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EFFECT OF PROTEINE CONTENT IN BOAR SEMINAL PLASMA ON THE SPERM MOTILITY IN DILUTED SEMEN STORED FOR 3 DAYS

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ABSTRACT

Recently, it was frequently demonstrated that fertility of sows after artificially inseminated is lower than after mating. This is associated with a reduced fertilization capacity of overdiluted insemination doses. The aim of this study was to investigate the sperm motility in the semen samples, forming from the ejaculates with high or low protein content, stored *in vitro* on 17°C for 3 days. Progressive motility was significantly higher ($p<0.01$) in the ejaculates with high, compared to the ejaculates with low protein content (82% vs. 76%). After 3 days of storage, in the1:4 dilution proportion, the average progressive motility was significantly ($p<0.01$) decreased in relation to this value in native semen from the boars with high (82% to64%), as well from the boars with low protein content in seminal plasma (76% to48%). However, the average diluted semen progressive motility was significantly greater ($p<0.01$) in the boars with high (64%), compared to the boars with low protein content in seminal plasma (48%). The number of good diluted semen samples (≥65% progressive motility), was also significantly ($p<0.01$) greater in the boars with high (41%), compared to the boars with low protein content in seminal plasma (12%). These results show that seminal plasma proteins play an important role in maintaining the sperm progressive motility of diluted semen *in vitro* stored for 3 days.

Keywords: Semen, protein, dilution, storage, motility, boar.

INTRODUCTION

About 99% of worldwide pig artificial insemination (AI) is performed with extended liquid semen, stored at 15 to 20°C for 0 to 5 days, with 85% of AI doses using within the day of collection or on the following day **(Kommisurd et al., 2002; Stančić and Dragin, 2011; Kalifa et al., 2014)**.In the classic intracervical AI,inseminationdosevolume80ml to100mlof extended liquid semen, with 3×10^9 to6×10^9motile spermatozoa(mean4×10^9) are used **(Johnson et al., 2000; Khalifa et al., 2014),** with about 1,200 to 1,500 AI doses per boar per year **(Glossop, 1998; Stančić, 2000; Singleton, 2001; Stančić and Dragin, 2011).**These number of annually doses production per boar has been more often defined as insufficient in modern industrial pig production **(Singleton, 2001; Stančić et al.,2009)**.This is the reason for the creation of a larger number of AI doses per ejaculate, which requires a higher degree ofejaculate dilution. Recently, overdilution of ejaculate is frequently demonstrated as a reason for reduced fertility in the artificially inseminated, compared to naturally inseminated sows **(Spronket al., 1997; Zvekić, 2003; Gadea, 2005; Almin et al., 2006)**. Namely, overdilution of semen, reduce the concentration of bioactive substances in seminal plasma, which play an important role in maintaining the sperm fertility *in vitro* **(Maxwell et al., 2007; Caballero et al., 2008; Stančić et al., 2012)**, as well as their transport, survival and fertilization capacity in the female reproductive tract **(Langedijak et al., 2002; Strzeżek et al., 2005; Muiño-Blanco et al., 2008; Juyena and Calogero, 2012; Madej et al., 2013)**. It has been shown that seminal plasma is important for sperm progressive motility **(Kommisurd et al., 2002)**. It has been also found that overdilution of seminal plasma, for example in overdiluted AI doses, reduce the sperm progressive motility **(Maxwell et al. 2007; Caballero et al., 2008; Stančić et al., 2012)**. It is the result of reduction the protein concentration in seminal plasma **(Strzeżek et al., 2005; Garcia et al., 2009).** Significant variation of protein content in the seminal plasma between individual boars was also found **(Flowers, 2001; Novak et al., 2010)**.

Therefore, the aim of this work was to determine whether there is a difference in the progressive motility level of the diluted semen samples, made from ejaculates with high or low protein content in seminal plasma, stored *in vitro* for 3 days.

MATERIAL AND METHODS

Ejacualate collection

Ejaculates from the four Swedish Landrace boars, aged between 18 and 22 months, were collected on one industrial farm in AP Vojvodina (Serbia). Gel-free fractions ejaculates were used. All the equipment used during the semen collection, was sterile and disposable. Ejaculate volume was measured on farm. Semen sample, about 60ml,was placed in a sterilepolyethylene100 ml volume bottle. Bottles with the semen samples was placed in a thermo-box ata temperature of17°C and transported to the laboratory for Animal Reproduction, Faculty of Agriculture in Novi Sad, within 2 to 4 hours after collection. The total of 192 ejaculates was used in experiment (4 boars × 4 ejaculates per week × 12 month).

Preparing and examination of semen samples

Immediately after arrived to the laboratory, ejaculates were heated in water-bathonthe37°C, for 45minutes. After that, basic parameters of semen quality were determined. Motility was assessed using a phase contrast microscope at 100 × magnification and a heating stage on 37°C.Sperm concentration ($\times10^6$/ml of semen) and total sperm count($\times10^9$)in the ejaculate was assessed by using photometric method (Photometer SDM5, Minitübe, Tifenbach, Germany). The ejaculates with similar quality parameters were used for the formation of diluted semen samples. Only good ejaculates were used (volume ≥120 ml, progressive motility ≥65% and sperm concentration $\geq200\times10^6$/ml). Protein content (%) in semen samples were determined according to AOAC Official Method 2001.11. All diluted semen samples were divided in to two groups. First group: samples

formed from ejaculates with high protein content in seminal plasma(average4.3%). Second group: samples formed from ejaculates with low protein content(average2.4%). Ejaculate samples were extended in Beltsville Thawing Solution - BTS1(Minitübe, Tifenbach, Germany). From the each ejaculate, a sample of 100times smaller than the volume of native ejaculate, were made. For example, if the volume of native ejaculate was 250ml, the semen sample was 2.5ml. This sample was diluted in the 1:4 and stored in a thermo-box for 72h, at a temperature of17^{o}C. Diluted semen samples were mixed every 24h. After 72h, the samples were heated in water-bath onthe37^{o}C, for 45minutes, and progressive motility (%) were assessed by using a phase contrast microscope at 100 × magnification and a heating stage on 37^{o}C.

Statistical analysis

The data were analyzed by the software package "Statistics 12". Mean, standard deviation, minimum and maximum values of the studied traits were determined. T-test were used for testing the significance of differences between the means of investigated values.

RESULTS AND DISCUSSION

Average sperm progressive motility in native semen, immediately before dilution, diluted semen progressive motility and the number of good semen samples, after 3 days storage in 1:4 dilution proportion, are shown in Table 1.

Table 1 Progressive motility in the semen sample diluted 1:4 and stored 72h on +17^{o}C

Parameter		Protein content level in seminal plasma		Total
		High - HPC (4.3%)	Low - LPC (2.4%)	
Number of samples examined		96[1]	96[1]	192
Progressive motility of native semen (%)[2]		82±5.49^{AX}(70-90)	76±6.29^{BX}(65-90)	79(65-90)
Progressive motility of all examined diluted semen samples (%)		64±7.56^{AY} (50-90)	48±10.49^{BY} (65-90)	56 (65-90)
Good diluted semen samples[3]	n	39/96	12/96	51/192
	%	41±3.87^{A}	12±1.04^{B}	26
Progressive motility of good diluted semen samples (%)[3]		71±4.31^{A} (65-80)	66±1.94^{B} (65-70)	70 (65-80)

[1]Twoboarsper48 samples per high and low level of seminal plasma protein; [2]Imediately before dilution; HPC – High protein content in native seminal plasma; LPC – Low protein content in native seminal plasma;[3]Progressive motility ≥ 65%; Min. and max. values in parenthesis.
Values with different superscripts, within the same row, significant differ ($^{A, B}$ p<0.01; $^{a, b}$ p<0.05).
Values with different superscripts, within the same column, significant differ ($^{X, Y}$ p<0.01; $^{x, y}$ p<0.05).

Average progressive motility of native semen were significantly higher (p<0.01) in the semen with high protein content - HPC (82%), compared with low protein content – LPC semen (76%). After 72h preservation on 17^{o}C, in 1:4 dilution proportion, progressive motility was significant (p<0.01) decrease compared with native semen progressive motility immediately before dilution (82% vs. 64% in the HPC and 76% vs. 48% in the LPC semen). Average progressive motility of all examined diluted semen samples was significant (p<0.01) greater (64%) in the samples made from the HPC semen, compared with samples made from the LPC semen (48%). The number of good diluted semen samples (progressive motility ≥ 65%), after 72h preservation in 1:4 dilution proportion, was significant (p<0.01) greater in the samples made from the HPC semen (39/96, 41%), compared with samples made from the LPC semen (12/96, 12%). Progressive motility of good diluted semen samples, was higher in the samples made from the HPC semen (71%), compared with samples made from the LPC semen (66%) (Table 1). These results clearly demonstrated that seminal plasma protein concentration significant influence the progressive motility, both in the native and diluted semen.

It has been frequently demonstrated that the use of preserved semen for artificial insemination, which often involves extensive dilution or removal of seminal plasma, results in lower fertility rates than with natural mating **(Spronk et al., 1997; Tummaruk et al., 2000; Zvekić, 2003; Gadea, 2005; Alm et al., 2006)**. It has been found that transcervical infusion of seminal plasma prior to insemination are effective method for optimizing fertilization conditions in the sows **(Waberski et al., 1996)**. This evidence suggests that components of seminal plasma participate in key events related to sperm function, fertilization, and embryo development in the female reproductive tract **(Juyena and Calogero, 2012)**. There are several factors that might influence fertility of stored boar semen. It has been previously shown by **Harrison et al. (1978)** that transfer of sperm cells from seminal plasma to artificial thawing solution, decrease sperm motility, which indicates that seminal plasma might be of importance to maintain sperm fertilizing capacity during storage. Individual boar variation concerning the amount of seminal plasma, might be one of the factors influencing fertility of stored semen, because seminal plasma is important for sperm progressive motility **(Kommisurd et al., 2002)**. It has been shown that protein content in seminal plasma, play a major role to maintain the sperm progressive motility in diluted semen **(Strzeżek et al., 2005; Garcia et al., 2009)**. It has been also found a significant variation of protein content in the seminal plasma between individual boars **(Flowers, 2001; Novak et al., 2010)**. Native seminal plasma addition into the highly diluted boar semen, significantly increase sperm progressive motility **(Garcia et al., 2010)**. Addition of seminal plasma with high protein content, in to the sperm from ejaculates with low content of seminal plasma protein, significantly increase the progressive motility (67% vs. 48%) of the sperm in the semen diluted in 1:2 and 1:4 proportion, and stored for 3 day, at 17^{o}C **(Stančić et al., 2012)**. Significantly (p<0.01) greater sperm progressive motility in diluted semen with a native seminal plasma (65.6%), compared with the diluted semen containing washed sperm - without natural seminal plasma (6.4%), were found by **Chuita et al. (2014)**. It was found that several seminal plasma proteins as a markers of ejaculate quality and boar fertility **(Novak et al., 2010; Centurion et al., 2003)**. Because, the quantification of seminal plasma proteins could be a useful tool to detection ejaculates with reduced fertility and selection the boars prior to using for artificial insemination **(Flowers, 2001; Juyena and Calogero, 2012).**

CONCLUSION

Based on the obtained results in this work, it can be concluded that ejaculates with high protein content in seminal plasma, has a significantly greater sperm progressive motility in the native and diluted semen. This fact should be taken in mind, when determining the degree of ejaculate dilution proportion to AI doses formation. It may also be useful parameter to selection boars with higher fertility for using in artificial insemination.

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