Journal of Reproduction and Development, Vol. 60, No 5, 2014

—Technology Report—

Is the Function of the Porcine Sperm Reservoir Restricted to the Ovulatory Period?

Klaus-Peter BRÜSSOW[1)], Istvan EGERSZEGI[2)] and Jozsef RÁTKY[2)]

[1)]*Institute of Reproductive Biology, Leibniz Institute for Farm Animal Biology (FBN), 18196 Dummerstorf, Germany*
[2)]*Research Institute for Animal Breeding, Nutrition and Meat Science, National Agricultural Research and Innovation Centre, 2053 Herceghalom, Hungary*

Abstract. The uterotubal junction (UTJ) and caudal isthmus are recognized as a functional pre-ovulatory sperm reservoir (SR). Spermatozoa are released from the SR in a complex and concerted action. However, whether this functionality is restricted only to the ovulatory period is still open to debate. Our study was aimed to analyze the presence of spermatozoa within the UTJ (SR), isthmus (ISTH) and ampulla (AMP) after laparoscopic intrauterine insemination (LIUI) either in the peri- (PERI) or post-ovulatory (POST) period or at mid cycle (MID). Each uterine horn of estrus synchronized gilts (n=12) was inseminated with 20 ml sperm (29.5×10^6 cells/ml). Oviducts were recovered 7 h after LIUI and separated into the UTJ, ISTH and AMP, and sections were flushed with 10 ml PBS+EDTA solution. After centrifugation, the sperm pellet was evaluated by Čeřovský staining. The median sperm numbers in the PERI, POST and MID groups were 578, 171 and 789 in the UTJ; 545, 233 and 713 in the ISTH; and 496, 280 and 926 in the AMP, respectively, and there were differences between the POST and MID groups ($P<0.05$) but not between the oviductal sections of each group ($P>0.05$). Compared with the MID group, the percent of intact sperm cells was higher ($P<0.01$) in the PERI and POST groups (32.8 *vs.* 66.4 and 76.8%). Also, the percentages of aberrations in the acrosome and tail were higher ($P<0.05$) in the MID group. Based on this, it can be assumed that the sperm reservoir is active during different phases of the estrus cycle. However, the mid-cycle oviduct environment considerably impairs sperm cell quality.

Key words: Female pig, Laparoscopic intrauterine insemination, Oviduct, Spermatozoa, Sperm reservoir

(**J. Reprod. Dev. 60:** 395–398, 2014)

In the pig, the uterotubal junction (UTJ) and caudal isthmus act as a functional tubal sperm reservoir (SR) that ensures the availability of viable spermatozoa for fertilization [1, 2]. In the SR, most spermatozoa maintain a normal ultrastructure and viability [3, 4]. A number of concerted factors may explain formation of the SR, including the narrowed lumen [5], viscous mucus [6], lower temperature [7], local enzymatic and ionic milieu [8], selective binding of spermatozoa to the epithelium [9] and specific tubal fluid components [10].

The sequential release of spermatozoa from the SR towards the site of fertilization (i.e., ampulla-isthmic-junction) is suggested to be a complex and concerted process including opening of the lumen by a decrease in hormonally driven endosalpingeal edema, dissolution of hyaluronan (HA)-rich mucus, hyperactive sperm motility, increased flow of tubal fluid and redirected oviductal contractions [11]. However, oocyte signals, follicular fluid components and temperature gradients are also involved in the process of (peri-) ovulatory sperm release in pigs [7, 12–15].

It remains unknown, if the function of the SR is restricted only to the ovulatory period. Therefore, the aim of our study was to analyze the presence of spermatozoa within the UTJ, isthmus (ISTH) and ampulla (AMP) either in the peri- (PERI) or postovulatory (POST) period or at mid cycle (MID). Also, the quality of spermatozoa was assayed in terms of their morphology and acrosome integrity. Since insemination of sows out of estrus bears some difficulties, we used laparoscopic intrauterine insemination (LIUI) to ensure appropriate application of semen at these different time points of the estrus cycle. The LIUI approach has been successfully applied in previous studies [12, 16, 17].

Altogether, 12 gilts were successfully inseminated by means of LIUI, and altogether 24 oviducts were surgically recovered and dissected. Of 72 oviduct sections, 71 could be flushed, and sperm cells were recovered from all oviduct sections except for two (97.2%).

There was a considerable variation in the number of sperm cells recovered from different oviduct sections (Table 1). There were significantly ($P<0.05$) increased mean and median cell numbers in all segments of the oviduct after insemination in the peri-ovulatory period and at mid-cycle compared with those for postovulatory LIUI. However, the number of spermatozoa did not differ between the oviduct sections (UTV *vs.* ISTH *vs*. AMP) of each insemination group. However, we found a tendency ($P=0.079$) for an increasing median number of sperm cells towards the ampulla in the POST group.

Insemination during the luteal phase of the estrus cycle resulted in a lower percent ($P<0.05$) of intact spermatozoa compared with LIUI in the peri- and postovulatory periods (Table 2). As well, aberrations of the acrosome (swollen or lacking acrosome) and tail (folded, bent or broken tail) were more often present in the MID group.

Received: April 2, 2014
Accepted: May 20, 2014
Published online in J-STAGE: June 24, 2014

Correspondence: K-P BRÜSSOW (e-mail: bruessow@fbn-dummerstorf.de)

Table 1. Sperm concentration (mean, median, minimum and maximum values) in different oviduct sections after LIUI in the peri- (PERI) and postovulatory (POST) periods and at mid cycle (MID)

Oviduct section	Sperm concentration	PERI	POST	MID
UTJ	Mean ± SE	3932 ± 2348	676 ± 390[a]	9221 ± 8269[b]
	Median	578	171[a]	789[b]
	Min – max	0–17261	41–3141	97–67063
ISTH	Mean ± SE	952 ± 382	402 ± 213[a]	1770 ± 842[b]
	Median	545	233[a]	713[b]
	Min – max	37–3205	15–1830	78–6850
AMP	Mean ± SE	1022 ± 456	353 ± 106[a]	2172 ± 1460[b]
	Median	496	280[a]	926[b]
	Min – max	0–3604	73–973	97–12320

[a,b] $P<0.05$ for comparisons within rows.

Table 2. Assessment of sperm quality at different time points (PERI, POST and MID)

	Intact spermatozoa (%)	Damaged acrosome (%)	Tail aberration (%)
PERI	66.4 ± 6.0[a]	29.3 ± 6.5[a,b]	17.4 ± 4.0[a,b]
POST	76.8 ± 3.5[a]	16.2 ± 2.6[a]	7.0 ± 1.7[a]
MID	32.8 ± 3.9[b]	38.6 ± 2.7[b]	28.2 ± 4.3[b]

[a,b] $P<0.05$ for comparisons within columns.

There is not too much data regarding the sperm cell distribution within the porcine oviduct after mating or insemination. Rigby [18] found an average of 10^6 spermatozoa in the UTJ and only about 1500 in the isthmus 6 h after insemination. Viring [19] reported on 500, 2.6 and 0.3 $\times 10^3$ sperm cells in the UTJ, isthmus and ampulla, respectively, at the same time point. In other studies [20–22], a larger proportion of spermatozoa was found in the UTJ compared with other oviduct sections. In these studies, insemination was performed in the preovulatory period, but oviducts were flushed at different intervals (24 h after insemination [21, 22] or 6-8 h before ovulation, during ovulation or 6-8 h after ovulation [20]). Therefore, there is some difficulty in comparing the real number of sperm cells counted between the previous and present studies. Furthermore, there is great variation between gilts in the number of spermatozoa that can be attributed to the boar used [20] but also to the different methods of sperm number analysis (Bürker or Neubauer hemocytometer compared with sperm cell count after Čeřovský staining in our study). No significant differences were found in our study regarding the (mean and median) number of sperm cells within the oviduct section, regardless of the time point of insemination. However, the majority of spermatozoa (63.5, 47.3 and 70.0% in the PERI, POST and MID groups, respectively) remained in the UTJ. A larger proportion of spermatozoa was also present in the UTJ and the lower isthmus in other studies [20, 22, 23]. These results confirm that these sections act as a functional tubal sperm reservoir.

It was previously shown that spermatozoa are located at specific sites within the SR environment [24] and that there are two sperm subpopulations, one with epithelial contact and one without such contact [4]. Since a large number of spermatozoa can be recovered by flushing, these authors suggest that most of the spermatozoa are in the oviduct lumen and loosely attached to the luminal surface. Thus, it can be assumed that the majority of the spermatozoa present in the oviduct were flushed out in our present study.

The question of whether the function of the SR is restricted to the ovulatory period can be answered only using a special insemination protocol. We used the laparoscopic intrauterine insemination (LIUI) approach [15, 16]. This allowed us to ensure application of sperm into the genital tract even at mid cycle. Interestingly, we found similar numbers and distributions of sperm cells when insemination was done at mid cycle compared with the peri-ovulatory period. Additionally, the sperm concentrations in all oviduct sections were higher ($P<0.05$) after MID insemination in comparison to postovulatory sperm application. So we can speculate first of all that the nature of the SR to store and to release spermatozoa is active during the whole estrus cycle. Differences in the sperm concentration within the oviduct sections can be explained probably by differences in hormone milieu. It was previously shown that signals from the preovulatory follicle due to the transfer of steroids, prostaglandins and peptides via the subovarian plexus by a countercurrent mechanism are involved in the function of the SR [12, 25–27]. There is experimental evidence [25] that injection of progesterone into the oviductal serosa at the UTJ increased the release of spermatozoa, leading to polyspermic fertilization. However, it was previously shown [28] that the progesterone concentration within the oviductal fluid was similar prior to and after ovulation, i.e., during the peri- and postovulatory periods. In the systemic blood, the progesterone level was two times higher after ovulation, and the difference between the oviductal and blood plasma concentrations was about four times higher after ovulation. However, this cannot explain the lower number of spermatozoa in the postovulatory period. On the other hand, since the luteal phase of the porcine estrus cycle is characterized by increased progesterone levels [29, 30], these concentrations could mimic the signal to release (a higher number of) spermatozoa into the oviduct in the MID group.

Abnormal spermatozoa, e.g., those with a damaged acrosome, tail aberrations and protoplasmatic droplets, are to some extent common in boar semen [31]. In the SR, most of the sperm population with epithelial contact maintained intact plasma membranes during the preovulatory period and showed acrosome reacted-like membrane changes during the postovulatory period [4]. Flushing of oviducts

in the pre-, peri- and postovulatory period revealed that 68, 51 and 46% of spermatozoa had an intact plasma membrane [20]. In our study, the proportions of intact spermatozoa were 66 and 77% after peri- and postovulatory insemination, respectively, whereas after mid-cycle insemination, the proportion fell to 33%, ($P<0.05$). Acrosome and tail abnormalities were also significantly higher in the MID group. Without doubt, the histo-architecture [32] and the milieu of the porcine oviduct diverge in different periods of the estrus cycle [33–37]. Therefore, the mid-cycle oviduct environment is not physiologically proper for sperm cells, and as a result, the quality of spermatozoa is influenced. However, further studies should highlight in more detail the functionality of the SR and of sperm-oviduct interaction.

In conclusion, our study revealed that the sperm reservoir functions during different phases of the estrus cycle. However, mid-cycle oviduct environment considerably impairs sperm cell quality.

Methods

Animals and animal treatment

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Agricultural Ministerial Department of Mecklenburg-Vorpommern, Germany.

Altogether, 12 Landrace gilts (9 months old, with mean body weights of 138 kg) were included in the trial. Estrus was synchronized in all gilts by 15 days of feeding with Regumate® (16 mg altrenogest/day/gilt; MSD Animal Health, Unterschleissheim, Germany). Twenty-four hours after the last Regumate® feeding (0800 h), each animal received a single intramuscular injection of 850 IU equine chorionic gonadotropin (eCG; Pregmagon®, IDT Biologika, Dessau-Tornau, Germany). Ovulation was induced 80 h later by administration of 500 IU human chorionic gonadotropin (hCG; Ovogest®, MSD Animal Health, Unterschleissheim, Germany).

Laparoscopic insemination and oviduct and sperm recovery

Laparoscopic intrauterine insemination (LIUI) into each uterine horn was performed with 20 ml of extended, fresh boar semen (29.5×10^6 spermatozoa/ml; motility 80%; extender: AndroStar® Plus, Minitüb, Tiefenbach, Germany). Semen was collected from the same proven AI Pietrain boar (AI Station BVN, Malchin, Germany). LIUI was performed as described previously [15, 17]. Briefly, general anaesthesia in gilts was induced with ketamine (17.25 mg/kg BW, Ursotamin®, Serumwerk Dessau, Germany) and azaperone (1.2 mg/kg BW, Stresnil®, Elanco Animal Health, Bad Homburg, Germany) and animals were fixed in a dorsal position. A pneumoperitoneum with CO_2 was automatically produced (Endo Tech, Munich, Germany). Thereafter, three trocar cannulas (Karl Storz, Tuttlingen, Germany) were inserted into the abdomen for 0° optics (ETB, Berlin, Germany) and grasping forceps (NeoMed, Gutach/Bleibach, Germany). All laparoscopic handling was observed on a video monitoring system (NeoMed, Gutach/Bleibach, Germany). For insemination, the uterine horn was carefully fixed with atraumatic forceps, and the uterine wall was punctured approximately 10 cm caudal to the uterotubal junction with a trocar that was 2.5 mm in diameter. Under visual control, a 2.2 mm catheter (RÜSCH feeding tube, W. Rüsch AG, Kernen, Germany) connected to a 20 ml syringe was inserted through the trocar cannula about 3 cm into the uterine lumen in the direction towards the tip of the uterine horn, and then semen was deposited. The insemination procedure was repeated at the opposite uterine horn.

LIUI was performed at three different time points of the estrus cycle, i.e., in the peri-ovulatory (PERI; 31 h post hCG, n=4 gilts) or postovulatory period (POST; 79 h post hCG, n=4) or at mid cycle (MID; day 9 of the estrus cycle, n=4). Seven hours after LIUI, gilts were subjected to ovariohysterectomy, and the oviducts (n=24) were dissected into three segments: the caudal isthmus and uterotubal junction (UTJ), cranial isthmus (ISTH) and ampulla (AMP). Each section was flushed with 10 ml PBS containing 1.78 mM EDTA (Sigma-Aldrich, St. Louis, MO, USA). Flushed fluids were centrifuged twice at 500 *g* (for 10 and for 7 min). Supernatants were removed, and the volume of sperm pellets was measured with a pipette. From each sample, 10 µl of the sperm pellet were taken to prepare smears. Smears were stained according to the method of Čeřovský [38] and the total number of spermatozoa was counted from stained smears of each 10 µl sample. The total sperm cell number per flushing was determined based on this number and the volume of the sperm pellet, respectively. Sperm cell morphology (intact and acrosome and tail aberrations) and acrosome integrity were evaluated by analyzing at least 200 spermatozoa.

Statistical analysis

Two replications with six gilts each were performed. Calculation of means and standard deviations, as well as of median values and 25th and 75th percentiles was carried out using the software package SigmaPlot 11.0 (Systat Software, San Jose, CA, USA). One-way ANOVA followed by Tukey's test was used to compare the results. Differences of $P<0.05$ were considered significant.

References

1. **Rodríguez-Martínez H, Saravia F, Wallgren M, Tienthai P, Johannisson A, Vázquez JM, Martínez E, Roca J, Sanz L, Calvete JJ.** Boar spermatozoa in the oviduct. *Theriogenology* 2005; **63**: 514–535. [Medline] [CrossRef]
2. **Brüssow K-P, Rátky J, Rodriguez-Martinez H.** Fertilization and early embryonic development in the porcine fallopian tube. *Reprod Domest Anim* 2008; **43**(Suppl 2): 245–251. [Medline] [CrossRef]
3. **Rodriguez-Martinez H, Nicander L, Viring S, Einarsson S, Larsson K.** Ultrastructure of the uterotubal junction in preovulatory pigs. *Anat Histol Embryol* 1990; **19**: 16–36. [Medline] [CrossRef]
4. **Mburu JN, Rodriguez-Martinez H, Einarsson S.** Changes in sperm ultrastructure and localisation in the porcine oviduct around ovulation. *Anim Reprod Sci* 1997; **47**: 137–148. [Medline] [CrossRef]
5. **Hunter RHF.** Pre-ovulatory arrest and peri-ovulatory redistribution of competent spermatozoa in the isthmus of the pig oviduct. *J Reprod Fertil* 1984; **72**: 203–211. [Medline] [CrossRef]
6. **Johansson M, Tienthai P, Rodriguez-Martinez H.** Histochemistry and ultrastructure of the intraluminal mucus in the sperm reservoir of the pig oviduct. *J Reprod Dev* 2000; **46**: 183–192. [CrossRef]
7. **Hunter RHF, Nichol R.** A preovulatory temperature gradient between the isthmus and ampulla of pig oviducts during the phase of sperm storage. *J Reprod Fertil* 1986; **77**: 599–606. [Medline] [CrossRef]
8. **Rodriguez-Martinez H, Ekstedt E, Ridderstråle Y.** Histochemical localization of carbonic anhydrase in the female genitalia of pigs during the oestrous cycle. *Acta Anat (Basel)* 1991; **140**: 41–47. [Medline] [CrossRef]
9. **Fazeli A, Duncan AE, Watson PF, Holt WV.** Sperm-oviduct interaction: induction of capacitation and preferential binding of uncapacitated spermatozoa to oviductal epithelial cells in porcine species. *Biol Reprod* 1999; **60**: 879–886. [Medline] [CrossRef]
10. **Tienthai P, Johannisson A, Rodriguez-Martinez H.** Sperm capacitation in the porcine oviduct. *Anim Reprod Sci* 2004; **80**: 131–146. [Medline] [CrossRef]

11. **Rodriguez-Martinez H, Larsson B, Pertoft H, Kjellen L.** GAG's and spermatozoon competence in vivo and in vitro. *In*: Lauria A, Gandolfi F, Enne G, Gianaroli L (eds.), Gametes: Development and Function. Serono Symposium, Serono; 1998: 239–272.
12. **Hunter RHF, Cook B, Poyser NL.** Regulation of oviduct function in pigs by local transfer of ovarian steroids and prostaglandins: a mechanism to influence sperm transport. *Eur J Obstet Gynecol Reprod Biol* 1983; **14**: 225–232. [Medline] [CrossRef]
13. **Brüssow K-P, Rátky J, Torner H, Sarlós P, Solti L.** Contribution of porcine follicular fluid in the process of fertilization in vivo. *Reprod Domest Anim* 1999; **34**: 139–145. [CrossRef]
14. **Brüssow K-P, Rátky J, Torner H, Solti L.** Possible role of follicular fluid on porcine in vivo fertilization. In: Sato E, Miyamoto H, Manabe N (eds.), Animal Frontiers. Hokuto Pub Co. Ltd, Kyoto; 2003: 89–95.
15. **Brüssow K-P, Torner H, Rátky J, Manabe N, Tuchscherer A.** Experimental evidence for the influence of cumulus-oocyte-complexes on sperm release from the porcine oviductal sperm reservoir. *J Reprod Dev* 2006; **52**: 249–257. [Medline] [CrossRef]
16. **Brüssow K-P, Torner H, Rátky J.** Sperm migration in pigs after deep intrauterine and intraperitoneal insemination. *J Reprod Dev* 2011; **57**: 342–345. [Medline] [CrossRef]
17. **Brüssow K-P, Vernunft A, Kempisty B, Rátky J.** Single fixed-time laparoscopic intrauterine insemination as a tool to obtain low-diversity porcine embryos. *Vet Med (Praha)* 2013; **58**: 412–416.
18. **Rigby JP.** The persistence of spermatozoa at the uterotubal junction of the sow. *J Reprod Fertil* 1966; **11**: 153–155. [CrossRef]
19. **Viring S.** Distribution of live and dead spermatozoa in the genital tract of gilts at different times after insemination. *Acta Vet Scand* 1980; **21**: 587–597. [Medline]
20. **Mburu JN, Einarsson S, Lundeheim N, Rodriguez-Martinez H.** Distribution, number and membrane integrity of spermatozoa in the pig oviduct in relation to spontaneous ovulation. *Anim Reprod Sci* 1996; **45**: 109–121. [Medline] [CrossRef]
21. **Sumransap P, Tummaruk P, Kunavongkrit A.** Sperm distribution in the reproductive tract of sows after intrauterine insemination. *Reprod Domest Anim* 2007; **42**: 113–117. [Medline] [CrossRef]
22. **Tummaruk P, Sumransap P, Techakumphu M, Kunavongkrit A.** Distribution of spermatozoa and embryos in the female reproductive tract after unilateral deep intra uterine insemination in the pig. *Reprod Domest Anim* 2007; **42**: 603–609. [Medline] [CrossRef]
23. **Brandt Y, Lang A, Madej A, Rodriguez-Martinez H, Einarsson S.** Impact of ACTH administration on the oviductal sperm reservoir in sows: the local endocrine environment and distribution of spermatozoa. *Anim Reprod Sci* 2006; **92**: 107–122. [Medline] [CrossRef]
24. **Fléchon J-E, Hunter RHF.** Distribution of spermatozoa in the utero-tubal junction and isthmus of pigs, and their relationship with the luminal epithelium after mating: a scanning electron microscope study. *Tissue Cell* 1981; **13**: 127–139. [Medline] [CrossRef]
25. **Hunter RHF.** Local action of progesterone leading to polyspermic fertilization in pigs. *J Reprod Fertil* 1972; **31**: 433–444. [Medline] [CrossRef]
26. **Hunter RHF.** Ovarian endocrine control of sperm progression in the fallopian tubes. *Oxf Rev Reprod Biol* 1995; **17**: 85–124.
27. **Hunter RHF, Petersen HH, Greve T.** Ovarian follicular fluid, progesterone and Ca^{2+} ion influences on sperm release from the fallopian tube reservoir. *Mol Reprod Dev* 1999; **54**: 283–291. [Medline] [CrossRef]
28. **Brüssow K-P, Rátky J, Schneider F, Torner H, Kanitz W, Solti L.** Effects of follicular fluid on the transport of porcine oocytes into the oviduct at ovulation. *Reprod Domest Anim* 1999; **34**: 423–429. [CrossRef]
29. **Egerszegi I, Schneider F, Rátky J, Soós F, Solti L, Manabe N, Brüssow K-P.** Comparison of luteinizing hormone and steroid hormone secretion during the peri- and postovulatory periods in Mangalica and Landrace gilts. *J Reprod Dev* 2003; **49**: 291–296. [Medline] [CrossRef]
30. **Brüssow K-P, Schneider F, Tuchscherer A, Egerszegi I, Rátky J.** Comparison of luteinizing hormone, leptin and progesterone levels in the systemic circulation (Vena jugularis) and near the ovarian circulation (Vena. cava caudalis) during the oestrous cycle in Mangalica and Landrace gilts. *J Reprod Dev* 2008; **54**: 431–438. [Medline] [CrossRef]
31. **Čeřovský J, Frydrychová S, Lustyková A, Rozkot M.** Changes in boar semen with a high and low level of morphologically abnormal spermatozoa. *Czech J Anim Sci* 2005; **50**: 289–299.
32. **Yaniz JL, Lopez-Gatius F, Hunter RHF.** Scanning electron microscopic study of the functional anatomy of the porcine oviductal mucosa. *Anat Histol Embryol* 2006; **35**: 28–34. [Medline] [CrossRef]
33. **Iritani A, Sato E, Nishikawa Y.** Secretion rates and chemical composition of oviduct and uterine fluids in sows. *J Anim Sci* 1974; **39**: 582–588. [Medline]
34. **Rodriguez-Martinez H, Petroni A, Einarsson S, Kindahl H.** Concentrations of prostaglandin F2 alpha in the pig oviductal fluid. *Prostaglandins* 1983; **25**: 413–424. [Medline] [CrossRef]
35. **Wollenhaupt K, Brüssow K-P.** Determination of acid phosphatase activity in oviduct tissue of gilts with spontaneous oestrus and synchronized ovulation. *Arch exp. Vet Med* 1987; **41**: 51–57.
36. **Nichol R, Hunter RHF, Gardner DK, Leese HJ, Cooke GM.** Concentrations of energy substrates in oviductal fluid and blood plasma of pigs during the peri-ovulatory period. *J Reprod Fertil* 1992; **96**: 699–707. [Medline] [CrossRef]
37. **Carrasco LC, Romar R, Avilés M, Gadea J, Coy P.** Determination of glycosidase activity in porcine oviductal fluid at the different phases of the estrous cycle. *Reproduction* 2008; **136**: 833–842. [Medline] [CrossRef]
38. **Čeřovský J.** Metoda barvení kančích spermií pro morfologické hodnocení. *Živoč Výr* 1976; **21**: 361–366.