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REGULAR ARTICLE

EFFECTS OF BREED, SPERMATOZOA CONCENTRATION, AND STORAGE ON PROGRESSIVE MOTILITY OF EXTENDED BOAR SEMEN

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ABSTRACT

The classic technology of artificial insemination (AI) often requires insemination doses to be kept for more than 24 hours, with a requirement that the degree of progressive motility at the moment of insemination not be below 65%. The aim of this paper was to determine the influence of breed, spermatozoa concentration, and storage time on the fertilization capacity of extended semen from native ejaculates of boars. The research included the following boar breeds: Duroc (n=34), Hampshire (n=30), Large White (n=42) and Swedish Landrace (n=32), from large pig farms in Vojvodina (Republic of Serbia). Two ejaculates were collected from each boar once monthly for 12 months (a total of 24 ejaculates per boar). There was statistically significant ($p<0.01$) influence of breed on the number of spermatozoa samples that maintained ≥ 65% progressive motility during 48 hours of storage in 1:4 dilution. There was also an influence of spermatozoa concentration on progressive motility. As spermatozoa concentration increased during storage, ≥ 65% progressive motility declined ($P\leq0.01$) within 24 hours. The results show that it is necessary to determine the adequate dilution rate and storage time for each ejaculate, while taking into account spermatozoa concentration in the native semen.

Keywords: breed, spermatozoa concentration, liquid extended semen, motility, storage time, boar.

INTRODUCTION

For artificial insemination procedures in swine, insemination doses most commonly consist of 100 ml of liquid extended semen with a minimum of 3 – 5 x 10^9 progressively motile spermatozoa **(Almin et al., 2006; Stančić et al., 2009)**. On average, one boar ejaculate provides approximately 21 insemination doses **(Singleton, 2001)**. Ideally, insemination doses are stored at 17°C for 1 to 2 days **(Johnson et al., 2000)**. In order to exploit genetically superior boars, under production conditions it is often necessary to store semen for longer periods. However, it is important to maintain satisfactory semen quality to insure adequate fertilization. One measurement of semen quality is progressive motility of spermatozoa. At the end of storage, i.e. at the time of insemination, it is recommended that ≥65% progressive motile spermatozoa are required for adequate fertility rates. However, it is well-known that there are significant variations between individual boars of the same breed, as well as between boars of different breeds in terms of fertilization capacity of insemination doses, depending on the storage time of insemination doses. In addition, there are several other factors that can affect spermatozoa fertility during storage. One of the most prominent factors is the ratio of the amount of semen plasma to spermatozoa concentration in an ejaculate. Semen plasma has a significant impact on the degree of progressive motility. If artificial extenders are added to the native semen, the percentage of progressively motile spermatozoa decreases, while the extent of agglutination increases. This suggests that seminal plasma might protect the cell membrane of spermatozoa, thus maintaining its fertilization capacity during storage **(Waberski et al., 1994; Stančić, 2002; Kommisrud et al., 2002; Wolf and Smital, 2009)**.

The objective of this study is to determine the influence of breed, spermatozoa concentration, and storage time in native ejaculate of boars on the fertilization capacity of extended semen.

MATERIAL AND METHODS

Boar breeds used in this study included Duroc (n=34), Hampshire (n=30), Large White (n=42) and Swedish Landrace (n=32), which are used for artificial insemination on several

large pig farms in Vojvodina (Republic of Serbia). Two ejaculates were collected monthly from each boar for 12 months (a total of 24 ejaculates per boar).

Within 2 hours after collecting ejaculates, the following parameters were determined in the laboratory: volume, spermatozoa concentration per 1 ml, a total number of spermatozoa per ejaculate and progressive motility (%). Spermatozoa concentration was determined using a photometric method (Photometer SDM5, Minitüb), whereas the progressive motility was determined by a microscopic method. Each ejaculate was diluted in the ratio of 1:4 with the extender BTS1 (Minitüb) and stored for 3 days at a temperature of 17^{o}C in a refrigerated box for boar semen (34l, Minitüb). Progressive motility of the extended semen was evaluated at 24, 48 and 72 hours after semen dilution, depending on the boar breed and spermatozoa concentration in the native ejaculate. Prior to determination of progressive motility, native and extended spermatozoa samples were heated at 37.5^{o}C in a water bath for 30 to 40 minutes.

The resulted were processed in the "Statistica 9" software.

RESULTS

The average volume of the native ejaculate from all the boars was 272.2 ml, ranging from 205 (Duroc) to 304 ml (Hampshire), while the average spermatozoa concentration was 203.3 x 10^{6}/ml, ranging from 176.6 (Hampshire) to 235.3 (Duroc) (Table 1).

Table 1 Parameters of native ejaculates of four boar breeds (mean ± SE)

Ejaculate parameters	Boar breeds					Breed effect (P-value)
	Duroc (n=34)	Hampshire (n=30)	Large White (n=42)	Swedish Landrace (n=32)	Total (n=138)	
Volume (ml)	205.2±8.98	304.4±13.36	289.0±14.20	291.4±21.14	272.2±8.17	0.001**
Sptz. concentration (x10^{6}/ml)	235.3±19.24	176.6±7.38	190.5±12.06	211.3±14.50	203.3±7.26	0.023*
Total sptz. number (x10^{9})	45.9±3.29	51.7±2.19	54.5±4.11	58.7±4.51	53.4±1.91	0.1979 ^{ns}
Progressive motility (%)	82±1.13	85±1.23	84±1.19	85±0.91	85±0.57	0.1126 ^{ns}

Legend : Sptz – Spermatozoa, SE – Standard error; **(p<0,01); * (p<0,05); ^{ns} Not significant.

The results in Table 1 indicate that the breed of boars has highly significant ($p<0.01$) effect on ejaculate volume and significant ($p<0.05$) effect on spermatozoa concentration in 1 ml of an ejaculate. The total number and progressive motility of spermatozoa showed no significant variations ($p\leq 0.05$) in relation to the breed.

The number of extended (diluted 1:4) semen samples that maintained ≥ 65% progressive motility after 24 hours of storage was influenced by breed (Table 2). A highly significant difference ($p<0.01$) was determined between the Duroc and Large White breeds (47% vs. 71.4%), while a significant difference ($p<0.05$) was determined between the Duroc and Swedish Landrace breeds (47% vs. 68.8%), Large White and Hampshire (71.4% vs. 53.3%) and Swedish Landrace and Hampshire (68.8% vs. 53.3%). After 48hours of storage, a significant difference ($p< 0.05$) was found between the Duroc and Large White breeds (38.2% versus 47.6%), Duroc and Swedish Landrace (38.3% vs. 50%), Large White and Hampshire (47.6% vs. 33.3%), and Swedish Landrace and Hampshire (50% vs. 33.3%). After 72 hours of storage, no effect of breed was observed. It is important to note that only around 20% of insemination doses of all the studied boars maintained the minimum required progressive motility after 72 hours of storage.

Table 2 Effect of breed on the number of extended semen samples that maintained ≥ 65% progressive spermatozoa motility

Breed	Extended semen storage		
	24 h	48 h	72 h
Duroc	47.0% (16/34)^{B}	38.2% (13/34)^{b}	17.6% (6/34)^{A}
Large White	71.4% (30/42)^{A}	47.6% (20/42)^{A}	21.4% (9/42)^{A}
Swedish Landrace	68.8% (22/32)^{a}	50.0% (16/32)^{A}	21.9% (7/32)^{A}
Hampshire	53.3% (16/30)^{b}	33.3% (10/30)^{b}	20.0% (6/30)^{A}
Total	60.8% (84/138)	42.7% (59/138)	20.3% (28/138)

Legend: $^{Aa, Bb}$ Different capital and small letter ($P<0.05$); different capital letters ($P<0.01$); the same letters ($P>0.05$), within the same columns.

The number of insemination doses that maintain ≥ 65% progressive motility declined as the spermatozoa concentration in the native ejaculate increased during the first 72 hours of storage (Table 3). However, a significant decrease of this value was observed only during the first 24 hours of storage. The highest values of this parameter (76.6% and 66.7%) were observed in doses from ejaculates with the lowest spermatozoa concentration (≤ 150 x 10^6/ml and 151-201 x 10^6/ml). The percentage of ≥65% progressive spermatozoa motility was

significantly higher ($p< 0.01$) after 24 hours of storage than the high concentration (202-302 x 10^6/ml and ≥ 303 x 10^6/ml) spermatozoa concentration in doses (54.8% and 48.8%, respectively).

Table 3 Effect of spermatozoa concentration in ejaculates on the number of extended semen samples that maintained ≥ 65% progressive motility during 72 hours of storage

Sperm concentration (x10^6/ml)	Extended semen storage time		
	24 h	48 h	72 h
≤ 150 (Average = 121)	76.6% (23/30) ^{A}	56.7% (17/30) ^{A}	26.7% (8/30) ^{A}
151- 201 (Average = 175)	66.7% (24/36) ^{a}	44.4% (16/36) ^{A}	22.0% (8/36) ^{A}
202- 302 (Average = 273)	54.8% (17/31) ^{B}	38.7% (12/31) ^{A}	19.3% (6/31) ^{A}
≥ 303 (Average = 377)	48.8% (20/41) ^{B}	34.1% (14/41) ^{A}	14.6% (6/41) ^{A}
Total (Average = 203)	60.8% (84/138)	42.7% (59/138)	20.3% (28/138)

Legend : $^{Aa, Bb}$Different capital and small letter ($P<0.05$); different capital letters ($p<0.01$); same letters ($P>0.05$), within the same columns.

DISCUSSION

The results of this study clearly show that boar breed has a significant effect on semen volume and spermatozoa concentration of the native ejaculate. Our results are in agreement with others for the influence of the breed on parameters of boar ejaculate **(Gerfen et al., 1994; Ciereszko et al., 2000; Jankevičiute and Žilinskas, 2002; Stančić et al., 2002; Stančić et al., 2003; Smital et al., 2004; Chukwuemeka et al., 2005; Wolf and Smital, 2009)**. This study and others indicate that the Duroc breed has the lowest ejaculate volume with the highest spermatozoa concentration. However, the average number of spermatozoa per ejaculate for boars of all the studied breeds was 53 x 10^9 which was not significant different between breeds.

The results of this research showed that the boar breed has an influence on maintenance of spermatozoa progressive motility in insemination doses during the first 48 hours. Although the Duroc breed had the highest spermatozoa concentration, it had the lowest ($p<0.05$) number of doses (47%) with ≥65% progressive motility after 24 hours of storage.

The highest ($p < 0.05$) number of doses (71.4% and 47.6%) was found in the Large White breed. After 72 hours of storage, there were no significant differences ($P > 0.05$) between breeds in the values of ≥65% progressive motility. At that time, all breeds had approximately 20% of samples with ≥65% progressive motility. We also found that an increase of spermatozoa concentration in the native ejaculate significantly reduces progressive motility in the extended semen doses after 72 hours of storage at 17°C. Spermatozoa concentration has effect on the amount of seminal plasma which encloses every spermatozoon, both in the native and extended semen. Consequently, the increase of spermatozoa concentration results in the decreasing amount of seminal plasma which encloses every individual spermatozoa **(Kommisrud et al., 2002)**. It has been reported that spermatozoa concentration and storage time of extended semen are factors that significantly affect the degree of semen spermatozoa progressive motility in insemination doses **(Stančić et al., 2002; Kommisrud et al., 2002; Stančić et al., 2003ab; Grafenau et al., 2003ab; Katanić, 2004; Boe-Hansen et al., 2005; Stančić et al., 2009)**. What is important to note is that in this study only 20% of insemination doses maintained ≥65% progressive motility after 72 hours of storage. It is reported that extended semen from only 20 to 30% of boars has the ability to withstand 72 hours of storage without a significant loss in progressive motility **(Weitze, 1990; Stančić et al. 2003ab)**.

Seminal plasma has a significant influence on spermatozoa motility **(Kommisrud et al., 2002)** and dilution reduces the content of protein, natural antioxidants, and other natural components of seminal plasma, which are necessary for normal functioning and integrity of the spermatozoa membrane. The reduction of fertilisation potential, due to spermatozoa aging, cannot be avoided during the storage of extended semen. However, such aging process of spermatozoa can be decreased by adequate control of native semen/spermatozoa parameters, adequate dilution rate of native ejaculate, as well as by application of appropriate conditions and storage time of extended semen **(Boe-Hansen et al., 2005)**. Our results indicate that it is necessary to determine the adequate dilution rate and storage time for each ejaculate, while taking into account the spermatozoa concentration in the native semen.

CONCLUSION

Based on our results, it can be concluded:

1. There was statistically significant influence of breed on the number of semen samples that maintained ≥ 65% progressive motility during 48 hours of storage in 1:4 dilution.

2. Spermatozoa concentration influence spermatozoa progressive motility. As spermatozoa concentration increased during storage, ≥65% progressive motility declined within 24 hours.
3. It is necessary to determine the adequate dilution rate and storage time for each ejaculate, while taking into account spermatozoa concentration in the native semen.

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