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Sow fertility after insemination with varying doses of volume and spermatozoa count

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Abstract: The aim of the present study was to investigate the possibility of increasing boar's reproductive exploitation by using AI doses of doubly reduced volume and sperm count in the intrauterine AI procedure. The experiment was conducted at a commercial pig farm in Serbia in 2014. Classic intracervical insemination (ICI) was performed by using 50 mL or 100 mL volume doses containing 4×10^9 or 2×10^9 progressively motile spermatozoa. The same volumes and sperm numbers per dose were used with intrauterine insemination (IUI). Each dose combination was used to inseminate 30 sows. Intrauterine insemination with AI doses of reduced volume (50 mL) and sperm count (2×10^9) did not produce a statistically significant difference ($P < 0.05$) in the farrowing rate (76.7%) as compared with 4×10^9 spermatozoa in the same volume (83.3%) or with insemination by doses of 100 mL with a 2×10^9 (83.3%) or a 4×10^9 sperm count (86.7%). The number of live-born piglets (10.82) was larger following IUI using a 50 mL volume dose with a 2×10^9 sperm count as compared with ICI with the same AI dose volume and sperm count (9.85). The results show that the use of reduced AI dosages provides an opportunity for the swine industry to considerably exploit the reproductive potential of genetically superior boars.

Key words: Artificial insemination, dose, fertility, number, sow, spermatozoa, volume

1. Introduction

About 99% of pig artificial insemination (AI) worldwide is performed with diluted liquid semen, stored at 15 to 20 °C for 0 to 5 days, with 85% of AI doses used within the day of collection or on the following day (1,2). In the classic intracervical AI, an insemination dose volume of 80 to 100 mL of diluted liquid semen, with 3×10^9 to 5×10^9 motile spermatozoa (a mean of 4×10^9), is used (2). On average, a single boar produces about 1200 of such insemination doses annually (3). In terms of today's production needs and expenses, the number of annual insemination doses produced per boar ejaculate is inefficient (3–5). This is the reason for increasing the number of AI doses per ejaculate, which often leads to a higher dilution rate of ejaculate. However, it has been frequently shown that using overdiluted AI doses is the main reason for reduced fertility in artificially inseminated animals as compared with naturally mated sows (6). To overcome this problem, AI doses of reduced volume and spermatozoa numbers delivered by the intrauterine route have received considerable attention. However, an overriding consideration in using reduced AI doses and new delivery techniques is that when compared with the classic intracervical insemination, sow fertility rates should not be compromised (7–12). Due to the high cost of high genetic quality boars used in the Serbian commercial pig production, it is necessary to increase the number of insemination doses per boar per year, while maintaining the sow fertility rate. We assumed that this can be achieved using intrauterine insemination with reduced doses of volume and sperm count.

Therefore, the objective of this study was to evaluate sow fertility rates when comparing the classic intracervical AI technique with intrauterine AI procedures that use reduced doses of volume and spermatozoa.

2. Materials and methods

The experiment was conducted at a commercial farm unit in Vojvodina, Serbia, during a regular production cycle.

2.1. Farm performances

In the breeding herd there were about 1500 sows (75% Swedish landrace and 25% Large white), with a relatively

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low sow farrowing rate (an average of 78% in 2012/2013). High genetic quality boars (Swedish landrace, Large white, Duroc, and Hampshire) of domestic or foreign origin were used for on farm AI. Lactation lasted for 28 days. The weaned sows stayed in crates for 30 days after AI, and then went to open group pens. In the herd there were clinical symptoms of PRSA, but the disease was not laboratory confirmed in the experimental period. Vaccination was carried out against hog cholera and swine erysipelas.

2.2. Experimental procedure

Classic intracervical insemination was performed using 50 mL or 100 mL volume doses containing 4×10^9 or 2×10^9 progressively motile spermatozoa (80% of the total spermatozoa number per dose). The same volumes and sperm counts per dose were used with transcervical intrauterine insemination (into the uterine body). Each dose combination was used to inseminate 30 Swedish landrace sows (a total of $30 \times 2 \times 4 = 240$) of second to fifth farrowing parity, in which the estrus was detected on the fourth or fifth day after weaning. Detection of estrus was performed twice daily using a full boar contact at 12 h intervals (morning and evening), starting on the second day after weaning. The CASA system for the semen production lab (Sperm Vision Production, Minitübe, Germany) was used for progressive motility assessment. For each insemination fresh diluted semen was used from the same Swedish landrace boar. Three boars were used for AI of all experimental sows. Boars were of the same age (20 to 23 months) and average ejaculate parameters (volume: 250 mL to 300 mL; total sperm count: 60×10^9 to 65×10^9; progressive motility: 80% to 90%). Approximately the same number of sows underwent intracervical or intrauterine insemination with AI doses from each boar by the same operator. Insemination was performed about 3 to 4 h after semen collection. After cleaning the vagina, intracervical inseminations were performed using sterile disposable Foamtip Safe Blue catheters (Minitübe), and intrauterine inseminations were performed using sterile disposable "Verona" Foamtip catheters (Minitübe), according to accepted industry protocols with commercially reared pigs. For short term storage of liquid extended semen, BTS1 extender (Minitübe) was used. The first insemination was performed at 12 h, and the second one 36 h after standing estrus detection. Experimental sows stayed in crates for 30 days after AI, and then were housed in open group pens, separated from other production sows. The sows were considered to have returned to estrus (rebreeding) when they had full boar contact twice a day, which was detected at day 14 after AI.

2.3. Statistical analysis

The farrowing rate, expressed as the ratio of number of sows farrowed/number of sows inseminated or as % of sows inseminated, was analyzed for effects of insemination methods, dose volume, and sperm count using Fischer's exact test. Litter size, expressed as the number of total (live and dead) piglets farrowed/sow, were analyzed for effects of insemination methods, dose volume, and sperm count by general analysis of variance and mean comparisons using Fischer's protected LSD, using Statistica 12 software (StatSoft Inc., Tulsa, OK, USA). The mathematical model can be given as follows:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

where Y_{ij} = observed value on the treatment; μ = population mean; α_i = effect of treatment; and e_{ij} = error term.

3. Results

Classical intracervical insemination with 100 mL volume doses of 2×10^9 spermatozoa produced farrowing rates of 76.7%, which was not significantly ($P > 0.05$) lower when compared with the 83.37% rate produced by doses of 4×10^9 spermatozoa, as shown in Table 1.

The farrowing rates of intrauterine insemination with doses of the same 100 mL volume were 83.3% and 86.7% for spermatozoa numbers of 2×10^9 and 4×10^9, respectively,

Table 1. Effect of insemination method, dose volume, and sperm count on farrowing rate.

Insemination dose parameters		Farrowing rate, %[1]	
Volume, mL	Sperm per dose	Intracervical insemination	Intrauterine insemination
50	2×10^9	66.7^{ax} (20/30)	76.7^{az} (23/30)
	4×10^9	73.3^{ay} (22/30)	83.3^{az} (25/30)
100	2×10^9	76.7^{ay} (23/30)	83.3^{az} (25/30)
	4×10^9	83.3^{ay} (25/30)	86.7^{az} (26/30)

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

[x,y,z]Within a column, means without a common superscript differ ($P < 0.05$).

[1]Values in parenthesis = number farrowed/number inseminated.

and were not significantly different (P > 0.05) from each other. The intrauterine insemination procedure at 100 mL volume and 2×10^9 or 4×10^9 spermatozoa numbers did not significantly increase (P > 0.05) farrowing rates (83.3% or 86.7%) when compared with the intracervical procedure (76.7% or 83.3%) using the same volume and number of spermatozoa.

The intracervical procedure at volumes of 50 mL with 2×10^9 (66.7%) showed significantly reduced (P < 0.05) farrowing rates as compared with intracervical insemination at 50 mL volume with 4×10^9 (73.3%), as well as at 100 mL volumes with 2×10^9 (76.7%) or 4×10^9 spermatozoa (83.3%). However, farrowing rates following intracervical insemination with 4×10^9 spermatozoa in volumes of 50 mL (73.3%), and with 2×10^9 or 4×10^9 spermatozoa in volumes of 100 mL (76.7% or 83.3%, respectively), were not significantly different (P > 0.05) from each other. The farrowing rates of intrauterine insemination with doses of 50 mL were 76.7% and 83.3%, for spermatozoa numbers of 2×10^9 and 4×10^9, respectively, and were not significantly different (P > 0.05) from each other (Table 1).

The average number of live-born piglets per litter ranged from 9.85 to 10.27 piglets in the classic intracervical insemination, and 10.04 to 10.82 piglets in the intrauterine insemination (Table 2). The method of insemination and the number of spermatozoa contained in a dose had a highly significant influence (P < 0.01) on the average number of live born piglets and the total number of piglets farrowed per litter, as shown in Table 2.

The volume of the dose had no effect (P > 0.05) on the number of live piglets farrowed. The number of dead born pigs did not significantly differ (P > 0.05) depending on the applied method of insemination, dose volume, and number of spermatozoa in a dose.

4. Discussion

The average farrowing rate following the classic intracervical AI on commercial pig farms in Serbia is lower (average 77%) than that in the developed European countries (85% to 90%) and the reproductive exploitation of high genetic quality boars is inefficient (4,5). Increasing the number of AI doses per ejaculate is the most commonly used method for improving the efficiency of boar reproductive exploitation. However, this often leads to ejaculate overdilution, which has been shown as the main reason for reduced fertility in artificially inseminated animals as compared with naturally mated sows (6,13). Therefore, we assumed that increasing the number of insemination doses per boar per year, without decreasing sow fertility rates, can be achieved using intrauterine insemination with reduced doses of volume and sperm count. We considered the farrowing rate over 77% as an acceptable value according to the present production conditions on Serbian pig farms.

Our results clearly show that intrauterine insemination provides technologically acceptable farrowing rates (≥80%), in the Serbian farm practice, with doses of reduced volume (50 mL) and number of spermatozoa (4×10^9) and with typical volumes (100 mL) and 2×10^9 and

Table 2. Effect of insemination method, dose volume, and sperm count on litter size at farrowing ($\bar{x}$ ± SD).

Insemination dose parameters		Litter size, piglets/sow farrowed (means ± standard deviation)					
		Intracervical insemination			Intrauterine insemination		
Volume, mL	Sperm per dose	Live	Dead	Total	Live	Dead	Total
50	2×10^9	$9.85^{axy} \pm 0.31$	0.50 ± 0.13	$10.35^{ax} \pm 0.35$	$10.82^{bx} \pm 0.28$	0.48 ± 0.12	$11.30^{bx} \pm 0.32$
	4×10^9	$10.27^{ay} \pm 0.30$	0.50 ± 0.12	$10.77^{ay} \pm 0.34$	$10.04^{ax} \pm 0.28$	0.44 ± 0.12	$10.48^{ay} \pm 0.32$
100	2×10^9	$10.17^{axy} \pm 0.29$	0.52 ± 0.12	$10.70^{axy} \pm 0.33$	$10.25^{ax} \pm 0.28$	0.64 ± 0.11	$11.16^{axy} \pm 0.32$
	4×10^9	$10.16^{axy} \pm 0.28$	0.48 ± 0.11	$10.64^{axy} \pm 0.32$	$10.31^{ax} \pm 0.27$	0.54 ± 0.11	$10.85^{axy} \pm 0.31$

P-values for the main effects	Live	Dead	Total
Insemination methods	0.0055	0.5290	0.0073
Insemination volume	0.2640	0.2215	0.2235
Sperm number in dose	0.0086	0.4002	0.0096

[a,b]Within a row, means without a common superscript differ (P < 0.05).
[x,y]Within a column, means without a common superscript differ (P < 0.05).

4×10^9 sperm counts. This is in contrast to the results of the intracervical procedure, which achieved a maximal 83.3% farrowing rate only with 4×10^9 spermatozoa in doses of 100 mL. Furthermore, intrauterine insemination allows the application of AI doses with doubly reduced volume and sperm count, without decreasing sow fertility, as compared with conventional intracervical AI. This is very important for increasing the reproductive efficiency of high genetic quality boars.

The finding of the present study confirm the results of others in that intrauterine insemination with doses of various volumes (100, 85, 50, 30, and 20 mL) and spermatozoa counts (4, 3, 1.5, and 1×10^9), produced farrowing rates ranging from 78% to 96% with litter sizes of 9 to 12 live-born piglets (7–14). Additionally, a key feature in the success of intrauterine insemination is the deposition of semen in the cranial parts of the female reproductive system (body of uterus, uterine horns, uterotubal junctions, or oviducts). By the use of this technique, the insemination dose volume and the number of spermatozoa in a dose can be radically reduced, whereas the fertility of sows remains the same or improves as compared with that of the classic intracervical insemination technique (7,11,12). Several factors can explain the success of intrauterine insemination. First, the deposition of semen in the body of the uterus or uterine horns reduces the possibility of losing the sperm through polymorphonuclear leukocyte phagocytosis (15,16). Secondly, there is little or no loss of spermatozoa caused by semen backflow from the female reproductive system, which quite often happens immediately after intracervical insemination (8,17). However, the number of spermatozoa in a dose necessary to achieve the optimum farrowing rate and litter size is significantly linked with the interval from insemination to ovulation, as well as with the number of performed inseminations (7,9,14,18). Previous studies reported that optimal farrowing rates in sows could be achieved if insemination is performed around 24 h before ovulation, with doses containing 2×10^9 spermatozoa. An increase in the number of spermatozoa above this value has no impact on the achieved fertility, whereas a reduction in the number of spermatozoa below 2×10^9 results in lowered fertility in inseminated sows (4,10,19). Those studies showed that with intrauterine insemination, doses of one-half of the volume and the number of spermatozoa that are employed with classic intracervical insemination will produce a satisfactory farrowing rate and litter size. With these methods, it is possible to obtain twice as many doses per ejaculate, while not increasing the level of ejaculate dilution. For example, producing twice as many doses of a 100 mL volume with 4×10^9 spermatozoa from the same ejaculate requires its double dilution. Conversely, if one-half of the volume (50 mL) and spermatozoa number (2×10^9) are used, it negates the need for extra dilution. This can become a major factor because the semen of a large number of boars does not tolerate an increased extension rate. Only 20% to 30% of boars produce semen that maintains ≥65% progressive motility after 72 h of storage when extended at a 1:4 dilution (20,21). Also, increasing the amount of artificial extenders in semen leads to a decrease in progressive motility and agglutination of spermatozoa (22–24). Dilution reduces the protein content and natural antioxidants, as well as other natural ingredients of seminal plasma necessary for normal functioning and the integrity of the sperm cell membrane (1,25,26). Additionally, seminal plasma plays an important role in regulating capacitation, the establishment of an oviductal sperm reservoir (27), the modulation of the uterine immune response (15), and sperm transport in the female genital tract, as well as in gamete interaction and fusion (28–30).

In summary, we showed that intrauterine insemination with one-half the volume and number of spermatozoa is capable of producing higher fertility rates in sows when compared with classic intracervical insemination under present production conditions on commercial pig farms in Serbia. Utilizing these techniques, the number of insemination doses per boar ejaculate is increased without increasing the ejaculate dilution rate. This procedure allows the Serbian swine industry to considerably exploit the reproductive potential of genetically superior boars without decreasing sow fertility rates.

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