THE FIELD REPRODUCTIVE PERFORMANCE OF PIGS AFTER ARTIFICIAL INSEMINATION WITH SEMEN FROZEN IN STATIC VAPOURS OF LIQUID NITROGEN

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Abstract

The aim of the study was to estimate the effect of packaging forms (0,5 ml midi and 5 ml maxi straws) and two freezing methods of boar semen on reproductive performance of sows and gilts. The study was carried out on 150 crossbreed females (75 multiparous sows and 75 pubertal gilts) and 4 boars. Females have been randomly divided into ten research groups: fifteen pigs in each group. The sows and gilts in experimental groups were inseminated with frozen whilst in the control groups with liquid semen. Each ejaculate was divided into two samples after collection. First sample was frozen according to the method A, whilst second part was frozen according to the method B. Semen was packaged in 0,5 and 5 ml straws and frozen at uncontrolled rate in liquid nitrogen vapours in polystyrene boxes. The females were post cervically inseminated, before AI at every insemination dose 5 mg of PGF2α was added. The occurring of ovulation and pregnancy diagnosis were performed through ultrasonography. In sows inseminated with semen frozen according to the method A and B in 0,5 and 5 ml straws farrowing rate was 80 and 67% vs. 80 and 67%, whilst total litter size was: 10,1 and 9,1 vs. 10,2 and 9,2 respectively. In gilts inseminated with frozen semen according to the method A and B in 0,5 and 5 ml straws farrowing rate was: 73 and 67% vs. 67 and 60%, whilst total litter size was: 9,1 and 8,3 ($P \leq 0,01$) vs. 8,8 and 7,3 ($P \leq 0,01$), respectively. In sows and gilts inseminated with liquid semen farrowing rate and total litter size was: 93 and 93%, 12,6 and 10,7 ($P \leq 0,01$), respectively. Reproductive performance in females inseminated with semen frozen according to the method A were not significantly higher than in pigs inseminated with semen frozen according to the method B, except total litter size in gilts inseminated with semen packed maxi straws (8,3 vs. 7,3) ($P \leq 0,01$) where the difference was statistically significant. Comparing effect of midi and maxi straws there was no significantly influence of packaging form on reproductive performance of sows, whilst in gilts, females inseminated with semen packaged in 0,5 ml straws, gave significantly higher total litter size than gilts inseminated with semen confectioned in 5 ml maxi straws and frozen according to the method A and B (9,08 vs. 8,3 and 8,8 vs. 7,3) ($P \leq 0,01$).

Introduction

Today, various packages have been used for deep freezing of boar semen in order to obtain semen with acceptable post-thaw survival and fertility. In our center we have prepared the protocols A and B which are our modifications of the methods described originally by Westendorf et al. (1975) and by Pursel and Park (1987), respectively. Nowadays applied boars semen freezing protocols use a programmable freezers. Spermatozoa are cooled in these devices in optimal, controlled rate. Freezer has also disadvantage which is expensive price.

The most valid assessment of freezing methods of semen and of boar´s fertility is to obtain viable pregnancies and normal offspring following in vivo insemination (Johnson et al., 1981; Erikkson et al., 2002). Application of PGF2α to semen doses, post cervical inseminations and ultrasonography were used for the enhancement of freezing technique assessment (Pena et al.,1998). The aim of this work was to estimate the influence of packaging forms and freezing methods of boar semen on reproductive performance both sows and gilts.

Materials and methods

Experiments were carried out from 2004 to 2007 on 150 crossbreed animals of Polish Landrace and Polish Large White (75 multiparous sows and 75 of spontaneously ovulating gilts in a big farm of swine flock.

Both the sows and the gilts have been randomly divided into ten research groups: fifteen pigs in each group. Ejaculates from 4 Pietrain × Duroc boars were divided after collection into 2 equal aliquots and frozen according to the method A and B in 5 ml maxi and 0,5 ml midi straws which were frozen in polystyrene box onto static LN nitrogen vapour (Bielas et al., 2003; Bielas, 2006). The sows in groups 1,2,3,4 and the gilts in group 1´, 2´ 3´, 4´ were inseminated with frozen semen whilst in the control groups 5 (sows) and 5' (gilts) with liquid semen. The sows in group 1, 2 and the gilts in groups 1´ and 2´ were inseminated with semen frozen using the method A. The female in the group 3, 4, 3´ and 4´ were inseminated with the semen frozen according to the method B. The animals in even groups 2, 2´, 4 and 4´ were inseminated with semen confectioned in 5 ml maxi straws. The females in odd groups (1, 1´, 3 and 3´) were inseminated with semen packaged in 0.5 ml straws. One insemination dose containing 5 billions of spermatozoa was placed either into one of 5 ml maxi straw or into ten of 0.5 ml midi straws. The sows and the gilts in all experimental groups were post cervically inseminated three times every ten hours from the start of a standing reflex with disposable catheter (IMV, France). Before AI at every insemination dose 5 mg of PGF2α (1 ml of Dinolytic, Pharmacia Animal Health) was added (Pena et al., 1998).

The occurring of ovulation and pregnancy diagnosis were performed through ultrasonography (5-MHz sector mechanical scanner, Dramiński, Poland). The results were analyzed statistically using Statica 6,0 Pl computer software with the use of a single-factor analysis of variance in the orthogonal system. Differences between means were determined by Duncan or Tukey´s test.

Results and discussion

The fresh semen from the boars used for freezing was of acceptable quality (mean motility 80% and 95% normal acrosoms). The in vitro evaluation showed that quality of spermatozoa decreased after thawing of straws. Freezing and packaging of boar semen according to the method A both in 0,5 ml and 5 ml straws gave the best motility (45 and 37%) and alive sperms (58 and 52%) after thawing in comparison to the quality of semen after thawing of 0,5 and 5 ml straws frozen according to the method B (40 and 33% motility and 51 and 47% of alive sperms). (table 1) A total of 120 females were inseminated with frozen semen and in these animals the overall average farrowing rate and the total piglets born were 70% and 9,0 ±1,76 (females inseminated with liquid semen, as control: 93,33% and 11,67 ± 1,76), respectively. In sows inseminated with semen frozen according to the method A and B in 0,5 and 5 ml straws farrowing rate was 80 and 67% vs. 80 and 67%, whilst total litter size was: 10,1 and 9,1 vs. 10,2 and 9,2, respectively (table 2). Sows inseminated with liquid semen gave a significantly higher value of the total litter size than sows inseminated with semen frozen according to the method A in 0,5 and 5 ml straws, respectively (12,64 ± 3,1 vs. 10,08 ± 3,7 and 9,10 ± 1,87) and to the method B in 0,5 and 5 ml straws, respectively (12,64 ± 3,1 vs. 10,2 ± 5,5 and 9,2 ± 4,4) (P≤0,01) (table 2). Also gilts gave a significantly higher total litter size after insemination with liquid semen in comparison with semen frozen according to the method A as well as B both in 0,5 and 5 ml straws, respectively: (10,71 ± 1,45 vs. 9,08 ± 2,08 and 8,3 ± 0,45) and (10,71 ± 1,45 vs. 8,8 ± 1,3 and 7,33 ± 0,75) (P≤0,01) (table 3).

Gilts inseminated with semen frozen according to the method B and packaged in 5 ml straws gave a significant poor response for litter size (7,33 ± 0,75), both than gilts from groups 1´ (9,08 ± 2,08) and 3´ (8,8 ±1,3) (p≤0,01) which were inseminated with semen packaged in 0,5 ml straws (table 3). While there were no significant differences both in the farrowing rate and the litter size between the sows inseminated with semen frozen according to the method A as well as semen frozen according to the method B both in 0,5 and 5 ml straws (table 2). In sows inseminated with semen packaged in 0,5 ml straws were observed not significantly higher litter size than in sows inseminated with semen packaged in 5 ml straws which were frozen according to the method A (10,8 ±3,2 vs. 9,1±1,8) as well as according to the method B (10,25±5,6 vs. 9,2±0,4), respectively (table 2). However in gilts inseminated with semen packaged in 0,5 ml straws total litter size was significantly higher than in gilts inseminated with semen packaged in 5 ml straws which were frozen both according to the method A (9,08±2,8 vs. 8,3±0,45) and to the method B (8,8±1,28 vs. 7,3±0,75), respectively (P≤0,01) (table 3). In sows and gilts inseminated with frozen semen the farrowing rate was 73,33 vs. 68,33% and the total piglets born was 9,7 ±1,9 vs. 8,43 ±1,2, respectively (table 2, 3). In sows and gilts inseminated with liquid semen farrowing rate and total litter size was 93 and 93%+ 12,6 and 10,7 (P≤0,01), respectively (table 2, 3). These conception and fertility obtained in pigs after insemination with frozen thawed boar semen are similar to the results received by Johnson et al. (1981) and Erikkson et al. (2002) and are quite satisfactory. These results indicate that under good conditions (insemination strategy) frozen boar semen can give results that approach those obtained with fresh semen (Erikkson et al., 2002). However, it seems that frozen boar semen does not yet have the quality needed to achieve adequate results under the broad range of conditions in routine practical application. Moreover, the variation among boars in the freezability of the semen is still a significant problem (Woelders et al., 2005). Field trials with frozen-thawed semen have shown low fertility rates probably due to the difficulty in determining the correct time of AI (Johnson et al., 1981). Better fertility rates were obtained when AI was carried out close before to the time of ovulation (Erikkson et al., 2002; Scheid et al., 1990). But Almlid and Hofmo (1996) presented data which indicated that is possible to achieve litter size more than 10 piglets and satisfactory farrowing rates using frozen semen if special attention is paid to heat detection and timing of insemination related to ovulation. The most valid assessment of boar fertility is to obtain viable pregnancies and normal offspring following in vivo insemination. However, field trials of semen fertility are also imprecise because of the high variability associated with the female and with the conditions of insemination. Technical expertise in oestrus detection and competence to carry out the insemination can have a major impact on the success rates. The study presented by Pena et al. (1998) has shown that fertility and litter size can be significantly improved by the injection of 5 mg of PGF2α at the moment of AI in sows mated during the low fertility season. The improvement in conception rate and litter size was attributed to the enhancement of sperm transport along the female reproductive tract due to stimulation of PGF2α the contractions of the myometrium and oviducts in pig and advanced ovulation. Nowadays, approximately 25 million AIs are registered worldwide every year. More than 99% of these inseminations are made with semen extended in a liquid state. The remaining 1% of the inseminations is made with frozen-thawed spermatozoa at doses of 5000-6000 x 10^6 spermatozoa. Despite the large sperm number per dose, the fertility is substantially lower than that obtained with cooled semen (9). In sows inseminated intra cervically with 3000 x 10^6 cooled spermatozoa, only approximately 1 x 10^3 reach the sperm reservoir in the isthmus of the oviduct. The number of functionally frozen-thawed spermatozoa available in the oviduct is usually 10-fold lower compared with cooled semen. That´s why deposition of frozen-thawed semen in the anterior regions of the reproductive tract should allow a larger sperm population to reach the uterotubal junction.

The lower fertility compared with cooled semen obtained after intra-cervical artificial insemination, together with the large number of spermatozoa required per dose, constrains the widespread commercial application of frozen-thawed spermatozoa in the pig industry (Roca et al., 2006). The fertility success for frozen-thawed semen in AI programmes is highly dependent of the interval between AI and ovulation. At present, the methods for forecasting the time of ovulation are limited to the assessment of the average duration of oestrus, because spontaneous ovulation takes place when two-thirds of the standing oestrus period has passed (Roca et al., 2006). Reproductive performances of pigs such as: conception rate, farrowing rate, litter size were higher in the groups of females inseminated with semen frozen in the 0,5 ml straws in comparison to the 5 ml straws. Therefore, in this study is seen evident good effect (especially in gilts) of the midi straws in comparison to the maxi straws on reproductive indexes of pigs inseminated with frozen semen.

Improved reproductive performance of females inseminated with semen packaged in 0,5 ml straws was apparently due to the better quality of thawed semen deriving from this packages. Comparing effect of the freezing method A to the method B, the first mentioned method seems to have better influence on conception and fertility of inseminated pigs. The values of reproductive rates for sows inseminated with semen frozen according to the method A, were no significantly higher than the values of corresponding indexes of the method B. But in gilts inseminated with semen frozen according to the method A and packaged in midi straws total litter size was significant higher in comparison to the corresponding feature of the method B. The results obtained in this study could indicate that under good conditions (insemination strategy) boar semen frozen at uncontrolled rate in liquid nitrogen vapours in polystyrene boxes can give reproductive performance that approach nearly those obtained with fresh semen.

Table 1. Quality of spermatozoa from 4 boars after thawing and 0.5 h inkubation at 38 °C in BTS

Method	A		B	
Type of the straws	0,5 ml	5 ml	0,5 ml	5 ml
% MOT (CASA)	45	37	40	33
% DAR (Giemsa stain)	35	42	39	45
% alive sperms (SYBR 14/IP)	58	52	51	47

Table 2. Efficacy and efficiency of inseminations of sows with frozen and liquid semen with respect to the experimental group (mean, ±SE ; n = 75)

Number of group, method, packaging	Conception rate %	Farrowing rate %	Total litter size	Number of stillbirth
1, A, 0,5 ml	93,33	80,00	10,08 B 3,17	1,33 0,26
2, A, 5 ml straws	86,67	66,67	9,10 B 1,87	1,33 0,33
3, B, 0,5 ml	93,33	80,00	10,25 B 5,65	1,14 0,14
4, B, 5 ml straws	80,00	66,67	9,20 B 4,40	1,66 1,33
5, liquid semen	93,33	93,33	12,64 A 3,01	1,42 0,28
Total N = 75	83,33	77,33	10,14 5,19	1,34 0,31

Table 3. Efficacy and efficiency of inseminations of gilts with frozen and liquid semen with respect to the experimental group (mean, ±SE ; n = 75)

Number of group, method, straws	Conception rate %	Farrowing rate %	Total litter size	Number of stillbirth
1´, A, 0,5 ml	86,67	73,33	9,08 BD 2,08	1,25 0,25
2´, A, 5 ml	86,67	66,67	8,30 B 0,45	1,00 0,00
3´, B, 0,5 ml	100	66,67	8,80 BD 1,28	1,14 0,14
4´, B, 5 ml	66,67	60,00	7,33 BC 0,75	1,50 0,50
5´, liquid semen	93,33	93,33	10,71 A 1,45	1,33 0,25
Total n = 75	86,67	72,00	9,01 2,49	1,21 0,17

A,B Those values sharing the same superscript, within a parameter, do not differ from each other at p value ≤ 0,01

Conclusion

In conclusion, although the straws were frozen in Styrofoam box onto static liquid nitrogen vapours, the present freezing protocols of boar semen gave acceptable results of the field fertility trials. It probably was achieved because special attention was paid to heat detection and timing of insemination related to ovulation, addition of PGF2α to each insemination dose, good freezability of the sperms donors and improved of the freezing-thawing methods. The method B is simpler and less time consuming for preparation than method A. These fertility results obtained with frozen-thawed boar semen are quite satisfactory. These results indicate that under good conditions (insemination strategy) frozen boar semen can give results that approach those obtained with fresh semen.

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