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UTILIZATION OF BOAR SEMEN BY ALTERNATIVE TECHNIQUES OF SWINE ARTIFICIAL INSEMINATION (A REVIEW)

IOANNIS A. TSAKMAKIDIS, ELENI D. TZIKA[1]

SUMMARY: Conventional artificial insemination (CAI) has been highly contributed in swine global industry development. However, new boar semen technologies have been presented, such as sex sorted, encapsulated and sperm mediated gene transfer spermatozoa. Moreover, the improvement of frozen-thawed boar semen fertilizing ability is a scientific topic under investigation. New technologies must be supported by alternative insemination procedures in order to be economically performed in pig industry. For these purposes, deep intrauterine insemination (DUI), as well as, intra-oviductal insemination (IOI) by laparoscopy, has been applied and their efficiency has been studied. One of the main targets of the aforementioned techniques is to benefit the potential advantages of the high genetic value boars by using the minimal number of spermatozoa needed to achieve a high fertilization rate following artificial insemination. Although CAI is an indispensable method for the commercial pig farms, the application of alternative techniques is feasible to be performed in selected animals of high genetic value. This review discusses the suitability of the available insemination procedures for the efficient of biotechnological applications.

Key words: *swine, pig, laparoscopic, intra-uterine, artificial insemination, biotechnology.*

INTRODUCTION

Conventional intra-cervical swine artificial insemination (CAI) is an indispensable method for the fertilization of the sows in commercial pig farms. The use of liquid diluted boar semen by the performance of traditional AI has contributed a lot in the development of swine global industry improvement. It is a simple technique that involves the deposition of high number of spermatozoa within the posterior portion of the cervical canal by means of a catheter that engages with the folds of the cervix, simulat-

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[1]Ioannis A. Tsakmakidis PhD, lecturer, Eleni D. Tzika PhD, Dipl. ECPHM, assistant professor, Clinic of Farm Animals, Faculty of Veterinary Medicine, Thessaloniki, Greece.
Corresponding author: Ioannis A. Tsakmakidis, e-mail: iat@vet.auth.gr, tel.: +30 2310994467.

ing the corkscrew tie of the boar's penis. However, in nowadays the demand for semen from genetically superior boars has become bigger and can only be satisfied by using more boars for semen collection. A solution for this requirement could be the decrease of spermatozoa's number per insemination dose, without reduction of fertilization rate. The minimum threshold number of spermatozoa for maximum fertilization depends not only on the boar, but also on the sperm manipulation process applied before insemination. On the other hand, new sperm technologies have already been developed, such as frozen-thawed sperm, or are into the developing process, such as sex-sorted spermatozoa, sperm mediated gene transfer (SMGT), encapsulated spermatozoa or freeze-dried spermatozoa. How quickly these technologies can be utilized in the pig breeding industry depends upon their efficiency after wide application.

After insemination, the terminal stage of spermatozoa's pathway is the oviduct. Spermatozoa migrate through the uterus, the horns and the utero-tubal junction (UTJ) into the oviduct. In the ampulla of the oviduct, sperm cells interact with the oocyte, penetrate the zona pellucida and fertilize the oocyte. However, the volume of the insemination dose is quickly reduced because only a little fraction of the inseminated spermatozoa reach the oviductal ampulla (Scott and Overstreet, 1999). Polymorphonuclear leucocytes eliminate the sperm population within 30 min, up to 60%, by phagocytosis (Woelders and Matthijs, 2001). Further sperm limitation is caused by the adhesion of sperm cells to epithelial cells of endometrium and by migration into uterine glands (Hadjisavas et al. 1994). Consequently, over 90% of the inseminated spermatozoa are eliminated from the reproductive tract of the female pig within 2–3 h after insemination (Roca et al. 2006).

Keeping in mind the physiological events regulating spermatozoa's transport inside female genital tract, we can understand that more sensitive and stressed boar spermatozoa, such as sorted and frozen-thawed, should be depopulated more in the case that they inseminated intra-cervical using CAI. Freezing process of boar sperm has a detrimental effect on spermatozoa membranes (Guthrie and Welch, 2005), reduces motility and viability (Hernandez et al. 2007), impairs sperm chromatin integrity (Fraser and Strzezek, 2005) and induces oxidative stress and production of reactive oxygen species (Chatterjee and Gagnon, 2001). Moreover, sex sorted spermatozoa have short lifespan because sperm membrane is adversely affected by the flow cytometry and sorting processes, which limits the viability, storage capability and fertilization ability of spermatozoa (Parilla et al. 2005). Furthermore, flow cytometric sorting is related to the low number of spermatozoa obtained, since the number of sexed spermatozoa produced per unit time is limited for commercial use by conventional methods of use (Garcia et al. 2007). However, swine biotechnological applications have recently improved and a need for their proper use exists. All the aforementioned comments lead in the requirement for the use of a low dose insemination technique, in relation with semen deposition closer to the oviduct. For this purpose, a non-surgical, deep intra-uterine insemination (DUI), as well as, a surgical, laparoscopic intra-oviductal insemination (IOI), method have been development and successfully used with both frozen/thawed and sex sorted semen.

DEEP INTRA-UTERINE INSEMINATION (DUI)

Transrectal ultrasonography application, before the performance of DUI is very useful for the determination of sow's ovarian status. A classification of the sows in 3

groups (pre-ovulatory, peri-ovulatory, and post-ovulatory) can be take place. According to Bolarin et al. (2006), pre-ovulatory insemination result in higher pregnancy rate, farrowing rate and litter size. Moreover, in order to avoid the damage of genital tract, DUI must be performed in sows of parity ≥2.

Using DUI, semen should be deposited into the uterine horn. A usual successfully dose for frozen-thawed boar semen is $1000x10^6$ spermatozoa in a volume of 7.5 ml (Vazquez et al. 2008). A flexible catheter with length more that 1.50m (usually 1,80m), external diameter 4 mm, internal diameter 1.80 mm must be inserted in genital tract of the sow through the hole of the classic part of the catheter which must be locked firmly into the cervix. Afterwards, the flexible part of the catheter must be moved slowly and carefully through the cervical canal and be gently pressed forward along the one uterine horn as far as possible. Before and after semen insemination, a little volume of semen-free extender can be administrated to lubricate the catheter and to force any remaining spermatozoa, respectively. Mezalira et al. (2005) reported that 12.6-17.1% of inseminated spermatozoa by DUI can be loss because of the backflow 60 min after insemination. The total time of insemination process is about 4-5 min.

After the removal of the catheter, it must be observed carefully for possible hurt of the genital tract, bleeding, remaining semen etc.; events that could affect the future reproductive efficiency of the inseminated sow. However, the incidence of bleeding in sows after DUI is low (<2%) and similar to that observed in sows inseminated with the traditional AI method (Wongtawan et al. 2006). Bolarin et al. (2006) did not found significant changes of sows' reproductive productivity after the application of DUI in 407 sows. Inserting of the catheter into the left or into the right horn is randomly. However, the fertilization of the oocytes takes place in both of the horns. Martinez et al. (2005) reported that spermatozoa are moving through the uterus and can fertilize oocytes in the contralateral oviduct. Investigating the same issue, Tummaruk et al. (2007) 24 h after DUI application, flushed with BTS extender both of the oviducts and the horns in 5 sows. They found spermatozoa only in one side of the oviducts and/or horns (left side in 3 sows, right side in 2 sows). However, a second experiment of them in 5 sows was carried out. Early embryos were found in both of the horns, 48 up to 72 h after DUI, confirming that the fertilization takes place in both oviducts, independently of the deposition of semen in one horn. Two different pathways of spermatozoa have reported to explain the fertilization in the contralateral oviduct: trans-peritoneal and intrauterine pathway. According to Martinez et al (2005) the trans-peritoneal pathway is effective in a very small percentage of the sows (<5%), while the intrauterine pathway seems be the predominant route (>75% of the sows). However, Brussow et al. (2011) studied sperm migration ways and provided experimental evidence that intrauterine, but not trans-peritoneal, sperm migration occurs after intrauterine insemination with low number of boar spermatozoa. The efficiency of DUI as a low dose insemination technique was studied by Martinez et al. (2002). They reported that DUI comes up to conventional AI results, when $150x10^6$ spermatozoa are inseminated 36 h after the onset of hormonal controlling estrous (table 1).

Table 1. Comparison between CAI and DUI in 519 sows (Martinez et al., 2002)

Technique	Dose: sptz. x106	Pregnancy (%)*	Farrowing rate (%)	Litter size	Live born piglets
DUI Once (1)	10	39,1 a	39,1 a	9,44±0,36	9,03±0,38
	25	51,7 a	46,7 a	9,3±0,35	8,75±0,37
	50	77,8 b	76,2 b	9,4±0,19	8,91±0,20
	150	86,3 b	82,9 b	9,7±0,19	9,30±0,20
CAI Twice (2)	6.000 (2x3.000)	86,4 b	83,0 b	9,97±0,17	9,4±0,18

Dissimilar letters (a, b) denote a significant difference ($p<0.001$). * Detected in 24 - 28 days

LAPAROSCOPIC INTRA-OVIDUCTAL INSEMINATION (IOI)

Laparoscopic oviductal insemination is a minor surgical procedure that allows semen to be deposited into the site of fertilization, the oviduct, decreasing the loss of spermatozoa by phagocytosis or backflow and increasing the probability of fertilization. Therefore, it is not a widespread method, but it is the best choice when a low number of spermatozoa with high value, short lifespan and special characteristics, such as sex-sorted and SMGT should be used. After the hormonal synchronization of estrus, the ovaries of the sows are scanned by transrectal ultrasonography. Sows showing multiple preovulatory follicles (diameter >6 mm) are selected for insemination. A sedation of the sows is required, while the laparoscopic insemination must be performed under general anesthesia. The animals are placed in supine position in a laparoscopy cradle at an angle of approximately 20°-30° above horizontal. Laparoscopy allows the operator to locate the exact point where the insemination needle should be inserted. Generally, sperm must be deposited into the two oviducts, as close as possible to the utero-tubal junction UTJ without forcing it. The complete minor surgery lasts about 15 min. Fertilization rate higher than 90% have been reported after laparoscopic IOI with low number of boar spermatozoa 10-15x10^6 per horn (Table 2), (Fantinati et al., 2005).

Table 2. Fertilization rate in gilts after laparoscopic insemination with different doses of semen (Fantimanti et al., 2005)

Semen dose: spermatozoa x106	Number of inseminated gilts	Mean fertilization rate
1500	4	94.5±2.1a
15	4	91.2±3.2a
10	4	92.3±2.6a
5	6	81.9±6.2a
1	6	50.5±10.1b

Dissimilar letters (a, b) denote a significant difference ($p<0.05$)

Johnson et al. (1991) demonstrated encouraging results after IOI with 3x10^5 sex sorted boar spermatozoa (Table 3).

Table 3. Intra-Oviductal insemination with sex sorted semen (Johnson, 1991)

Spermatozoa	Number of inseminated sows	Number of farrowings	Live born piglets	Litter size
X	8	4	37	9,3
Y	10	5	34	6,8
Un-sorted	11	5	40	8,0

More recently Vazquez et al. (2005) achieved notable fertilization percentage and low rate of polyspermy by the performance of IOI with $3x10^5$ or $6x10^5$ sex sorted spermatozoa (Table 4).

Table 4. Intra-Oviductal insemination with sex sorted semen (Vazquez et al., 2005)

Number of spermatozoa	Fertilization (%)	Polyspermy (%)
3x105 sex sorted	72,6	5,1
3x105 un-sorted	74,4	6,7
6x105 sex sorted	72,7	7,1
6x105 un-sorted	69,2	11,1

CONCLUDING REMARKS

The alternative techniques of swine artificial insemination can assist the development of other biotechnological applications. Non-surgical deep intra-uterine and laparoscopic intra-oviductal insemination can optimize the use of boar ejaculates, as well as, be advantageous for the utilization of frozen, sexed and SMGT boar treated spermatozoa. Both methods can be an efficient tool for obtaining a high rate of fertilization using low doses of semen of genetically improvement boars, while the use of the aforementioned sperm products seems to be more feasible. Thus, alternative artificial insemination methods provide more choices in swine industry to increase its productivity.

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UPOTREBA SPERME NERASTA KOD ALTERNATIVNIH TEHNIKA VEŠTAČKOG OSEMENJAVANJA SVINJA

IOANNIS A. TSAKMAKIDIS, ELENI D. TZIKA

Izvod

Konvencionalno veštačko osemenjavanje (CVO) je verlo mnogo korišteno za unapređenje globalne industrije svinja. Sada se koriste nove tehnologije, kao što su sexing, enkapsulacija i transfer gena spermatozoida. Osim toga, unapređenje upotrebe duboko zamrznutog-otopljenog semena nerasta se sve više naučno istražuje. Ove nove tehnologije moraju biti praćene novim, alternativnim, procedurama veštačke inseminacije, radi povećanja ekonomičnosti njihove industrijske primene. Zbog toga se, sve više izačavaju mogućnosti primene novih VO tehnologija, kao što su duboko intrauterino osemenjavanja i depozicija inseminacione doze u ovidukt. Ove tehnologije, naime, pružaju mogućnost upotreba inseminacionih doza znatno manjeg volumena i broja spermatozoida, što značajno povećava efikasnost reproduktivnog iskorištavanja genetski superiornih nerastova. U ovom radu su opisane mogućnosti primene novih tehnologija veštačkog osemenjavanja svinja.

Ključne reči: svinje, laparoskopija, intra-uterino, veštačko osemenjavanje, biotehnologija.

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ACTINOMYCOTIC GRANULOMA IN HIGHLY PREGNANT SOW (CASE REPORT)

TIHOMIR PETRUJKIĆ, VLADIMIR KUKOLJ, BRANKO PETRUJKIĆ, BRANISLAV STANKOVIĆ[1]

SUMMARY: This paper presents a case of udder actinomicotic granuloma in high-pregnant sows, which was surgically treated. The sow is from a private pig farm in eastern Serbia. Landrace sows, about 3 years old, weighing about 200 kg, it is normal eating and behaving normally. During the first examination, the mammary gland tumor was established, in the size of a fist. In another review, 81 days after mating the sow, the tumor was the size of the balloon of 5 liters. The tumor was surgically removed, and a sample was sent for histopathological diagnosis. The clinical picture and histopathological findings confirmed chronic purulent-granulomatous actinomicotic inflammation of the mammary gland. The postoperative course was uneventful. The sow farroved 6 live piglets, 32 days after surgery (113 days gestation). All 6 piglets are weaned after 6 weeks. After weaning the litter, sows were surgically ovariectomised. The metastatic formations was not observed. Applied surgical procedure and postoperative treatment, show that the surgical treatment of mammary tumors can be successfully applied in practice.

***Ključne reči**: high pregnant sow, actinomycosis, mammary gland, surgical trtetment.*

INTRODUCTION

Actinomycosis is described as chronic granulomatous disease by cattle and pigs and rarely other animals and humans (Šamanc, 2001 and 2009). The cause of this disease is sometimes *A.bovis* and *A.isreali*. Acording to the some authors, swine actinomycosis occurs sporadically (Sofrenović et.al., 1979; Naglić et.al., 2005). In the sows, the most common changes are observed on udder, and rarely on the other parts of skin and

Case report / *Prikaz slučaja*

[1]Tihomir Petrujkić, DVM, PhD, full professor, Vladimir Kukolj, DVM, PhD, asisstant, Branko Petrujkić, DVM, PhD, asisstant, Faculty of Veterinary Medicine, Belgrade, Serbia. Branisalv Stanković, DVM, PhD, assistant, Faculty of Agriculture, Belgrade- Zemun, Serbia.
Corresponding author: Tihomir Petrujkić, e-mail: tihomirpetrujkic@hotmail.com; phone: + 381 11 268-41-84.

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