Veterinarski Glasnik 2017, 71 (2), 98-106
UDC: 636.4.082.453.5
https://doi.org/10.2298/VETGL1702098V

Full Research Article

ANALYSIS OF REPRODUCTIVE PARAMETERS AFTER INTRAUTERINE INSEMINATION OF SOWS WITH SEMEN STORED FOR DIFFERENT TIME PERIODS

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Received 27 September 2017; Accepted 23 October 2017
Published online: 30 November 2017

Abstract

Introduction. Artificial insemination of sows with cooled semen has become a routine practice in the swine industry and has almost completely ruled out natural mating. The storage time of the cooled semen and preservation of its qualitative parameters are of the upmost importance and depend partly on the semen extender used. The aim of this study was to determine the quality of cooled semen during storage in a thermobox with a commercial extender by assessment of sperm motility, cytomorphology, and assessment of reproductive parameters in sows after intrauterine insemination.

Materials and Methods. The semen was preserved with Duragen® (Magapor, Spain) extender and stored at 17±1°C. A total of 110 sows were included in the study and randomly divided into four groups: K1 (n=25) , K2 (n=25), K3 (n=30), and K4 (n=30). The sows were inseminated twice with semen (>1x10^7/ml spermatozoa) which had been stored for 1 day (S1), 3 days (S3), 5 days (S5), or 7 days (S7). Pregnancy was diagnosed by imaging ultrasound.

Results and Conclusions. The best results were obtained when the sows were inseminated with semen stored for one day. However, there were no statistically significant differences in the number and vitality of newborn piglets when semen stored for 3 or 5 days was used. Intrauterine insemination resulted in a satisfactory number of farrowings and piglets despite the fact that a relatively low number of spermatozoa was determined in each dose used. The use of semen with a higher dilution rate and its intrauterine application ensured a larger number of obtained doses from one ejaculate and indicates this would lead to a more profitable use of boars.

Key Words: boar, cooled semen, intrauterine insemination, piglets

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INTRODUCTION

Boar spermatozoa are susceptible to the effects of temperatures below zero, and hence, in the swine industry, the use of only slightly chilled boar semen for artificial insemination is common. The storage time of semen chilled at 16-18ºC and preserved with extenders is from 5 to 7 days. After this time, spermatozoa undergo unwanted changes which are reflected as a decreased number of mobile and viable sperm cells and subsequently decreased fertility (Mezalira et al., 2005). Semen fertility is dependent upon the quality of the sampled semen, the extender medium, and storage conditions. Progressive motility decreases during storage from 89-90% in fresh sperm samples to 65-75% after 24 h. Eventually, after 72h it decreases below 60% regardless of the type of semen expender used. This modern trend in swine reproduction allows storage of extended semen at temperatures between 16 and 18°C (Kuster and Althouse, 1999).

Insemination of sows and gilts is done during the estrus, prior to ovulation i.e. when the animal is in heat and the sow is displaying its willingness to mate (positive lumbar test), which occurs mainly 24-36 h from the beginning of estrus. A higher fertility rate is obtained when the sow is inseminated twice (Petrujkić et al., 2011). With one boar ejaculate, a total of 1500 insemination doses for intracervical insemination (3x 109 progressively mobile sperm cells in each dose), 100 ml each, can be obtained. In a high quality herd, 600 sows can be inseminated with an average of 2.5 doses per successful insemination (Belestra, 2004). Sows and gilts can also be inseminated intra utero. Intensive production demands a significantly higher number of offspring from genetically superior boars. It has been shown that both the volume and semen dose can be significantly decreased when semen deposition is administered deeper, i.e. towards the cranial portion of the uterus. A number of authors have estimated that shallow intrauterine insemination with doses containing 0.5 x 109 spermatozoa has results which are similar to classical intracervical insemination (Watson et al., 2002, Belstra, 2004, Mezalira et al., 2005).

The aim of this study was to determine the reproductive indicators in sows inseminated intra utero with boar semen which had been diluted with a commercial extender and stored in a thermobox at 17±1°C for one, three, five or seven days. The number of pregnant sows, total number of piglets, and number of live and still born piglets were taken into consideration as reproductive indicators.

MATERIALS AND METHODS

A total of 110 clinically healthy Large Yorkshire sows, from private pig farms in the region of Srem, Serbia, were included in the trial. The animals were from 2 to 4 years of age, parity from 2 to 5 and farmed under similar ambient, dietary and farming conditions.

Sampling, testing, scoring and dilution of boar semen

Boar ejaculates of approximately 200ml each were obtained by masturbation with the technique of manual fixation. For artificial insemination of 110 sows, four ejaculates were taken. The estimated number of spermatozoa was approximately 4 x 10^8/ml in each of the four ejaculates. The semen was conserved with Duragen® (Magapor, Spain) at a ratio of 1:8 and stored in a thermobox at 17±1°C. After dilution, 60 doses were obtained, each dose having a volume of 90 ml and number of spermatozoa >1x10^7/ml. The total and progressive sperm motility was estimated under a coverslip on a microscope slide at 37°C. Spermatozoa concentration was measured in 1 ml of semen with the aid of a chemocytometer. For estimation of the numbers of live spermatozoa, and subpopulations of pathologically altered sperm cells, the permanent eosin-nigrosin stain method was applied.

The selected sows were allotted to one of the following experimental groups: K1 (sows inseminated with semen stored for 1 day, n=25), K3 (sows inseminated with semen stored for 3 days, n=25), K5 (sows inseminated with semen stored for 5 days, n=30), and K7 (sows inseminated with semen stored for 7 days, n=30). Sows were inseminated twice, the first time 6 h and the second 24 h after the first sign of estrus (standing reflex) was noticed, with semen stored for 1 day (S1), 3 days (S3), 5 days (S5) or 7 days (S7) with an intrauterine catheter for single use (Spirette safe blue, MINI-TUBE, Germany). The catheters were coated with an antibiotic lubricant and wrapped in a closed plastic casing. For intrauterine insemination, the tip of the catheter was introduced into the cervical canal. Thereon, through this catheter, a thinner but longer catheter was introduced. When the tip of the inner catheter reached the uterus (20-30 cm from the tip of the main catheter) the semen dose was deposited.

Examination of pregnant sows

Pregnancy was diagnosed with a 3.5 MHz ultrasound probe (Control AC1) 25 days after insemination. Sows which disclosed images which corresponded to embryos and fetal membranes were considered pregnant. A second check-up was done after farrowing, when other reproductive parameters were also determined.

Statistical data analysis

The included descriptive statistical parameters were: arithmetical mean, standard deviation, standard error, variation interval and coefficient of variation. For testing the significance of difference between experimental groups, two tests were used. ANOVA was used to reveal significant differences between treatments. Tukey's test was used to analyze the difference between individual parameters. Significance levels 1% and 5% were considered to be statistically significant. Statistical analysis of the results was performed with PrismaPad 4.0 and MS Excel software packages.

RESULTS

Estimation of spermatozoa motility in semen with different storage times

The S1 boar semen tested showed overall motility of 90% and progressive motility was estimated at 85%. S3 semen displayed an overall motility of 80% and progressive motility was 75%. The diluted S5 semen contained 75% motile spermatozoa and an estimated progressive motility of 70%. After seven days of storage (S7), the total motility decreased to 70% and the progressive motility was 65%.

Eosin-nigrosin staining disclosed 10% dead spermatozoa after one day of storage, 20% after three days, 25% after five days and 30% after seven days of storage (Table 1).

Table 1. Progressive motility and percentage of live spermatozoa in the diluted semen examined on days 1 (S1), 3 (S3), 5 (S5) and 7 (S7) of storage

	S1	S3	S5	S7
Total motility	90%	80%	75%	70%
Progressive motility	85%	75%	70%	65%
Percentage live/dead spermatozoa	90%/10%	80%/20%	75%/25%	70%/30%

Ultrasound examination 25 days after insemination determined the success of the insemination procedure. The percentage of pregnant sows inseminated with S1 semen was 94.7%, S3 semen 88.7%, S5 semen 78.6% and S7 semen 71.4%.

Number of piglets in a litter

The average number of piglets per litter in group K1 was 11.48±1.64, that is significantly higher ($p<0.05$) compared to the average number of piglets in group K7 (9.88±1.83) (Table 2).

Table 2. Descriptive statistical parameters for the total number of piglets in the litters

Semen	$\overline{X}$	SD	Sx	Min	Max	CV
S1	11.48[a]	1.64	0.3272	9.00	15.00	14.25
S3	10.80	2.22	0.4435	6.00	14.00	20.53
S5	10.60	1.44	0.2887	7.00	14.00	13.62
S7	9.88[a]	1.83	0.3666	7.00	14.00	18.55

a, $P\leq0.05$

Between all the other groups, there was no statistically significant difference in piglet numbers per litter. The highest degree of variation in the number of piglets in a litter

was in group K3 (20.53%), one of the reasons being that some sows in this group gave birth to only six piglets. The lowest coefficient of variation (13.62%) was recorded in group K5.

Data on the number of liveborn piglets

The group of sows inseminated with S1 semen gave birth to the highest number of liveborn piglets (11.04±1.49), which was significantly higher ($p<0.05$) than the number delivered by sows inseminated with S3 semen (9.80±1.90), and sows inseminated with S7 semen (9.64±1.87) (Table 3). Between all other groups, there was no statistically significant difference in number of liveborn piglets. The highest coefficient of variation (19.38%) was recorded in the group inseminated with semen stored for seven days. The lowest coefficient of variation (11.52%) was reported in the group of sows inseminated with semen stored for five days.

Table 3. Descriptive statistical parameters for the number of liveborn piglets per litter

Semen	$\overline{X}$	SD	Sx	Min	Max	CV
S1	11.04^{ab}	1.49	0.2971	9.00	14.00	13.46
S3	9.80^{a}	1.90	0.3786	6.00	13.00	19.32
S5	10.12	1.17	0.2332	7.00	12.00	11.52
S7	9.64^{b}	1.87	0.3736	6.00	14.00	19.38

Same letter indicate a significant difference at a, P≤0.05; b, P≤0.01

Total number of weaned piglets

In the group of sows inseminated with semen stored for one day, the number of weaned piglets was higher than in all other groups (9.48±1.99; Table 4). Insemination with semen stored for five days resulted in the lowest number of weaned piglets (8.72±1.17) (Table 4). Between these two groups, there was no statistically significant difference in the number of weaned piglets. Analysis of the variations in the number of weaned piglets revealed that it was almost equal within groups, the lowest being 13.46% in sows inseminated with semen stored five days, and highest in sows inseminated with semen stored for three or seven days.

Table 4. Descriptive statistical parameters for the number of weaned piglets in the litters

Semen	$\overline{X}$	SD	Sx	Min	Max	CV
S1	9.48	1.39	0.2776	7.00	12.00	14.64
S3	9.04	1.34	0.2676	6.00	12.00	14.80
S5	8.72	1.17	0.2347	7.00	11.00	13.46
S7	8.92	1.32	0.2641	6.00	12.00	14.80

DISCUSSION

One of the aims of this study was to verify the economic impact and advantages of intrauterine insemination of sows with a lower number of spermatozoa compared with classical intracervical insemination or natural mating. Experimental sows underwent intrauterine insemination with a dose of 90 ml containing $1x10^9$ spermatozoa. Application of even smaller semen doses was described by Weberski et al. (1994), Kuster and Althouse (1999), Martinez et al. (2001), Watson and Behan, (2002), Dimitrov et al. (2007), Fitzgerald et al. (2008), and Pelland et al. (2008), while Kruger and Rath (2000) used a very low number of spermatozoa in each dose ($1x10^6$/ml). Similar results on the fertility of semen were obtained by Estienne et al. (2007) when nine different semen extenders were used (Beltsvill Thawing Solution, Merck III, Androhep-lite, Sperm Aid, MR-A, Modena, X-Cell, VSP, Vital) in a dose of $35x10^6$ spermatozoa/ml stored at 18°C for seven days. Boonkusol et al. (2010) investigated semen diluted with extenders Beltsvill, Merch III and Androhep stored for 0, 1, 3, 5, and 7 days at 15°C. The motility of spermatozoa stored for 3 days after dilution was for Betsvill 60.9±6.5%, Merch III 70±7%, and Androhep 72.3±5.8%, which is in agreement with our results. In addition, Karageorgiou et al. (2016) were examined spermatozoa motility in semen diluted with Biopig, Optin I.A., or Duragen extenders during 48 h. The best results were obtained with Biopig extender (66.5%), while motilities in Duragen and Optin I.A. were 66.3% and 50.7%, respectively. In that study, semen stored for 5 and 7 days had lower spermatozoa motility values than the ones disclosed in our study (Betsvill 25.8±7.2%, Merch III 23.3±0% and Androhep 43.3±9.6%). However, better results were reported by Huo et al. (2002), who observed the effects of four different diluents (Beltsvill Thaw solution-BTS, Androhep, Zorlesco and Kiev). After seven days of storage, only dilution with Kiev resulted in semen with progressive motility of 57.93%, while it was higher in all other tested diluents (BTS 76.18%, Zorlesco 77.18%, Androhep 83.33%). Recently, Berg et al. (2014) examined motility of spermatozoa in TRIXcell+ extender after storage time of 10 days, and there were more than 60% progressive motility of spermatozoa.

With regard to the number of piglets born in a litter to sows inseminated with semen stored for one (11.48), three (10.80), five (10.60) or seven days (9.88), these results from our study are in agreement with the results published by Wabarski et al. (1994). Gomez et al. (2009) obtained a higher number of piglets by using the diluent Duragen which had been stored for 1, 12, and 15 days (15.4±0.88; 14.4±1.14; 9.1±0.85). These results can be explained by the way in which the study was carried out.

The number of liveborn piglets in sows inseminated with semen S1 was 11.4, semen S3 (9.8), semen S5 (10.12), and semen S7 (9.64) in the current study. Similar results were obtained by Johanson et al. (1988), who used cooled semen stored for four days and with different diluents, and obtained the following results: 10.7 (Betsvill), 10.5 (Modified) and 9.4 (MR-A). Contrary to expectations, Fitzgerald et al. (2008) and Dimitrov et al. (2007) obtained a lower number of piglets per litter after intrauterine

insemination: 8.97±0.54 and 8.88±0.41, respectively. Using Duragen diluent, Berg et al. (2014) obtained a high number of liveborn piglets (14.2±0.7) with cooled semen stored for 10 days.

CONCLUSION

Intrauterine insemination with a lower number of spermatozoa in each insemination dose gave satisfactory results (percentage of farrowings and litter size). By using more diluted semen and by intrauterine application, a larger number of insemination doses can be obtained from each ejaculate, thus enabling a more profitable use of reproductive boars.

Acknowledgements

This investigation was done within the project of interdisciplinary research III46002, finaced by Ministry ofEducation and Science of The Republic of Serbia.

Authors contributions

VS participated in the design of the study, conceived of the study, and participated in its design and coordination and helped to draft the manuscript. MV participated in the design of the study, conceived of the study, and participated in its design and coordination and helped to draft the manuscript. MM participated in the design of the study, conceived of the study, and participated in its design and coordination and helped to draft the manuscript. Milorad Mirilovic participated in the design of the study and performed statistical analyses of obtained data. SDj participated in the design of the study and performed statistical analyses of obtained data. AJ helped in drafting the manuscript and technicaly prepared manuscript for submission. BV performed clinical examination, artificial insemination and drafted the manuscrip.

Conflict of interest

The authors hereby declare that they have no competing interests

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ANALIZA REPRODUKTIVNIH PARAMETARA INTRAUTERINO OSEMENJENIH KRMAČA U ZAVISNOSTI OD VREMENA ČUVANJA RAZREDJENOG SEMENA

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Kratak sadržaj

Uvod. Veštačko osemenjavanje svinja rashlađenim semenom je postala rutinska praksa u industrijskoj proizvodnji svinja, pa je u razvijenim zemljama skoro potpuno zamenilo prirodno parenje. U zavisnosti od vrste upotrebljenog razređivača, dužina čuvanja rashlađenog semena i očuvanje njegovih kvalitativnih parametara je od izuzetne važnosti. Cilj istraživanja je analiza kvaliteta rashlađenog semena tokom njegovog skladištenja u termoboksu pri 17±1°C.

Materijal i metode. Seme je konzervirano upotrebom razređivača Duragen® (Magapor, Španija) i skladišteno pri temperaturi od 17±1°C. U ogled je uključeno 110 krmača, podeljenih u četiri grupe (K1, K3, K5, K7). Krmače su osemenjivane dvokratno semenom skladištenim 1 dan (S1), 3 dana (S3), 5 dana (S5) i 7 dana (S7). Ultrazvučnim pregledom je vršena dijagnostika graviditeta. Kvalitet semena je ocenjivan ispitivanjem pokretljivosti spermatozoida i citomorfološkim pregledom i praćenjem reproduktivnih parametara kod intrauterino osemenjenih krmača.

Rezultati i zaključak. Najbolji rezultat je postignut osemenjavanjem krmača semenom starim jedan dan. Međutim, nije bilo značajne razlike u broju i vitalnosti oprašene prasadi korišćenjem rashlađenog semena starog 3 i 5 dana. Intrauterinim osemenjavanjem su dobijeni zadovoljavajući rezultati (procenat prašenja i veličina legla) sa manjim brojem spermatozoida u inseminacionoj dozi. Upotrebom semena sa većim razređenjem i njegovim intrauterinim deponovanjem, omogućeno je dobijanje većeg broja inseminacionih doza semena od jednog ejakulata i rentabilnije korišćenje nerastova u reprodukciji.

Ključne reči: nerast, rashlađeno seme, intrauterino osemenjavanje, prasad