

Do Motility and Sperm Dose Count Affect In Vivo Fertility in Boar? [†]

Radimir Savić ^{1,*}, Dragan Radojković ¹, Marija Gogić ², Mladen Popovac ¹, Aleksandra Petrović ¹
and Čedomir Radović ²

¹ Faculty of Agriculture, University of Belgrade, 11080 Belgrade, Serbia; radodrag@agrif.bg.ac.rs (D.R.); mlp@agrif.bg.ac.rs (M.P.); aljeksaljeks@gmail.com (A.P.)

² Institute for Animal Husbandry, 11080 Belgrade, Serbia; gogic.marija@gmail.com (M.G.); cedomirradovic.izs@gmail.com (Č.R.)

* Correspondence: savic@agrif.bg.ac.rs

[†] Presented at the 1st International Online Conference on Agriculture—Advances in Agricultural Science and Technology (IOCAG2022), 10–25 February 2022; Available online: <https://iocag2022.sciforum.net/>.

Abstract: The objective of this research was to determine whether a mass sperm motility in native ejaculate and a sperm dose-count (SDC) affect in vivo boar fertility. Total of 983 ejaculates taken from Landrace ($n = 16$ animals), Large White ($n = 19$ animals) and Duroc ($n = 7$ animals) breed boars has been analysed. Concentration of native sperm was assessed by means of a colorimeter and the evaluation of mass sperm motility was done by a subjective assessment using a microscope. On average 20.70 doses for insemination were obtained per ejaculate and they were divided into three classes (SDC = ≤ 2.50 ; 2.51–4.00; $\geq 4.01 \times 10^9$). The insemination of 7661 breeding females has been performed twice. The assessment of the effect was performed by means of General Linear Model in a SAS 9.1.3 statistical package, using a model that includes a breed fixed effect and linear regression effect of motility nested within the class of sperm count in a dose. Average values of return rate (%), farrowing rate (%) and litter size at birth accounted for: 15.53%, 73.41% and 12.65 live piglets, respectively. All the traits of in vivo fertility varied under the effect of breed ($p < 0.001$). Increase of motility by one unit (%) resulted in decreased percentage of return rate by 0.11% ($p < 0.001$) in all three SDC classes. On the other hand, farrowing rate increased by 0.12–0.13% ($p < 0.001$) depending on SDC class. When litter size at birth is in question a linear regression effect of motility was determined inside SDC with the highest sperm count ($b = 0.01$; $p < 0.05$).

Keywords: boar; sperm; return rate; farrowing rate; litter size

Citation: Savić, R.; Radojković, D.; Gogić, M.; Popovac, M.; Petrović, A.; Radović, Č. Do Motility and Sperm Dose Count Affect In Vivo Fertility in Boar?

Proceedings **2022**, *4*, x.

<https://doi.org/10.3390/xxxxx>

Academic Editor(s): Bin Gao

Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

An artificial insemination represents a primary way of fertilization in intensive farm production [1]. Productivity control and assessment of boar fertility is an indispensable part of reproductive management [2]. The evaluation of reproductive performance in boar is estimated on the basis of farrowing rate and number of live born piglets at birth [3].

A direct effect of boar explains 5.3% of total variability of farrowing rate, and it involves the effects of: breed (22%), individual (29%), age (0.3%), spermatozoa motility (9%), with 40% variability not being determined [4]. Spermatozoa motility is the most important parameter of sperm fertilizing potential [5]. Volume of ejaculate, concentration and number of live, progressively motile spermatozoa are essential for determining a maximum dilution of ejaculate [6].

The research of Ruiz-Sánchez et al. [7] indicates a great variability of in vivo fertility in boar: conception rate (73–98%), farrowing rate (71–98%) and litter size at birth (8.8–12.0 piglets). Timely identification of boars that are able to produce larger litters at birth is necessary for improving pig production [2].

The objective of this research was to determine whether a mass sperm motility in native ejaculate and a sperm dose-count affect in vivo boar fertility.

2. Material and Methods

The research was conducted on a pig farm that owns its reproductive and commercial breeding herd. Boars were housed in a separate facility, in the boxes of 8 m², with a partially slatted concrete flooring. Microclimatic conditions were semi-automatic regulated by vertical and horizontal ventilation. Nutrition was based on balanced feed mixtures while fresh water was available *ad libitum*.

A total of 983 ejaculates of Landrace boars ($n = 16$ animals), Large White boars ($n = 19$ animals) and Duroc boars ($n = 7$ animals) was analysed, a minimum number of ejaculates per boar being 5. The ejaculates were collected by a standard manual method, introducing a boar into a room with a sow-phantom. A volume of ejaculate was measured by a graduated cylinder with precision of ± 2 mL. A concentration of native sperm was estimated by means of a photo-colorimeter and motility of spermatozoa mass was assessed by a subjective estimation under a microscope.

Commercial diluters providing possibility of storing doses up to seven days were used. Doses for insemination were standardized to the volume of 100 mL. The ejaculates ($n = 266$, $n = 441$ and $n = 276$) were diluted to: ≤ 2.50 , 2.51–4.00 and $\geq 4.01 \times 10^9$ spermatozoa dose count (SDC). On average 20.70 insemination doses per ejaculate were obtained. Up to the moment of insemination the doses were stored at 17 °C and used within 96 h of preparation. Sows were inseminated twice and of total of 7661 matings the 5665 farrowings were realized.

Impact assessment was carried out by applying the General Linear Model procedure of the statistical package SAS 9.1.3 (SAS Inst. Inc., USA), using the following model:

$$y_{ijk} = \mu + B_i + b(C_j) + e_{ijk},$$

where: y_{ijk} Error! Bookmark not defined.—is an analysed fertility trait, μ —general population average, B_i —effect of boar breed ($i = 1,2,3$), $b(C_j)$ —linear regression effect of spermatozoa mass motility nested within the class of sperm count in a dose ($j = 1,2,3$) and e_{ijk} —random error. The comparison of the Least Square Means values of fertility traits was done by *t*-test.

3. Results and Discussion

The average values obtained for return rate (%), farrowing rate (%) and size of litter at birth were: 15.53%, 73.41% and 12.65 live piglets, respectively. All the traits of in vivo fertility varied under the effect of breed (Table 1). Large White breed boars had highest return rate which was by 5.52 and 4.02% higher in relation to Duroc and Landrace boars. The best reproductive efficiency (lowest return rate and highest farrowing rate) was obtained by Duroc boars compared to the other two meaty, fertile breeds. One possible reason is that Duroc boars primarily mated with females of F1 genotype, due to which there occurred the expression of heterosis effect. The largest number of live piglets at birth was realized by Landrace boars in relation to the other two studied breeds.

Table 1. Phenotypic differences between breed.

Trait	Landrace	Large White	Duroc	RMSE ¹
Return rate (%)	13.90 ^{a,A}	17.92 ^B	12.50 ^{b,A}	4.57
Farrowing rate (%)	75.29 ^A	70.40 ^B	77.71 ^C	5.07
Litter size at birth	12.95 ^A	12.52 ^B	12.05 ^C	0.85

¹ RMSE- Root Mean Square Error; Differences in phenotypic values of traits inside rows designated by different letters are statistically significant: a,b— $p < 0.05$ A,B,C— $p < 0.001$.

A consequence of the increased motility of spermatozoa mass seems to be a decrease in return rate by 0.11% ($p < 0.001$) in all three SDC (Figure 1). On the other hand, farrowing rate increased by 0.12–0.13% ($p < 0.001$) depending on SDC (Figure 2). The results obtained suggest the significance of sperm motility for semen in vivo effectiveness. When the size of litter at birth is in question (Figure 3), a linear regression effect of motility was determined inside SDC with the highest number of spermatozoa ($b = 0.01$; $p < 0.05$).

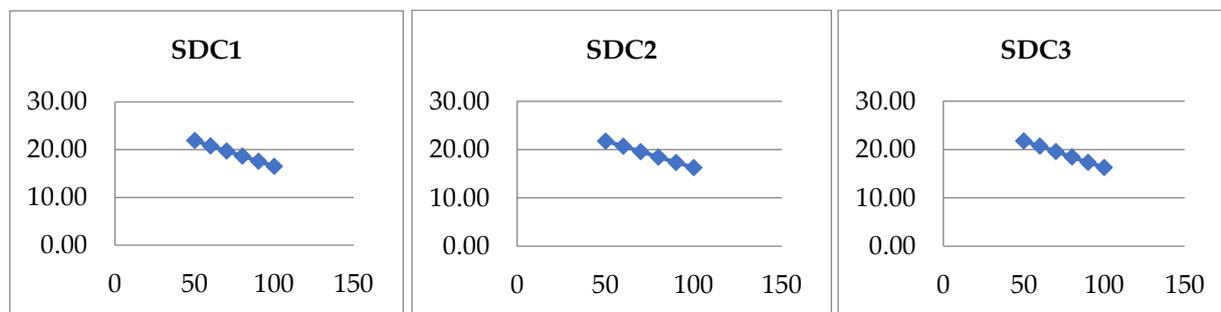


Figure 1. Return rate (%) depending on the motility (%) nested within the sperm count in a dose (SDC1: $\leq 2.50 \times 10^9$; SDC2: $2.51\text{--}4.00 \times 10^9$; SDC3: $\geq 4.01 \times 10^9$).

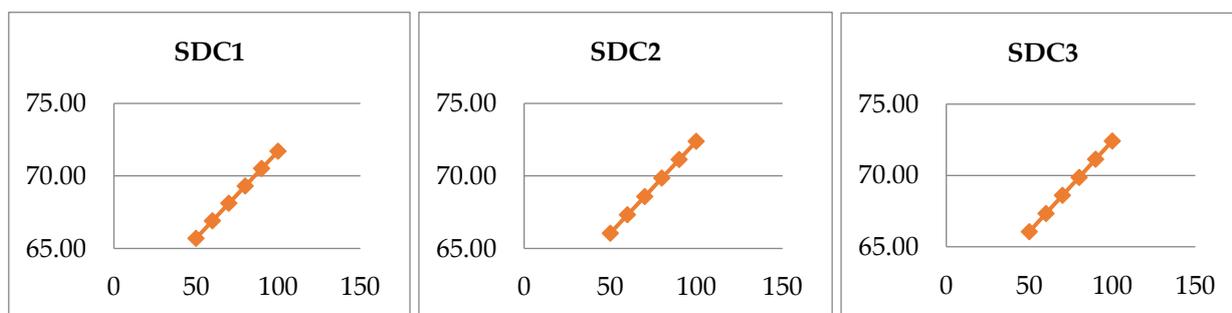


Figure 2. Farrowing rate (%) depending on the motility (%) nested within the sperm count in a dose (SDC1: $\leq 2.50 \times 10^9$; SDC2: $2.51\text{--}4.00 \times 10^9$; SDC3: $\geq 4.01 \times 10^9$).

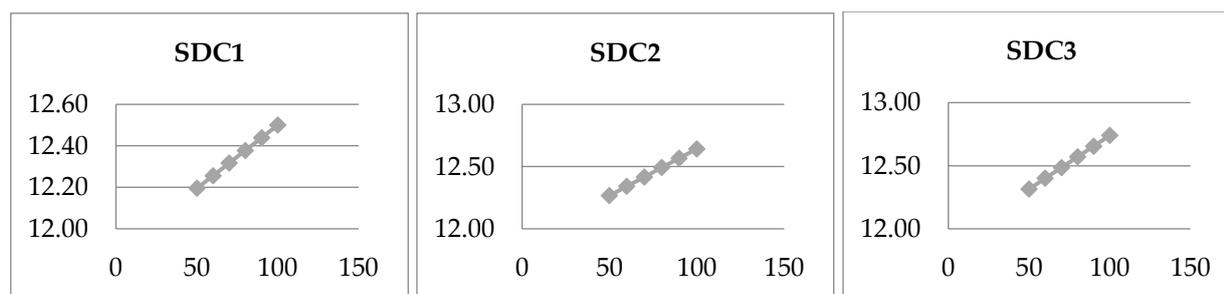


Figure 3. Litter size depending on the motility (%) nested within the sperm count in a dose (SDC1: $\leq 2.50 \times 10^9$; SDC2: $2.51\text{--}4.00 \times 10^9$; SDC3: $\geq 4.01 \times 10^9$).

The research of Tremoen et al. [8] showed that there are differences between the breeds of Norwegian Landrace and Duroc boars regarding CASA variables and the effect on a total number of piglets at birth the motility being the most frequently used parameter of sperm quality. Differences between breeds in in vivo fertility might be a consequence of certain mechanisms at a molecular level. In this respect, research of Xu et al. [9] suggests multiple changes at the level of proteins and status of phosphorylation between the sperm of Large White and of Duroc breed suggesting a correlation between reproductive efficiency and fertilizing potential. They have identified different novel molecular mechanisms which might help to better understand spermatogenesis, spermatozoa motility, energy metabolism and a process of joining spermatozoa and ovum.

The analysis of sperm in production conditions leads to discovering ejaculates of poor quality which are connected with poor fertility [10]. The results of this research comply with the statement of Savić et al. [2] that suggests that sperm qualitative traits can effect in vivo fertility, therefore an individual assessment of each boar ejaculate used for artificial insemination is imperative. The evaluation of sperm standard characteristics enables identification of ejaculates which are potentially poorly fertile but efficiency of estimation of boar fertility based on these characteristics is not enough [11]. Motility is one of the most important traits that affects the in vivo fertility of boars: sperm penetration, farrowing rate and litter size [2] which was confirmed by this research. Some studies [12] indicate that the number of live born piglets and litters with more piglets (more than 12 piglets) are associated with the parameters of sperm motility. It was shown in certain studies mentioned by Flowers [13] that if doses with three or more billions of spermatozoa are used the correlation between motility and in vivo fertility of breeding females is asymptotic, the point at which fertility no longer increases significantly being the increase of progressive motility from 60 to 70%.

4. Conclusions

The traits of in vivo fertility varied under the effect of breed. Duroc boars realized best reproductive efficiency while Landrace boars had largest size of litter at birth. Motility of sperm affects return rate and farrowing rate in all three classes of sperm dose count. The size of litter at birth increases with the increase of sperm motility inside sperm dose count with the highest number of spermatozoa.

Author Contributions: Conceptualization, R.S., D.R.; methodology, R.S.; software, R.S.; validation, R.S., D.R.; formal analysis, M.G.; investigation, A.P.; resources, Č.R.; data curation, M.G.; writing—original draft preparation, R.S.; writing—review and editing, all authors; visualization, M.P.; supervision, R.S.; project administration, D.R.; funding acquisition, R.S., D.R. All authors have read and agreed to the published version of the manuscript.

Funding: The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2022 no. 451-03-68/2022-14/200116 and 451-03-68/2022-14/200022.

Institutional Review Board Statement: This study did not require ethical approval, since trial was conducted within a standard technological process on the farm.

Informed Consent Statement: Not applicable.

Data Availability Statement: The results from data analyses performed in this study are included in this article and its tables/figures.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lopez Rodriguez, A.; Van Soom, A.; Arsenakis, I.; Maes, D. Boar management and semen handling factors affect the quality of boar extended semen. *Porcine Health Manag.* **2017**, *3*, 1–12.
2. Savić, R.; Davidović, V.; Božičković, I. Assessment of fertility of boars—Different approaches. In Proceedings of the 13th International Symposium “Modern Trends in Livestock Production”, Belgrade, Serbia, 6–8 October 2021; pp. 149–162.
3. Flowers, W.L. Triennial Reproduction Symposium: Sperm characteristics that limit success of fertilization. *J. Anim. Sci.* **2013**, *91*, 3022–3029.
4. Broekhuijse, M.L.W.J.; Šoštarić, E.Č.; Feitsma, H.; Gadella, B.M. Application of computer assisted semen analysis to explain variations in pig fertility. *J. Anim. Sci.* **2012**, *90*, 779–789.
5. Kunowska-Słószarz, M.; Makowska, A. Effect of breed and season on the boar’s semen characteristics. *Ann. Wars. Univ. Life Sci.-SGGW Anim. Sci.* **2011**, *49*, 77–86.
6. Kanokwan, K. Association and Expression Study of CD9, PLCz and COX-2 as Candidate Genes to Improve Boar Sperm Quality and Fertility Traits. Inaugural-Dissertation, Institut für Tierwissenschaft, Abt. Tierzucht und Tierhaltung der Rheinischen Friedrich-Wilhelms-Universität Bonn, Bonn, Germany, 2011.

7. Ruiz-Sánchez, A.L.; O'Donoghue, R.; Novak, S.; Dyck, M.K.; Cosgrove, J.R.; Dixon, W.T.; Foxcroft, G.R. The predictive value of routine semen evaluation and IVF technology for determining realtive boar fertility. *Theriogenology* **2006**, *66*, 736–748. 1
8. Tremoen, N.H.; Gaustad, A.H.; Andersen-Ranberg, I.; van Son, M.; Zeremichael, T.T.; Frydenlund, K.; Grindflek, E.; Våge, D.I.; Myromslien, F.D. Relationship between sperm motility characteristics and ATP concentrations, and association with fertility in two different pig breeds. *Anim. Reprod. Sci.* **2018**, *193*, 226–234. <https://doi.org/10.1016/j.anireprosci.2018.04.075>. 2
9. Xu, Y.; Han, Q.; Ma, C.; Wang, Y.; Zhang, P.; Li, C.; Cheng, X.; Xu, H. Comparative Proteomics and Phosphoproteomics Analysis Reveal the Possible Breed Difference in Yorkshire and Duroc Boar Spermatozoa. *Front. Cell Dev. Biol.* **2021**, *9*, 652809. <https://doi.org/10.3389/fcell.2021.652809>. 3
10. Gadea, J. Sperm factors related to in vitro and in vivo porcine fertility. *Theriogenology* **2005**, *63*, 431–444. <https://doi.org/10.1016/j.theriogenology.2004.09.023>. 4
11. Jung, M.; Rüdiger, K.; Schulze, M. In Vitro Measures for Assessing Boar Semen Fertility. *Reprod. Domest. Anim.* **2015**, *50* (Suppl. 2), 20–24. 5
12. Michos, I.; Tsantarliotou, M.; Boscós, C.M.; Tsousis, G.; Basioura, A.; Tzika, E.D.; Tassis, P.D.; Lymberopoulos, A.G.; Tsakmakidis, I.A. Effect of Boar Sperm Proteins and Quality Changes on Field Fertility. *Animals* **2021**, *11*, 1813. <https://doi.org/10.3390/ani11061813>. 6
13. Flowers, W.L. Selection for boar fertility and semen quality-the way ahead. *Soc. Reprod. Fertil.* **2009**, *66*, 67–78. 7