Original Article

Distribution of Spermatozoa in the Reproductive Tracts of Sows after Intra-uterine Insemination Using Frozen-thawed Boar Semen

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Abstract

The aims of the present study were to determine the number of spermatozoa in the reproductive tract of sows after intra-uterine insemination (IUI) with frozen-thawed (FT) boar semen and to investigate the influence of adding seminal plasma in the thawing medium on the sperm transport in the female reproductive tract. Fourteen multiparous sows were divided into 3 groups: group I (n= 5), insemination with 80 ml of extended fresh semen, group II (n= 4), insemination with 40 ml of FT semen diluted with Modena™ extender and group III (n= 5), insemination with 40 ml of FT semen diluted with combination of Modena™ and seminal plasma. All the sows were inseminated once with $2x10^9$ motile spermatozoa at 36.1±2.8 hours after human Chorionic Gonadotropin (hCG) administration. The reproductive tract was collected from the sows at 12.4±2.2 hours after insemination and was divided into 5 parts, i.e. ampulla, cranial isthmus, caudal isthmus, utero-tubal junction (UTJ) and cranial uterine horn. All parts of the reproductive tract were flushed by phosphate buffer solution and the number of spermatozoa was determined using a Neubauer hemocytometer. Spermatozoa were found in all parts of the sows' reproductive tracts at 12.4 hr after IUI using either fresh (group I) or FT boar semen (group II and III). Most of the spermatozoa were found in the UTJ (47.8%, 47.8% and 38.1% in group I, II and III, respectively, $p > 0.05$) and the caudal isthmus (27.2%, 26.4% and 28.1% in group I, II and III, respectively, $p > 0.05$). The total number of recovered spermatozoa in group III (409,420 sperm) tended to be higher than group I (286,750 sperm, $p = 0.109$) and II (287,000 sperm, $p = 0.139$). Number of spermatozoa in the cranial isthmus in group III (76,400 sperm) tended to be higher than that in group I (35,750 sperm) and II (33,333 sperm) ($p > 0.05$). It can be concluded that at 12.4 hr after IUI using FT semen, spermatozoa were found in all parts of the reproductive tracts of the sows similar to that using extended fresh semen. Supplementation of seminal plasma in the thawing medium of FT boar semen tended to increase the transportation of spermatozoa toward the cranial isthmus. This might be due to the suppression of PMN cells in the female reproductive tract and the reduction in cryoinjury of the FT boar sperm caused by seminal plasma.

Keywords: frozen semen, intra-uterine insemination, reproductive tract, sow

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บทคัดย่อ

การกระจายตัวของอสุจิในท่อทางเดินระบบสืบพันธุ์แม่สุกรภายหลังการผสมเทียมแบบสอดท่อเข้ามดลูกด้วยน้ำเชื้อพ่อสุกรแช่แข็ง

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วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อตรวจประเมินจำนวนเซลล์อสุจิในท่อทางเดินระบบสืบพันธุ์แม่สุกรภายหลังการผสมเทียมแบบสอดท่อเข้ามดลูก (IUI) ด้วยน้ำเชื้อพ่อสุกรที่ผ่านการแช่แข็งและทำละลาย (FT) และเพื่อศึกษาผลของการเสริมเซมินอลพลาสม่าในน้ำยาทำละลายต่อการขนส่งอสุจิในท่อทางเดินระบบสืบพันธุ์เพศเมีย แม่สุกรนางจำนวน 14 ตัว ถูกแบ่งเป็น 3 กลุ่ม ได้แก่ กลุ่มที่ 1 (5 ตัว) ผสมเทียมด้วยน้ำเชื้อสดปริมาตร 80 มล. กลุ่มที่ 2 (4 ตัว) ผสมเทียมด้วยน้ำเชื้อแช่แข็งที่ทำละลายด้วยสารละลาย Modena™ ปริมาตร 40 มล. และกลุ่มที่ 3 (5 ตัว) ผสมเทียมด้วยน้ำเชื้อแช่แข็งที่ทำละลายด้วยสารละลาย Modena™ ผสมกับเซมินอลพลาสมาในสัดส่วน 1:1 โดยปริมาตรปริมาตร 40 มล. แม่สุกรทุกตัวถูกผสมเทียมเพียงครั้งเดียวด้วยน้ำเชื้อที่มีจำนวนอสุจิ $2x10^9$ ตัว ที่เวลา 36.1±2.8 ชั่วโมง หลังการเหนี่ยวนำการตกไข่ด้วยฮอร์โมน hCG ทำการเก็บท่อทางเดินระบบสืบพันธุ์แม่สุกรภายหลังการผสมเทียม 12.4±2.2 ชั่วโมง และแบ่งเป็น 5 ส่วน ได้แก่ แอมพูลล่า อิสมัสส่วนต้น อิสมัสส่วนท้าย ยูทีเจ และ ส่วนต้นของปีกมดลูก แต่ละส่วนถูกนำมาชะล้างท่อด้านในด้วยสารละลายพีบีเอสแล้วทำการตรวจนับเซลล์อสุจิที่ล้างออกมาด้วยเครื่องนับเม็ดเลือด (hemocytometer) อสุจิถูกตรวจพบในทุกส่วนของท่อทางเดินระบบสืบพันธุ์แม่สุกรภายหลังการผสมเทียม 12.4 ชั่วโมง ทั้งในกลุ่มที่ผสมเทียมด้วยน้ำเชื้อสด (กลุ่มที่ 1) และกลุ่มที่ผสมเทียมด้วยน้ำเชื้อแช่แข็ง (กลุ่มที่ 2 และ 3) อสุจิส่วนใหญ่ถูกพบในท่อยูทีเจ (47.8% 47.8% และ 38.1% ในกลุ่มที่ 1 2 และ 3 ตามลำดับ $p> 0.05$) จำนวนอสุจิที่ตรวจพบทั้งหมดในกลุ่มที่ 3 (409,420 เซลล์) มีแนวโน้มสูงกว่า กลุ่มที่ 1 (286,750 เซลล์ $p= 0.109$) และ กลุ่มที่ 2 (287,000 เซลล์ $p= 0.139$) จำนวนอสุจิในท่ออิสมัสส่วนต้นในกลุ่มที่ 3 (76,400 เซลล์) มีแนวโน้มสูงกว่ากลุ่มที่ 1 (35,750 เซลล์) และกลุ่มที่ 2 (33,333 เซลล์) ($p> 0.05$) การศึกษาครั้งนี้สรุปว่าเซลล์อสุจิถูกตรวจพบในทุกส่วนของท่อทางเดินระบบสืบพันธุ์แม่สุกรที่เวลา 12.4 ชั่วโมง ภายหลังการผสมเทียมแบบสอดท่อเข้ามดลูกด้วยน้ำเชื้อพ่อสุกรแช่แข็ง การเสริมเซมินอลพลาสมาในสารทำละลายน้ำเชื้อแช่แข็งมีแนวโน้มเพิ่มการขนส่งอสุจิไปจนถึงท่ออิสมัสส่วนต้นได้มากขึ้น สาเหตุอาจเกิดจากเซมินอลพลาสมาสามารถกดการทำงานของเซลล์ที่ทำหน้าที่เก็บกินสิ่งแปลกปลอมภายในเยื่อบุโพรงมดลูกของสุกร และ ช่วยลดการเสียหายจากการถูกแช่แข็งของน้ำเชื้อพ่อสุกรได้

คำสำคัญ: น้ำเชื้อแช่แข็ง การผสมเทียมแบบสอดท่อเข้ามดลูก ท่อทางเดินระบบสืบพันธุ์ สุกรนาง

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Introduction

Frozen-thawed (FT) boar semen has a lower viability and shorter lifespan than extended fresh semen due to a significant increase in sperm damage occurring during cooling and freezing processes (Waberski et al., 1994; Bailey et al., 2008). To achieve an acceptable fertility after artificial insemination (AI) with FT boar semen, an optimal number of spermatozoa per dose, a timing of insemination in relation to ovulation, as well as an insemination technique are needed to be carefully determined. In general, the FT boar spermatozoa can survive for only 4-6 hours after AI (Waberski et al., 1994). Therefore, the timing of ovulation should be precisely controlled (Wongkaweewit et al., 2012). In addition, an alternative insemination technique, i.e. intrauterine insemination (IUI) has been developed (Watson and Behan, 2002; Sumransap et al., 2007). Using IUI, the semen volume and the number of spermatozoa per dose can be reduced. Recently, Buranaamnuay et al. (2010[a]) demonstrated that multiple doses (2-3 doses) of IUI with $1.0x10^3$ spermatozoa/dose resulted in 65% farrowing rate and 9.4 total number of piglets born per litter in multiparous sows induced the ovulation by 750 IU of human chrorionic gonadotrophin (hCG).

Earlier studies have demonstrated that seminal plasma in the boar semen is associated with the inflammatory response in the female genital tract after AI (Rozeboom et al., 2000), an increase in the uterine contraction and a reduction of the interval from onset of oestrus to ovulation (Waberski et al., 1995). The expression of some cytokines in the porcine endometrium (e.g. interleukin (IL)-1β, IL-6, IL-10, granulocyte-macrophage colony stimulating factor and transforming growth factor (TGF)- β1) as well as the polymorphonuclear neutrophilic granulocytes (PMNs) can be moderated by either artificial

insemination or the inseminated components (Jiwakanon et al., 2011). For instance, cytokine TGF-β1 was suppressed by the semen extender (i.e. BTS) and insemination with seminal plasma alone suppressed PMNs cell infiltration in the endometrium (Jiwakanon et al., 2011). In addition, the suppressive effect of seminal plasma *in vitro* was dose-dependent (Rozeboom et al., 2001). A recent study has demonstrated that the boar seminal plasma contains TGF- β1 and IL-10 with high individual variation and the first 10 ml of the sperm-rich fraction of the ejaculated semen contained the highest amount of TGF-β1 (Jiwakanon and Dalin, 2012). A supplementation of 10% v/v seminal plasma or 50% v/v seminal plasma to the thawing medium improves the FT sperm motility, sperm viability and pregnancy rate (Garcia et al., 2009; Okazaki et al., 2009; Kaeoket et al., 2011). On the other hand, Abad et al. (2007[a]) demonstrated that 10% v/v seminal plasma supplementation for conventional AI with FT boar semen failed to improve both litter size and farrowing rate in multiparous sows. Furthermore, the number of spermatozoa in the sperm reservoir at 8 hr after AI was lower in gilts inseminated with FT semen compared to fresh semen, which leads to the inability to form an effective sperm reservoir, and seminal plasma also did not affect the establishment of sperm reservoir after AI with FT semen (Abad et al., 2007[b]). Tummaruk and Tienthai (2010) have demonstrated that IUI with a reduced number of extended fresh sperm per dose resulted in the same amount of sperm deposited within the crypt of the utero-tubal junction (UTJ) and the caudal isthmus compared to conventional AI. However, studies on the formation of sperm reservoir as well as the sperm distribution in the reproductive tract of sows after IUI with FT semen in relation to the seminal plasma supplementation have not been done.

As for extended fresh semen, the sperm distribution in the female reproductive tract has been comprehensively investigated in both gilts and sows using conventional AI (Hunter, 1981; Kunavongkrit et al., 2003), IUI (Sumransap et al., 2007) or deep intra-uterine insemination techniques (Tummaruk et al., 2007). The mechanism of sperm transport from the site of semen deposition to the sperm reservoir is complicated and is regulated by many factors (Langendijk et al., 2002; Mburu et al., 1996; Tummaruk et al., 2010; Tummaruk and Tienthai, 2011). In pig, the UTJ and caudal isthmus are sperm reservoir and act as a selective barrier of the inseminated sperm and also serve as a sperm trapper before facilitating towards the site of fertilization (Hunter, 1981; Waberski et al., 2006). The aims of the present study were to determine the number of spermatozoa in the reproductive tract of sows after IUI with FT boar semen and to investigate the influence of adding seminal plasma in the thawing medium on the sperm transport in the female reproductive tract.

Materials and Methods

Animals: Fourteen crossbred Landrace×Yorkshire multiparous sows were purchased from a commercial swine herd on the day of weaning and were brought to the Department of Obstetrics, Gynaecology and Reproduction, Nakhon Pathom province, Thailand. The sows were kept in individual crate and were fed twice a day (approximately 4.0-5.0 kg per day) on a commercial feed (Starfeed176® BP Feed Co. Ltd, Saraburi, Thailand) containing 15.0% protein, 2.0% fat and 10.0% fiber. Water was provided *ad libitum* via water nipples. The sows were carefully detected for the onset of standing oestrus twice a day (am/pm) after weaning. The sows were divided into 3 groups: group I (n= 5), insemination with 80 ml of extended fresh semen, group II (n= 4), insemination with 40 ml of FT semen diluted with Modena™ extender and group III (n= 5), insemination with 40 ml of FT semen diluted with a combination of Modena™ extender and seminal plasma (1:1 v/v). The seminal plasma was collected from the supernatant of the semen extended in Modena™. The supernatant were centrifuged twice at 800x g for 10 min, collected into 50 ml centrifuge tube and stored at -20°C until use (Kaeoket et al., 2011; Rienprayoon et al., 2012). All the sows were inseminated with $2x10^9$ motile spermatozoa at 36.1±2.8 hours after hCG (Chorulon®, Intervet Ltd., Boxmeer, The Netherlands) administration.

***Detection of oestrus and ovulation*:** Oestrus detection was performed by using the back pressure test on the day after weaning (Day 1) onwards. The sows were allowed to have a fence line nose-to-nose contact with mature boars twice a day. Sows with a standing reflex were considered in oestrus. At the onset of standing oestrus, 750 IU of hCG was administrated intramuscularly to the sows to induce ovulation (Wongkaweewit et al., 2012). The time of ovulation was determined by monitoring the follicles every 8 hour using transrectal real time B-mode ultrasonography with 5-MHz linear transducer (Honda Electronics Co, Ltd, Tokyo, Japan). The first ultrasound scanning was performed at about 10-12 hour after the sows exhibited standing oestrus. The appearance of the follicles (anechoic structure with diameter of 0.5-1.0 cm) on the ovary of the sows was re-examined every 8 hour and once the follicles disappeared; the sows were defined as ovulated. The estimated ovulation time was defined as 4 hr before the follicles disappear.

Semen collection and dilution: Semen was collected from two proven boars using gloved-hand method. The fresh semen was evaluated (i.e. motility, sperm concentration and sperm morphology) before further process. Semen with motility at least 70%, concentration at least 150 spermatozoa/ml and percentage of normal sperm more than 85% was diluted using Modena™ extender. The fresh semen for group I contained $2,000x10^6$ motile spermatozoa extended in 80 ml of Modena™ and the FT semen for group II and III contained $2,000x10^6$ motile spermatozoa in 40 ml of Modena™ (group II) or a combination of Modena™ and seminal plasma 1:1 v/v (group III). The extended fresh semen was used immediately (group I). The pre-insemination extended fresh semen had to have a motility higher than 70%.

Semen freezing and thawing: The cryopreservation of boar semen was performed according to our previous protocol (Chanapiwat et al., 2009). Briefly, the semen was diluted (1:1 v/v) with extender I (Modena™, Swine Genetics International, Ltd., Iowa, USA) shortly after the collection. The diluted semen was transferred to 50 ml centrifuge tubes, equilibrated in cell incubator (Micom control system 20Q, Continental plastic CORP, Wisconsin, USA) at 15^0C and transported to the laboratory within 2 hour. After transportation, the semen was centrifuged at 800x g for 10 min; the supernatant was discarded. The sperm pellet was re-suspended (about 1-2:1) with extender II (80 ml of 11% lactose solution and 20 ml egg yolk) to a concentration of $1.5x10^9$ spermatozoa/ml. After cooling at 5^0C for 90 min, two parts of the semen were diluted with one part of extender III (89.5% of extender II with 9% glycerol and 1.5% Equex STM Paste; Nova Chemical Sales Inc., Scituate, MA, USA) to the final concentration of $1.0x10^9$ sperm/ml. The processed semen was loaded into 0.5 ml straws (Bio-Vet, Z.I. Le Berdoulet, France) and sealed with PVC powder. The straws were placed in a chamber and frozen by the controlled rate freezer (Icecube 14s, Sylab, Purkersdorf, Austria). Then, the straws were plunged into liquid nitrogen (-196^0C) for storage. The frozen semen was thawed at 50^0C for 12 sec. The thawed semen was diluted and prepared within 30 min before insemination. The FT semen was diluted with either Modena™ extender (group II) or combination of Modena™ and seminal plasma (group III). The diluted semen (40 ml) was incubated at 37^0C for 10 min and checked for subjective motility before insemination. FT semen with a motility of ≥40% was used for insemination.

Intra-uterine insemination: The IUI technique was carried out according to our previous study (Sumransap et al., 2007). Briefly, the IUI catheter (Magaplus®, Magapor, Ejea de los Caballeros, Spain) was inserted through the vagina into the cervix. The inner tube extended 20 cm more beyond the tip of the outer catheter residing in the uterine body or the posterior uterine horn. The sows were inseminated in individual crate with the presence of a mature boar. Each sow was inseminated with a single dose of either extended fresh semen (group I) or FT semen (group II and III) at 36.1±2.8 hours after hCG administration.

Recovery of spermatozoa: The sows were slaughtered at a commercial abattoir at 12.4±2.2 hours after insemination. Afterwards, the reproductive tracts were collected and transferred to the laboratory within 20 min. The number of corpora lutea (CL) was counted on both ovaries and was defined as the number of ovulation. Each side of oviducts and uterine horns was divided into five parts as: ampulla (2/3 of ampulla next to cranial isthmus), cranial isthmus (1/2 of isthmus next to ampulla), caudal isthmus (1/2 of isthmus next to cranial isthmus), UTJ (1 cm of the tip of uterine horn and 1 cm of caudal isthmus) and cranial uterine horn (1/3 of the uterine horn next to the UTJ). The method of sperm recovery was modified after Sumransap et al. (2007). The ampulla part was flushed with 1 ml of warm phosphate buffer solution (PBS) twice. The isthmus and the UTJ parts were flushed through the lumen with 0.5 ml of PBS twice. Spermatozoa were retrieved by two consecutive flushes into separate plastic vials. The final volume from each flushing was measured from each plastic vial. The uterine horn part was flushed with 20 ml of PBS twice into a petridish. The total number of spermatozoa in the flushing solution from each part was determined using Neubauer hemocytometer (Baeco, Hamburg, Germany). If spermatozoa were not found in the counting chamber, the flushing solution was centrifuged to remove supernatant and the total number of spermatozoa was re-examined.

Statistical analysis: The statistical analyses were performed using the Statistical Analysis Systems software package version 9.0 (SAS Institute Inc., 1996, Cary, N.C., USA). Descriptive statistics including the means and the standard deviations (SD) of parity number, weaning-to-oestrus interval (WOI), oestrus-to-ovulation interval (EOI), the interval from insemination to slaughter, the number of ovulation and the length of the uterine horn were calculated. The number of ovulation was compared among groups by ANOVA. The number of ovulation and the number of spermatozoa were compared between the left and the right sides by pair *t*-test. The numbers of spermatozoa (log-transformation) and the proportion of spermatozoa were compared among groups and among parts using the general linear model (GLM) procedure of SAS. The statistical model included the groups, parts of the reproductive tracts (ampulla, cranial isthmus, caudal isthmus, UTJ, cranial uterine horn) and the interaction between group and parts. Least-squares means were obtained from each class of the factors and were compared by using least-significant different test with Tukey-Kramer adjustment for multiple comparisons. Statistical significance was defined as $p< 0.05$.

Results

On average, WOI in groups I, II and III were 5.3±0.8, 5.8±0.5 and 4.8±0.5 days, respectively ($p> 0.05$). The individual motilities of the spermatozoa in group I, II and III were 80.0±3.5%, 43.8±2.5% and 42.0±2.7%, respectively. After slaughter at 12.4±2.2 hours after insemination, all of the sows showed ovulation. On average, the ovulation took place at 46.9±5.7, 47.3±3.9 and 50.3±2.7 hour after the onset of oestrus in groups I, II and III, respectively ($p> 0.05$). The interval from onset of oestrus to ovulation varied among sows from 38.0 to 51.0 hour (means±SD= 48.2±4.1 hours). The interval from hCG administration to ovulation was 40.0±4.4 hour (range 30.0-46.5 hours). On average, the ovulation took place at 6.4±3.3 hours (range -3.0 to 8.5 hours) after insemination. The number of ovulation of sows in groups I, II and III were 17.8, 17.5 and 17.8, respectively ($p> 0.05$). The length of the uterine horn (one side) in group I, II and III were 114.5, 127.5 and 108.3 cm, respectively ($p> 0.05$).

Table 1 Means number (cells) and proportion (%) of spermatozoa recovered from different parts of the reproductive tracts of sows 12 hour after insemination

Group	n	Mean of spermatozoa (cells) in the oviducts and uterine horns* (proportion (%))				
	Parts	1	2	3	4	5
I	5	2,750[a] (0.9[a])	35,750[a] (12.6[a])	78,500[b] (27.2[b])	135,000[b] (47.8[b])	34,750[a] (11.6[a])
II	4	3,000[a] (0.8[a])	33,333[a] (12.6[bc])	75,666[b] (26.4[ab])	149,666[b] (47.8[c])	25,333[a] (9.0[ab])
III	5	2,800[a] (0.7[a])	76,400[b] (19.4[a])	115,620[b] (28.1[b])	153,800[b] (38.1[b])	60,800[b] (13.6[a])

* 1. ampulla 2. cranial isthmus 3. caudal isthmus 4. UTJ 5. cranial uterine horn
[abc] One letter in common within row did not differ significantly ($p > 0.05$)

Spermatozoa were found in all parts of the reproductive tracts after IUI with either fresh (group I) or FT boar semen (groups II and III) in all sows (Table 1). Most of the spermatozoa were found in the UTJ (47.8%, 47.8% and 38.1% in groups I, II and III, respectively, $p > 0.05$) and the caudal isthmus (27.2%, 26.4% and 28.1% in groups I, II and III, respectively, $p > 0.05$). For all groups, less than 1.0% of the spermatozoa were found in the ampulla (Table 1). In groups I and II, the number of spermatozoa in the ampulla and the cranial isthmus was less than the caudal isthmus and the UTJ ($p < 0.05$), while in group III, the number of spermatozoa in the ampulla were less than the cranial isthmus, the caudal isthmus and the UTJ ($p < 0.05$) (Table 1). Number of spermatozoa found in the cranial isthmus in group III (76,400 sperm) tended to be higher than that in group I (35,750 sperm) and II (33,333 sperm) ($p > 0.05$).

Across the groups, 0.9%, 14.5%, 27.2%, 46.1% and 11.4% of the recovered sperm was found in the ampulla, the cranial isthmus, the caudal isthmus, the UTJ and the cranial uterine horns, respectively ($p < 0.001$). The proportion of spermatozoa flushed from each part were not different ($p > 0.05$) among groups (Table 2). The total number of inseminated sperm, the total number of recovered sperm and the proportion of sperm recovered from all parts of the reproductive tract in groups I, II and III are summarized in Table 2. As can be seen from the table, the total number of spermatozoa in group III (409,420 sperm) tended to be higher than that in group I (286,750 sperm, $p = 0.109$) and group II (287,000 sperm, $p = 0.139$).

Table 2 Total inseminated sperm number (cells), total recovered sperm (cells) and proportion (%) of spermatozoa recovered from the reproductive tracts of sows 12 hour after insemination

Group	Inseminated spermatozoa	Recovered spermatozoa	Proportion
I	$2x10^9$	286,750	0.01433
II	$2x10^9$	287,000	0.01435
III	$2x10^9$	409,420	0.02047

Discussion

This is the first study demonstrating sperm distribution in the reproductive tract of sows after IUI with FT boar semen. It is well established that the number of sperm in the sows reproductive tract influence subsequent fertility and fecundity of sows (Mburu et al., 1996; Tummaruk et al., 2007). Earlier studies demonstrated that IUI in sows allowed the reduction both in the volume and the number of spermatozoa per dose without any negative effect on the number of spermatozoa deposited in the reproductive tract of sows (Sumransap et al., 2007), the fertilization rate, the number of embryos (Tummaruk et al., 2007), farrowing rate and litter size at birth in sows (Watson and Behan, 2002). This is due to the fact that IUI markedly reduces the semen backflow when a high volume of semen (80-100 ml) is used for insemination (Bennemann et al., 2004). The IUI has already been implemented for FT boar semen under field conditions (Chanapiwat et al., 2009; Buranaamnuay et al., 2010). For instance, previous study has demonstrated that the use of IUI with $1,000x10^6$ and 20 ml volume of FT semen in a commercial pig farm resulted in 65% farrowing rate and 9.4 total number of piglets born per litter (Buranaamnuay et al., 2010[a]). However, the sperm distribution in the reproductive tract of sows after IUI with FT boar semen has never been investigated before. The present study revealed that the use of IUI for FT boar spermatozoa resulted in spermatozoa deposited in the reproductive tract in all sows comparable to that using extended fresh semen.

To our knowledge, only one study on the sperm distribution using FT semen has been done in gilts during the past 10 years (Abad et al., 2007[b]), and none has been done in sows. In gilts, the previous study demonstrated that $8.6\text{-}19.3x10^3$ spermatozoa was found in the sperm reservoir at 8 hour after conventional AI with a single dose (80 ml) of $3x10^9$ FT sperm (Abad et al., 2007[b]). This number was lower than that was found in our study. This difference might be due to the semen volume, the total number of spermatozoa per dose, and the length of the uterine horns. Kaeoket et al. (2010) found that using FT boar semen with a volume of 40-60 ml was more suitable than 80-100 ml. The recommended number of total spermatozoa for FT boar semen varies between $1,000\text{-}5,000x10^6$ sperm (Okazaki et al., 2009; Buranaamnuay

et al., 2010b; Kaeoket et al., 2010; Spencer et al., 2010). In our study, 40 ml with a total number of 2,000x10^6 of FT spermatozoa was used. This resulted in an accumulation of spermatozoa in all parts of the reproductive tract of sows similar to that using conventional extended fresh semen.

In addition, the effect of using seminal plasma as a thawing medium on sperm distribution was investigated. It was found that the supplementation of seminal plasma in the thawing medium of the FT semen tended to increase the number of spermatozoa deposited in the cranial isthmus as well as the whole reproductive tracts of sows. The reason might be due to the suppression of PMN cells in the female reproductive tract by seminal plasma (Rozeboom et al. 2001; Jiwakanon et al., 2011), therefore the phagocytosis of spermatozoa by the PMN cells might be reduced. Furthermore, it was found that the supplementation of seminal plasma in the cryopreserved boar sperm was able to reduce the cryoinjury and improve FT sperm parameters, e.g. acrosome integrity and membrane disorder (Vadnais et al., 2005). It was demonstrated that the supplementation of 20% v/v of seminal plasma decrease the percentage of capacitated sperm from 59.5% to 26.8% (Vadnais et al., 2005). However, the effect of seminal plasma on sperm capacitation was dependent on both temperature and composition of the semen extender. It was found that no effect of seminal plasma was observed when egg yolk remained in the semen or when the temperature was maintained at 17^{0}C (Vadnais et al., 2005). In the present study, egg yolk was not washed before AI and it might remain during seminal plasma supplementation. In addition, the concentration of seminal plasma used in the present study might not be sufficient. Furthermore, the quality of the seminal plasma may be different among boars (Rodriguez-Martinez et al., 2011; Jiwakanon and Dalin, 2012). Therefore, the supplementation of seminal plasma to enhance the sperm transport and the deposition of the boar sperm in the female reproductive tract may depend on the difference of seminal plasma contents among boars (Jiwakanon and Dalin, 2012) and the concentration of the seminal plasma used in each insemination dose.

In general, seminal plasma consists of a variety of protein components which is important for boar spermatozoa (Rodriguez-Martinez et al., 2011). However, the role of the seminal plasma proteins in the function of the boar spermatozoa remains unclear. It has been hypothesized that the variation in the concentration of seminal plasma proteins maybe responsible for its influence on the boar spermatozoa (Maxwell and Jonhnson, 1999). In boar, spermadhesins are most (approximately 90%) of total seminal plasma proteins and these proteins are associated with the fertilization process. Spermadhesins are divided into heparin binding proteins (AQN-1, AQN-3, AWN) and non-heparin binding proteins (PSP-I/PSP-II). PSP-I/PSP-II is 50% of the total seminal plasma proteins which play a major role in the modulation of uterine immune activity by preventing the possible infections of the lower genital tract and providing the optimal uterine environment for the early embryo attachment (Rodrigurez-Martinez, 2005; Garcia et al., 2006). It has been reported that high variability exists in the seminal plasma composition and concentration among breeds, individual males, ejaculates within same males and even sperm fractions within ejaculate (Zhu et al., 2000; Caballero et al., 2004, 2008; Garcia et al., 2009). Therefore, the variation in the composition and concentration of seminal plasma proteins may affect the differences in effect (protective or harmful) on the sperm function and male fertility (Killian et al., 1993; Maxwell and Johnson, 1999). Garcia et al. (2009) found that the functional proteins in seminal plasma of sperm rich fraction appeared to enhance sperm survival rate compared to seminal plasma of the rest of ejaculation. Therefore, the quality of the seminal plasma used in our study might be different among boars and ejaculates. The evaluation of seminal plasma proteins as well as other components is needed to be carefully evaluated before further implementation.

This study demonstrated that the number of recovered sperm in each segment did not differ between extended fresh semen and FT semen. The pattern of sperm distribution observed herein is in close agreement with previous reports in sows inseminated with extended fresh semen (Mburu et al., 1996; Sumransap et al., 2007). Mburu et al. (1996) have demonstrated that the plasma membrane integrity of the sperm in the sperm reservoir was significantly changed during peri- and post ovulatory period as compared to pre-ovulatory period. In our study, the sperm population that was determined was in only post-ovulatory periods. In general, sperm capacitation is essential for successful fertilization (Suzuki et al., 2002). Cryopreservation of boar sperm resulted in a capacitation like changes (Green and Watson, 2001; Vadnais et al., 2005). Recent studies found that the supplementation of seminal plasma in the FT semen was able to reverse cryocapacitation, leading to extend sperm survival and increased ability of FT sperm to enter the sperm reservoir close to the site of fertilization (Suzuki et al., 2002; Kirkwood et al., 2008; Okazaki et al., 2009). Moreover, seminal plasma also facilitates sperm transport to UTJ by uterine contraction and by modulating inflammatory immune response in the uterus (Waberski et al., 1996; Rozeboom et al., 2000). In the sows with long oestrus duration, the sperm storage is important because fertilization takes several hours after mating/insemination. Thus, the oviduct should be able to provide the optimal environment for fertilizing capability of sperm (Tienthai et al., 2004).

It can be concluded that at 12.4 hr after IUI using FT boar semen, spermatozoa were found in all parts of the reproductive tract of sows similar to that using extended fresh semen. Supplementation of seminal plasma in the thawing medium of FT boar semen tended to increase the number of spermatozoa accumulated in cranial isthmus. This might be due to the suppression of PMN cells in the female reproductive tract and the reduction in the cryoinjury of the FT boar sperm caused by seminal plasma.

However, optimal concentration as well as specific proteins in the boar seminal plasma should be further analyzed.

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