number of gilts	3	

In all these three experiments the numbers of spermatozoa recovered were larger in the posterior half of the oviduct than in the anterior half regardless of the medium used.

DISCUSSION

To minimize the differences in sperm distribution within the genital tract between gilts, one uterine horn was in each case used as a control, inseminated with spermatozoa suspended in a buffer solution. In Experiments I and II no ligation of the uterine horns posterior to the deposition site was done. In Experiment III, however, the anterior part of each uterine horn was ligated, preventing backflow of semen. When these studies were carried out, no information was available concerning transuterine transport of semen in the pig. Recent experiments, however, have demonstrated that such transuterine transport takes place (*Viring et al.* 1980). It is therefore likely that seminal plasma and TESNaK-glucose buffer became mixed in both uterine horns in gilts belonging to Experiments I and II. The mixing ratio is, however, not immediately 1:1 in the uterus. Five min after deposition of radiolabelled substances in the uterine horns, only a small part of the inseminate had been transported to the contrary uterine horn.

The mean number of spermatozoa recovered from the oviducts was higher when the adjacent uterine horn was inseminated with spermatozoa suspended in seminal plasma. These results were independent of the techniques used (Tables 1, 2 and 3). The varying results among gilts belonging to Experiments I and II might be due to a variation in rate of transuterine transport after deposition.

In in vivo studies it was recently demonstrated that seminal plasma caused a relaxation of the muscular activity of the isthmic part of the oviduct in pigs (*Viring & Einarsson* 1980). The decreased muscular activity of the porcine isthmus might facilitate sperm transport into the oviducts. It is therefore suggested that boar seminal plasma promotes sperm transport into the oviducts of oestrous pigs.

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SAMMANFATTNING

Galtspermaplasmans inverkan på spermiedistributionen i könsorganen hos gylta.

Avsikten med föreliggande undersökning var att studera galtspermaplasmas inverkan på spermietransporten i könsorganen hos brunstiga svin med speciell hänsyn till spermietransporten in i äggledarna. Tre experiment utfördes med sammanlagt 17 gyltor.

Experiment I. Hos nio gyltor injicerades 10^{10} spermier med spermaplasma i ena livmoderhornet och 10^{10} spermier med TESNaK-glykosbuffert i det andra livmoderhornet via kirurgisk teknik. Spermadeponeringen gjordes under allmän anestesi. Gyltorna slaktades 1—2 respektive 4—6 timmar efter deponeringen. Könsorganen togs ut och spermieantalet bestämdes i äggledarna och livmoderhornen.

Experiment II. Inseminationsdoserna preparerades som i experiment I. Katetrar opererades in i vardera livmoderhornet cirka 24 timmar före beräknad insemination. Katetrarna mynnade ventralt om blygden. Inseminationsdoserna injicerades samtidigt och långsamt via katetrarna efter det att ståreflexen framkallats. Gyltorna slaktades en timme efter inseminationen och antalet spermier i de honliga könsorganen beräknades.

Experiment III. Under allmän anestesi ligerades livmoderhornen 10 cm från „uterotubal junction" hos tre gyltor. Spermadoserna, som innehöll 2×10^9 spermier, preparerades som i experiment I och deponerades i livmoderhornen framför ligaturerna via kanyl. Gyltorna slaktades en timme efter insemination och antalet spermier i äggledarna och de ligerade livmoderavsnitten beräknades.

I alla tre experimenten återfanns i medeltal flera spermier i de äggledare, som stod i förbindelse med livmoderhorn inseminerade med spermier och spermaplasma. I experiment I och II var detta fallet hos 10 av 14 gyltor och i experiment III hos alla tre gyltorna. Av resultaten framgår sålunda att spermaplasma underlättar spermiernas transport in i äggledarna hos brunstiga honsvin. De bakomliggande orsakerna diskuteras.

(Received September 12, 1980).

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