HETEROSPERMIC INSEMINATION PERFORMED BY FROZEN-THAWED AND LIQUID STORED BOAR SPERM

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Abstract

The objective of this study was to verify the fertility of cryopreserved boar sperm by heterospermic insemination performed by a mix of a liquid stored and post-thaw sperm. The second objective of this study was to utilize the benefits of a liquid stored insemination doses to reduce a negative impact on fertility results after post-thaw insemination performed by cryopreserved sperm. Eight sperm rich fraction from seven fertile hybrid boars with verified fertilizing ability were collected by the gloved-hand technique. Semen from five boars was cryopreserved and semen from two boars was liquid stored. Post-thaw sperm with 20-30% progressive motility and liquid stored sperm evaluated as a sperm with substandard quality were used to produce heterospermic insemination doses. In total, 14 sows were inseminated using the intrauterine technique. Piglet paternity was determined by molecular-genetic analysis of microsatellite markers. The results of paternity showed that the sires of all the born piglets were boars used as producers of liquid stored sperm. As expected, the negative impact of insemination performed by cryopreserved sperm on sow fertility results was reduced by heterospermic insemination performed by a mix of a liquid stored and post-thaw sperm. However, no piglets from cryopreserved sperm were born. Cryopreserved sperm was unable to compete with liquid stored sperm in heterospermic insemination doses.

Key Words: Boar semen, heterospermy, artificial insemination, cryopreservation

The use of heterospermic artificial insemination (mixing of spermatozoa from two or more boars) is common in commercial swine farms (Ferreira et al., 2014). Differences in sperm fertilizing capacity of males often remain undetected by routine semen parameters. Heterospermic insemination with equal numbers of spermatozoa from 2 males is an accurate method for assessing differences in fertility (Stahlberg et al., 2000) and prevents decreases in performance when conducted with stored semen (Haugan et al., 2005). Artificial insemination (AI) with frozen-thawed semen in pigs is not a routine technique; its use is usually restricted to specific cases, such as preservation of valuable genetic material (germplasm banks), safety strategies in case of natural disasters, long-distance transport of sperm, and in combination with sex-sorting (Yeste et al., 2017). Frozen-thawed semen exhibit much lower fertilizing capacity (Knox, 2015) and Cerolini et al. (2001) also recorded that poor survival rate of spermatozoa and, as a consequence, the high concentration is required in the insemination dose (ID). Cryopreservation of boar semen is still the subject of world research. The objective of this study was to verify the fertility of cryopreserved boar sperm by heterospermic insemination performed by a mix of a liquid stored and post-thaw sperm. The second objective of this study was to utilize the benefits of a liquid stored insemination doses to reduce a negative impact on fertility results after post-thaw insemination performed by cryopreserved sperm.

Material and Methods

Eight sperm rich fraction from seven fertile hybrid boars with verified fertilizing ability were collected by the gloved-hand technique. The following parameters were evaluated in fresh native boar semen: semen volume, progressive sperm motility, sperm concentration and morphologically abnormal spermatozoa. The progressive sperm motility was assessed with the use Computer Assisted Semen Analysis (CASA). The sperm concentration was determined by a cytometric method using Bürker's chamber. Morphologically abnormal spermatozoa (MAS) were assessed according to the staining method of

Čeřovský (1976) and evaluated microscopically under oil immersion at the 1500× magnification.

Semen from five boars (ranging from 1 to 5) was diluted by Safecell Plus extender (IMV, France) in dilution ratio of 1+1.5 and stored at the temperature 17°C. Progressive sperm motility was evaluated 24 hours after semen dilution. Semen was cryopreserved using the straw freezing procedures describe by Westendorf et al. (1975) and modified by Minitübe. Straws were thawed in a water bath at 38°C for 40 seconds.

As a compensation for reduced spermato-analytical parameters of post-thaw sperm, two boars characterized by producing substandard semen quality were chosen for liquid stored sperm preservation. Boars were marked as boar A, boar B and B2 (B2 is ejaculate from boar B used in the 2nd period insemination). Ejaculates of these two boars were processed and diluted in Safecell Plus extender to the final concentration of 3.0×10^9 spermatozoa/ID. Diluted semen was stored at the temperature 17°C in the thermobox and was used within 72 hours. Just before every insemination, heterospermic ID containing 6.0×10^9 spermatozoa/ID was prepared by mixing post-thaw sperm (3.0×10^9) and liquid stored sperm (3.0×10^9).

Fourteen selected sows ranging from 4 to 8 litters were chosen for insemination of heterospermic ID. Insemination was performed by the intrauterine technique. Insemination was divided into two periods. In the 1st period 9 sows were inseminated and in the 2nd period 5 sows were inseminated. Sows were inseminated at an average four times during their oestrus.

The sperm of boars, skin of sows and piglets were taken to the paternity determination. The paternity was determined by molecular-genetic analysis of microsatellite markers in laboratory of Agrogenomics in Brno (LAMGen s. r. o., Czech Republic).

Basic statistical analyse of the results were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The initial semen quality parameters of five boars used for cryopreservation are presented in the Table 1. In addition there are also values of sperm motility during cryopreservation and after thawing. Three boars had post-thaw progressive sperm motility 30%. In the Czech Republic 30% of progressive motility is suggested as a minimum for AI performed by cryopreserved semen. The other boars had progressive sperm motility 20 and 25%. Semen of boars used as producers of liquid stored ID was characterized by a high occurrence of morphologically abnormal spermatozoa and low sperm concentration (Table 2). There was noted a high incidence of morphologically abnormal spermatozoa in ejaculates from both boars. Boar A had 72% MAS, boars B and B2 (52% MAS and at the 2nd period 68.5% MAS). The highest portion of morphological abnormalities was represented by proximal protoplasmatic droplet followed by distal protoplasmatic droplet. Progressive sperm motility in time of insemination (after 24h, 48h and 72h of storage time) was at substandard quality ranging from 40% to 55%. Minimum of 50% progressive sperm motility is suggested for AI in the Czech Republic. Fourteen sows were inseminated by heterospermic insemination doses containing mix of post-thaw sperm and liquid stored sperm (Table 3). Sows were inseminated with intrauterine technique in their oestrus and the number inseminations were between 3 and 5 per sow. Two sows aborted on the 51st and the 60th day of their pregnancy. Samples for assessment of piglet paternity were taken after farrowing. The results of paternity showed that the sires of all the born piglets were boars used as producers of liquid stored sperm. Although liquid sperm with substandard quality was used, no piglets from cryopreserved sperm were born. Flowers et al. (2016) recorded in their study that heterospermic insemination and subsequent paternity testing is an effective technique for defining relationships between common semen quality tests and fertility, especially in situations where reproductive performance of all the boars is high. Stahlberg et al. (2000) supports the hypothesis that the viability of sperm cells in the female reproductive tract might contribute to differences in the proportion of offspring after heterospermic insemination.

Table 1. Boar semen quality parameters before and after freezing (n=5)

Boar	1	2	3	4	5
Progressive sperm motility (%)	75	65	80	70	50
Semen volume (ml)	170	330	130	160	220
Morphologically abnormal spermatozoa (%)	9	2,5	10	10,5	25,5
Sperm concentration($\times 10^3/mm^3$)	327.5	165	420	492.5	252.5
Progressive sperm motility 1+1.5 in SCP (%)	70	70	75	70	50
Progressive sperm motility after 24h (%)	70	70	70	60	40
Post-thaw progressive sperm motility (%)	30	20	30	25	30

Table 2. Quality parameters of liquid stored of boar semen A, B and B2

Boar	A	B	B2
Progressive sperm motility (%)	65	70	75
Semen volume (ml)	380	550	390
Morphologically abnormal spermatozoa (%)	72	52	68.5
Sperm concentration($\times 10^3/mm^3$)	125	180	210
Progressive sperm motility 0h (%)	60	65	55
Progressive sperm 24h (%)	40	50	55
Progressive sperm motility 48h (%)	40	40	50
Progressive sperm motility 72h (%)	40	40	40

B2 – boar B in the 2nd period insemination

Table 3. The results of heterospermic insemination according to the used pairs of boars

Pairs of boars liquid /cryopreserved	A/1	B2/2	B/3	B/4	B2/5
Sows inseminated (n)	3	3	2	4	2
Average number of insemination/sow	4	5	3	3.5	5
Conception rate (%)	100	100	100	100	100
Farrowing rate (%)	100	100	50	75	100
Number of total born piglets/litter	10	12.67	12	12.33	14
Number of live born piglets/litter	8	12.33	11	11.67	12.5
Paternity of piglets	A	B2	B	B	B2

B2 – boar B in the 2nd period insemination

Conclusion

The objective of this study was only partially achieved. As expected, the negative impact of insemination performed by cryopreserved sperm on sow fertility results was reduced by heterospermic insemination performed by a mix of a liquid stored and post-thaw sperm. However, the most important part of this experiment was unsuccessful. No piglets from cryopreserved sperm were born. In this study, cryopreserved sperm was unable to compete with liquid stored sperm in heterospermic insemination doses despite of the substandard quality of the liquid stored sperm used.

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