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INFLUENCE OF SEASON, BOARS BREED AND AGE ON PROTEIN CONTENT VARIATION IN SEMINAL PLASMA*

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Summary: Previous studies demonstrated that protein content in boar seminal plasma were highly correlated with fertility of boars. The purpose of the present study was to determine if protein content in seminal plasma significantly varies between boars, as well as if season of the year, boar breed or age significantly influence protein content in seminal plasma. In the first experiment, semen was collected weekly for 12 months, from 4 boars (48 ejaculates per boar). In the second experiment, two ejaculate per boars were taken from 106 boars (total 212 ejaculates) at commercial pig farm in Serbia. Total protein content in seminal plasma was determined for each collection. Protein content in seminal plasma considerably varies between individual boars (from 1.5% to 4.6%). More than 50% of boars had less than 3% protein in the seminal plasma. Protein content in seminal plasma is quite constant in the ejaculates of the same boar, at the one ejaculate per week collection frequency condition, with very little variations between months during the year. Boar breed or age did not have a significant ($P > 0.05$) impact on the protein content in seminal plasma (Landrace=2.98%; Large White=2.92% and Duroc=3.01%; ≤ 16 months=3.04%, 17 to 20 months=3.14%, 21 to 24 months=3.03 and ≥ 25 months=2.74%). These data indicated that the seminal plasma protein content could be genetically determined for each single boar. Consequently, these findings support the assumption that quantification of protein content in seminal plasma could serve as a powerful tool to improve boar fertility and reproductive performance.

Key words: breed, age, season, protein, seminal plasma, boar.

INTRODUCTION

Conventional intracervical artificial insemination (AI) is performed in about 99% of worldwide intensive pig production, using expensive genetically superior boars (Singleton, 2001; Kommisurd et al., 2002; Stančić and Dragin, 2011; Stančić et al., 2013; Kalifa et al., 2014). When expensive genetically superior boars is used, the prediction of sperm fertilizing ability, to selection the high fertility boars, has a great economic importance (Gadea et al., 2005).

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It has been shown that seminal plasma is important for boar semen fertility *in vitro* (Maxwell et al., 2007; Juyena and Calogero, 2012; Stančić et al., 2012), and that seminal plasma proteins play a key role in this phenomenon (Kommisurd et al., 2002; Strzeżek et al., 2005; Caballero et al., 2008; Garcia et al., 2009). Namely, it has been found that over dilution of seminal plasma, for example in over diluted AI doses, reduce the sperm progressive motility and increase number of sperm with damaged acrosome or disintegrated acrosomal membrane (Kommisurd et al., 2002; Maxwell et al. 2007; Stančić et al., 2012). It is the result of protein concentration reduction 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). We hypothesized that quantification of seminal plasma proteins could be a useful method to selection the high fertility boars prior to using for artificial insemination, if the protein content in the individual boar seminal plasma is constant and does not vary depending on boars breed or age, as well as on the season of the year.

Therefore, the aim of this study was to determine the individual boar variation of protein content in seminal plasma, as well as to determine whether the protein content in seminal plasma significantly depends on the season, boar breed or age.

MATERIALS AND METHODS

Two separate experiments were conducted on six large farms for intensive pig production in Serbia (AP Vojvodina). Farm capacity is 1,000 to 2,000 Swedish Landrace and Large White sows. Artificial insemination (AI) is performed by Landrace, Large White and Duroc boars, domestic or foreign origin.

First experiment. The seasonal variations of protein content in seminal plasma was determined in four boars. Total of 48 ejaculates (gel-free fraction) were collected per boar per year (one ejaculate per week, four ejaculates per month). All equipment which was in contact with the semen was sterile and disposable. Ejaculate samples, volume about 60 ml, were transported from the farm to the laboratory in a thermo-box at 4°C, within 2 to 3 hours after on farm collection.

Second experiment. The influence of breed and age on protein content in seminal plasma was investigated in 106 boars (Landrace=38, Large White=44 and Duroc=24), average 23 months of age. Two ejaculates were taken from each boar (total 212 ejaculates). Equipment for ejaculate collection and transport conditions, were the same as in the first experiment.

Preparing and chemical analyses of seminal plasma. Immediately after arrived to the laboratory, 20 ml semen sample were centrifuged at 1,500×g for 20 minutes at 4°C. Then the seminal plasma was separated and re-centrifuged at 3,000×g for 15 minutes at 4°C minutes, to remove residual organic particles. Chemical analysis of seminal plasma samples was performed on the same or next day. From the moment of preparation until the time of analysis, seminal plasma samples were kept in a refrigerator at 4°C. Protein content (%) in semen samples was determined according to AOAC Official Method 2001.11.

Statistical analysis: The difference between obtained results was tested by Student's t-test. The data were analyzed by the software package "Statistics 12". Mean ± standard deviation, minimum and maximum values of the experimental data are presented.

RESULTS

The average protein content in seminal plasma per month, of four boars (2 with high and 2 with low protein content in seminal plasma) were shown in Figure 1. There was not significant ($P > 0.05$) variation in seminal plasma protein content between the months of the year, as in high protein content boars (3.6% to 4.6%), as well as in low protein content in investigated boars (1.6% to 2.6%).

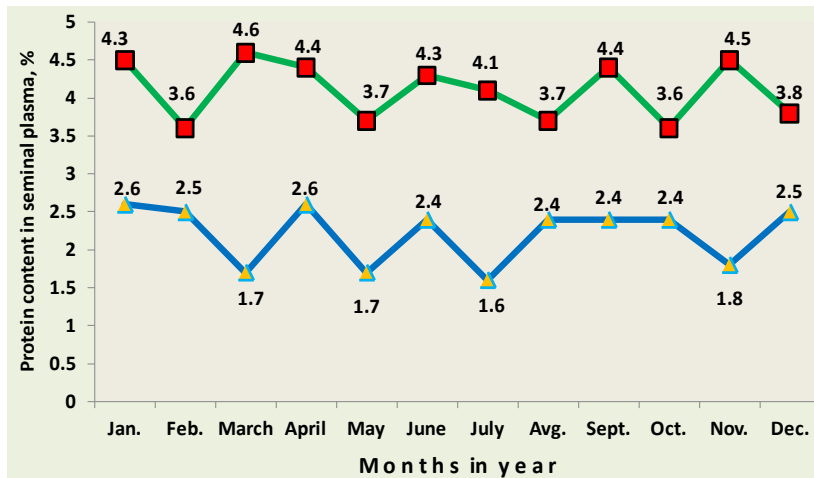


Figure 1. Variation of protein content in seminal plasma during the year in fourboars (Average values from 4 boars: 4 ejaculate per month per boar)

Distribution of protein content in 212 seminal plasma samples, obtained from 106 boars (2 ejaculates per boar), were shown in Figure 2. More than 50% of boarshad less than 3% protein in the seminal plasma, 15% of boars had 3to 3.5% protein, 22% of boars had 3.6 to4.1% protein, while seminal plasma in only 9% of the boars contains $\geq 4.2\%$ protein. The average protein contentin seminal plasma of all investigated boars was 2.97% (Table 1 and 2).

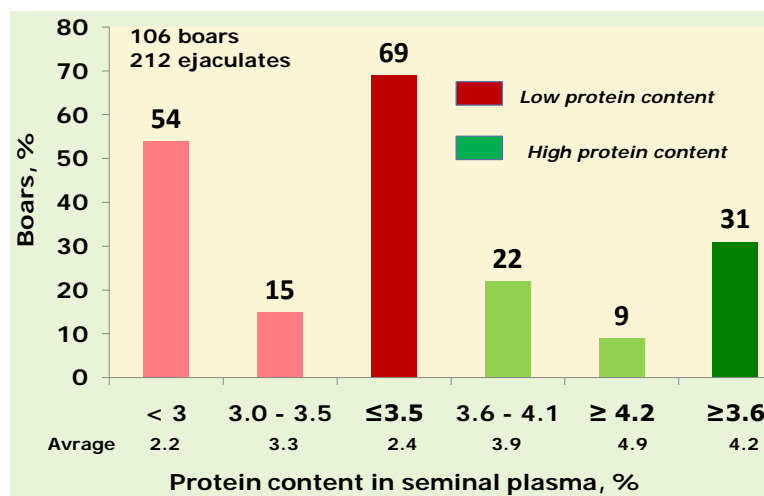


Figure 2. BI Distribution of boars according to protein content in seminal plasma

There are not significant ($P > 0.05$) variation of protein content in seminal plasma between ejaculates of one boar, as well as between the individual boars within high (3.4% to 4.4%) and low protein boars group (1.4% to 2.6%). However, average protein content in seminal plasma in high (4.0%) was significant ($P < 0.01$) greater, compared with 2.1% in low protein content boars (Figure 3).

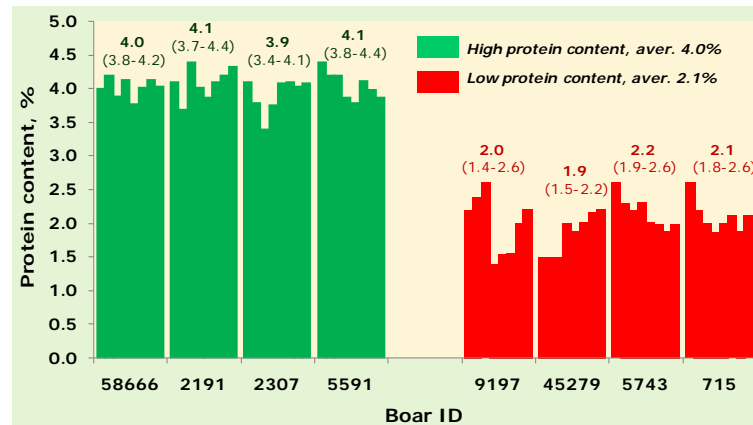


Figure 3. Variation of protein content in seminal plasma in the individual boars

The influence of boar breed on protein content in seminal plasma, were given in Table 1. Boars breed had no significant ($P>0.05$) effect on the protein content in seminal plasma (Landrace=2.98%; Large White=2.92% and Duroc=3.01%).

Also, there was no significant ($P>0.05$) variation in the average protein content in seminal plasma of different age boars (≤ 16 months=3.04%, 17 to 20 months=3.14%, 21 to 24 months=3.03 and ≥ 25 months=2.74%).

Table 1. Effect of boars breed on protein content in seminal plasma

Breed	Boars		Protein content (%)	
	n	%	Average \pm SD	min. – max.
Landrace	38	35.8	2.98 \pm 1.250a	1.00 – 6.50
Large White	44	41.5	2.92 \pm 1.005a	1.23 – 5.34
Duroc	24	22.7	3.01 \pm 0.869a	1.35 – 4.45
T o t a l	106	100.0	2.97	1.00 – 6.50

a, b, c, d Values in columns with different superscripts significantly differ ($P<0.05$).

Also, there was no significant ($P>0.05$) differences of the average protein content in seminal plasma between the boars of different age (≤ 16 months=3.04%, 17 to 20 months=3.14%, 21 to 24 months=3.03 and ≥ 25 months=2.74%) (Table 2).

Table 2. Effect of boars age on protein content in seminal plasma

Age (months)	Boars		Protein content (%)	
	n	%	Average \pm SD	min. – max.
≤ 16	33	31.1	3.04 \pm 1.017a	1.00 – 5.17
17 to 20	25	23.6	3.14 \pm 1.139a	1.53 – 6.5
21 to 24	20	18.9	3.03 \pm 1.031a	1.34 – 5.34
≥ 25	28	26.4	2.74 \pm 0.984a	1.23 – 5.05
T o t a l	106	100.0	2.97	1.00 – 6.50

a, b, c, d Values in columns with different superscripts significantly differ ($P<0.05$).

DISCUSSION

The results of the present study show that there was not significant ($P>0.05$) variation of protein content in seminal plasma between the months of the year (Fig. 1). More than 50% of boars had less than 3% protein in the seminal plasma, 37% of boars had 3% to 4.1%, and only 9% of boars had $\geq 4.2\%$ protein in the seminal plasma (Fig. 2). Protein content in seminal plasma was not significant ($P>0.05$) different between ejaculates of the same boar, at one ejaculate per week collection frequency condition (Fig. 3). Boars breed (Table 1) or age (Table 2) did not have a significant ($P>0.05$) impact on the protein content in seminal plasma.

Classic parameters of ejaculate quality significantly vary at each single boar, as well as between the boars. This variation is due to the strong influence of many factors (Stančić et al., 2003a; Smital, 2009). Most important among them are: frequency of semen collection (Singleton and Flowers, 2001; Wolf and Smital, 2009), boars age (Jankevičiūtė and Žilinskas, 2002; Smital, 2009; LópezRodríguez, 2012), boar breed (Ciereszko et al., 2000; Stančić et al., 2003a; Smital et al., 2004; Smital, 2009) and season of the year (Ciereszko et al. 2000; Okere et al., 2005; Smital, 2009; Lapustet et al., 2011; Stančić et al., 2012; Savić et al., 2013). For these reasons, the classic parameters of semen quality are not sufficient indicators of fertility and reproductive performance in boars (Novak et al., 2010). On the other hand, it has been frequently shown that seminal plasma proteins play a key role in the processes related to sperm function *in vitro* (Kommisur et al., 2002; Maxwell et al., 2007; Caballero et al., 2008; Stančić et al., 2012), as well as to fertilization and embryo development in the female reproductive tract (O'Leary et al., 2004; Strzeżek et al., 2005; Waberski et al., 2006; Rodriguez-Martinez et al., 2011; Juyena and Calogero, 2012). Significant variation of protein content in the seminal plasma between individual boars was found by Flowers (2001) and Novak et al. (2010). However, breed depending protein content in seminal plasma between Chinese Fangjing, Chinese Meishan and American Yorkchire boars were not found (Grafen et al., 1994; Maxwell and Johnson, 1999). Proteins content in boars seminal plasma varies between 1.8% and 4.5% (Frunzã et al., 2008).

Results obtained by Flowers (1998) demonstrated that concentration of seminal plasma proteins were highly correlated with *in vitro* boar semen fertility. Further studies (Flowers, 2001) have shown that sows insemination with ejaculates that contained high level of seminal plasma protein always produced farrowing rates and litter sizes greater than inseminations with lower protein concentration in seminal plasma. However, we have assumed that it is important to determine whether the protein content in seminal plasma is constant in the same boar and is not influenced by frequency of semen collection, season of year, boar breed or age. If this is true, then quantification of these proteins in semen could serve as a useful tool in commercial pig production to improve boar fertility, as well as a marker to rank boars in terms of their fertility, prior to sows insemination (Flowers, 2001; Novak et al., 2009; Novak et al., 2010).

CONCLUSION

The findings of the present study indicated that the seminal plasma protein content could be genetically determined for each single boar. This support our assumption, based on the results of others, that quantification of protein content in seminal plasma could serve as a powerful tool for selection the boars with high fertilizing potential. Consequently, it can improve boar reproductive performance. However, in our opinion, further studies should be undertaken to determine the heritability of protein content in boars seminal plasma. In addition, the physiological mechanism of the relationship between seminal plasma proteins content and boar semen fertility *in vitro*, as well as between seminal plasma proteins content and sows fertility rate, needs to be elucidated.

REFERENCES

- CABALLERO, I., VAZQUEZ, J.M., GARCÍA, E.M., PARRILLA, I., ROCA, J., CALVETE, J.J., SANZ, L., A.E. MARTÍNEZ, A.E.: Major proteins of boar seminal plasma as a tool for biotechnological preservation of spermatozoa. *Theriogenology*, 70:1352–1355, 2008.
- CIERESZKO, A., OTTOBRE, J.S., GLOGOWSKI, J.: Effects of season and breed on sperm acrosin activity and semen quality of boars. *Anim. Reprod. Sci.*, 64:89–96, 2000.
- FLOWERS, W.L.: Boar fertility and artificial insemination. Proc. 15th IPVC Congress, Birmingham, England, 5-9 July, 1998, Vol. 1, Pp 45-51, 1998.
- FLOWERS, W.L.: Relationship between seminal plasma proteins and boar fertility. *Swine News (USA)*, 6:1-4, 2001.
- FRUNZĂ, I., CERNESCU, H., KORODI, G.: Physical and chemical parameters of boar sperm. *Lucrări Stiințifice Medicină Veterinară (Timisoara)*, 41:634-640, 2008.
- GADEA, J.: Sperm factors related to *in vitro* and *in vivo* porcine fertility. *Theriogenology*, 63:431–444, 2005.

- GARCIA, M.E., CALVETE, J.J., SANZ, L., ROCA, J., MARTINEZ, E.A., VAZQUEZ, J.M.: Distinct Effect of Boar Seminal Plasma Fractions Exhibiting Different Protein Profiles on the Functionality of Highly Diluted Boar Spermatozoa. *Reproduction of Domestic Animals*, 44:200-205, 2009.
- GERFEN, W.R., WHITE, B.R., COTTA, A.M., WHEELER, M.B.: Comparison of the semen characteristics of fengjing, meishan and yorkshire boars. *Theriogenology*, 41:461-469, 1994.
- JANKEVIČIŪTĖ, N., ŽILINSKAS, H.: Influence of some factors on semen quality of different breeds of boars. *Veterinarija ir Zootechnika*, 19(41)1-3, 2002.
- JUYENA, S.A., CALOGERO, S.: Seminal Plasma: An Essential Attribute to Spermatozoa (A Review). *Journal of Andrology*, 33(4)536-551, 2012.
- KOMMISRUUD, E., PAULENZ, H., SEHESTED, E., GREVLE, S.I.: Influence of Boar and Semen Parameters on Motility and Acrosome Integrity in Liquid Boar Semen Stored for Five Days. *Acta Vet. Scand.*, 43:49-55, 2002.
- LAPUSTE, C., DIACONESCU, S., HINCU, M., PASCOVEANU, G.: Influence of Season on the Quantity and Quality of Boar Semen. *Animal Science and Biotechnologies*, 44(1)270-272, 2011.
- LÓPEZ RODRÍGUEZ, A.: Fresh boar semen: quality control and production, PhD Thesis. Faculty of Veterinary Medicine, Ghent University, 2012.
- MAXWELL, W.M.C., DE GRAAF, P.S., GHAOUI, E.-H.R., EVANS, G.: Seminal plasma effects on sperm handling and female fertility. *Soc. Reprod. Fertil., Suppl.*, 64, 13-38, 2007.
- MAXWELL, M.W. C., JOHNSON, L.A.: Physiology of spermatozoa at high dilution rates: the influence of seminal plasma. *Theriogenology*, 52:1353-1362, 1999.
- NOVAK, S., RUIZ-SANCHEZ, A., DIXON, W.T., FOXCROFT, G.R., DYCK, M.K.: Seminal Plasma Proteins as Potential Markers of Relative Fertility in Boars. *Journal of Andrology*, 27:1-24, 2009.
- NOVAK, S., RUIZ-SANCHEZ, A., DIXON, W.T., FOXCROFT, G.R., MICHAEL, G.R., DYCK, K.: Seminal Plasma Proteins as Potential Markers of Relative Fertility in Boars. *Journal of Andrology*, 31(2)188-200, 2010.
- O'LEARY, S., JASPER, M.J., WARNES, M.G., T.D. ARMSTRONG, D.T., ROBERTSON, S.A.: Seminal plasma regulates endometrial cytokine expression, leukocyte recruitment and embryo development in the pig. *Reproduction*, 128:237-247, 2004.
- OKERE, C., JOSEPH, A., EZEKWE, M.: Seasonal and genotype variations in libido, semen production and quality in artificial insemination boars. *Journal of Animal and Veterinary Advances*, 4(10)885-888, 2005.
- RODRIGUEZ-MARTINEZ, H., KVIST, U., ERNERUDH, J., SANZ, L., CALVETE, J.J.: Seminal plasma proteins: what role do they play? *Am. J. Reprod. Immunol.*, 66:11-22, 2011.
- SAVIĆ, R., PETROVIĆ, M., RADOJKOVIĆ, D., RADOVIĆ, Č., PARUNOVIĆ, N.: The effect of breed, boar and season on some properties of sperm. *Biotechnology in Animal Husbandry (Bgd., Srb.)*, 29(2)299-310, 2013.
- SINGLETON, L.W., FLOWERS, B.: Managing Boars in Artificial Insemination Centers. *Pork Information Gateway*, Purdue University & North Carolina State University, pp. 1-7, 2001.
- SMITAL, J., DE SOUSA, L.L., MOHSEN, A.: Differences among breeds and manifestation of heterosis in AI boar sperm output. *Anim. Reprod. Sci.*, 80:121-130, 2004.
- SMITAL, J.: Effects influencing boar semen. *Anim. Reprod. Sci.*, 110:335-346, 2009.
- STANČIĆ, B., GAGRČIN, M., RADOVIĆ, I.: Influence of season, boar breed and age on semen quality. 1. Native semen. *Biotechnology in Animal Husbandry (Bgd., Srb.)*, 19(1-2)17-23.
- Stančić I. and S. Dragin, 2011. Modern technology of artificial insemination in domestic animals. *Contemporary Agriculture (Novi Sad, Srb.)*, 60(1-2)204-214, 2003a.
- STANČIĆ, B., BOŽIĆ, A., STANČIĆ, I., DRAGIN, S., RADOVIĆ, I., PETROVIĆ, M.: Effect of worm and cold period of the year on boar semen quality parameters. *Contemporary Agriculture (Novi Sad, Srb.)*, 61(1-2)163-168, 2012.
- STANČIĆ, B., ZVEKIĆ, D., RADOVIĆ, I., BOŽIĆ, A., ERDELJAN, M.: Increasing reproductive exploitation of AI boars (a review). *Proc. 23rd Internat. Symp. „New Technologies in Contemporary Animal Production”*, Novi Sad (Serbia), 19. – 21. Jun, 2013, pp 141-143, 2013.
- STRZEZEK, J., WYSOCKI, P., KUKLINSKA, M.D.: Proteomics of boar seminal plasma – current studies and possibility of their application in biotechnology of animal reproduction. *Reproductive Biology*, 5:279-290, 2005.
- WABERSKI, D., DÖHRING, A., ARDON, F., RITTER, N., ZERBE, H., SCHUBERT, H.-J., HEWICKER-TRAUTWEN, M., WITZE, K.F., HUNTER, R.H.F.: Physiological routes from intra-uterine seminal contents to advancement of ovulation. *Acta Vet. Scand.*, 48(13)1-8, 2006.
- WOLF, J., SMITAL, J.: Quantification of factors affecting semen traits in artificial insemination boars from animal model analyses. *J. Anim. Sci.*, 87: 1620-1627, 2009.

UTICAJ SEZONE, RASE I STAROSTI NERASTOVA NA VARIRANJE SADRŽAJA PROTEINA U SPERMALNOJ PLAZMI

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Izvod: Ranija istraživanja su pokazala da je sadržaj protein u spermalnoj plazmi u visokoj korelaciji sa fertilitetom nerastova. Cilj ovih istraživanja je da se ustanovi da li sadržaj protein u spermalnoj plazmi značajno varira između nerastova, kao i da li godišnja sezona, rasa ili starost nerastova značajno utiču na sadržaj protein u spermalnoj plazmi. U prvom eksperimentu, sperma je uzimana nedeljno, tokom 12 meseci, od 4 nerasta (48 ejakulata po nerastu). U drugom eksperimentu, uzeta su dva ejakulata od 106 nerastova (ukupno 212 ejakulata). Kod svakog ejakulata je određen sadržaj protein. Ustanovljeno je da sadržaj proteina značajno varira između pojedinih nerastova (1.5% do 4.6%). Više od 50% nerastova ima manje od 3% proteina u spermalnoj plazmi, dok je sadržaj proteina u spermalnoj plazmi jednog istog nerasta dosta konstantan, sa minimalnim varijacijama između pojedinih meseci u godini. Rasa ili starost nerastova nisu imali značajan ($P>0.05$) uticaj na sadržaj protein u spermalnoj plazmi (Landras=2.98%; Veliki Jorkšir=2.92% i Durok=3.01%; ≤ 16 meseci=3.04%, 17 to 20 meseci=3.14%, 21 to 24 months=3.03 i ≥ 25 meseci=2.74%). Ovi rezultati pokazuju da je sadržaj protein u spermalnoj plazmi genetski determinisan za svakog nerasta. S tim u vezi, ovi rezultati podržavaju mišljenje da kvantifikacija protein u spermalnoj plazmi može poslužiti kao snažno sredstvo za povećanje fertiliteta i reproduktivne performance nerastova.

Ključne reči: rasa, starost, sezona, protein, spermalna plazma, nerast

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