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Theriogenology

A Study on the Number of Recovered Spermatozoa in the Uterine Horns and Oviducts of Gilts, after Fractionated or Non-Fractionated Insemination

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ABSTRACT. The objective of this study was to compare the number of recovered spermatozoa, in different parts of the uterine horn and oviduct in gilts, after insemination with fractionated (experiment) and non-fractionated (control) liquid stored semen. The number of spermatozoa and volume of backflow was also investigated. Twenty three cross-bred gilts were used in the study. They were divided into 2 groups, a control group (non-fractionated liquid stored semen, n=10) which were inseminated with 100 ml of liquid stored semen containing 3,000 million spermatozoa per dose and an experimental group (fractionated liquid stored semen, n=10) which were inseminated with 50 ml of liquid stored semen, with 3,000 million spermatozoa per dose and followed by another 50 ml of semen dilutor (Beltville Thawing Solution, BTS). Thereafter, backflow semen was collected and measured every 15 min for a period of 1 hr. Three or 12 hr after insemination, 5 gilts from each group had the uterus, the horn of the uterus, the oviducts and the ovaries removed under general anaesthesia. The horn of uterus and the oviducts were separated by ligation into 6 segments. All 6 segments were flushed with BTS to collect all spermatozoa within the segment. Recovered spermatozoa were counted, using a haemocytometer and the volume recorded. It was seen that the percentage of spermatozoa in the backflow semen in the experimental group was less than in the control group. The difference was not significant in the gilts that were operated on 3 hr after insemination, the mean number of spermatozoa in the uterine horn and the utero-tubular junction (UTJ) was more in the experimental than in the control group, but less in the isthmus and the ampulla of the oviduct. The gilts which were operated on 12 hr after insemination, had relatively more ovulating gilts in the control group than in the experimental group (3 of 4 gilts compare to 3 of 5 gilts). The control group had more spermatozoa in the oviduct than the experimental group, but less in UTJ and in the horn of the uterus. Again the difference was not significant. It can be concluded that fractionated (experimental) or non-fractionated (control) insemination of semen with the same number of spermatozoa provides no significant difference in the number of spermatozoa either in the horn of the uterus, the UTJ or the oviduct of gilts.

KEY WORDS: insemination, spermatozoa distribution, swine.


Artificial insemination (AI) in pigs can improve breeding values faster than natural service. It is also able to distribute improved genetics ten times faster than by natural service. It has become popular to use AI in pigs since it can also reduce the number of boars on the farm, which lessens the feed and maintenance costs of the boars. AI techniques can also lower the risk of spreading reproductive diseases as well as reducing the number of workers needed during mating [9].

Semen quality is of vital importance for efficient artificial insemination in Thailand. Insemination techniques are also very important when the inseminators are pressed for time during insemination which can result in more than 50% of sperm being lost because of semen backflow during the first hour following insemination. This could well affect fertility because too few spermatozoa enter the reproductive tract [8] resulting in only 5–10 percent remaining 1–2 hr after insemination [6, 10]. In the Ukraine, the use of fractionated liquid stored semen has been shown to reduce the number of spermatozoa that flowed back out after insemination [2]. Non-fractionated liquid stored semen was conventionally prepared and diluted to provide a 100 ml dose. Fractionated liquid stored semen was diluted to provide the same number of spermatozoa in a 50 ml dose and followed by 50 ml of pure diluent. It was anticipated that fractionated insemination should ensure better distribution of spermatozoa throughout the reproductive tract.

The aim of this study was to compare the number of recovered spermatozoa in the uterine horns and oviducts of gilts 3 and 12 hr after insemination with either fractionated or non-fractionated liquid stored semen.

MATERIALS AND METHODS

Animal and sample selection: Twenty healthy three breed crossed gilts (Duroc x Large White x Landrace), which had shown at least one oestrus or were in the pro-oestrous period, were brought to the Faculty of Veterinary Science, Chulalongkorn University and kept in individual pens near adult boars. All gilts were checked for oestrous signs twice a day by using the back pressure test and a teaser boar. Oestrous gilts were ranked according to the time of oestrus and allocated into 2 groups using simple random sampling, which is considered a reliable statistical method.

Control group (non-fractionated insemination): The 10 gilts in this group were inseminated with 100 ml of liquid-
stored semen containing a total of $3 \times 10^9$ spermatozoa.

**Experimental group (fractionated insemination):** The 10 gilts were inseminated with 50 ml liquid-stored semen containing a total of $3 \times 10^9$ spermatozoa, immediately followed by 50 ml of diluent.

**Detection of oestrus:** The gilts were observed for signs of pro-oestrus twice a day, between 6:00–8:00 a.m. and 4:00–6:00 p.m. After expressing signs of pro-oestrus, the gilts were examined for the onset of standing oestrus every 4 hr, by using the back pressure test in the presence of an adult boar, until such time as when the gilts ceased showing standing oestrus. The onset of oestrus was decided to be 2 hr prior to the first time that the gilts showed standing oestrus and the end of oestrus was determined to be 2 hr after the gilts showed the last standing oestrus. The duration of the standing oestrus time was then recorded. Subsequently, the gilts were examined for signs of pro-oestrus twice a day and for standing oestrus every 4 hr to identify the onset of the second oestrus. Ovulation time was expected to occur two-third into the oestrus period as calculated from the onset of oestrus [3]. The insemination time was based upon the length of the first oestrus period. The gilts were inseminated at the second oestrus period 6–8 hr before the anticipated ovulation time. The interval between the two oestrus period and interval from onset of the second oestrus to insemination was recorded.

**Collection and dilution of semen:** The semen was collected from an adult boar, by the glove-hand method. The semen quality of the boar had been previously checked every 1–2 months. After the ejaculate had been collected, it was examined for sperm motility and sperm concentration [7]. The ejaculate was diluted using a Beltsville Thawing Solution diluent (BTS) to provide $3 \times 10^9$ spermatozoa in a total volume of 50 ml. This was used to prepare the non-fractionated dose by adding a further 50 ml of diluent. The seminal plasma in both groups was therefore the same. The diluted liquid semen was kept at 15–200°C over a period no greater than 48 hr. The diluted semen was then warmed to 35–37°C for 15 min and checked for motility before being used. Motility needed to be greater than 60% and viability more than 80% to be acceptable.

**Insemination and collection of semen backflow:** The gilts were artificially inseminated with the diluted liquid semen. Collection and recording of the semen backflow was carried out 4 times: during the first 15 min after AI, 16–30 min after AI, 31–45 min after AI and 46–60 min after AI. The volume and sperm numbers of the backflowing semen were measured and the total number of spermatozoa were calculated.

**Recovery of spermatozoa:** The gilts were anesthetized and laparotomised either 3 or 12 hr after AI (5 gilts per time and per treatment). General anesthesia was induced by azaperone (2 mg/kg) and after 10 min lidocaine (5 mg/gilt) was injected into lumbosacrum and while gilts were given thiopental sodium (10 mg/kg) to induce general anaesthesia. After opening the abdomen the uterine horns were clamped where they join the uterus and ligated, while the broad liga-ment and the cervix were prepared for ovariohysterectomy. The genital organs were taken out, and the tip of the oviducts were ligated. After the supporting ligaments were cleaned, the uterine horn and the oviduct on each side were divided into 6 parts (Fig. 1) and ligated

1. Ampulla part (2/3 of ampulla next to the cranial isthmus part)
2. Cranial isthmus part (1/2 of isthmus next to the ampulla part)
3. Caudal isthmus part (1/2 of isthmus next to the UTJ)
4. Utero-tubal junction (UTJ) (1 cm of the tip of uterine horn and 1 cm of isthmus)
5. Cranial uterine horn part (1/3 of uterine horn next to the UTJ)
6. Caudal uterine horn part (2/3 of uterine horn next to the cranial uterine horn)

The number of old corpora lutea, follicles (8–10 mm in diameter) and new corpora lutea were recorded. The ampulla section was flushed twice with 1.0 ml of BTS, the isthmus parts and the UTJ part were flushed twice with 0.5 ml of BTS in plastic Eppendorf vials. Each uterine horn part was flushed with 20.0 ml of BTS twice into a flask. The total number of spermatozoa in the flushing from each segment were determined using a Neubauer haemocytometer. If spermatozoa were not found inside the counting chamber, the flushing media was gently centrifuged to remove the supernatant and the sediment was recounted.

**Statistical analysis:** Data was analysed using the SPSS program (Statistical Package for the Social Sciences). Differences between groups of gilts for the duration of first oestrus, the interoestrous interval, the duration of the second proestrus, the interval from the onset of second oestrus to insemination, the number of follicles, corpora lutea and the volume of backflow fluid between the groups were tested, using a t-test. The total number of spermatozoa in the semen backflow was converted to a percentage and the differences between the groups were analysed using a t-test. Finally for each of the 6 parts, the distribution data (number of sperma-
FRACTIONATED INSEMINATION IN GILTS

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FRACTIONATED INSEMINATION IN GILTS

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tozoa) in the genital tract was transformed using logarithmic transformation (log10) and the differences between control and treatment group were analysed with a t-test.

The data regarding the total number of spermatozoa from the ampulla to the caudal uterine horn were multiplied by a factor of 6 for scoring purposes. They were then summarized and adjusted logarithmically. Similarly, the data regarding the total number of sperm from the ampulla to the UTJ were multiplied by 4. They were then summarized and adjusted logarithmically. The differences between the two groups were analysed using a t-test.

RESULTS

One gilt from the control group was excluded from the study 12 hr after AI because of excessive semen backflow during insemination. The results from this study were therefore reduced to 19 gilts. The length of the first oestrus, the interoestrous interval, the duration of the second pro-oestrus, the interval from the onset of the second oestrus until insemination, the total number of follicles, the total number of fresh corpora lutea and the total number of old corpora lutea, 3 hr and 12 hr after AI showed no significant differences between the two groups.

The volume of semen backflow and the number of spermatozoa in the semen backflow: The volumes of backflow at 0–15, 0–30, 0–45 and 0–60 min after AI were not significantly different between the two groups (Table 1). Similarly, the percentages of recovered spermatozoa compared with the number inseminated, 0–15, 0–30, 0–45 and 0–60 min after A.I. was not significantly different between the two groups (Table 1). There was, however, a trend that the experimental group had a lower percentage of recovered sperm than the control group (26.6 VS 39.1).

Number and distribution of spermatozoa in the reproductive organs: The number of total spermatozoa in the segments of cranial and caudal uterine horns, UTJ, cranial and caudal isthmus and ampulla were not significantly different between the two groups, either 3 hr or 12 hr after A.I. (Tables 2 and 3).

For both groups combined, the mean number of spermatozoa in the ampulla, the cranial and caudal isthmus and the UTJ were not significantly different 3 and 12 hr after A.I., but there were significantly fewer spermatozoa in the cranial and caudal uterine horns 12 hr after A.I., compared with those at 3 hr (p<0.05) (Table 4).

The score of total sperm: The logarithmic score of total spermatozoa in the ampulla-caudal uterine horn and the ampulla-UTJ were not found to be significantly different (p>0.05).

Table 1. Volume of semen backflow (Mean ± S.D.) and the percentages of sperm out of the number inseminated in the semen backflow (Mean ± S.D.) at 0–15, 0–30, 0–45 and 0–60 min after AI

<table>
<thead>
<tr>
<th>Group</th>
<th>The periods of collected semen backflow (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–15</td>
</tr>
<tr>
<td>Control</td>
<td>Volume (ml)</td>
</tr>
<tr>
<td>(n=9)</td>
<td>Spermatozoa (%)</td>
</tr>
<tr>
<td>Experiment</td>
<td>Volume (ml)</td>
</tr>
<tr>
<td>(n=10)</td>
<td>Spermatozoa (%)</td>
</tr>
</tbody>
</table>

Table 2. Distribution of total spermatozoa (Geometric mean ± S.D.) in the oviducts and uterine horns, 3 hr after AI

<table>
<thead>
<tr>
<th>Group</th>
<th>Ampulla</th>
<th>Cranial isthmus</th>
<th>Caudal isthmus</th>
<th>UTJ</th>
<th>Cranial uterine horn</th>
<th>Caudal uterine horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>55 ± 38</td>
<td>223 ± 22</td>
<td>2.041 ± 3</td>
<td>107,151 ± 1</td>
<td>6,456,542 ± 3</td>
<td>13,803,842 ± 3</td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment</td>
<td>19 ± 60</td>
<td>56 ± 40</td>
<td>1.318 ± 2</td>
<td>147,910 ± 1</td>
<td>14,454,397 ± 17</td>
<td>33,884,415 ± 24</td>
</tr>
<tr>
<td>(n=10)</td>
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<td></td>
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<td></td>
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</tbody>
</table>

Table 3. Distribution of total spermatozoa (Geometric mean ± S.D.) in the oviducts and uterine horns, 12 hr after AI

<table>
<thead>
<tr>
<th>Group</th>
<th>Ampulla</th>
<th>Cranial isthmus</th>
<th>Caudal isthmus</th>
<th>UTJ</th>
<th>Cranial uterine horn</th>
<th>Caudal uterine horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>144 ± 31</td>
<td>123 ± 25</td>
<td>4,365 ± 1</td>
<td>81,283 ± 1</td>
<td>398,107 ± 3</td>
<td>446,683 ± 8</td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment</td>
<td>3 ± 18</td>
<td>15 ± 43</td>
<td>1,513 ± 2</td>
<td>128,824 ± 2</td>
<td>707,945 ± 4</td>
<td>104,712 ± 812</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Semen backflow: The percentage of spermatozoa in the backflow semen was less in the experimental group (fractionated insemination) than in the control group (non-fractionated insemination). It could be that in the experimental group the diluent used for the last part of the insemination was different. It is worth mentioning that the experimental group was given twice the concentration of semen than that given to the controls, in the primary process was responsible. However, the difference was not significant.

There was a trend indicating that the backflow volume in the experimental group was less than in the controls. Again the difference was not significant. It is worth mentioning that the experimental group was given twice the concentration of semen than that given to the controls, in the primary 50 ml dose, which could have provided a greater amount of semen oestrogen. Uterine contractions are directly related to this hormone [1]. More oestrogen in the first 50 ml of semen given to the experimental groups, might have stimulated prostaglandin release and uterine contractions, which can make the semen move rapidly through the uterine horns [13]. This could have caused less backflow in the experimental group than in the controls.

The number of spermatozoa in the reproductive tract: There are many factors affecting the distribution of spermatozoa in the reproductive tract, including semen quality [10], semen volume and the oestrus interval [1]. This is especially so before and after ovulation, when the sperm distribution in the oviduct is very different [4]. From this study, the experimental group, three hours after insemination, tended to have spermatozoa in the UTJ and further up the horn of the uterus, the UTJ and the oviduct of gilts is counted. It has been anticipated that the fractionated semen might have given better results but this was seen not to be the case.

ACKNOWLEDGEMENTS. This study was financially supported by the Research Fund of the Faculty of Veterinary Science, Chulalongkorn University. Semen laboratory and surgical facilities was supported by the Department of Obstetrics Gynaecology and Reproduction and the Farm Animal Hospital of the Faculty of Veterinary Science, Chulalongkorn University, Thailand. The authors would like to thank Associated Professor Dr. Nils Lundehiem for his advise on statistical methods and analysis.

REFERENCES


Table 4. Distribution of total spermatozoa (Geometric mean ± S.D.) for both groups combined in the oviducts and uterine horns between 3 and 12 hr after AI

<table>
<thead>
<tr>
<th>Time after AI (h)</th>
<th>Ampulla</th>
<th>Cranial isthmus</th>
<th>Caudal isthmus</th>
<th>UTJ</th>
<th>Cranial uterine horn</th>
<th>Caudal uterine horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>32 ± 40</td>
<td>112 ± 27</td>
<td>1,633 ± 2</td>
<td>125,689 ± 1</td>
<td>9,653,837 ± 8</td>
<td>21,537,733 ± 10</td>
</tr>
<tr>
<td>12</td>
<td>18 ± 34</td>
<td>39 ± 33</td>
<td>2,409 ± 2</td>
<td>105,365 ± 2</td>
<td>548,529 ± 3</td>
<td>200,909 ± 143</td>
</tr>
</tbody>
</table>

a), b) and c), d) value in columns followed by different letters indicate significant differences (p<0.05).


