FULL PAPER *Theriogenology*

Conception Rate and Litter Size in Multiparous Sows after Intrauterine Insemination Using Frozen-Thawed Boar Semen in a Commercial Swine Herd in Thailand

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ABSTRACT. The aim of the present study was to determine the conception rate and litter size in sows after fixed time intra-uterine insemination using frozen-thawed boar semen in a commercial swine herd in Thailand. Sixty-nine Landrace multiparous sows were randomly allocated into two groups, including control (n=36) and treatment (n=33). The control sows were inseminated with extended fresh semen (3×10^9 motile sperm/dose, 100 m*l*) at 24, 36 and 48 hr after the onset of estrus. The treatment sows were inseminated with frozen-thawed semen (2×10^9 motile sperm/dose, 20 m*l*) at 24 and 36 hr after induction of ovulation by human chorionic gonadotropin. All inseminations were carried out by using an intra-uterine insemination technique. The time of ovulation was determined by using transrectal real-time B-mode ultrasonography. The conception rate, farrowing rate, total number of piglets born/litter (TB) and number of piglets born alive/litter (BA) were evaluated. The sows inseminated with extended fresh semen yield a higher TB (10.8 versus 9.0 piglets/l, P=0.015) and tended to have a higher conception rate (88.9% versus 75.8%, P=0.150) than sows inseminated with frozen-thawed semen. In conclusion, insemination using frozen-thawed boar semen can be practiced with convinced fertility under field conditions by fixed-time intrauterine insemination with 2×10^9 sperm/ dose of 20 m*l* at 24 and 36 hr after the onset of estrus.

KEY WORDS: conception, frozen semen, litter size, reproduction, swine

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Nowadays, artificial insemination has been used in the swine industry worldwide. Most of the artificial insemination in pigs was performed by using extended fresh semen, while the use of frozen-thawed semen accounts for less than 1% of the artificial insemination worldwide [7]. In general, the extended fresh semen can be used immediately after being extended in the semen extender or storage at 18°C for 3–5 days depending on the type of semen extender. The main limitation of the extended fresh semen is the short timing of semen storage. Therefore, the semen cannot be transported for a long distance. This limits the chance of distributing a good genetic resource across countries. Furthermore, because the age of the boar is generally limited to 3–5 years, the use of semen from superior genetic boars is not efficient. The development of frozen semen is, therefore, established to keep the semen of superior genetic boars in the swine industry. Furthermore, the advantage of using frozen-thawed boar semen in the swine industry includes the preservation of good genetic boars that have passed the progeny test, the distribution of genetic material more rapidly than fresh semen, the increased possibility to transport semen across countries and the reduction in disease transmission among herds. These make the insemination management easier. The success of cryopreservation technology initiated after the discovery of glycerol as a cryo-protectant. However, the process of producing frozen-thawed boar semen is much more complicated than the extended fresh semen. Moreover, artificial insemination using frozen-thawed boar semen requires a higher number of sperm than the conventional artificial insemination. This is due to the fact that the sperm viability of the frozen-thawed semen is relatively low [2]. The loss of sperm was mainly due to cold shock. During the past decade, researchers are still investigating the optimal technique to increase the conception rate and litter size of pigs using frozen-thawed boar semen [3, 4, 7, 8, 14, 22].

In practice, the intra-cervical artificial insemination in pigs with a high number (5 to 6×10^9 per dose) of frozen-thawed sperm often results in a decrease of 20 to 30% in farrowing rates and 2 to 3 in number of total piglets born per litter (TB) compared to extended fresh semen [8, 9]. Therefore, the use of frozen-thawed boar semen for artificial insemination in commercial swine herds is limited [3, 7]. In an attempt to obtain satisfactory fertility results using low numbers of sperm per dose, non-surgical deep insemination procedures to deposit semen into the uterine body (intra-uterine insemination) have been developed [15]. Using frozen-thawed semen, insemination outside of the optimal insemination-to-ovulation period (i.e., 4–6 hr before ovulation) significantly decreased the farrowing rate and TB [14]. In addition, intrauterine

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insemination is an insemination technique that has been developed to reduce the number of sperm per insemination dose [19]. It has been demonstrated that an approximately 3-fold reduction in the number of sperm for extended fresh semen can be used with intrauterine insemination without affecting the farrowing rate and TB, compared to conventional artificial insemination [19]. To our knowledge, no fertility data are available using fixed-time intrauterine insemination with frozen-thawed boar semen under field conditions in Thailand. The present study was performed to evaluate fertility results after fixed time intrauterine insemination with reduced numbers of frozen-thawed boar sperm in induced ovulating weaned sows.

MATERIALS AND METHODS

Animals: The inseminations were conducted between November and December of 2008 in a commercial swine herd in the middle part of Thailand. In total, 69 Landrace multiparous sows (parity numbers 2 to 6) with weaning-to-estrus intervals of 3 to 6 days were included in the experiment. The sows were allocated individual pens adjacent to adult boars and fed with a corn-soybean-fish based commercial feed, containing approximately 15.0% crude protein as a feed-basis, twice a day. Water was provided *ad libitum* via a water nipple.

Semen collection and cryopreservation: The sperm-rich fraction of ejaculates was collected from 10 Yorkshire boars aged between 1 and 3 years. The boars were housed in an artificial insemination center for the herds and were routinely used as semen donors for artificial insemination in the herds. The percentage of sperm motility was evaluated subjectively under a light microscope at 400 × magnification. Only ejaculates with ≥70% motility were cryopreserved, using the straw freezing procedure [5]. Briefly, semen was extended (1:1, v/v) in a semen extender (Modena™, Swine Genetics International, Ltd., Cambridge, IA, U.S.A.) and cooled down to 15°C for 2 hr. After centrifugation at 800 × g for 10 min, the pellets were diluted in lactose-egg yolk (LEY) extender (80 m*l* of 11.0% lactose solution and 20 m*l* egg yolk) to a concentration of 1.5×10^9 cells per m*l*. After further cooling to 5°C over a 90-min period, the diluted sperm were resuspended with extender III (LEY extender, 9.0% glycerol and 1.5% Equex STM Paste; Nova Chemical Sales Inc., Scituate, MA, U.S.A.) to a final concentration of 1×10^9 sperm per m*l*. The processed sperm were packed into 0.5 PVC-french straws (Bio-Vet, Z.I. Le Berdoulet, France) and frozen by placing in liquid nitrogen (LN_2) vapor approximately 3 cm above the level of LN_2 for 20 min. The frozen doses were plunged into LN_2 until thawed.

Thawing procedure and evaluation of the post-thaw sperm quality: Thawing was achieved by immersing the straws in 50°C-water for 12 sec. Immediately after thawing, the semen was diluted (1:4) with Modena™ extender. The extended, thawed semen was incubated in a 38°C water bath for 30 min, and the post-thaw sperm quality was evaluated. The subjective sperm motility was assessed using a bright-field microscope (400×). Sperm concentration was evaluated using a Bürker hemocytometer (Boeco, Hamburg, Germany), and the viability of sperm was determined by eosin-nigrosin staining [2]. The frozen-thawed semen used for insemination must have a sperm motility of at least 40.0%. Before insemination, the qualified frozen-thawed semen was diluted with 20 m*l* of Modena™ extender. The diluted semen was incubated at 38°C for 10 min and checked for the post-thaw motility before insemination.

Detection of estrus and ovulation: Estrus detection was performed twice daily (8 AM/ 4 PM), starting from the day after weaning, by allowing the females to have direct contact with a mature boar and the back pressure test. Sows that exhibited a standing reflex were considered to be in estrus. For the sows inseminated with frozen-thawed semen, the onset of estrus was decided to be 4 or 8 hr before the first time when the standing reflex was detected. The occurrence of ovulation was investigated every 8 hr after estrus by using real-time B-mode ultrasonography (HS-2000, Honda Electronics Co., Ltd., Toyohashi, Japan). Briefly, after the removal of feces from the rectum, a 5-MHz multiple angle transducer was carefully introduced into the rectum. The appearance of follicles on the ovaries was observed. The ovulation time was defined as 4 hr before the first time when no follicles were visible [21].

Intra-uterine insemination: The control sows were inseminated with extended fresh semen (3×10^9 motile sperm/dose, 100 m*l*) at 24, 36 and 48 hr after the onset of estrus, and the treatment sows were inseminated with frozen-thawed semen (2×10^9 motile sperm/dose, 20 m*l*) at 24 and 36 hr after the induction of ovulation. Ovulation induction was performed by injecting 750 IU of human chorionic gonadotropin (hCG) (Chorulon®, Intervet/Schering Plough, Boxmeer, The Netherlands) intramuscularly at first detected estrus. The inseminations were carried out in gestation crates. Briefly, the intrauterine insemination device (Deep goldenpig™ catheter, IMV Technologies, L'Aigle, France) was inserted through the vagina into the cervix. The inner tube extended 200 mm beyond the tip of the outer catheter lying in the uterine body or the posterior uterine horn. Then, extended semen was inseminated. For the frozen semen group, a 2 m*l* Modena™ extender was flushed to force the remaining sperm from the device.

Management, pregnancy detection and reproductive data: The sows were carefully detecting estrus symptoms twice a day from about 17 days after insemination onwards using a mature boar. The sows were kept in individual crates from insemination until 105 days of gestation before moving them to the farrowing pen at about one week before farrowing. The sows had free access to water nipples. Feed was provided twice a day. At about 24 days after insemination, a 24-day non-return rate was recorded. Conception was also determined by trans-rectal real-time B-mode ultrasonography (HS-2000, Honda Electronics Co., Ltd.). At farrowing, the TB, the number of piglets born alive per litter (BA), the number of stillborn piglets per litter and the number of mummified fetuses per litter were evaluated. Additionally, the one-year (i.e., from November 2007 to October 2008) reproductive performance data (i.e., litter size at birth, conception rate and farrowing rate) of multiparous sows (party numbers 2–7) were also collected from the database of the

Table 1. Reproductive data of sows inseminated using intrauterine insemination with extended fresh semen (control) compared with frozen-thawed semen (mean ± SD)

Parameters	Group	
	Control (n=36)	Frozen-thawed semen (n=33)
Parity number	4.8 ± 1.7 (2–8)	4.6 ± 2.0 (2–8)
Body condition score	2.5 ± 0.4 (2–3)	2.5 ± 0.5 (2–3)
Weaning-to-estrus interval (days)	3.6 ± 1.2 (2–6)	3.6 ± 0.6 (2–4)
Subjective motility (%)	NA	47.6 ± 3.7 (40–50)
Estrus-to-ovulation (hr)	NA	43.0 ± 4.9 (38.0–50.0)
Hormone treatment to ovulation (hr)	NA	36.4 ± 3.7 (33.0–42.5)
Last insemination to ovulation (hr)	NA	4.7 ± 5.7 (1.5–17.5)

NA=not available; number in parenthesis is the range of the data.

Table 2. Reproductive performance of multiparous sows (parity numbers 2–7) during one year before the experimental period (n=1,980) and in sows inseminated with extended fresh semen (control, n=36) compared with frozen-thawed semen (n=33)

Parameters	Reproductive performance[1]	Experimental group	
		Control	Frozen-thawed semen
Conception rate (%)	95.7	88.9[a] (32/36)[2]	75.8[a] (25/33)
Farrowing rate (%)	90.9	61.1[a] (22/36)	66.8[a] (22/33)
Total number of piglets born/ litter	11.5 ± 2.5	10.8 ± 2.2[a]	9.0 ± 2.5[b]
Number of piglets born alive/ litter	10.4 ± 2.1	9.5 ± 3.4[a]	8.5 ± 2.3[a]
Stillborn piglets (%)	6.8	3.9[a]	4.3[a]
Mummified fetuses (%)	1.5	10.9[a]	0[a]

a,b: Different superscripts within a row differ significantly ($P<0.05$); [1]One year reproductive performance; [2]number of observations is displayed in parentheses.

herd (n=1,980 observations).

Statistical analyses: The statistical analyses were carried out by using SAS (SAS version 9.0, Cary, NC, U.S.A.). Descriptive statistics including the means, standard deviations (SD) and ranges of all reproductive performance data were calculated. The conception rate and farrowing rate were analyzed by Chi-square tests. The TB and BA were analyzed by one-way ANOVA. Groups of sows (control and treatment) were included as independent variables. Least-squared means were obtained and compared by using the Student's *t*-test. A $P<0.05$ was considered as statistically significant.

RESULTS

Estrus and ovulation: On average, individual motility of the frozen-thawed semen used for intrauterine insemination was 47.6% (range 40 to 50%). The interval from the onset of estrus to ovulation (EOI) was 43.0 ± 4.9 hr (range 38 to 50 hr), and the interval from hCG injection to ovulation (HOI) was 36.4 ± 3.7 hr (range 33 to 45 hr). The interval between the latest insemination and ovulation (IOI) was 4.7 ± 5.7 hr (range 1.5 to 17.5 hr) (Table 1).

Conception rate: On average, the conception and farrowing rates of sows during one year before the experiment were 95.7 and 90.9%, respectively. During the experiment, the conception rate of sows inseminated with extended fresh semen (control) and frozen-thawed semen was 88.9 and 75.8% ($P=0.150$), respectively. The farrowing rate of sows between the control (61.1%) and the frozen-thawed semen (68.8%) groups did not differ significantly (Table 2). In the control group, four out of 36 sows (11.1%) were not pregnant, and eight out of 33 sows (24.2%) in the frozen-thawed semen group were not pregnant. In the frozen-thawed semen group, the interval from last insemination-to-ovulation was 4.3 ± 5.4 hr (range 1.5 to 17.5 hr) in the pregnant sows, and this interval was 6.1 ± 7.6 hr (range 2.0 to 17.5 hr) in the non-pregnant sows ($P=0.591$). Likewise, the post-thawed sperm motility (48% and 46%), weaning-to-estrus interval (3.6 and 3.7 days), estrus-to-ovulation interval (43.4 and 41.1 hr) and hormonal treatment-to-ovulation interval (36.6 and 35.6 hr) did not differ significantly between pregnant and non-pregnant sows, respectively ($P>0.05$). Nevertheless, for the sows inseminated with frozen-thawed semen, the conception rate was 81% in those that ovulated within 6 hr after insemination, while it was 67% in those that ovulated more than 6 hr after insemination.

Litter size: Litter size at birth of all inseminated sows is presented in Table 2. The TB was significantly higher in the sows inseminated with extended fresh semen compared to sows inseminated with frozen-thawed semen (10.8 and 9.0 piglets/*l*, $P=0.015$). Nevertheless, the BA was not significantly different between sows inseminated with extended fresh semen and those inseminated with frozen-thawed semen (9.5 and 8.5 piglets/*l*, $P=0.283$).

DISCUSSION

The present study demonstrated that fixed-time intra-

uterine insemination can be applied for frozen-thawed boar semen using a low volume and sperm number in sows under field conditions. The fixed-time intrauterine insemination with frozen-thawed boar semen resulted in an acceptable fertility and fecundity (i.e., 75.8% conception rate, 9.0 TB and 8.5 BA). Nevertheless, this is still lower than that inseminated by extended fresh semen within the same herd (i.e., 88.9% conception rate, 10.8 TB and 9.5 BA). However, the present study obtained a better fertility outcome from the frozen-thawed boar semen than earlier studies in Thailand [3, 4]. In Spain, Roca *et al.* [14] found that deep intrauterine insemination with 1×10^9 sperm/dose of the frozen-thawed boar semen resulted in a 70% conception rate and 9.25 TB. Recently, a retrospective study based on field fertility data of frozen-thawed boar semen in the U.S.A. found that the farrowing rate has been improved from 62.0 to 89.5%, and the TB was improved from 9.7 to 14.1 piglets/l after implementing frozen-thawed boar semen in a swine nucleus herd for over four years (2007–2011) [7]. This indicates the importance of proper management and the skill of using frozen-thawed boar semen on fertility results under field conditions. Therefore, a good fertility could be obtained from frozen-thawed boar semen when the practitioners are familiar with a complex semen-freezing process and proper management practice.

In the present study, intrauterine insemination was chosen for insemination instead of a conventional artificial insemination technique, because the conventional artificial insemination requires a higher number of sperm per dose (i.e., 3×10^9 sperm/dose), and the sperm loss in the female reproductive tract was relatively high [15, 16]. On the other hand, for deep intrauterine insemination, the distribution of sperm in the female reproductive tract may not be enough in some sows, if too low of a number of sperm is used ($<0.15 \times 10^9$ sperm/dose). Furthermore, it has been demonstrated that the progesterone receptor in the sperm reservoir of sows inseminated with deep intrauterine insemination with a low number of sperm differs from that inseminated by a conventional artificial insemination technique [18]. This may also influence fertility and/or fecundity of the sows. Therefore, the intrauterine insemination technique is an effective and practical method for frozen-thawed boar semen under field conditions.

The present study found that the interval from the onset of estrus-to-ovulation of sows averages 43.0 hr. This is consistent with a previous study (i.e., 41.4 hr) [22]. Nevertheless, the variation of the ovulation time in the present study (i.e., 38–50 hr) was less than that reported in the previous study (i.e., 22–72 hr) [22]. The reason is because the ovulation induction protocol uses hCG [21]. It has been demonstrated that frozen-thawed sperm can survive for only a short period in the female reproductive tract compared to fresh semen [20]. The timing of ovulation in relation to insemination is, therefore, extremely important. The most optimal period of insemination for frozen-thawed boar sperm is 4–6 hr before ovulation [1, 20]. Insemination at too long of a time prior to ovulation leads to an increase in the proportion of unfertilized oocytes and/or early embryonic loss and low litter size at birth [14]. Therefore, insemination by using frozen-thawed boar semen should be conducted with a control ovulation protocol.

In the present study, a fixed time intrauterine insemination with frozen-thawed sperm at 24 and 36 hr after hCG treatment (2×10^9 sperm/dose) resulted in a 75.8% conception rate and 66.8% farrowing rate. This protocol is better and more practical than an inseminating protocol previously reported [3, 14, 22]. For instance, Wongtawan *et al.* [22] had a 40.0% conception rate after applying deep intrauterine insemination using frozen-thawed semen with 0.40×10^9 sperm. In addition, Roca *et al.* [14] achieved a 72.5% conception rate after deep intrauterine insemination using frozen-thawed semen with 1×10^9 sperm. In addition, the optimal number of frozen-thawed sperm per dose is important for fertility results. In pigs, the number of sperm that accumulate in the sperm reservoir influences the fertilization rate [17]. Therefore, an optimal number of sperm ensures the fertility outcome. However, the optimal number of sperm for frozen-thawed boar semen has not been precisely determined. Recently, Chanapiwat *et al.* [6] found that approximately 300,000 to 400,000 sperm were found in the sperm reservoir at 12 hr after insemination by either extended fresh semen or frozen-thawed semen. Furthermore, the supplementation of seminal plasma in the thawing medium resulted in a higher number of sperm detected in the sperm reservoir at 12 hr after insemination [6]. The reasons might be due to the suppression of leucocytes in the female reproductive tract and the reduction in cryo-injury of the frozen-thawed boar sperm of seminal plasma. Therefore, both the timing of insemination and the number of sperm per dose are important. Based on the present results, the fixed-time insemination at 24 and 36 hr after hCG treatment using 2×10^9 sperm/dose can be practically recommended for frozen-thawed boar semen.

In addition to the number of sperm, the semen volume is also an important factor in determining the success of artificial insemination. This is because the semen volume stimulates the dilation of the uterus after artificial insemination. The optimal semen volume enhances the uterine contraction by stimulating the release of oxytocin [12]. This results in an increase in uterine contraction. The contraction of the uterus is the first physiological response of the female to the sperm [11]. The uterine contraction occurs from many factors, e.g., the stimulation of the cervix by the artificial insemination catheter [10, 11]. Furthermore, the supplementation of seminal plasma has been shown to enhance the viability of the frozen-thawed boar sperm and increase the conception rate [13]. These factors may affect the conception rate and litter size in sows. Based on our result, a semen volume of at least 20 m*l* per dose is recommended for fixed-time intrauterine insemination using frozen-thawed semen. Additional substances that can enhance sperm transport should be further investigated.

In the present study, decreases in farrowing rate in both control (61.1%) and treatment (66.8%) groups were found in comparison with the averaged reproductive performance of the herd (90.9%) (Table 2). The reason is not known, but it might be possibly caused by one or combination of factors, such as seasonal influences, fluctuation of feed quality (e.g., quality of raw materials and contamination of mycotoxin), quality of stock persons and health problems. Since the

present study was performed under field conditions, the reproductive performances of the sows can vary depending on many factors. Therefore, the conception rate was determined shortly after insemination. It was found that the conception rate of the sows in the control group (88.9%) was comparable to the average farrowing rate of the herd (Table 2). Although it is important to evaluate the cause of low farrowing rate of the sows in both groups, it is rather difficult to discuss precisely on the reproductive performances without enough information concerning all the factors involved. Nevertheless, the sows in the present study was inseminated during winter season (i.e., November and December) and was expected to farrow in hot season (i.e., March and April). In general, the average outdoor temperature in Thailand is hottest in April. Therefore, the pregnant sows in both groups might have suffered from moderate to severe heat stress during hot season and, partially, caused reproductive failures.

In conclusion, the present study revealed that artificial insemination using frozen-thawed boar semen can be established in a commercial swine herd. Intrauterine insemination is an insemination technique that is suitable and practical for frozen-thawed boar semen. Using frozen-thawed boar semen, a volume of 20 m*l* containing 2×10^9 sperm/ dose is recommended for fixed-time intrauterine insemination. Moreover, a hormonal induction of ovulation should be done in the female at the onset of standing estrus to ensure an optimal time of ovulation. This is a model for increasing the efficacy of utilizing boar semen in commercial herds.

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