Acta Veterinaria (Beograd), Vol. 60, No. 2-3, 257-262, 2010.

DOI: 10.2298/AVB1003257S UDK 619:636.4.082.454

# FERTILITY OF SOWS AFTER INTRACERVICAL OR INTRAUTERINE INSEMINATION WITH DIFFERENT SPERMATOZOA NUMBER IN REDUCED VOLUME DOSES

STANČIĆ B*, RADOVIĆ I*, STANČIĆ I**, DRAGIN S*, BOŽIĆ A* and GVOZDIĆ D***

**Faculty of Agriculture, Department of Animal Husbandry, Novi Sad, Serbia*
***High School of Agronomy, Department of Veterinary medicine, Šabac, Serbia*
****Faculty of Veterinary Medicine, Department of Pathophysiology, Belgrade, Serbia*

(Received 21st January 2010)

*Modern intensive pig production demands an increasing number of insemination doses per ejaculate of genetically superior boars. In order to achieve such a result the possibility of producing insemination doses in both reduced volume and spermatozoa count without decreasing the fertility of sows is studied. In this trial we studied the effect of insemination with reduced volumes of semen (50 mL) and varied spermatozoa count (4, 2 or 1x10$^9$). Insemination was performed by the classical (intracervical) or by the new (intrauterine) techniques and the basic fertility parameters (farrowing % and litter size) were measured. The farrowing value decreased with decreasing spermatozoa numbers after intracervical and intrauterine insemination. However, the farrowing value, regardless of spermatozoa numbers, was significantly higher after intrauterine insemination (83.3%, 76.7%, 66.7%) compared to the classical intracervical insemination (73.3%, 66.7% and 50%). Litters size did not vary significantly depending on the applied insemination procedure or spermatozoa number. These results indicate that application of the new intrauterine insemination procedure enables the use of doses with a smaller volume and spermatozoa number, at the same time obtaining a satisfactory farrowing and litter size. This opens the possibility of obtaining a significantly higher number of doses per ejaculate when compared to the classical intracervical insemination procedure. In such a way can be increased significantly the degree of reproductive exploitation of genetically superior boars.*

*Key words: AI, dose, fertility, intracervical, intrauterine, sow, spermatozoa number, volume*

## INTRODUCTION

Artificial insemination is a biotechnological procedure which can not be avoided in intensive pig production. Above all this supposes the possibility of obtaining the highest possible number of insemination doses per year from each

boar. This can practically be obtained by increasing the number of insemination doses per ejaculate (Glossop, 2000; Stančić, 2000; Stančić *et al*., 2002; Stančić *et al*., 2009).

During classical intracervical insemination 100 mL volumes of diluted sperm containing from 2 to $5x10^9$ progressively motile spermatozoa are applied. According to EU data, in the last years, the insemination dose volume is about 100 mL, containing on average $3.7x10^9$ spermatozoa. Thus, from one boar are obtained on average 1200 insemination doses per year (Flowers, 1998; Singleton, 2001; Rozeboom, *et al*., 2004). However, such a production does not satisfy the increasing needs in intensive pig production, especially when genetically superior boars are considered. With this in mind, are considered the possibilities of applying insemination doses of smaller volume and spermatozoa number in order to obtain a higher number of doses per ejaculate (Belstra, 2002). At the same time special care has to be given in order not to decrease the fertility (farrowing value and litter size) of sows. The achievement of such a scope is mirrored in the new technology of transcervical superficial intrauterine insemination with small volume doses and decreased spermatozoa number (Hunter, 1995; Rath, 2002; Stančić *et al*., 2003; Mezalira *et al*., 2005; Stančić *et al*., 2009).

The aim of this study was to publish the obtained results in sow fertility after classical (intracervical) insemination and new (intrauterine) artificial insemination technologies with reduced volume and spermatozoa number doses.

## MATERIAL AND METHODS

A total of 180 sows in the second and fifth parity were artificially inseminated in the first estrus. Lactation lasted on average for 30 days. Sows were inseminated by the classical intracervical (n=90) or by new intrauterine procedure (n=90). Diluted sperm doses 50 mL in volume (half the volume usually applied) were inseminated with 4, 2, or 1 $x10^9$ progressively motile spermatozoa. The sperm diluent used was BTS-1 (Minitüb, Germany). Counting the number of sperm cells in the native semen, as well as the calculation of the degree of dilution, was performed by the photometric method with the aid of SDM5 photometer (Minitüb, Germany). Progressive motility was determined under a light microscope. The doses of diluted sperm were used within 2 to 3 hours after dilution. Disposable sterile catheters Foamtip Safe Blue were used for intracervical insemination and Foamtip Safe Blue- Verona catheters (Minitüb, Germany) were used for intrauterine insemination. Detection of estrus was twice in 24 hours, with 10 – 12h intervals. The first insemination was done after 12h, and the second 24h after the sow's standing reflex was detected.

Statistical data analysis was done with the software packet “Statistica 8” within which descriptive statistics and testing of significance levels within the observed treatments were calculated. Distribution of the obtained results was observed in order to establish within which data values are determined.

## RESULTS AND DISCUSSION

Decreased spermatozoa number, in reduced volume doses, results in a decline in farrowing values in both intracervical and intrauterine insemination. Within the observed values there was no statistical significance. However, regardless of the number of spermatozoa in a dose farrowing values were higher after intrauterine (83.3%, 76.7% and 66.7%), compared to intracervical insemination (73.3%, 66.7% and 50%) (Table 1). Statistically significant differences in farrowing were between intracervical insemination with 1 x $10^9$ spermatozoa per dose and intrauterine insemination with 2 x $10^9$ ($p<0.05$) and 4 x $10^9$ ($p<0.01$) spermatozoa per dose.

Table 1. Farrowing rate in experimental sows

| Insemination method | Experimental sows | | Spermatozoa number per dose (x$10^9$) | | |
|---|---|---|---|---|---|
| | | | 4 | 2 | 1 |
| Intracervical | Inseminated | n | 30 | 30 | 30 |
| | Farrowed | n | 22 | 20 | 15 |
| | | % | 73.3 | 66.7 | $50.0^{AB}$ |
| Intrauterine | Inseminated | n | 30 | 30 | 30 |
| | Farrowed | n | 25 | 23 | 20 |
| | | % | $83.3^{A}$ | $76.7^{B}$ | 66.7 |

$^{A}$Values with the same superscript are significantly different ($p<0.01$)
$^{B}$Values with the same superscript are significantly different ($p<0.05$)

Variations in litter size depending on the insemination method and spermatozoa numbers were recorded (Table 2).

Table 2. Litter size in experimental sows

| Insemination method | Average litter size (n) | Spermatozoa number per dose (x$10^9$) | | |
|---|---|---|---|---|
| | | 4 | 2 | 1 |
| Intracervical | Liveborn | 10.27 | 9.85 | $9.67^{AB}$ |
| | Stillborn | 0.50 | 0.50 | 0.97 |
| | Total | 10.77 | 10.35 | $10.54^{CD}$ |
| Intrauterine | Liveborn | $10.04^{A}$ | $10.82^{B}$ | 10.50 |
| | Stillborn | 0.44 | 0.48 | 0.45 |
| | Total | $10.48^{C}$ | $11.30^{D}$ | 10.95 |

Values with the same superscript are significantly different ($p<0.05$)

A statistically significant difference ($p<0.05$) was measured between the number of liveborn piglets after intrauterine insemination with 2 x $10^9$ and 4 x $10^9$ spermatozoa per dose compared to the number of liveborn piglets after intracervical insemination with 1 x $10^9$ spermatozoa per dose (10.04:9.67 and 10.82:9.67). A statistically significant difference between the total number of piglets after intrauterine insemination with 2 x $10^9$ and 4 x $10^9$ spermatozoa per dose compared to the total number of piglets born after intracervical insemination with 1 x $10^9$ spermatozoa per dose (10.48:10.54 and 11.30:10.54).

There are physiological presumptions that the number of spermatozoa in a dose progressively significantly decreases if the semen is deposited in the cranial portions of the reproductive tract (corpus uteri, uterine horns, uterus – oviduct connection, isthmus or oviduct ampulla) (Hunter, 1973; Hunter, 1995; Krüger *et al*., 2000; Rath, 2002; Wongtawan *et al*., 2005), but at the same time similar or higher sow fertility is achieved (Watson *et al*., 2002; Belstra, 2004; Mezalira *et al.*, 2005; Grafenau *et al*., 2004; Stančić *et al*., 2007; Stančić *et al*., 2008). Thus the reason for which there is an ever increasing interest to study the influence of the depth of intrauterine insemination with reduced volume and spermatozoa number doses on the fertility of sows. The aim is to significantly increase the number of insemination doses per ejaculate at a yearly level.

The results published in this paper clearly indicate that after superficial intrauterine insemination with reduced volume and spermatozoa number doses (50 mL and 2 x $10^9$) compared to classical parameters (100 mL and 4 x $10^9$) a farrowing value of 77% can be obtained. This percentage is close to the values obtained under practical filed conditions after intracervical insemination (76 – 80%) (Stančić *et al*., 2009). Similar results were reported by a number of authors. Vanscicle (2002) reported that after inseminating sows with 50 mL doses containing 1.5 x $10^9$ spermatozoa the farrowing rate was 92.8%. Mezalira *et al*. (2005) obtained a farrowing rate of 88.9% after using 20 mL of semen containing 1 x $10^9$ spermatozoa. In a recently published paper we reported a farrowing rate of 83.3% after inseminating the sows with 100 mL semen containing 2.5 x $10^9$ progressively mobile spermatozoa (Stančić *et al.*, 2007).

Despite the fact that statistically significant differences between litter size and semen volume and spermatozoa number, as well insemination methods applied, is obvious that intrauterine insemination gives a good average litter size. Similar results were reported by Vanscikle, 2002; Rozeboom *et al*., 2004; Mezalira *et al*., 2005; Stančić *et al*., 2007.

In intensive pig production in the Vojvodina region the average volume of semen used is 100 mL containing a total of 4 to 5 x $10^9$ spermatozoa. Previous studies indicate that the average ejaculate volume on farms in Vojvodina is 278 mL with 80% progressively mobile spermatozoa. Out of such ejaculates 10 insemination doses are produced (Stančić *et al*., 2002). If new superficial intrauterine insemination technology was applied, using a volume of 50 mL with 2 x $10^9$ sperm cells, 22 insemination doses could be manufactured out of an average ejaculate (Stančić *et al.*, 2009). In such a mode the price of a single dose would be smaller which would significantly improve the degree of reproductive exploitation of genetically superior boars.

ACKNOWLEDGEMENTS:
The work is a part of the research on the project TP-20087, financed by the Ministry of Sciences and Technological Development, Republic of Serbia, from 2008. to 2011.

Address for correspondence:
Prof. dr Blagoje Stančić
Faculty of Agriculture
Trg D. Obradovića 8
21000 Novi Sad, Serbia
E-mail: blagoje.stancic@gmail.com

## REFERENCES

1. *Belstra AB,* 2002*,* Review: Intrauterine (transcervical) and fixed-time artificial insemination in swine, *Swine Reprod*, 3, 1-6*.*
2. *Belstra AB,* 2004, Artificial Insemination Equipment Developments. *International Pig Topics*, 19, 4, 37-41.
3. *Flowers LW*, 1998, Boar fertility and artificial insemination, Proc. 15th IPVC Congress, Birmingham, England, 5-9 July, 45-51.
4. *Glossop CE*, 2000, Animal welfare and the artificial insemination industry, Boar Semen Preservation IV, Beltsville, Maryland, 207-11.
5. *Grafenau P sen, Grafenau P jr*, 2004, Intrauterinná Inseminácia u prasnic, Odborny seminár: Nove Trendy v inseminácii hospodárskych zvierat, VUŽV Nitra, 25. maj, 18-21.
6. *Hunter RHF*, 1973, Transport, migration and survival of spermatozoa in the female genital tract: species with intrauterine deposition of semen, In: Hafez E, Thibault C (eds.), Sperm Transport, Survival and Fertilizating Ability*, INSERM*, 26, 309-42.
7. *Hunter RHF,* 1995, Ovarian endocrine control of sperm progression in the Fallopin tubes, *Oxf Rev Reprod Biol,* 17, 85-124.
8. *Krüger C, Rath D,* 2000, Intrauterine insemination in sows with reduced sperm number, *Reprod Fertil Develop,* 12,113-7.
9. *Mezalira A, Dallanora D, Bernardi LM, Wentz I, Bortolozzo FP,* 2005, Influence of Sperm Cell Dose and Post-insemination Backflow on Reproductive Performance of Intrauterine Inseminated Sows, *Reprod Dom Anim*, 40, 1-5.
10. *Rath D*, 2002, Low Dose Insemination in the Sow – A Review, *Reprod Dom Anim*, 37, 201-5.
11. *Roseboom JK, Reicks LD, Wilson EM*, 2004, The reproductive performance and factors affecting on-farm application of low-dose intrauterine deposition of semen in sows, *J Anim Sci,* 82, 2164-8.
12. *Singelton WL*, 2001, State of the art in artificial insemination in the Uniteted States, *Theriogenelogy,* 56,1305-10.
13. *Stančić B, Božić A, Radović I, Grafenau P, Pivko J, Hrenek P, Stančić I*, 2007, Veštačko osemenjavanje svinja dozama sa samanjenim brojem spermatozoida (pregled), *Savremena poljoprivreda,* 56, 1-2, 1-11.
14. *Stančić B, Grafenau P jr, Radović I, Petrović M, Božić A*, 2009, Intenzitet iskorištavanje sperme nerastova u Vojvodini i mogućnost njegovog povećanja, *Savremena poljoprivreda*, 58, 1-2, 19-26.
15. *Stančić B, Petrović M, Radović I, Gagrčin M,* 2008, Povećanje reproduktivne efikasnosti nerastova primenom nove tehnologije veštačkog osemenjavanja svinja, *Biotechnol Anim Husb,* 24 (spec. issue), 175-84.
16. *Stančić B, Radović i, Božić A, Gagrčin M*, 2009, Fertilitet krmača posle klasičnog vo inseminacionim dozama različitog volumena i broja spermatozoida, *Savremena poljoprivreda*, 58, 1-2, 62-6.

17. *Stančić B, Radović I, Stančić I, Gagrčin M, Božić A, Kragić S, Grafenau P, Chrenek P, Pivko J*, 2006, Fertilitet krmača posle intracervikalne i intrauterine inseminacije dozama redukovanog volumena, *Biotechnol Anim Husb,* 22 (special issue) 273-81.
18. *Stančić B, Grafenau P, Pivko J, Gagrčin M*, 2002, Influence of dilution rate and preservation time on the boar sperm quality, Proc XVIII Int Conf Farm Anim Reprod Liptovsky Jan (Slovakia), May 30-31, 110-2.
19. *Stančić B, Pivko J, Kubovičova E, Grafenau P,* 2003, Variranje progresivne pokretljivosti u razređenoj spermi nerastova tokom godine, *Savremena poljopoprivreda,* 52, 3-4, 263-8.
20. *Stančić B*, 2002, Kvalitet sperme nerastova na vojvođanskim farmama, *Biotechnol Anim Husb*, 18, 5-6, 103-7.
21. *Stančić B*, 2000, Savremeni principi tehnologije veštačkog osemenjavanja svinja (pregled), 3. Simpozijum "Uzgoj i zaštita zdravlja svinja", Vršac, 21-23. jun, Zbornik radova, 35-41.
22. *Vansickle J,* 2002, New Insemination Tool Still Unproven, *Nat Hog Farm*, 7, 1-3.
23. *Watson PF, Behan JR*, 2002 Intrauterine insemination of sows with reduced sperm numbers: results of a commercially based field trial, *Theriogenology*, 57, 6, 1683-93.
24. *Wongtawan T, Saravia F, Wallgren M, Caballero, Rodriquez-Martinez H*, 2005, Fertility after deep intra-uterine artificial insemination of concentrated low-volume boar semen doses, *Theriogenology,* 62, 1, 1-2.

## FERTILITET KRMAČA POSLE INTRACERVIKALNOG ILI INTRAUTERINOG OSEMENJAVANJA RAZLIČITIM BROJEM SPERMATOZOIDA U DOZAMA REDUKOVANOG VOLUMENA

STANČIĆ B, RADOVIĆ I, STANČIĆ I, BOŽIĆ A i GVOZDIĆ D

SADRŽAJ

Savremena intenzivna proizvodnja svinja zahteva dobijanje što većeg broja inseminacionih doza po ejakulatu genetski superiornih nerastova. Radi realizacije ovog cilja, istražuju se mogućnosti pravljenja inseminacionih doza redukovanog volumena i broja spermatozoida, ali da inseminacija ovakvim dozama ne rezultuje smanjenim fertilitetom krmača. U ovom radu je ispitivan uticaj osemenjavanja dozama duplo redukovanog volumena (50 ml) i različitiog broja spermatozoida u dozi (4, 2 ili $1x10^9$), posle klasične (intracervikalne) i nove (intrauterine) tehnologije osemenjavanja, na osnovne parametre fertiliteta krmača (% prašenja i veličina legla). Vrednost prašenja je opadala sa smanjenjem broja spermatozoida u dozi, kako posle intracervikalnog, tako i posle intratuterinog osemenjavanja. Međutim, vrednost prašenja je, bez obzira na broj spermatozoida u dozi, bila znatno veća posle intrauterinog (83,3%, 76,7% i 66,7%) u odnosu na klasično intracervikalno osemenjavanje (73,3%, 66,7% i 50%). Veličina legla nije značajno varirala u zavisnosti od primenjene metode osemenjavanja ili broja spermatozoida u dozi. Ovi rezultati ukazuju da je, primenom nove tehnologije intrauterinog osemenjavanja, moguće koristiti doze znatno redukovanog volumena i broja spermatozoida, a da se, pri tome, postignu zadovoljavajuće vrednosti prašenja i veličine legla. Ovo stvara mogućnost dobijanja značajno većeg broja doza po ejakulatu, u odnosu na klasičnu tehnologiju intracervikalnog osemenjavanja. Time se može značajno povećati stepen reproduktivne eksploatacije genetski superiornih nerastova.