**Conception Rate, Farrowing Rate and Litter Size after Intrauterine Insemination with Cryopreserved Boar Semen in Spontaneous and Induced Ovulation Sows**

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**Introduction**

The procedure for artificial insemination (AI) in pigs using cryopreserved semen involves the deposition of a high number of cryopreserved spermatozoa (5-6x10^9 cells) in a large volume of diluent (80-100 ml) into the cervix. Despite inseminating with this high sperm number, low fertility levels are usually obtained (about 20-30% conception and farrowing rates and 2-3 total piglets born per litter lower than those achieved by fresh semen) (1, 2). Therefore, the use of cryopreserved semen in commercial AI swine herds is still limited (3).

Recently, a procedure for non-surgical intrauterine insemination (IUI) in sows, which allows the transcervical deposition of semen into the uterine body, has been developed (4, 5). The fertility rates achieved by IUI with fresh semen using 1x10^9 spermatozoa per dose were comparable to those of conventional AI with 3x10^9 spermatozoa per dose (4). Furthermore, it has been demonstrated that the number of spermatozoa in the sperm reservoir at about 24 h after insemination was not significantly different between IUI and conventional AI (8). However, no fertility data are available using IUI with cryopreserved boar semen. The present study was conducted to evaluate fertility of sows after IUI with cryopreserved semen under field conditions.

**Materials and Methods**

**Animals**: Twenty weaned sows (10 Yorkshire; Y and 10 Landrace; L) with parity number between two and six were used in the present study. Semen collection and cryopreservation: The sperm-rich fractions from four purebred boars (2 L and 2 Y) aged between 1 and 3 yrs old were used. The boars were of proven fertility and held in a commercial herd in Lopburi province, Thailand. The ejaculates were collected with a minimum of a one-week interval using the gloved-hand technique. Fresh semen that had a minimum of 70% sperm motility was used for further processing (6). Shortly after collection, the semen was diluted with isothermal Beltsville thawing solution (BTS; Minitüb, Germany) extender at a ratio of 1:1 (v/v). Diluted semen was placed at 15°C for 2 h and later centrifuged at 800xg for 10 min. The supernatant was discarded and the pellet was re-suspended (about 1 to 2:1) with lactose-egg yolk (LEY) extender (80 ml of 11% lactose solution and 20 ml egg yolk). After further cooling to 5°C over a 90-min period, two parts of semen were mixed with one part of extender III (LEY extender and 9% glycerol with or without 1.5% Equex-STM®). The processed semen (with 1.5% Equex-STM®) was loaded into 0.5 ml straws (Bio-Vet, Z.I. Le Berdoulet, France). The final concentration of sperm frozen was approximately 1x10^9 sperm/ml with 3% glycerol. The straws were sealed with PVC powder before placing in liquid nitrogen (LN2) vapor at 3 cm above the level of LN2 for 20 min and then plunged into LN2.

**Thawing procedure**: Thawing was achieved by immersing the straws in water at 50°C for 12 sec (6). Immediately after thawing, the semen was diluted in 20 ml of BTS extender. The motility of post-thaw spermatozoa was evaluated. A minimum of 35% sperm motility contained in the frozen-thawed (FT) semen was used for insemination.

Detection of estrus and ovulation: Estrus detection was performed twice daily (AM/PM), starting from the day after weaning, by allowing the females to have direct contact with a mature boar and back pressure test. Sows that exhibited a standing reflex were considered to be in estrus. At the onset of estrus, the sows were inseminated at 24 h after hormonal treatment (41 h after 750 IU human chorionic gonadotrophin hormone (hCG; Chorulon; Intervet International B.V., Boxmeer, The Netherlands) at the onset of estrus and IUI was performed once at approximately 41 h after hormonal treatment.

**Fertility parameters**: Conception rate at 24 d after insemination (CR), farrowing rate (FR), number of total pigs born per litter (TB) and number of live born piglets per litter (BA) were evaluated.

**Statistical analyses**: The statistical analyses were carried out using Fisher’s exact test and Student’s
t-test of SAS (SAS Inst. V. 9.1, Cary, NC, USA). The differences with \( P<0.05 \) were considered as statistical significance.

Results and Discussion

On average, the interval from weaning-to-estrus was 4.4±0.8 d (range 3-6) and 3.6±1.4 d (range 1-6) in group I and II, respectively \( (p=0.13) \). The interval between onset of estrus and ovulation (EOI) was 39.8±8.0 h (range 28-52) and 41.5±4.7 h (range 32-44) in group I and II, respectively \( (p=0.58) \).

On average, FT semen used for IUI in both groups had the sperm motility of 38.5% (range 35-45%) and the viability of 58% (range 41-74%). The number of insemination per sow was 2.2±0.4 times (range 2-3) and 1.0 time in group I and II, respectively. The interval between last insemination and ovulation (IOI) was 3.8±1.8 h (range 0-7.5 h) and 3.9±2.5 h (range 2-9 h) in group I and II, respectively \( (p=0.96) \). The number of sows with IOI ≤6 h was 9 sows and 8 sows in group I and II, respectively. All of the sows that farrowed had an IOI of ≤6 h.

The fertility results including CR, FR, TB and BA of the sows in group I and II are presented in Table 1.

**Table 1** Estrus to ovulation interval (EOI), conception rate (CR), farrowing rate (FR), total number of piglets born/litter (TB) and number of piglets born alive/litter (BA) of sows in group I (n=10) and II (n=10) after IUI with FT semen

<table>
<thead>
<tr>
<th>Group</th>
<th>EOI (h)</th>
<th>CR (%)</th>
<th>FR (%)</th>
<th>TB (%)</th>
<th>BA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>39.8±8.0</td>
<td>60</td>
<td>40</td>
<td>9.3±2.2</td>
<td>8.8±3.2</td>
</tr>
<tr>
<td>II</td>
<td>41.5±4.7</td>
<td>20</td>
<td>20</td>
<td>7.5±3.5</td>
<td>6.5±4.9</td>
</tr>
</tbody>
</table>

Means±SD, Different superscripts within column are significantly different \( (p<0.05) \).

No differences in all fertility parameters measured between the groups \( (p=0.17, p=0.63, p=0.48 \) and \( p=0.52 \) for CR, FR, TB and BA, respectively).

TB after IUI with FT semen varied from 6 to 11 piglets/litter in group I and from 5 to 10 piglets/litter in group II. Totally, 37 piglets were born from four sows in group I and 15 piglets were born from two sows in group II.

In the present study, the EOI of group II was in agreement with previous reports \( (7, 8) \). Moreover, the variation of EOI within group was more pronounced in group I than group II sows. These indicated that the application of hCG at the onset of estrus in weaned sows could control/synchronize the time of ovulation. In the present study, low fertility results were obtained in both groups. The reasons might be due to insufficient numbers of spermatozoa per dose in both groups. In addition, it could also be speculated that the number of insemination in group II might be insufficient.

In the present study, approximately 800x10^6 motile spermatozoa per dose (~40% post-thaw motility) were used. This is clearly not to be an adequate number of functional spermatozoa to colonize in the reservoir. It has been demonstrated that low numbers of spermatozoa per insemination dose decrease the number of functional spermatozoa that colonize in the oviductal sperm reservoir and hence decrease fertilization rate and litter size \( (9, 10) \). In addition, it has been reported that one insemination with low numbers of spermatozoa in gilt resulted in fewer embryos, more unfertilized oocytes and lower fertilization rate compared to two inseminations with high numbers of spermatozoa even though precise time relative to ovulation has been practiced \( (11) \). In the present study, 4 of 9 sows in group I and 6 of 8 sows in group II that were inseminated at the optimal time in relation to ovulation \( (IOI=6 \) h) were not pregnant. We suggested that the number of FT spermatozoa per dose as well as the number of insemination during standing estrus should be increased in order to improve fertility results. Interestingly, group II sows had about 2 piglets/liter lower than group I sows, although the results were not significant. It has been indicated that the injection of hCG at the onset of estrus influenced embryonic survival and fetal developments in pregnant gilts \( (12) \). The injection of hCG at the onset of estrus resulted in an increase of progesterone at 48 h post estrus and shortened the interval from onset of estrus to LH surge, which is not good for the embryonic survival in Meishan pig \( (12) \).

In conclusion, IUI using 800x10^6 motile FT spermatozoa is not sufficient to provide acceptable fertility results in both spontaneously ovulating and induced ovulating sows under field conditions.

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References