



## Seasonal changes in fresh semen quality and freezability in boar semen

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### ABSTRACT

The objective of this study was to investigate the effect season on sperm quality in fresh and cryopreserved boar sperm. Ejaculates (59) of fertile boars were divided into 4 groups according to season: winter, spring, summer and autumn. Semen volume, sperm motility, sperm concentration, percentage of morphologically abnormal spermatozoa, total number of spermatozoa per ejaculate, sperm motility after 24h storage time and the AST activity before cryopreservation and after thawing in supernatant was assessed. Samples of diluted sperm in a semen-dilution rate of 1+1.5 in Androhep were stored at 17°C until the next day. Sperm was cryopreserved. Straws were thawed in a water bath at 38°C for 40 sec and sperm motility was evaluated immediately. It was recorded that only about 49% of boars were suitable for cryopreservation. Their motility was higher than 30% which is tolerable for AI. Season affected the semen volume, fresh sperm motility, percentage of morphologically abnormal spermatozoa, sperm motility after 24h storage time, AST activity in supernatant, AST activity after thawing and post-thaw sperm motility which was the highest in spring. A significant negative correlation coefficient was noted between sperm motility and AST activity. There was no significant effect of season on sperm concentration and total number of spermatozoa per ejaculate. These data support the notion that cryopreservation of boar semen in spring may yield sperm with greater post-thaw sperm motility.

**Key words:** Boar, Cryopreservation, Seasonal effect, Semen quality

Artificial insemination (AI) is still the world's most used methods in pig breeding (Riesenbeck 2011). The fertilizing potential of a semen dose is inherently linked to the quality of the spermatozoa (Tsakmakidis *et al.* 2010). Selecting boars that consistently produce high-quality ejaculates is crucial for AI centres, to ensure the production of high numbers of semen doses per ejaculate with good fertilizing ability throughout the year (Ciereszko 2000). Several studies indicated that the reproductive performance and the sperm quality of a boar depends on the following factors: heritability, testicular size, nutrition, age, breed, sexual exploration intensity, collection method, handling of semen post-collection, temperature, photoperiod, social environment, sexual behavior and systemic diseases (Marchev *et al.* 2003, Chenoweth 2005, Smital 2009). However, to maximize the advantages of AI, it is important to include cryopreserved spermatozoa, as this is the only practical alternative for global transport of sperm and long-lasting preservation of genetic material (Roca *et al.* 2006b). Cryopreservation of boar semen is rarely performed in commercial practice. The main reasons for this are the poor survival rate of spermatozoa and, as a consequence, the high concentration required in the insemination dose (Cerolini *et*

*al.* 2001). The objective of this study was to evaluate routine parameters of sperm quality across season in both fresh and cryopreserved semen and find out the optimal time for cryopreservation of semen.

### MATERIALS AND METHODS

Ejaculates from 59 fertile boars of the different breed were collected the gloved-hand technique during 2 years. The ejaculates were divided into 4 groups according to season period of the year: winter (1st-3rd month), spring (4th-6th month), summer (7th-9th month) and autumn (10th-12th month).

Semen volume, sperm motility, sperm concentration, percentage of morphologically abnormal spermatozoa (MAS) and total number of spermatozoa per ejaculate (TNS) were assessed. The volume of the sperm-rich fraction of the ejaculate was determined using a graduated cylinder. The sperm motility was subjectively assessed using phase contrast microscopy with a heating stage (38°C) at 100× magnification. Each sample was examined for three different microscopic fields and motility was expressed as percentage of sperm showing normal forward progressive movement. The sperm concentration was determined by a cytometric method using Bürker's chamber. MAS were assessed according to the staining method of Čerovský (1976) and evaluated microscopically under oil immersion and 1 500× magnification. Number of spermatozoa with

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proximal protoplasmic droplet, distal protoplasmic droplet, bent tail, folded tail, coiled tail, acrosome defect, swelling acrosome and with the other defects was determined from 200 counted spermatozoa per slide.

The semen was diluted using extender Androhep in dilution ratio of 1+1.5 and stored at the temperature 17°C. Sperm motility was evaluated 24h after semen dilution. Ejaculates were cryopreserved using the straw freezing procedure describe by Westendorf *et al.* (1975) and modified by Minitüb. Straws were thawed in a water bath at 38°C for 40s and extended in a dilution ratio of 1+2 in Androhep (38°C). Post-thaw sperm motility was evaluated immediately.

The aspartate aminotransferase (AST) activity was measured with a BIOLATEST kit and with an ENCORE spectrophotometer and calculated per  $10^9$  spermatozoa. A 2 ml sample of diluted semen was centrifuged at 1,700 rpm for 10min and the supernatant was used to assess the AST activity.

Basic statistical characteristics of the results arithmetic means, standard deviations and significance (P) were calculated by the QC Expert program. Statistical significance was checked by the analysis of variance ANOVA – Fisher's test at significance levels of  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ .

## RESULTS AND DISCUSSION

The initial parameters of boar semen quality before freezing in this study were following (mean±SD): semen volume  $204.75 \pm 64.23$  ml, motility of fresh semen  $78.47 \pm 7.61\%$ , sperm concentration  $460.17 \pm 184.03 \times 10^3/$

$\text{mm}^3$ , TNS  $91.13 \pm 37.28 \times 10^9$ , MAS  $17.74 \pm 14.55\%$ , sperm motility after 24h storage time  $61.53 \pm 11.00\%$  and AST activity in supernatant  $70.49 \pm 64.14$  mU/ $10^9$  spermatozoa. The proportions of the various MAS were represented (mean±SD):  $4.80 \pm 5.67\%$  spermatozoa with proximal protoplasmic droplet,  $5.65 \pm 5.11\%$  with distal protoplasmic droplet,  $1.11 \pm 0.96\%$  bent tail,  $6.50 \pm 10.25\%$  folded tail,  $0.84 \pm 0.41\%$  coiled tail,  $0.84 \pm 0.41\%$  acrosome defect,  $1.72 \pm 1.54\%$  acrosome swelling and  $2.51 \pm 2.57\%$  other defects. The percentage of MAS was not higher than 25%, which is tolerable for AI in the Czech Republic. The mean sperm motility after thawing was  $24.59 \pm 13.47\%$ . High variability exists in freezability of boar semen. In this work noted that only about 49% of boars were suitable for cryopreservation with average sperm motility after thawing  $35.36 \pm 4.80\%$ , values sperm motility after thawing higher than 30% are tolerable for AI in the Czech Republic. Similarly Chanapiwat *et al.* (2009) reported that more than half of boars represented less than 30% motile sperm after thawing. Causes of variability in sperm after thawing are still searching (Holt *et al.* 2005). Variability in freezability sperm may be related, e.g. to the proportion of fatty acids in membranes (Waterhouse *et al.* 2006), genetic factors (Holt *et al.* 2005) or methods of cryopreservation (Roca *et al.* 2006a). The mean values of semen volume, sperm motility, sperm concentration, TNS and MAS are presented in Table 1. Sperm motility after 24h storage time, sperm motility after thawing, AST activity in supernatant before and after thawing are presented in Table 2.

Season affected the semen volume when significantly the highest was ( $P < 0.05$ ) in autumn  $245.00 \pm 100.14$  ml vs

Table 1. Effect of season on boar semen quality

| Months    | n  | Semen volume        |        | Sperm motility     |       | Sperm concentration |        | Total number of spermatozoa per ejaculate |       | Morphologically abnormal spermatozoa |       |
|-----------|----|---------------------|--------|--------------------|-------|---------------------|--------|-------------------------------------------|-------|--------------------------------------|-------|
|           |    | ml                  |        | %                  |       | $10^3/\text{mm}^3$  |        | $\times 10^9$                             |       | %                                    |       |
|           |    | Mean                | SD     | Mean               | SD    | Mean                | SD     | Mean                                      | SD    | Mean                                 | SD    |
| 1st–3rd   | 15 | 190.00 <sup>a</sup> | 40.13  | 78.00              | 9.78  | 439.73              | 161.89 | 83.15                                     | 34.06 | 17.07                                | 15.60 |
| 4th–6th   | 17 | 217.65              | 68.92  | 81.18 <sup>a</sup> | 3.32  | 441.85              | 134.53 | 93.20                                     | 35.78 | 11.29 <sup>c</sup>                   | 9.29  |
| 7th–9th   | 19 | 187.89 <sup>b</sup> | 50.59  | 78.68              | 5.74  | 523.19              | 235.45 | 95.15                                     | 41.42 | 24.53 <sup>d</sup>                   | 16.66 |
| 10th–12th | 8  | 245.00 <sup>b</sup> | 100.14 | 73.13 <sup>b</sup> | 11.32 | 387.75              | 160.55 | 92.15                                     | 47.79 | 16.56                                | 11.37 |
| Total     | 59 | 204.75              | 64.23  | 78.47              | 7.61  | 460.17              | 184.03 | 91.13                                     | 37.28 | 17.74                                | 14.55 |

Table 2. Effect of season on boar semen quality

| Months    | n  | Sperm motility after 24h storage time |       | Post-thaw sperm motility |       | AST- supernatant       |        | AST- supernatant after thawing |       |
|-----------|----|---------------------------------------|-------|--------------------------|-------|------------------------|--------|--------------------------------|-------|
|           |    | %                                     |       | %                        |       | mU/ $10^9$ spermatozoa |        | mU/ $10^9$ spermatozoa         |       |
|           |    | Mean                                  | SD    | Mean                     | SD    | Mean                   | SD     | Mean                           | SD    |
| 1st–3rd   | 15 | 66.67                                 | 11.13 | 24.50 <sup>f</sup>       | 13.93 | 69.88                  | 28.33  | 121.95                         | 78.85 |
| 4th–6th   | 17 | 69.12 <sup>d</sup>                    | 4.76  | 30.74 <sup>f</sup>       | 10.22 | 49.60 <sup>a</sup>     | 26.54  | 84.21 <sup>a</sup>             | 31.63 |
| 7th–9th   | 19 | 68.95 <sup>d</sup>                    | 6.58  | 26.32 <sup>f</sup>       | 12.12 | 73.99                  | 55.95  | 153.00                         | 97.21 |
| 10th–12th | 8  | 60.00 <sup>c</sup>                    | 8.86  | 7.63 <sup>c</sup>        | 8.33  | 106.12 <sup>b</sup>    | 122.59 | 155.22 <sup>b</sup>            | 97.88 |
| Total     | 59 | 67.20                                 | 8.27  | 24.59                    | 13.47 | 70.49                  | 64.14  | 118.22                         | 75.04 |

a,b,c,d,e,f Means bearing different superscripts in a row differ; significantly a,b  $P < 0.05$ , c,d  $P < 0.01$ , e,f  $P < 0.001$ .

190.00±40.13 ml in winter and 187.89±50.59 ml in summer. Sperm motility was significantly the lowest ( $P<0.05$ ) in autumn 73.13±11.32 ml vs. 81.18±3.32 ml in spring. Savić *et al.* (2013) noted similar results in September and October when the ejaculates had values of sperm volume above average and sperm motility was the lowest. Janett *et al.* (2005) also found out that the semen volume was significantly higher in autumn. This is contrary to the findings of Wolf and Smítal (2009), who found that sperm motility was relatively constant throughout the year. Percentage of MAS was significantly the highest in summer 24.53±16.66% vs. 11.29±9.29% in spring ( $P<0.01$ ). This could have been caused by variation of temperature in the summer and in the autumn season. Several studies have shown that elevated ambient temperature, heat stress and/or hot weather have negative effects on semen production (Colenbrander *et al.* 1993, Frydrychová *et al.* 2007, Wolf and Smítal 2009, Frydrychová *et al.* 2011) as well as semen quality (Sonderman *et al.* 2008) in boars, and especially an increased proportion of MAS (Lipenský *et al.* 2010). On the other hand, this could also have been an influence of the genetic background (Rothschild 1996, Čeřovský *et al.* 2005). Sperm motility after 24h storage time was significantly the lowest ( $P<0.01$ ) in autumn 60.00±8.86% vs. 69.12±4.76% in spring and 68.95±6.58% in summer. The mean values of quality boar semen during year according to months are in Figs 1, 2.

Seasonal fluctuations of reproduction effectiveness are to a large extent determined by variation in quantitative and qualitative properties of an ejaculate, as well as the mating activity of boars. Wysokińska *et al.* (2009) states that the summer months, semen quality deteriorates and, as a result, there is observed poorer fertilisation effectiveness.

Post-thaw sperm motility was significantly the lowest ( $P<0.001$ ) in autumn 7.63±8.33% vs 24.50±13.93% in winter, 30.74±10.22% in spring and 26.32±12.12% in summer. Since the recovery rates of functional sperm after thawing are usually low, the initial semen quality of ejaculates is used to determine which are accepted for

cryopreservation (Roca *et al.* 2006a). Our results also showed how important is initial effect of semen quality particularly high proportion of motile sperm in the ejaculate before freezing where the highest sperm motility was in spring and post-thaw sperm motility was also the highest in spring. The same results noted Cerolini *et al.* (2001) where high sperm viability before freezing is indicative of both high viability and motility after freezing and thawing.

AST is an intracellular enzyme of spermatozoa and increased level of this enzyme in seminal plasma is considered to the sperm cell membrane damage which was especially evident after thawing of spermatozoa. The lowest values of the enzyme after thawing in the spring correspond to the highest value of sperm motility after thawing. A significant negative correlation was found between sperm motility and AST activity  $r = -0.36$  ( $P<0.01$ ). Season did not affect ( $P>0.05$ ) in sperm concentration and the total number of spermatozoa per ejaculate. The average sperm concentration values were relatively constant throughout the season. Similar results were reported Frydrychová *et al.* (2011) where the highest value for sperm concentration determined in September but other studies found significantly the lowest values of sperm concentration in summer and autumn vs. in winter and in spring (Frydrychová *et al.* 2007). Ciereszko *et al.* (2000) reported that semen quality varied with season, including high production of spermatozoa in autumn and winter and low production in summer. Wolf and Smítal (2009) also noted the highest concentration of sperm in the winter and early spring (December to April) and the lowest concentration of sperm was in the late summer and early autumn (August to October).

In conclusion, the results of this study show how is important the influence of the season and the initial quality of boar semen for cryopreservation. These data support the view that the cryopreservation of boar semen in spring may yield sperm with greater post-thaw sperm motility. Freezability prediction of boar semen according seasonal has considerable importance for artificial insemination and also in the area of genetic resources.

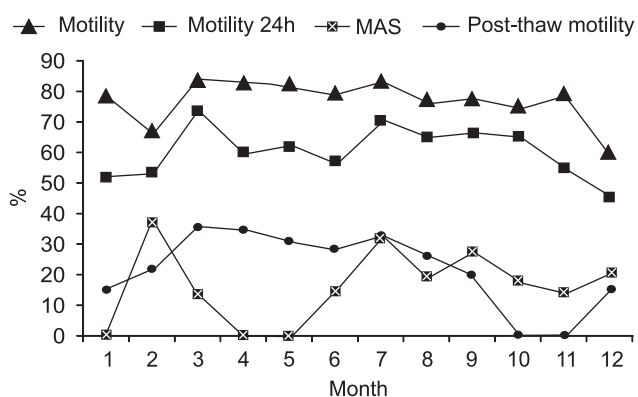


Fig. 1. Mean values of fresh sperm motility, motility after 24h storage time, post-thaw sperm motility and morphologically abnormal spermatozoa (MAS) during year according to months.

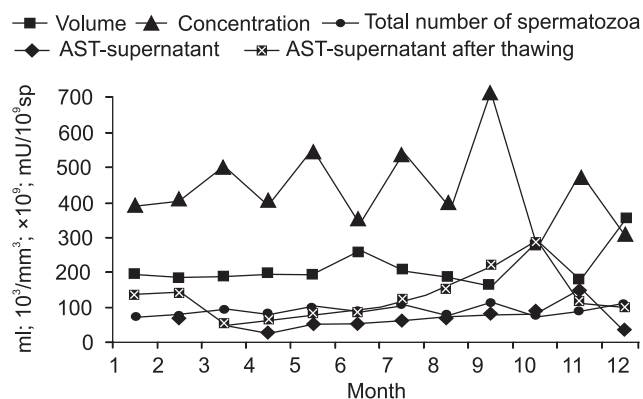


Fig. 2. Mean values of semen volume sperm concentration, total number of spermatozoa per ejaculate and AST activity before and after thawing during year according to months.

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