

# The Type of Container and Filling Method Have Consequences on Semen Quality in Swine AI Doses

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## Abstract

The automatic filling of semen doses for artificial insemination in swine shows economic advantages over the old-style, manual filling. However, no data could be found regarding the impact, if any, of this packing method on semen quality. This study aimed to compare two types of containers for boar semen, namely the automatically-filled tube and the manually-filled bottle, in terms of preserving the quality of boar semen. Five ejaculates from five different boars were diluted with the same extender and then divided in two aliquots. First aliquot was loaded in tubes filled by an automatic machine while the second was loaded manually in special plastic bottles. The semen was stored in liquid state at 17°C, regardless of the type of container and examined daily, for five days of storage by means of a computer-assisted sperm analyzer. Both types of containers maintained the semen within acceptable values, but after five days of storage significant differences ( $p < 0.05$ ) between the container types were observed in terms of all selected kinetic parameters. The tube showed better values for sperm motility and velocity, while the bottle showed superior values for straightness and linearity of sperm movement. The automatically-filled tubes offered better sperm motility in every day of the study. Given the fact that sperm motility is still the main criterion in assessing semen quality in semen production centers, the main conclusion of this study is that the automatic loading in tubes is superior and recommended over the old-style manual loading in bottles.

**Keywords:** AI dose, boar, container, sperm

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## 1. Introduction

The commercial units are always looking for improved efficiency and this applies also to swine semen production centers. In the past, the swine AI doses were filled and closed manually and this method is still used in some small centers. However, the automatic filling of semen doses shows an important economic advantage over the old-style, manual filling. It shortens the time necessary to process the semen and makes it possible to load a much higher number of semen doses within a day of work. As the contact of the semen with the air is shortened and the risk of contamination is decreased but on the other hand

the sperm get in contact with the parts of a filling machine that require rigorous hygiene and are pumped in tubes at a high speed which could cause additional stress, a fair question would be whether or not the automatic filling can influence the quality of preserved semen. Unfortunately, no data could be found regarding the impact, if any, of this packing method on semen quality in comparison with the old, manual method. Efforts have been made to assess the efficiency of different types of containers, both in swine [1] and other species [2-4] but no study could be found comparing these methods and respectively these types of container for boar semen.

This study aimed to compare two types of containers for boar semen, namely the automatically-filled tube and the manually-filled

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bottle, in terms of preserving the quality of boar semen during the conventional period of storage.

## 2. Materials and methods

Five ejaculates from five healthy and sexually mature boars were collected using manual method [5, 6] within a commercial unit specialized in swine AI doses production. The ejaculates passed the general exam and were diluted using the BTS® short-term extender (Minitube, Tiefenbach, Germany). Further, they were divided in two aliquots and the extended semen was loaded in two different types of containers, both commonly used for boar semen preservation in liquid state, at 17°C. First type was the old-style 100 ml plastic bottle, filled and closed manually (figure 1 –A), while the second type was the modern 100 ml plastic tube, filled and closed automatically (figure 1 –B). Both types of containers used within this study were produced by the same manufacturer (Minitube, Tiefenbach, Germany), in order to avoid the exposure of sperm to different chemical environments.

The examination of extended semen was performed in the day of dilution (Day 0) as well as after one (Day 1), two (Day 2), three (Day 3), four (Day 4) and five (Day 5) days of preservation in liquid state, at 17°C. For the quality evaluation a CASA system was used, namely SpermVision version 3.7 (Minitube of America - MOFA®, Verona, WI, USA) connected to an optical microscope Zeiss Axio Scope.A1 (Carl Zeiss MicroImaging GmbH, Göttingen, Germania) equipped with warming stage at 38°C. The following parameters were determined: total motility (TMot), progressive motility (PMot), straight line velocity (VSL), average velocity (VAP), curvilinear velocity (VCL), linearity (LIN), straightness (STR), wobble (WOB), lateral

head displacement (ALH), beat cross frequency (BCF). For “total motility” were considered all the sperm showing any type of movement, while for “progressive motility” were considered only the sperm showing at least 25µm/s average speed and at least 0.3 linearity.



**Figure 1 –A -B.** The types of containers used for preservation of liquid state boar sperm: bottle (A) and tube (B)

The collected data were processed using IBM SPSS® Statistics version 21 (IBM® Corporation, Chicago, IL, USA). The results are shown as mean values ± standard deviation (SD). To highlight the significant differences between the two types of container, the „Independent t-test” was applied. The differences were considered to be statistical significant if  $p < 0.05$ .

## 3. Results and discussion

After five days of storage, the semen still showed acceptable values for the kinetic parameters, regardless of the type of container (table 1). Progressive motility remained >60%, a value often considered an acceptance threshold before using the semen for AI [7].

**Table 1.** The values of seminal parameters after five days of preservation, according to the type of container that was used

Type of container		TMot	PMot	VAP	VCL	VSL	STR	LIN	WOB	ALH	BCF
Bottle	Mean	68.60 <sup>a</sup>	64.49 <sup>a</sup>	69.27 <sup>a</sup>	102.99 <sup>a</sup>	62.19 <sup>a</sup>	0.89 <sup>a</sup>	0.60 <sup>a</sup>	0.67 <sup>a</sup>	2.42 <sup>a</sup>	34.43 <sup>a</sup>
	SD	11.93	12.78	11.50	16.25	10.51	0.02	0.05	0.06	0.35	3.21
Tube	Mean	74.43 <sup>b</sup>	70.38 <sup>b</sup>	73.73 <sup>b</sup>	118.07 <sup>b</sup>	65.51 <sup>b</sup>	0.88 <sup>b</sup>	0.56 <sup>b</sup>	0.63 <sup>b</sup>	2.63 <sup>b</sup>	37.69 <sup>b</sup>
	SD	10.54	11.54	8.61	19.12	7.60	0.02	0.06	0.06	0.37	2.06

Within the same column, different superscripts indicate a significant difference between the types of container ( $p < 0.05$ )

Interestingly, all the calculated parameters showed significant differences ( $p < 0.05$ ) after five days of storage, according to the type of container (table 2). Thus, TMot, PMot, VAP, VCL, VSL, ALH and BCF showed higher values in case of automatically filled tube, while STR, LIN and

WOB showed higher values in case of manually filled bottles (tables 1 and 2). This could mean that the sperm move to a larger extent and with a higher speed when stored in automatically filled tubes, but their movement is less linear.

**Table 2.** The output of Independent t-test analyzing the values offered by the two types of containers, after five days of storage (equal variances assumed)

Parameter	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	df	Sig. (2-tailed)	Mean Difference (bottle-tube)
TMot	2.33	0.13	138	<b>.003</b>	-5.83
PMot	1.67	0.20	138	<b>.005</b>	-5.89
VAP	5.51	0.02	138	<b>.010</b>	-4.46
VCL	4.77	0.03	138	<b>.000</b>	-15.08
VSL	8.14	0.01	138	<b>.034</b>	-3.32
STR	0.91	0.34	138	<b>.026</b>	0.01
LIN	0.00	0.96	138	<b>.000</b>	0.04
WOB	0.40	0.53	138	<b>.000</b>	0.04
ALH	0.37	0.55	138	<b>.001</b>	-0.21
BCF	9.73	0.00	138	<b>.000</b>	-3.26

**The effect of container on the percentage of motile spermatozoa**

Monitoring the percentage of motile spermatozoa represents an useful and frequently used tool to evaluate the efficiency of preservation methods, in example the efficacy of an extender [8] or a container [1]. The motility of sperm is still one of the main criteria in evaluating the quality of semen, particularly in AI centers [9], as it is well known that a high value of this parameter is essential for fecundation [10].

In this experiment the percentage of motile sperm was monitored daily, in order to better observe the effect of container type on the kinetic potential of the sperm. The dynamics of the values of motility percentages during the five days of preservation are shown in figures 1 and 2.

During evaluation, total motility represented the total percentage of sperm showing any type of movement, while progressive motility referred only to the sperm with clear forward movements (average speed of at least 25  $\mu\text{m/s}$  and straightness of at least 0.3).

Total and progressive sperm motility showed superior values when the automatically filled tubes were used, in all of the five days of storage, suggesting that this type of container protects the biological value of sperm better. It's difficult to mention the cause for this phenomenon. Both

types of container were produced by the same manufacturer and from the same material, so the chemical factors are excluded. Among the potential causes that remain would be the shape of the container, the type of filling and the type of closing. The bottles are filled manually while the tubes are filled automatically. In case of manual filling, the process takes longer causing a prolonged stress on the sperm. In addition, the contact of semen with the ambient air is extended leading to an increase of the bacterial contamination, which could have negative effects such as sperm agglutination, impaired motility, acrosome disruption or loss of membrane viability [11]. As regards the type of closing, the bottles are provided with lids that need to be closed manually by rotation, while the tubes are automatically closed by heat and pressure at the open end, after loading the semen. Thus, the tubes are certainly closed airtightly, while for the bottles there is still some probability of air penetration, if the closing was not performed correctly. The penetration of atmospheric air is practically equivalent to the presence of a high volume of air inside the AI dose, which enables the gases exchange and the increase of seminal pH, leading to a decrease in sperm motility [12].

The effects of container type on progressive sperm motility during preservation have been also

observed by other authors. In example, for dog semen, Pinto et al [13] reported major differences after 48 h of storage, while Lopes et al [2] observed significant differences at 48 and 72 hours of storage both for progressive motility and membrane integrity. For stallion semen, some authors [3, 14] observed that sperm motility is protected better in some particular container type in comparison with other types available on the market, while other authors did not obtain major differences after 8 [15] or 24 [4, 16] hours of storage.

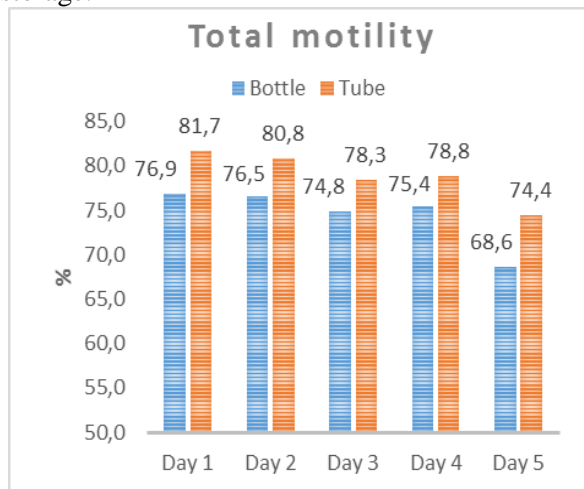


Figure 1. The variation of total sperm motility during preservation, according to the type of container

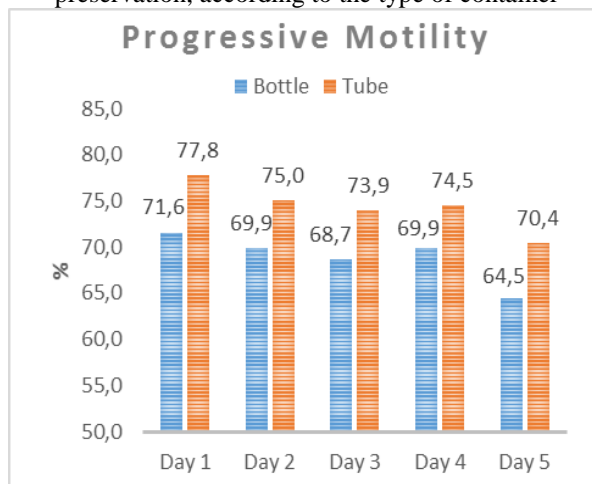


Figure 2. The variation of progressive sperm motility during preservation, according to the type of container

### The effect of container on the sperm velocity during storage

CASA systems can calculate not only the percentage of motile sperm within a sample, but also the speed of their movement, which can be defined using three parameters: average path

velocity (VAP), curvilinear velocity (VCL) and straight line velocity (VSL).

The effect of sperm speed on the fertilizing capacity is still controversial. Some previous studies reported both negative correlations with parturition rate [17, 18] and positive correlations with fertility [19], prolificacy [20] and *in-vitro* penetration rate [21] but also the lack of correlations with fertility indices [22]. A faster movement could help in faster covering the distance to the oviduct increasing the chances of fertilizing, but could also reflect a more intense metabolism of the seminal cell, leading to a faster exhaustion.

In this study, the curvilinear velocity (VCL) showed approximately similar values, without significant differences ( $p>0.05$ ) between the two types of container during the first four days of storage but significant higher values ( $p<0.05$ ) for the tube type after the fifth day of storage (figure 3). The lack of the dependence of VCL on the container type at the beginning of the storage period was also observed by other authors, for stallion semen [16].

Similarly, the average path velocity (VAP) was approximately equal after the first four days of storage for the two types of container. However, after the fifth day the spermatozoa stored in bottles showed a higher average speed compared to those stored in tubes ( $p<0.05$ ) (figure 4).

Remarkably, there was a gradual increase of the sperm average velocity from Day 1 until Day 4 (with the exception of Day 2 for tubes) (figure 4), although the curvilinear velocity remained relatively constant.

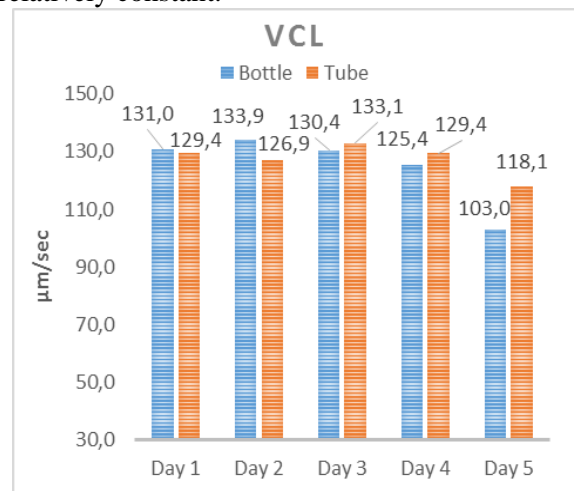
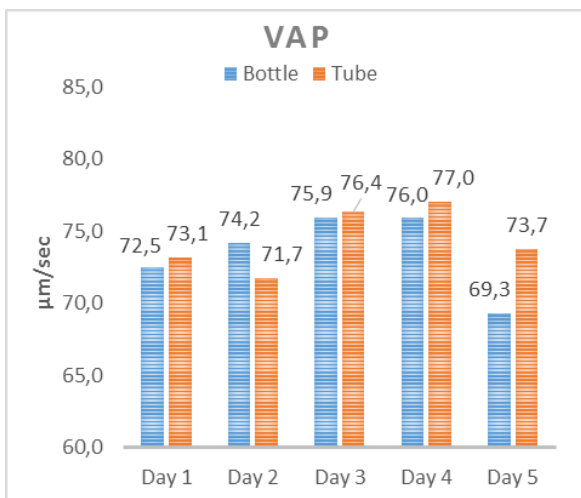
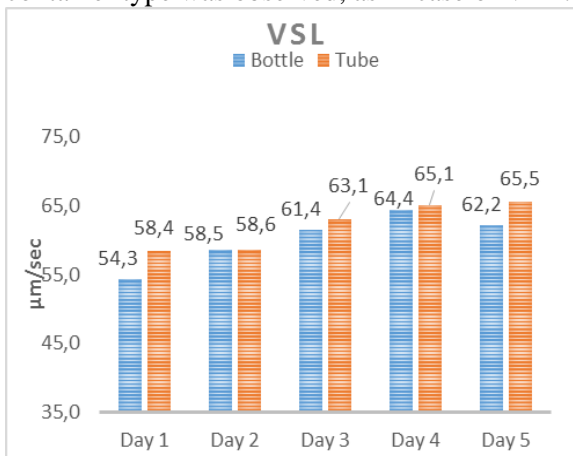


Figure 3. The variation of sperm curvilinear velocity during preservation, according to the type of container



**Figure 4.** The variation of average path velocity during preservation, according to the type of container

Straight line velocity (VSL) showed significantly higher values on sperm stored in tubes compared to those stored in bottles in days 1 and 5 ( $p < 0.05$ ) and close values, without significant differences during the other three days (figure 5). The same gradual increase in the values regardless of the container type was observed, as in case of VAP.

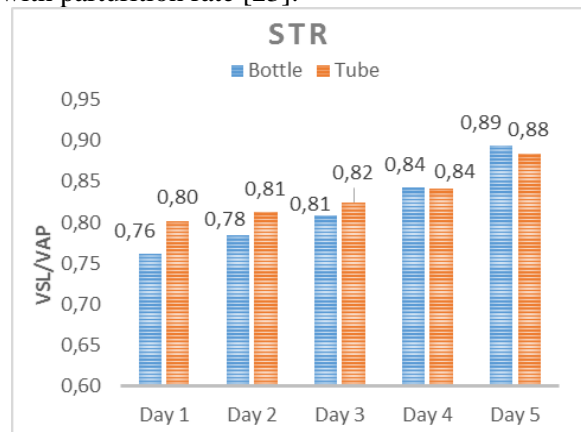


**Figure 5.** The variation of straight line velocity during preservation, according to the type of container

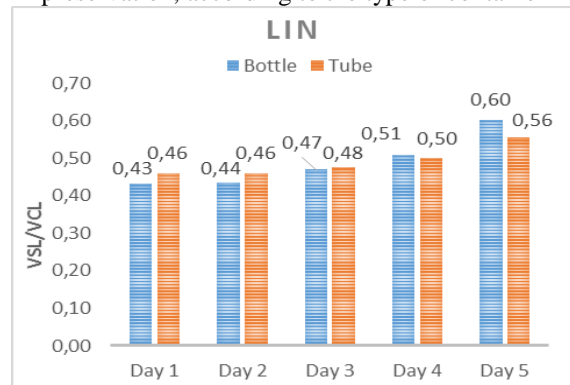
The results suggest that the two types of container do not have a different effect on the sperm velocity during the first four days of storage; however, significant differences can be observed after the fifth day in terms of all three velocity parameters, with superior values in case of tube containers. This could mean that the tubes filled and closed automatically protect better the metabolism of seminal cells during storage compared to the bottles filled and closed manually.

**The effect of container on the sperm movement uniformity during storage**

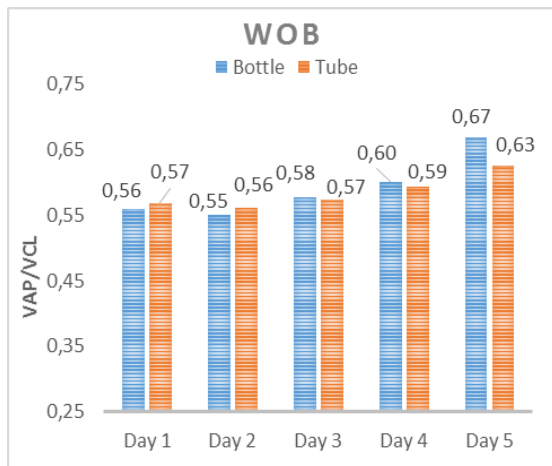
CASA systems can also calculate some indicators reflecting the direction and uniformity of sperm movement, as follows: straightness ( $STR = VSL/VAP$ ), linearity ( $LIN = VSL/VCL$ ), wobble ( $WOB = VAP/VCL$ ), amplitude of lateral head displacement ( $ALH = \text{mean distance traveled by the spermatozoon's head from its average path in a transversal direction}$ ), beat cross frequency ( $BCF = \text{the frequency of the head of spermatozoon intersecting its average path}$ ). Based on these, it can be stated that the sperm movement is more rectilinear or more uniform when STR, LIN and WOB show higher values, while higher values of ALH denote a more corrugated trajectory. Usually, when ALH is higher then BCF is lower and vice versa, because a longer distance traveled by the spermatozoon from its average path would mean a longer time necessary for the spermatozoon to return to it and thus a lower number of crossings per time unit. As stated by some authors, WOB and BCF correlate positively with parturition rate [23].



**Figure 6.** The variation of straightness during preservation, according to the type of container



**Figure 7.** The variation of linearity during preservation, according to the type of container



**Figure 8.** The variation of wobble during preservation, according to the type of container

The straightness coefficient showed significant higher values when the tubes were used during days 1 and 2, non significant differences during days 3 and 4 and significant higher values when the bottles were used after the fifth day of storage (figure 6).

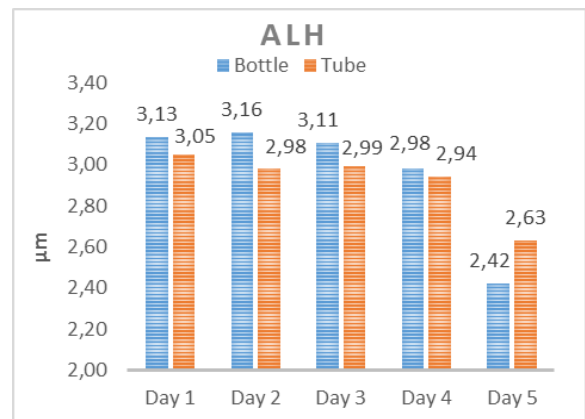
The linearity coefficient showed significantly higher values after in Day 1 in case of tubes, non significant differences in the days 2, 3 and 4, while in Day 5 the values were significantly higher for semen stored in bottles (figure 7).

The wobble coefficient showed no significant differences between the two types of containers during the first four days of storage. However, in Day 5 WOB was superior in case of semen stored in bottles (figure 8).

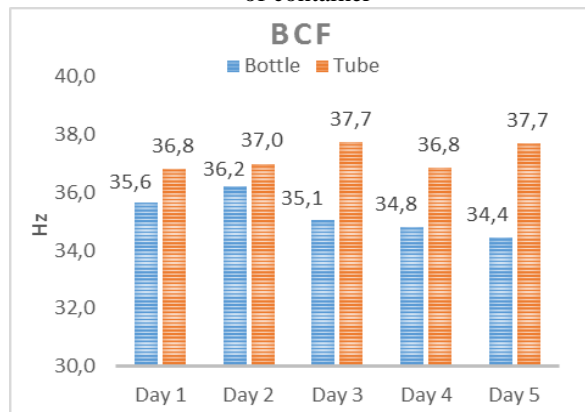
STR, LIN and WOB showed the same gradual increase of values, as observed in case of VAP and VSL (with the exception of WOB in Day 2). This could denote a streamlining of sperm movement over time. The actual velocity (VCL) remained relatively constant but the trajectory became less corrugated.

The amplitude of lateral head displacement was significantly higher on Day 2 in sperm preserved in bottles while on Day 5 it was superior in sperm preserved in tubes. In all the other days, no significant differences were observed (figure 9).

ALH reflects the mean distance traveled by the head of the spermatozoon transversally from its average path. Although a higher value of this parameter indicates a higher distance covered by the spermatozoon in a wrong direction, some studies found positive correlation between ALH and parturition rate [24].



**Figure 9.** The variation of amplitude of lateral head displacement during preservation, according to the type of container



**Figure 10.** The variation of beat cross frequency during preservation, according to the type of container

A high value of ALH (above 3.5 µm) could be a sign of sperm hyperactivation, particularly in association with high VCL [25]. Sperm hyperactivation is a prerequisite to fertilization [26], occurring physiologically in the oviduct and being associated with sperm capacitation [27]. However, if it occurs too early, it can compromise the success of fertilization through sperm exhaustion.

Beat cross frequency was, with the exception of Day 2, significantly higher in sperm stored in tubes (figure 10). As reported by some authors [23], BCF may positively correlate with the pregnancy rate, so a high value of it could be a sign of good quality.

#### 4. Conclusions

The results of this study show that both the two types of container are suitable for boar semen preservation, maintaining the main kinetic

parameters within acceptable values. However, it is to be noted that the tubes filled and closed automatically seems to protect better the sperm motility compared to the bottles filled and closed manually. During all days of storage, total and progressive motility were higher for sperm stored in tubes. As the percentage of motile sperm is still the main criterion in assessing semen quality before artificial insemination, we can consider that this type of container represents not only an economic solution, but also a way to maintain to biological value of sperm for a longer period of time. Nonetheless, the sperm preserved in manually filled bottles showed a more linear movement after five days of storage.

Surprisingly, there was a streamlining of sperm movement over time. Thus, the curvilinear velocity remained relatively constant during the study, while average path velocity and straight line velocity increased, showing the highest values after five days of storage. In this way the path followed by the sperm became more linear over time.

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### References

1. Eriksson B.M, Petersson H., Rodriguez-Martinez H., Field fertility with exported boar semen frozen in the new FlatPack container, *Theriogenology*, 2002, 58(6), 1065–1079. [http://dx.doi.org/10.1016/s0093-691x\(02\)00947-0](http://dx.doi.org/10.1016/s0093-691x(02)00947-0)
2. Lopes G., Simões A., Ferreira P., Martins-Bessa A., Rocha A., Differences in preservation of canine chilled semen using different transport containers, *Animal Reproduction Science*, 2009, 112, 158–163. <http://dx.doi.org/10.1016/j.anireprosci.2008.04.003>
3. Brinsko, S., Rowan, K., Varner, D., Blanchard, T., Effects of transport container and ambient storage temperature on motion characteristics of equine spermatozoa, *Theriogenology*, 2000, 53, 1641–1655. [http://dx.doi.org/10.1016/s0093-691x\(00\)00304-6](http://dx.doi.org/10.1016/s0093-691x(00)00304-6)
4. Webb G.W., Arns M.J., Harris M.A., Dekat C.L., Comparison of Two Containers Used for Shipment of Stallion Semen., *The Professional Animal Scientist*, 2005, 21(2), 133–137. [http://dx.doi.org/10.15232/s1080-7446\(15\)31182-7](http://dx.doi.org/10.15232/s1080-7446(15)31182-7)
5. Bogdan A.T., *Tratat de reproducție și însămânțări artificiale la suine*. Editura Tehnică Agricolă, București, 1999, pp 118-120.
6. Ciornei Ș.G., *Materialul seminal de vier și biotehnologii de reproducție la suine*. Editura “Ion Ionescu de la Brad”, Iași, 2012, pp 44-48.
7. Flowers W.L., Management of boars for efficient semen production, *J Reprod Fertil*, 1997. 52, 67–78.
8. Martin-Hidalgo D., Baron F.J., Robina A., Bragado M.J., Hurtado de Liera A., Garcia-Marin L.J., Gil M.C., Inter- and Intra-breed comparative study of sperm motility and viability in Iberian and Duroc boar semen during long-term storage in MR-A and XCell extenders, *Anim. Reprod. Sci.*, 2013, 139, 109-114. <http://dx.doi.org/10.1016/j.anireprosci.2013.04.001>
9. Lenz R.W., Kjelland M.E., Vonderhaar K., Swannack T.M., Moreno J.F., A comparison of bovine seminal quality assessments using different viewing chambers with a computer-assisted semen analyzer. *J Anim Sci.* 2011, 89(2), 383-8. <http://dx.doi.org/10.2527/jas.2010-3056>
10. Runceanu L, Cotea C, Drugociu D, Roșca P, *Reproducție, obstetrică și ginecologie veterinară*. Ed. “Ion Ionescu de la Brad”, Iași, 2007, pp 164-171.
11. Kuster C.E., Althouse G.C., The impact of bacteriospermia on boar sperm storage and reproductive performance *Theriogenology* 2016, 85, 21–26. <http://dx.doi.org/10.1016/j.theriogenology.2015.09.049>
12. Vyt P., Maes D., Sys S.U., Rijsselaere T., Van Soom A., Air contact influences the pH of extended porcine semen, *Reprod Dom Anim*, 2007, 42, 218 –20. <http://dx.doi.org/10.1111/j.1439-0531.2006.00733.x>
13. Pinto, C., Paccamonti, D., Eilts, B., Fertility in bitches artificially inseminated with extended, chilled semen, *Theriogenology*, 1999, 52, 609–616. [http://dx.doi.org/10.1016/s0093-691x\(99\)00156-9](http://dx.doi.org/10.1016/s0093-691x(99)00156-9)
14. Melo C.M., Alvarenga M.A., Zahn F.S., I. Martin, Orlandi C., Trinqué C.L.A., Dell’Aqua J.A. Jr., Papa F.O., Effect of the transport container and cryoprotectant on freezability of equine semen previously cooled for 24 h, *Animal Reproduction Science*, 2006, 94, 78–81. <http://dx.doi.org/10.1016/j.anireprosci.2006.03.093>
15. Maciel A.C., Castro F.S., Pires Neves A., Mattos R.C., Fiala Rechsteiner S., Preliminary results using a new container for cooled stallion semen transport, 6th ISSR Abstracts/Journal of Equine Veterinary Science 2012, 32, 475-518. <http://dx.doi.org/10.1016/j.jevs.2012.06.058>
16. Katila T., Combes G.B., Varner D.D., Blanchard T.L., Comparison of three containers used for the transport of cooled stallion semen, *Theriogenology*, 1997, 48(7), 1085–1092. [http://dx.doi.org/10.1016/s0093-691x\(97\)00341-5](http://dx.doi.org/10.1016/s0093-691x(97)00341-5)
17. Holt C., Holt W.V., Moore H.D.M., Reed H.C.B., Curnoc R.M., Objectively measured boar sperm

motility parameters correlate with the outcomes of on-farm inseminations: results of two fertility trials, *J Androl.*, 1997, 18, 312-323.

18. Park S., Effects of sow, boar, and semen traits on sow reproduction, Doctoral thesis, University of Nebraska-Lincoln, 2013.

19. Budworth P.R., Amann R.P., Chapman P.L., Relationships between computerized measurements of motion of frozen-thawed bull spermatozoa and fertility, *J Androl.*, 1988, 9, 41-54.

<http://dx.doi.org/10.1002/j.1939-4640.1988.tb01007.x>

20. Broekhuijse M.L.W.J., Sostaric E., Feitsma H., Gadella B.M., Application of computer assisted semen analysis to explain variations in pig fertility, *J. Anim. Sci.*, 2012, 90, 779-789.

<http://dx.doi.org/10.2527/jas.2011-4311>

21. Hirano Y., Shibahara H., Suzuki T., Takamizawa S., Yamaguchi C., Tsunoda H., Relationships between sperm motility characteristics assessed by the computer-aided sperm analysis (CASA) and fertilization rates in vitro, *J Assist Reprod Genet*, 2001, 18(4), 213-8.

22. Didion B.A., Computer-assisted semen analysis and its utility for profiling boar semen samples, *Theriogenology*, 2008, 70(8), 1374-1376.

<http://dx.doi.org/10.1016/j.theriogenology.2008.07.014>

23. Schulze M., Ruediger K., Mueller K., Jung M., Well C., Reissmann M., Development of an in vitro index to characterize fertilizing capacity of boar ejaculates. *Anim Reprod Sci.*, 2013, 140(1-2), 70-76.

<http://dx.doi.org/10.1016/j.anireprosci.2013.05.012>

24. Vyt, P., Maes D., Quinten C., Rijsselaere T., Deley W., Aerts M., de Kruif A., van Soom A., Detailed motility examination of porcine semen and its predictive value towards reproductive performance in sows, *Vlaams Diergeneesk. Tijdschr.* 2008, 77, 291-298.

25. Schmidt H., Kamp G., Induced hyperactivity in boar spermatozoa and its evaluation by computer-assisted sperm analysis, *Reproduction Research*, 2004, 128, 171-179.

<http://dx.doi.org/10.1530/rep.1.00153>

26. Chang H., Suarez S.S., Rethinking the relationship between hyperactivation and chemotaxis in mammalian sperm, *Biol. Reprod.*, 2010, 83, 507-513.

<http://dx.doi.org/10.1095/biolreprod.109.083113>

27. Armon L., Eisenbach M., Behavioral mechanism during human sperm chemotaxis: involvement of hyperactivation, *PLoS One*, 2011, 6(12), e28359.

<http://dx.doi.org/10.1371/journal.pone.0028359>