TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2024, vol. 9, No 1(16): 57–62

THE INFLUENCE OF AGE ON THE PARAMETERS OF EJACULATE IN BOARS

Igor Zdraveski*, Petar Dodovski, Pance Dameski, Natasa Pejcinovska, Nikola Karabolovski, Talija Hristovska, Maja Angelovska, Mimi Ristevski

University "St Kliment Ohridski" - Bitola, Faculty of Veterinary Medicine, Bitola, North Macedonia
E-mail: igor.zdraveski@uklo.edu.mk
ORCID: 0000-0001-9495-9906 I.Z.; 0000-0001-7824-3772 P.Dodovski; 0000-0002-0051-9916 P.Dameski; 0009-0003-1864-6870 N.P.; 0000-0004-8665-4319 N.K.; 0000-0003-3892-4375 T.H.; 0000-0003-3201-1516 M.A.; 0009-0009-7836-7842 M.R.
(Submitted: 19 March 2024; Accepted: 20 May 2024; Published: 25 June 2024)

ABSTRACT

The age of boars significantly influences the values of ejaculate parameters**.** The aim of this research was to determine the quality of boar ejaculates categorized by age groups: Group 1, up to 1 year, Group 2, up to 2 years, Group 3, up to 3 years, Group 4, 4 years and older. A total of 50 ejaculates from 50 boars from nine farms were examined, analysing the impact of boar age on ejaculate parameters (ejaculate volume (ml), progressive motility (%), sperm concentration ($x10^6$), total sperm count ($x10^9$), and percentage of total proteins). Analysis of ejaculate parameters in boars of different ages indicates that the most significant changes occur in sperm concentration and total sperm count, where significant differences were observed. These results suggest that age influences spermatogenesis, and with the aging of boars, there is a decrease in the intensity of spermatogenesis.

Key words*:* ejaculate parameters, boars age, seminal plasma.

Introduction

The age of the boar has a significant impact on both quantitative and qualitative characteristics of semen (Sutkevičienė and Žilinskas, 2004; Smital, 2009; Wolf and Smital, 2009a; Wolf and Smital, 2009b; Wierzbicki*et al.*, 2010). It has been established that boar semen, both quantitatively and qualitatively, consistently increases with testicular development, testosterone production, and an increase in libido until sexual maturity at 6 to 8 months of age, and later to a lesser extent until reaching adult body size (Rothschild and Ruvinsky, 2011).In general, seminal plasma is a complex mixture of proteins, hormones, electrolytes, nutrients, vitamins, and other bioactive substances (Centurion et al., 2003; Garcia *et al.*, 2009). All these components enable the normal survival and function of spermatozoa outside the male reproductive tract (in the female reproductive tract or during in vitro conditions). They also contribute to the normal physiological functions of the female reproductive tract, which are crucial for successful fertilization and early embryonic development (Stančić, 2005;Muiño-Blanco et al., 2008; Rodriguez-Martinez et al., 2011; López Rodríguez, 2012; Nasrini and Calogero2012).The impact of age on basic ejaculate parameters, which are indicators of semen quality, has been the subject of many studies (López Rodríguez 2012; Savić 2013).The results of these studies clearly demonstrate that age significantly affects spermatogenesis, ejaculate volume, and consequently, sperm concentration, total sperm count, and sperm motility. State that age positively influences ejaculate parameters, explaining that the maximum values of ejaculate parameters in boars are achieved at the age of 2.5 - 3.5 years (Savić et al., 2013).

In this investigation we analysed the basic parameters in boars categorised in groups which include young boars up to 1 year and the boars older than 4 years aiming to evaluate the alterations in ejaculate quality in different age category.

Materials and methods

The experiment was conducted at nine large commercial pig farms (Farm I, Farm II, Farm III, Farm IV, Farm V, Farm VI, Farm VII, Farm VIII, and Farm IX) with the capacity of 1000 sows in the breeding herd. Fifty boars aged between up to 1 year to 4 years old when the study period began, were selected and divided by age into 4 groups: Group I: up to 1 year (n=25), Group II: up to 2 years (n=8), Group III: up to 3 years (n=13), and Group IV: boars at 4 years of age and older (=4). Fifty sperm-rich ejaculate fractions were collected one per boar using the gloved hand method while the boar mounts a dummy sow. Semen samples were filtered through Minitube® filter immediately after collection. The volume of sperm (ml) was measured immediately after collection from the boar, using a graduated sperm collector at the farm. The fresh native semen samples, with volume range between 60 to 70ml taken from sperm rich fractions, prepared for regular use in AI, were placed in sterile plastic flasks with caps andstored in a thermo-box at +17^{o}C. Semen samples were transported to laboratory (within 2-4h after semen collection), forsemen quality assessment on CASA –computer assisted semen analysis. In the laboratorysperm was prepared and diluted for following parameters assessment: progressive motility (%), sperm concentration ($\times 10^6$/ml of ejaculate) and total sperm count in each ejaculate ($\times 10^9$). The average of all measurements per sample was used for data analysis according to (Kommisrud*et al.,* 2002). For protein content (%) in seminal plasmaassessment, semensamples were divided in to 20ml samples, placed in 50ml plastic tubes with caps and centrifuged at 1000×g for 10min at 4°C toremove the spermatozoa. The supernatant was recentrifuged (at 3000×g for 15min at 4°C) to purify the seminal plasma from any residual sperm and other organic particles. Samples were stored in a refrigerator at+4°C. The analysis was performed within 24hafter the ejaculates were collected at the farm at latest. The total protein content in the seminal plasma wasdetermined by the AOAC - Association of OfficialAnalytical Chemists as a chemical method (OfficialMethod 2001.11). Descriptive statistical analysis of data was performed using the software package “Statistics 12”. Analysis of variance (ANOVA) test with subsequent Kruskal Wallis test, Fisher exact two tailed test was used for comparison of mean values.

Results

The impact of age on the parameters of the examined boar ejaculates is presented in Table No. 1. Results of the research on ejaculate volume among the four analysed groups of boars showed that boars in the third group up to 3 years of age had the highest ejaculate volume (233 ± 108) compared to the other three groups, while the smallest volume was observed in boars of the fourth group at 4 years and older (145 ± 58). Statistical analysis did not reveal a statistically significant difference in ejaculate volume among the four analysed groups of boars. The highest average progressive motility of spermatozoa was in boars up to two years of age (79% ± 9.42), and the lowest in the group of boars up to one year of age (69% ± 19.93). The difference in progressive motility of spermatozoa was not statistically significant among the four analysed groups of boars. A statistically significant difference ($p = 0.049$) was observed during the analysis of the average sperm concentration values in the ejaculate of boars in the first group up to 1 year of age (367.36±174.69) compared to the third

group of boars up to 3 years of age (265.92±136.53). The total sperm count, on average, was significantly higher (p = 0.027) in the group of boars aged up to 1 year (73.60 ± 30.52), compared to the group of boars aged 4 years and older (39.20 ± 20.46). Results showed that the group up to 3 years of age had the highest value of total proteins (2.97 ± 0.8), while the lowest value was found in the group of boars up to 4 years and older (2.47 ± 0.83). Statistical analysis did not reveal a statistically significant difference in the percentage of total proteins between the groups of examined ejaculates (p > 0.05).

Table 1: Values of ejaculate parameters (ejaculate volume (ml), progressive motility (%), sperm concentration x 10^6, total sperm count x10^9, as well as the representation of total proteins (%) and statistical significance.

Age	up to 1 year	2 years	3 years	4 years and older	**P**
N	25	8	13	4	
	Mean value ± SD	Mean value ± SD	Mean value ± SD	Mean value ± SD	
Volume(ml)	215,44±72,37	215,62±63,66	233,07±108,42	145,00±58,02	p> 0,05 (NS)
Progressive motility(%)	69,24±19,93	79,37±9,42	70,38±17,84	73,75±17,01	p> 0,05 (NS)
Sperm concentration (×10^6/ml ejaculate)	367,36±174,69	309,87±40,24	265,92±136,53	256,50±88,82	p< 0,05 (I – III p - 0.049)
Total number of spermatozoa (×10^9/ml)	73,60±30,52	65,47±18,70	62,09±28,99	39,20±20,46	p< 0,05 (I – IV p - 0.027)
Percent of proteins (%)	2,81±0,94	2,71±0,65	2,97±0,77	2,47±0,70	p> 0,05 (NS)

Discussion

The analysis of ejaculate parameters in boars of different ages indicates that the most significant changes are observed in sperm concentration and total sperm count, where significant differences were also found. The results in our investigation suggest that age influences spermatogenesis, leading to a decrease in the intensity of spermatogenesis with the aging of individuals. Results obtained in this investigation corroborate with findings of other authors. Smital, (2009) reported that the age of the boar has a strong impact on the daily production of spermatozoa. This author states that this value increases until the age of 3.5 years. (Wolf and Smital 2009b) state that the increase in sperm concentration per 1 ml of ejaculate is constant up to 11 months of age. Earlier studies conducted by Kennedy and Wilkins (1984) showed that maximum values of sperm concentration can be obtained from boar ejaculates at the age of 24-29 months. Sperm concentration and thus spermatogenesis are associated with and follow the trend of testicular development and phenotypic testicular characteristics, which are conditioned by the age of the boar. Aging of the organism leads to a decline in testosterone concentration, affecting both the qualitative and quantitative characteristics of the ejaculate (Araujo et al., 2011). A good ejaculate should have a volume of 120 ml to 150 ml Rozeboom (2000). From the beginning of reproductive utilization, the ejaculate volume gradually increases by approximately 100 ml until the second year of age, after which it remains more or less constant (Wolf and Smital 2009a). The results in our study showed that the volume increases until third year of age but in boars of 4 years of age and older, there was a noticeable decrease of ejaculate volume compared to boars in other age categories, but these differences were not significant. (Jankevičiūtė and Žilinskas2002) found that the progressive motility of spermatozoa was highest in boars aged 18 to 24 months and lowest in boars older than 30 months. Progressive motility of

spermatozoa is strongly associated with the percentage of altered spermatozoa and the type of morphological changes in the ejaculate (Gil et al., 2009). The percentage of abnormal spermatozoa is higher in very young and old boars. Results from (Tsakmakidis et al., 2012) showed that the least morphometric changes and the lowest percentage of spermatozoa with chromosomal instability were observed in boars aged 18–33 months. Morphometric abnormalities in spermatozoa, as well as a higher percentage of chromosome damage, were identified in younger boars (7–10 months), which can be explained by issues during spermatozoa maturation. In older boars (51–61 months), these abnormalities were attributed to a high level of cell apoptosis in the epididymis, low testosterone levels, and increased oxidative stress in tissue (Jara et al., 2004). However, considering that the results in our investigation did not show statistically significant differences in terms of progressive motility between groups, it could be stated that age does not significantly affect progressive motility of spermatozoa and seminal plasma volume. Protein concentrations in the seminal plasma of examined boars did not differ significantly between boars of different age categories, which is in line with the understanding that the protein level in seminal plasma is not highly correlated with the age of boars (Gerfen et al., 1994; Maxwell and Johnson 1999; Apić2015; Stančićet al.,2015). The protein content in seminal plasma is relatively constant within the same boar; however, variations have been observed between boars and their age (Flowers 2001; Novak et al., 2010). However, it has been revealed that different percentage of certain fraction of seminal plasma proteins effects the boar ejaculates fertility potential (Stančić et al., 2019).

Conclusion

As a conclusionbased on the results obtained in this study, it can be said that age influences the intensity of spermatogenesis, and consequently, it likely leads to a reduction in ejaculate fertility especially in younger and the older category of boars.

References

1. Apić, J. (2015). *Effect of protein content in the boar seminal plasma on the diluted semen parameters and fertility of artificially inseminated sows (PhD Thesis).* Faculty of Agriculture, Department of Veterinary Medicine, University of Novi Sad, Serbia.
2. Araujo, AB., GA. Wittert. (2011). *Endocrinology of the aging male.* Best practice & research Clinical endocrinology & metabolism. 1;25(2):303–19. https://doi.org/10.1016/j.beem.2010.11.004.
3. Centurion, F., M.J. Vazquez, J.J.Calvete, J.Roca, L. Sanz, , I. Parrilla, M.E. Garcia, A. Emilio, A.E. Martinez. (2003). *Influence of Porcine Spermadhesins on the Susceptibility of Boar Spermatozoa to High Dilution.* Biology of Reproduction, 69:640–646.https://doi.org/10.1095/biolreprod.103.016527.
4. Flowers, WL. (2001). *Relationship between seminal plasma proteins and boar fertility*. Raleigh, NC: Annual swine news.1-4.
5. Garcia R., A. Pallas, R. Hernandez-Gil, S. Dimitrov. (2009). *The use of synthetic seminal plasma (Predil MR-A®) as a method to facilitate procedures with cervical and post-cervical artificial insemination of sows*. Agricultural Science and Technology.1(1):2–7.
6. Gerfen, RW., BR. White, MA. Cotta, MB. Wheeler. (1994). *Comparison of the semen characteristics of Fengjing, Meishan and Yorkshire boars.* Theriogenology. 1;41(2):461–70.https://doi.org/10.1016/0093-691X(94)90082-T.
7. Gil, MC., M.García-Herreros, FJ. Barón, IM. Aparicio, AJ. Santos, LJ. García-Marín. (2009). *Morphometry of porcine spermatozoa and its functional significance in relation with the motility parameters in fresh semen.* Theriogenology. 5;71(2):254–63.https://doi.org/10.1016/j.

8. Jankevičiūtė, N., H. Žilinskas. (2002). *Influence of some factors on semen quality of different breeds of boars*. Veterinarija ir zootechnika.19(41):15–9.
9. Jara, M., R. Carballada, P. Esponda. (2014). A*ge-induced apoptosis in the male genital tract of the mouse*. Reproduction.1;127(3):359–66.DOI: https://doi.org/10.1530/rep.1.0009
10. Kennedy, BW., JN. Wilkins. (1984). *Boar, breed and environmental factors influencing semen characteristics of boars used in artificial insemination.* Canadian Journal of Animal Science. 1;64(4):833-43.https://doi.org/10.4141/cjas84-09.
11. Kommisrud, E., H. Paulenz, E. Sehested, IS. Grevle. (2002). *Influence of boar and semen parameters on motility and acrosome integrity in liquid boar semen stored for five days.* Acta Vet Scand. 43(1): 49–55.https://doi.org/10.1186/1751-0147-43-49.
12. López Rodríguez, A. (2012). *Fresh boar semen: quality control and production (PhD Thesis)*. Faculty of Veterinary Medicine, Ghent University.
13. Maxwell, WM., LA. Johnson. (1999). *Physiology of spermatozoa at high dilution rates: the influence of seminal plasma*. Theriogenology. 1;52(8):1353–62.https://doi.org/10.1016/S0093-691X(99)00222-8.
14. Muiño-Blanco, B., R. Pérez-Pé, A.J. Cebrián-Pérez. (2008). *Seminal Plasma Proteins and Sperm Resistance to Stress*. Reprod. Dom. Anim., 43(4)18–31. https://doi.org/10.1111/j.1439-0531.2008.01228.x.
15. Nasrin, S.J., S. Calogero, (2012). *Seminal Plasma: An Essential Attribute to Spermatozoa (A Review)*. Journal of Andrology, 33(4)536–551. https://doi.org/10.2164/jandrol.110.012583.
16. Novak, S., A. Ruiz-Sánchez, W. Dixon, G. Foxcroft, M. Dyck. (2010). *Seminal plasma proteins as potential markers of relative fertility in boars*. Journal of Andrology. 4;31(2):188–200. https://doi.org/10.2164/jandrol.109.007583.
17. Rodriguez-Martinez, H., U. Kvist, J. Ernerudh, L. Sanz, J.J. Calvete. (2011). S*eminal plasma proteins: what role do they play?* Am. J. Reprod. Immunol., 66:11-22. https://doi.org/10.1111/j.1600-0897.2011.01033.x.
18. Rothschild, MF., A. Ruvinsky. (2011). (Eds.). *The genetics of the pig*. CABI.
19. Rozeboom, K. (2000). *Improving fertility with seminal plasma*. Swine News.23:15–7.
20. Savić, R., M. Petrović, D. Radojković, Č. Radović, N. Parunović. (2013). *The effect of breed, boar and season on some properties of sperm.* Biotechnology in Animal Husbandry. 29(2):299-310.https://doi.org/10.2298/BAH1302299S.
21. Savić, R., M. Petrović, D. Radojković, Č. Radović, N. Parunović, M. Pušić, R. Radišić. (2013). *Variability of ejaculate volume and sperm motility depending on the age and intensity of utilization of boars*. Biotechnology in Animal Husbandry, 29(4), 641–650.DOI: 10.2298/ BAH1304641S.
22. Smital, J. (2009). *Effects influencing boar semen*. Animal Reproduction Science. 110(3-4):335-46.https://doi.org/10.1016/j.anireprosci.2008.01.024.
23. Stančić, B. (2005). *Tehnologijaveštačkogosemenjavanjasvinja (monografija)*. Univerzitet u Novom Sadu, Poljoprivredni fakultet.
24. Stančić, B. (2005). *Reprodukcijasvinja (monografija)*. Poljoprivrednifakultet, Novi Sad.
25. Stančić, I., I. Zdraveski, S. Dragin, J. Apić, S. Vakanjac, P. Dodovski, S. Krstović, I. Galić. (2019). *The influence of different molecular weight seminal plasma protein content on some fertility parameters in boar s ejaculates.* Slovenian Veterinary Research, 56(4), pp.163–171.DOI: 10.26873/SVR-738-2019.
26. Stančić, I., J. Apić, I. Radović, S. Dragin, A. Božić, I. Žarković, B. Stančić. (2015). *Influence of season, boars breed and age on protein content variation in seminal plasma.* Cont Agri (Novi Sad, Srb.). 64(3–4)120–6.- po vancuver.
27. Sutkevičienė, N., H. Žilinskas. (2004). *Sperm morphology and fertility in artificial insemination boars*. 18(5.59):8.doi: 10.3390/ani11040920.

28. Tsakmakidis, IA., TA. Khalifa, CM. Boscos. (2012). A*ge-related changes in quality and fertility of porcine semen.* Biological research. 45(4):381–6.doi.org/10.4067/S0716-97602012000400009.

29. Wierzbicki, HE., IW. Gorska., AN. Macierzyńska., MA. Kmieć.(2010). *Variability of semen traits of boars used in artificial insemination*. Medycyna Wet.;66(11):765–9.

30. Wolf, J., J. Smital. (2009 a). *Effects in genetic evaluation for semen traits in Czech Large White and Czech Landrace boars*. Czech J. Anim. Sci. 1;54(8):349–58.DOI: 10.17221/1658-CJAS

31. Wolf, J., J. Smital. (2009b). *Quantification of factors affecting semen traits in artificial insemination boars from animal model analyses*. Journal of animal science. 1;87(5):1620–7.https://doi.org/10.2527/jas.2008-1373.