The Thai Journal of Veterinary Medicine

Volume 40
Issue 2 *June, 2010*

Article 10

6-1-2010

Intra-uterine and Deep intra-uterine Insemination using Cryopreserved Boar Semen in Spontaneously-ovulating Sows

Kakanang Buranaamnuay

Termpong Wongtawan

Sutthatip Masuwatana

Padet Tummaruk

Mongkol Techakumphu

Original Article

Intra-uterine and Deep intra-uterine Insemination using Cryopreserved Boar Semen in Spontaneously-ovulating Sows

Kakanang Buranaamnuay[1] Termpong Wongtawan[2] Sutthatip Masuwatana[3] Padet Tummaruk[1*] Mongkol Techakumphu[1]

Abstract

The present study was performed to investigate the *in vivo* fertility of frozen-thawed (FT) boar semen after intra-uterine (IUI) and deep intra-uterine insemination (DIUI) in spontaneously-ovulating sows. A total of 48 weaned sows were included. The sows were divided into three groups, i.e. natural mating (NM) (n=30), IUI (n=9) and DIUI (n=9). In the IUI and DIUI groups, the sows were inseminated twice, at 24 and 36 h after the detection of oestrous by IUI with $2x10^9$ spermatozoa/dose or DIUI with $1x10^9$ spermatozoa/dose. Transrectal ultrasonography was used to determine the time of ovulation after insemination. The results revealed that the conception as determined by a 24-day non-return rate of the sows was 96.6%, 88.8% and 66.6% (p=0.03) and the farrowing rate (FR) was 96.6%, 66.6% and 66.6% (p=0.01) in NM, IUI and DIUI groups, respectively. The numbers of total piglets born per litter were 9.4±2.8, 11.3±2.9 and 7.6±3.1 piglets in the NM, IUI and DIUI groups, respectively (p=0.10). These data indicate that the spontaneously-ovulating weaned sows inseminated with either IUI or DIUI using a relatively low numbers of FT spermatozoa resulted in a lower FR compared to NM. The total number of piglets born per litter after IUI was higher than DIUI.

Keywords: artificial insemination, frozen-thawed spermatozoa, ovulation, sow

[1]*Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.*
[2]*Faculty of Veterinary Medicine, Mahidol University, Salaya, Nakorn Pathom 73170, Thailand.*
[3]*Animal Breeding Extension Division, Military Development Office, Armed Forces Development Command, Royal Thai Armed Forces Headquarters, Chachoengsao 20140, Thailand.*
Corresponding author E-mail: *Padet.t@chula.ac.th*

บทคัดย่อ

การผสมเทียมแบบสอดท่อเข้าตัวมดลูกและปีกมดลูกด้วยน้ำเชื้อพ่อสุกรแช่แข็งในแม่สุกรที่ตกไข่ตามธรรมชาติ

คคนางค์ บูรณอำนวย[1] เติมพงศ์ วงศ์ตะวัน[2] สุทธาทิพย์ มาสุวัฒนะ[3] เผด็จ ธรรมรักษ์[1] มงคล เตชะกำพุ[1]

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อประเมินความสามารถในการผสมติดของแม่สุกรที่ได้รับการผสมเทียมด้วยน้ำเชื้อพ่อสุกรที่ผ่านการแช่แข็งและทำละลาย ด้วยวิธีการผสมเทียมแบบสอดท่อเข้าตัวมดลูก (IUI) และสอดท่อเข้าปีกมดลูก (DIUI) ในแม่สุกรที่มีการตกไข่ตามธรรมชาติ การทดลองใช้แม่สุกรนางหย่านม จำนวน 48 ตัว แบ่งออกเป็น 3 กลุ่ม ได้แก่ ผสมธรรมชาติ (NM) 30 ตัว และผสมเทียมด้วยวิธี IUI 9 ตัว และ DIUI 9 ตัว ในแม่สุกรที่ถูกผสมเทียม จะถูกผสม 2 ครั้ง ที่ 24 และ 36 ชั่วโมง หลังตรวจพบการเป็นสัด ด้วยวิธี IUI โดยใช้น้ำเชื้อ $2x10^9$ ตัว/โด๊ส หรือ วิธี DIUI ด้วยน้ำเชื้อ $1x10^9$ ตัว/โด๊ส ทำการตรวจอัลตราซาวด์ผ่านทางทวารหนักเพื่อตรวจเวลาการตกไข่หลังการผสมเทียม ผลการทดลองพบว่าอัตราการผสมติดซึ่งประเมินจากการไม่กลับสัดของแม่สุกรที่ 24 วันหลังการผสมเทียม เท่ากับ 96.6% 88.8% และ 66.6% (p=0.03) และอัตราเข้าคลอด เท่ากับ 96.6% 66.6% และ 66.6% (p=0.01) ในแม่สุกรกลุ่ม NM IUI และ DIUI ตามลำดับ จำนวนลูกสุกรแรกคลอดทั้งหมดต่อครอก เท่ากับ 9.4±2.8 11.3±2.9 และ 7.6±3.1 ตัว/ครอก ในกลุ่ม NM IUI และ DIUI ตามลำดับ (p=0.10) การทดลองครั้งนี้บ่งชี้ว่าอัตราการเข้าคลอดหลังการผสมเทียมแม่สุกรที่มีการตกไข่ตามธรรมชาติโดยใช้น้ำเชื้อพ่อสุกรแช่แข็งปริมาณต่ำด้วยวิธี IUI และ DIUI ต่ำกว่าการผสมธรรมชาติ และจำนวนลูกสุกรแรกคลอดทั้งหมดต่อครอกจากการผสมเทียมด้วยวิธี DIUI ต่ำกว่าการผสมเทียมด้วยวิธี IUI

คำสำคัญ: *การผสมเทียม น้ำเชื้อแช่แข็ง การตกไข่ แม่สุกร*

[1]ภาควิชาสูติศาสตร์ เธนุเวชวิทยา และวิทยาการสืบพันธุ์ คณะสัตวแพทยศาสตร์ จุฬาลงกรมหาวิทยาลัย กรุงเทพฯ 10330

[2]คณะสัตวแพทยศาสตร์ มหวิทยาลัย มหิดล ศาลายา นครปฐม 73170

[3]กองสนับสนุนและขยายพันธุ์สัตว์ สำนักงานทหารพัฒนา หน่วยบัญชาการทหารพัฒนา กองบัญชาการกองทัพไทย อำเภอพนมสารคาม จังหวัด ฉะเชิงเทรา 20140

***ผู้รับผิดชอบบทความ E-mail:** Padet.t@chula.ac.th*

Introduction

Conventional artificial insemination (AI) in pigs with a high number (5-6x10^9/dose) of frozen-thawed (FT) spermatozoa often results in a decrease of 20-30% in farrowing rates (FR) and 2-3 in number of total piglets born/litter (TB) compared to extended fresh semen (Johnson et al., 2000). Under field conditions, the use of FT boar spermatozoa for AI in commercial swine herds is limited (Wagner and Thibier, 2000). In an attempt to obtain satisfactory fertility results using low numbers of sperm/dose, non-surgical deep insemination procedures to deposit semen into the uterine body (intra-uterine insemination; IUI) or into the proximal third of one uterine horn (deep intra-uterine insemination; DIUI) have been developed (Martinez et al., 2001; Watson and Behan, 2002; Sumransap et al., 2007; Tummaruk et al., 2007). However, Day et al. (2003) found that DIUI in spontaneously-ovulating sow yields a lower litter size compared to conventional AI. Martinez et al. (2006) suggested that the reduction of litter size might be overcome by increasing the number of spermatozoa for DIUI. Using DIUI in induced ovulating weaned sows, 1x10^9 FT spermatozoa/dose can be inseminated without altering the reproductive performance (Roca et al., 2003). Nonetheless, DIUI in spontaneously-ovulating sows with 1x10^9 of FT spermatozoa resulted in 70% FR and 9.3 TB, which was lower than DIUI with 150x10^6 extended fresh spermatozoa (84% FR and 9.9 TB). Using FT semen, insemination outside of the optimal insemination-ovulation period (4 to 6 hrs before ovulation) significantly decreased FR and TB (Roca et al., 2003). In addition to DIUI, IUI is another insemination technique that has been developed to reduce the number of spermatozoa per insemination dose (Watson and Behan, 2002). It has been demonstrated that an approximately 3-fold reduction in the number of spermatozoa for extended fresh semen can be used with IUI without affecting FR and TB, compared to conventional AI (Watson and Behan, 2002). To our knowledge, no fertility data is available using IUI with FT boar semen and, certainly, there are no reports on evaluation of fertility results in sows inseminated with FT semen using IUI and DIUI. The latter should be conducted to determine the more

appropriate insemination procedure, indicated through the superior fertility results, to be used with FT boar semen particularly under commercial conditions. The present study was performed to evaluate fertility results after IUI or DIUI with reduced numbers of FT boar spermatozoa in spontaneously-ovulating weaned sows.

Materials and Methods

Sows, detection of oestrous and ovulation: The inseminations were conducted between January and June 2006 at the Animal Breeding Extension Division, Chachoengsao Province. A total of 48 weaned sows were included. The sows were divided into three groups, i.e. natural mating (NM) (n=30), IUI (n=9) and DIUI (n=9). The breed of the sows consisted of 22 Landrace (L), 17 Yorkshire (Y) and 9 crossbred LY. The average parity number of the sows was 2.8±2.0 (range 1 to 8) and the weaning-to-oestrous intervals varied from 3 to 7 days. A standing reflex was detected in weaned sows twice daily (0600 and 1800 hr) by allowing the females to have direct contact with a mature boar and the back pressure test. As the restraint of animals and the transrectal ultrasonography might induce stress and affect the process of sperm and/or oocyte transport (Tummaruk et al., 2007), the occurrence of ovulation was herein investigated every 12 hrs after oestrous detection by using real-time B-mode ultrasonography (Aloka, SSD 500, Japan) (Mburu et al., 1995). The ovulation time was defined as 6 hrs before the first time when no follicles were visible.

Semen cryopreservation and evaluation of the sperm quality: The sperm-rich fraction of ejaculates was collected from each of 4 L and 3 Y boars (1-3 year-old) once a week for 3 consecutive weeks. The boars were housed in a commercial herd in Nakorn Pathom Province and were being used as semen donors for AI in the farm. Only ejaculates with ≥ 70% motility were cryopreserved, using the straw freezing procedure (Gadea et al., 2004). The processed semen at a final concentration of $1x10^9$ sperm/ml was packed into 0.5-ml straws (Bio-Vet, Z.I. Le Berdoulet, France) and frozen by placing in liquid nitrogen (LN_2) vapor for 20 min before plunging into LN2. Thawing was achieved by immersing the straws in 50°C of water for 12 sec. The thawed semen was diluted (1:4) with Beltsville Thawing Solution (BTS; Minitüb, Abfüll-und Labortechnik GmbH & Co. KG, Germany) and was incubated at 38°C for 30 min. The post-thaw sperm quality was evaluated. The subjective sperm motility was assessed using a bright-field microscope (400x) (Buranaamnuay et al., 2009). Sperm concentration was evaluated using Bürker haemocytometer (Boeco, Germany) (Ax et al., 2000) and viability of spermatozoa was determined by eosin-nigrosin staining (Dott and Foster, 1972). Before insemination, the qualifying FT semen (≥35% motility) was diluted with 20 ml and 10 ml of BTS extender for IUI and DIUI, respectively. The diluted semen was incubated at 38°C for 10 min and checked for the post-thaw motility before insemination.

Intra-uterine and deep intra-uterine insemination and natural mating: The inseminations were performed in gestation crates. For the IUI group, $2x10^9$ spermatozoa diluted in 20 ml BTS were inseminated through the IUI device (Deep goldenpigTM catheter; IMV Technologies, L'Aigle, France) which was previously inserted through the vagina into the uterine body, and 2 ml of BTS extender were flushed before removing the catheter. For the DIUI group, a predetermined number of spermatozoa ($1x10^9$) in 10 ml of BTS extender were slowly infused into one uterine horn via the DIUI device (Firflex®, Magapor, Ejea de los Caballeros, Spain). The BTS extender (2 ml) was flushed to force the remaining spermatozoa out of the catheter. FT semen produced from each boar was used in both IUI and DIUI sows to minimize effect of individual boar variation. In each sow, double inseminations at 24 and 36 hrs after detection of oestrous were performed using FT semen from the same boar. The sows that were naturally mated were served as control. The mating procedure was performed according to the routine management of the herd. Briefly, the sows were mated 2 to 3 time every 12 hrs after the onset of oestrous.

Fertility data: Conception rate was determined by the proportion of sows that were not return to oestrous by 24 days after insemination/mating and the total number of sows inseminated. At farrowing, the proportion of sows that farrowed (farrowing rate, FR), TB and number of piglets born alive/litter (BA) was recorded.

Statistical analyses: The statistical analyses were carried out using SAS (SAS version 9.0, Cary, NC, USA.). Descriptive statistics including means, standard deviations (SD) and range of the data were calculated. Conception rate and FR were analyzed by Fisher's exact test. TB and BA were analyzed by one-way analysis of variance. Least-squares means were obtained from each group and were compared by using least-significant different test. The probability of $p<0.05$ were considered as statistically significant.

Results

The reproductive performances of all mated sows are presented in Table 1. Both IUI and DIUI resulted in a lower FR compared to NM ($p=0.009$) (96.6%, 66.6% and 66.6% for NM, IUI and DIUI, respectively). Conception rate in the NM group (96.6%) was higher than the DIUI group ($p=0.009$), but did not differed significantly compared to IUI group (88.8%) ($p=0.35$). The conception rate and FR were not significantly different between the IUI and DIUI groups ($p>0.05$). However, the DIUI group had a 3.6 TB (11.3 versus 7.7, p=0.03) and 2.5 BA (9.8 versus 7.3, $p=0.17$) lower than the IUI group (Table 1).

A total 11 ejaculates (6 ejaculates from the 4 L boars and 5 ejaculates from the 3 Y boars) were used for insemination. On average, the percentages of motile spermatozoa used for IUI and DIUI were 41.7±5.6% (range 35 to 50%) and 38.9±3.3% (range 35

to 45%), respectively (p=0.22). The FT sperm viability used for IUI and DIUI was 50.6±4.8% (range 43 to 59%) and 48.6±5.0% (range 43 to 55%), respectively (p=0.46). The interval from the onset of oestrous-to-ovulation (EOI) was 34.7±10.3 hrs (12-48 hrs) and 38.2±6.3 hrs (25-48 hrs) in the IUI and DIUI groups, respectively (p=0.39). The interval between the latest insemination-to-ovulation (IOI) was 6.0±5.0 hrs (0-18 hrs) and 3.6±2.4 hrs (0-6 hrs) in the IUI and DIUI groups, respectively (p=0.20).

Table 1. Reproductive performance of sows including non-return rate, farrowing rate, number of total piglets born/litter and number of piglets born alive/litter (means±S.D.) after intra-uterine insemination (IUI) and deep intra-uterine insemination (DIUI) with frozen-thawed boar semen compared with sows naturally mated (NM)

Parameters	NM	IUI	DIUI
Number of sows	30	9	9
Parity number	1.8±1.4	5.0±1.9	4.8±1.9
Weaning-to-oestrous interval (days)	NA	4.9±0.9	5.1±1.5
Conception rate (%)	29/30 (96.6%)[a]	8/9 (88.8%)[ab]	6/9 (66.7%)[b]
Farrowing rate (%)	29/30 (96.6%)[a]	6/9 (66.7%)[b]	6/9 (66.7%)[b]
Number of total piglets born/litter	9.4±2.8[ab]	11.3±2.9[a]	7.7±3.0[b]
Number of piglets born alive/litter	8.1±3.1[a]	9.8±3.6[a]	7.3±2.9[a]

[a,b] Different superscripts within row differed significantly (p<0.05)

Discussion

In the present study, insemination was performed every 12 hrs instead of 4-8 hrs because insemination twice a day is more practical and easier to be followed by farmers than insemination 3-4 times per day, and this trial was designed as a pilot study to determine whether insemination protocols utilized (e.g., number of sperm/dose, time and number of insemination/oestrous) were good enough to be used in commercial swine herds. In the present study, the FR of spontaneously-ovulating sows after DIUI with $1x10^9$ FT spermatozoa was comparable to previous reports (Roca et al., 2003). However, the sows in the DIUI group tended to have inferior TB and BA compared to the IUI group. Several factors may contribute to the successful fertility of FT boar semen but important ones in this study include the timing of insemination relative to ovulation time and the number of motile spermatozoa per insemination. As the life span of FT boar spermatozoa in the female genital tract is 4-8 hrs after insemination (Waberski et al., 1994), the timing of insemination relative to ovulation is essential for attaining optimal fertility. An inappropriate time of insemination results in low FR, TB and BA (Johnson et al., 2000). In the present trial, a sow that did not get pregnant ovulated at 12 hrs after the onset of oestrous, while insemination was begun at 30 hrs after the onset of oestrous (i.e., 18 hrs too early). This might be one reason for the unsuccessful fertilization in this sow. At a 12 hrs interval of ovulation detection, 18 sows used in this study ovulated at a range of 12 to 48 hrs after the onset of oestrous. Hence, double inseminations with FT semen at 24 and 36 hrs were somewhat too early for the late-ovulating sows and too late for the early-ovulating sows.

The number of spermatozoa per insemination relates to the number of functional spermatozoa colonized in the oviductal sperm reservoir and is important for achieving high fertility (Tummaruk et al., 2007). In the present study, the sperm motility used for DIUI was 38.9%; therefore, in the DIUI group, each weaned sow was inseminated with approximately $400x10^6$ motile sperm/dose. These relatively small numbers of functional spermatozoa inseminated were possibly inadequate for the establishment of sperm reservoirs and led to a unilateral and/or incomplete fertilization. Such events might be the crucial components of the low fertility after DIUI observed in this study. Therefore, based on our present results, the fertility of FT boar semen after DIUI in sows somehow needs to be improved. The ways for enhancing the in vivo fertility after DIUI with FT boar semen include increasing the number of viable spermatozoa per insemination dose (Martinez et al., 2006), enhancing the establishment of the sperm reservoir and/or the sperm transport by using seminal plasma (Abad et al., 2007) and induction of ovulation (Roca et al., 2003).

IUI with a reduced number of extended fresh boar spermatozoa has been studied earlier, and 85% FR and 10-12 TB could be received after insemination with $1x10^9$ spermatozoa/dose (Watson and Behan, 2002). To our knowledge, this study is the first report on IUI with a reduced number of FT spermatozoa in which the fertility obtained was acceptable and tended to be greater than results achieved after DIUI. Moreover, the fertility rates in the IUI sows also seemed to be comparable to those in the sows mated naturally with boars which this technique was routinely conducted at this place. These indicate that IUI could also be an optimal insemination method for FT semen under field conditions. In addition, our previous study has demonstrated that the expression of progesterone receptor in the oviductal tissue of the sows prior to fertilization after DIUI with a reduced number of spermatozoa was lower than that after conventional AI and IUI (Tummaruk et al., 2009). This might influence sperm transportation and the fertilization process and hence reduce in vivo fertility of the DIUI. Furthermore, it has been demonstrated that the number of spermatozoa in the crypt of the utero-tubal junction at 24 hrs after DIUI was lower than conventional AI and IUI (Tummaruk and Tienthai, 2008). Moreover, the spermatozoa could be obtained from both sides of the sperm reservoir after AI and IUI but in only one of five sows inseminated by DIUI (Tummaruk and Tienthai, 2010). These might lead to a lower fertilization rate of the DIUI compared to AI or IUI.

In conclusion, spontaneously-ovulating weaned sows inseminated with either IUI or DIUI using a relatively low numbers of FT spermatozoa resulted in a lower FR compared to NM. The total number of piglets born per litter after IUI was higher than DIUI. However, insemination using IUI and DIUI procedure should be investigated further due to that IUI and DIUI could be used with a reduced number of spermatozoa per dose and hence an increase the possibility of using semen from valuable boars.

Acknowledgements

This project received grants from the Research and Development Center for Livestock Production Technology, Chulalongkorn University, and the CHE-TRF Senior Research Fund (RTA5080010). K. Buranaamnuay is a grantee of the Royal Golden Jubilee (RGJ) Ph.D. Program, the Thailand Research Fund. Language editing of the manuscript has been coordinated by Chula Unisearch, Chulalongkorn University.

References

Abad, M., Sprecher, D.J., Ross, P., Friendship, R.M. and Kirkwood, R.N. 2007. Effect of sperm cryopreservation and supplementing sperm doses with seminal plasma on the establishment of sperm reservoir in gilts. Reprod. Domest. Anim. 42: 149-152.

Ax, R.L., Dally, M., Didion, B.A., Lenz, R.W., Love, C.C., Varner, D.D., Hafez, B. and Bellin, M.E., 2000. Semen evaluation In: Reproduction in Farm Animals. E.S.E. Hafez and B. Hafez (eds.), England, Blackwell Publishers 366-368.

Buranaamnuay, K., Tummaruk, P., Singlor, J., Rodriguez-Martinez, H. and Techakumphu, M. 2009. Effects of straw volume and Equex-STM® on boar sperm quality after cryopreservation. Reprod. Dom. Anim. 44: 69-73.

Day, B.N., Mathias, K., Didion, B.A., Martinez, E.A. and Caamano, J.N., 2003. Deep intrauterine insemination in sows: first field trial in USA commercial farm with newly developed device. Theriogenology 59: 213.

Dott, H.M. and Foster, G.C., 1972. A technique for studying the morphology of mammalian sperm which are eosinophilic in a differential live/dead stain. J. Reprod. Fert. 29: 443-445.

Gadea, J., Selles, E., Marco, M.A., Coy, P., Matas, C., Romar, R. and Ruiz, S. 2004. Decrease in glutathione content in boar sperm after cryopreservation; effect of the addition of reduced glutathione to the freezing and thawing extenders. Theriogenology 62: 690-701.

Johnson, L.A., Weitze, K.F., Fiser, P. and Maxwell, W.M.C. 2000. Storage of boar semen. Anim. Reprod. Sci. 62: 143-172.

Martinez, E.A., Vazquez, J.M., Roca, J., Lucas, X., Gil, M.A., Parrilla, I., Vazquez, J.L. and Day, B.N. 2001. Successful non-surgical deep intrauterine insemination with small numbers of spermatozoa in sows. Reproduction 122: 289-296.

Martinez, E.A., Vazquez, J.M., Parrilla, I., Cuello, C., Gil, M.A., Rodriguez-Martinez, H., Roca, J. and Vazquez, J.L., 2006. Incidence of unilateral fertilizations after low dose deep intrauterine insemination in spontaneously-ovulating sows under field conditions. Reprod. Domest. Anim. 41: 41-47.

Mburu, J.N., Einarsson, S., Dalin, A-M. and Rodriguez-Martinez, H. 1995. Ovulation as determined by transrectal ultrasonography in multiparous sows: relationships with oestrous symptoms and hormonal profiles. J. Vet. Med. A 42: 285-292.

Roca, J., Carvajal, G., Lucas, X., Vazquez, J.M. and Martinez, E.A. 2003. Fertility of weaned sows after deep intrauterine insemination with a reduced number of frozen-thawed spermatozoa. Theriogenology 60: 77-87.

Sumransap, P., Tummaruk, P. and Kunavongkrit, A. 2007. Sperm distribution in the reproductive tract of sows after intrauterine insemination. Reprod. Domest. Anim. 42: 113-117.

Tummaruk, P., Sumransap, P., Techakumphu, M. and Kunavongkrit, A., 2007. Distribution of spermatozoa and embryos in the female reproductive tract after unilateral deep intra uterine insemination in the pig. Reprod. Domest. Anim. 42: 603-609.

Tummaruk, P. and Tienthai, P., 2010. Number of spermatozoa in the crypts of the sperm reservoir at about 24 h after a low-dose intrauterine and deep intrauterine insemination in sows. Reprod. Domest. Anim. 45: 208-213.

Tummaruk, P., Tienthai, P., Manee-In, S. and Srisuwatanasakul, S. 2009. Expression of progesterone receptor in the utero-tubal junction after intra-uterine and deep intra-uterine insemination in sows. Reprod. Domest. Anim. (in press) doi: 10.1111/j.1439-0531.2009.01513.x

Waberski, D., Weitze, K.F., Gleumes, T., Schwarz, M., Willmen, T. and Petzoldt, R. 1994. Effect of time of insemination relative to ovulation on fertility with liquid and frozen boar semen. Theriogenology 42: 831-840.

Wagner, H.G. and Thibier, M., 2000. World statistics for artificial insemination in small ruminants and swine. Proc. 14th International Congress on Animal Reproduction, July, 2-6, 2000, Stockholm, Sweden, Abstracts volume 2: 77.

Watson, P.F. and Behan, J.R. 2002. Intrauterine insemination of sows with reduced sperm numbers: results of a commercially based field trial. Theriogenology 57: 1683-1693.