ASSESSMENT OF THE PLASMA MEMBRANE RESISTANCE OF BOAR SPERMATOZOA WITH USING SHORT HYPOOSMOTIC SWELLING TEST

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Abstract

The objective of this study was to assess the sperm membrane resistance in diluted boar semen with using short hypoosmotic swelling test (sHOS-test). sHOS-test evaluates the functional integrity of the spermatozoa plasma membrane, especially of the tail. sHOS-test involves incubation of spermatozoa in hypoosmotic solution 75 mOsm/l for 5 min. Eosin-nigrosine staining test was used with sHOS-test. Ejaculates from 21 fertile hybrid AI boars were collected by the gloved hand method. In the fresh boar semen were evaluated these parameters: semen volume, sperm motility, sperm concentration, morphologically abnormal spermatozoa, percentage of live sperm and sHOS-test. The boar semen was diluted in a semen-dilution rate of 1 + 4 in Androhep, Androstar, Androstar plus, MIII, LD and was stored at a temperature 17°C up to 96h. Samples of semen-dilution were kept at 38°C in water bath and resistance of spermatozoa was evaluated with sHOS-test before and after 1h incubation in water bath each day. The results of sHOS-test were included into four categories: swollen tail+negative eosin, nonswollen tail+negative eosin, swollen tail+positive eosin, nonswollen tail+positive eosin. A statistically significantly decrease were observed in categories swollen tail+negative eosin and nonswollen tail+negative eosin in all used extenders after 1h incubation spermatozoa in water bath ($P< 0.05$). This tendency also was recorded with the storage time. On the other hand category nonswollen tail+positive eosin had significantly increase after 1h incubation ($P< 0.001$). A statistically significantly decrease were found in category nonswollen tail+ negative eosin only in extender Androstar, Androstar plus after 72h storage time ($P< 0.05$). The results of this study lead to the conclusion that incubation of boar sperm during 1h, storage time and extenders participate in significantly decrease of spermatozoa membranes resistance ($P< 0.05$). The best resistance of sperm membrane was in extender Androhep and followed Androstar, Androstar plus, MIII and LD in terms of incubation and storage time.

Key Words: Boar; semen; short hypoosmotic swelling test; plasma membrane resistance

The integrity of the plasma membrane is fundamental for normal cell function. It is very important to analyze the structural and functional integrity of the sperm membrane because these characteristics are crucial for the viability and fertilizing ability of spermatozoa (Vazquez et al., 1997). The role of the plasma membrane in communication between the sperm cell and the external medium is essential (Calvete et al., 1996) and involves ion transport across the membrane (Kulkarni et al., 1997), the binding of different factors to specific receptors and the maintenance of the membrane potential (Zeng et al., 1995). One of the most widely used tests for evaluating spermatozoa membrane status is the hypoosmotic swelling test (HOS-test). HOS-test evaluates the functional integrity of the spermatozoa plasma membrane, especially of the tail. This test is based on the semi-permeability of the intact cell membrane that allows the sperm to swell under hypoosmotic conditions. When exposed to hypoosmotic solutions, biochemically active spermatozoa increase their volume to establish equilibrium between the fluid compartment within the spermatozoa and the extracellular environment. Swelling causes changes in both cell size and shape (Cabrita et al., 1999). This swelling process culminates in promoting a spherical expansion of the cell membrane covering the tail, thus forcing the flagellum to coil inside the membrane.

The short hypoosmotic swelling test (sHOS-test) is a modification of HOS-test (Pérez-Llano et al., 2001). Sperms are incubated in a 75 mOsm/l hypoosmotic solution for five minutes (Donadeu, 2004). Pérez-Llano et al. (2001) reported a significant correlation of sHOST with in vivo fertility and with farrowing rate. Only spermatozoa with intact plasma membranes and intact acrosome are able to fertilize an oocyte in vivo (Yanagimachi, 1994) and thus it is of interest to assess changes in these attributes over time during storage in different semen extenders. A large number of boar semen extenders is known but there exists a high variability of different diluents in terms of viability and fertilizing capacity of spermatozoa (Khan et al., 2006).

The objective of this study was to assess the sperm membrane resistance in diluted boar semen with using short hypoosmotic swelling test up to 96h storage time.

Material and Methods

Twenty-one ejaculates from 21 fertile hybrid AI boars aged 1 to 3 years were collected using the gloved-hand technique in autumn. The gel portion was removed using double gauze. The following parameters were evaluated in fresh native boar semen: semen volume, sperm motility, sperm concentration, morphologically abnormal

spermatozoa, percentage of viable spermatozoa, sHOS-test. The sperm motility was assessed subjectively using phase contrast microscopy with a heating stage (38°C) at 100x magnification. The sperm concentration was determined by a cytometric method using Bürker's chamber. Morphologically abnormal spermatozoa were assessed according to the staining method of Čeřovský (1976). The boar semen was diluted in a semen dilution rate of 1 + 4 in Androhep, Androstar, Androstar plus, M III (Minitüb, Germany), LD (Magapor, Spain) and was stored at a temperature of 17°C up to 96h.

Samples of semen-dilution were kept at 38°C in water bath and resistance of spermatozoa was evaluated with sHOS-test before (0h) and after 1h incubation in water bath each day (24h, 48h, 72h and 96h). sHOS-test was assessed by the method according to Pérez-Llano et al. (2003) with using the eosin-nigrosine staining technique (one drop from each sample was mixed with 1 drop of 1% eosin Y and 2 drops of 10% nigrosine were added after 30s). At least 200 spermatozoa were evaluated per slide under oil immersion and 1500x magnification. The results of sHOS-test were included into four categories: swollen tail+negative eosin, nonswollen tail+negative eosin, swollen tail+positive eosin, nonswollen tail+positive eosin.

Basic statistical characteristics of the results (arithmetic means, standard deviations and significance) were calculated by the QC Expert program. Statistical significance was checked by the analysis of variance ANOVA – Tukey method at significance levels of $P < 0.05$, $P < 0.01$, $P < 0.001$. An abnormal data were analysis by the Kruskal-Wallis test.

Results and discussion

The initial quality of semen collected for this study was as follows (mean±SD): sperm motility 70.24±8.79 %; semen volume 281.33±119.39 ml; sperm concentration 331.24 ± 154.71 x 10^3/mm^3; morphologically abnormal spermatozoa 26.55±19.81 %; viable spermatozoa 68.05±9.20 %; sHOS-test 63.57±11.94 % (total of swollen tails). The results of mean values of categories sHOS-test in 0h and 1h incubation in the water bath in different long-term extenders during storage time are show in Table 1.

Table 1. Mean values of categories sHOS-test in 0h and 1h incubation in the water bath in different long-term extenders during storage time

Extenders	Storage time	sHOS-test (%)							
		swollen tail				nonswollen tail			
		negative eosin		positive eosin		negative eosin		positive eosin	
		0h	1h	0h	1h	0h	1h	0h	1h
Androhep	24h	38.10	14.19^c	19.52	11.90^b	2.29	0.62	40.10	73.29^c
	48h	31.38	13.52^c	17.86	10.90^b	1.86	1.24	48.90	74.33^c
	72h	25.76	13.29^c	18.24	10.76^b	2.43	1.10	54.00	75.62^c
	96h	20.62	10.81^c	16.71	10.38^a	1.29	0.52	61.38	78.29^c
Androstar	24h	31.24	12.76^c	22.57	8.43^c	3.90	0.61	44.43	78.19^c
	48h	23.86	11.33^c	18.62	9.52^c	2.33	0.38	55.19	78.76^c
	72h	22.71	9.14^c	16.43	7.67^c	2.00	0.43^a	58.86	82.91^c
	96h	16.76	6.14^c	14.19	8.62^a	1.14	0.52	67.91	84.71^c
Androstar plus	24h	33.29	13.29^c	18.29	9.81^b	2.24	0.38	46.19	76.52^c
	48h	25.38	11.19^c	17.33	9.67^b	1.76	0.76	55.52	78.38^c
	72h	20.05	7.81^b	17.24	7.57^c	2.10	0.42^a	61.10	84.19^c
	96h	14.33	6.33^a	12.86	7.43^b	1.14	0.57	71.67	85.67^c
LD	24h	32.67	15.29^c	16.48	6.67^c	1.95	0.81	48.91	77.24^c
	48h	25.81	10.86^c	16.57	7.00^c	1.57	0.62	56.10	81.57^c
	72h	19.38	8.10^b	14.19	6.09^c	1.00	0.52	65.43	85.29^c
	96h	12.67	6.05^b	13.05	5.38^b	0.71	0.33	73.57	88.24^c
MIII	24h	31.38	13.95^c	19.48	11.00^c	2.14	0.33	47.86	74.71^c
	48h	24.95	11.52^c	18.29	10.57^b	2.05	0.52	55.19	77.52^c
	72h	18.86	7.67^b	16.62	9.28^b	1.38	0.62	63.14	82.43^c
	96h	12.86	5.57^a	16.57	8.76^b	1.14	0.57	69.43	85.10^c

^{a} $P < 0.05$, ^{b} $P < 0.01$, ^{c} $P < 0.001$

This investigation demonstrates that the sperm membrane resistance was gradually reduced during 1h incubation in all extenders until 96h of storage time. A statistically significantly decrease were observed in categories swollen tail+negative eosin and nonswollen tail+negative eosin in all used extenders after 1h incubation spermatozoa in water bath ($P< 0.05$). This tendency also was recorded with the storage time. On the other hand category nonswollen tail+positive eosin had significantly increase after 1h incubation ($P< 0.001$). Pérez-Llano et al. (2003) found that population spermatozoa response sHOS-test positive (swollen) is decreased during a 2h incubation and in parallel the percentage sHOS-test negative (nonswollen) spermatozoa is increased. A statistically significantly decrease were found in category nonswollen tail+negative eosin only in extenders Androstar, Androstar plus after 72h storage time ($P< 0.05$). Category sHOS-test swollen tail+negative eosin is very important because contains spermatozoa with the most resistant membrane. By contrast category sHOS-test nonswollen tail+positive eosin are dead spermatozoa.

We can see in the Table 1 that extender Androhep provided greater plasma membrane resistance (swollen tail+negative eosin) to adverse external conditions than the others extenders during the trial. sHOS-test selects spermatozoa with the most resistant membranes and could be an important indicator for choice of semen extender. According to Johnson et al. (2000) structural and functional changes in spermatozoa connected with the liquid storage of boar semen resemble a natural ageing process and may be influenced by the conditions and length of storage.

Conclusion

The present study found that incubation of boar sperm during 1h, storage time and extenders participate in significantly decrease of spermatozoa membranes resistance ($P< 0.05$). The best resistance of sperm membrane was in extender Androhep and followed Androstar, Androstar plus, MIII and LD in terms of incubation and storage time.

References

Calvete J.J., Sanz L., Enßlin M., Töpfer-Petersen E. (1996): Sperm surface proteins. Reproduction in Domestic Animals, 31, 101-105.

Cabrita E., Alvarez R., Anel E., Herr´aez M.P. (1999): The hypoosmotic swelling test performed with Counter:a method to assay functional integrity of sperm membrane in rainbow trout. Animal Reproduction Science, 55, 279-289.

Čeřovský J. (1976): Metoda barvení kančích spermií pro morfologické hodnocení. Živočišná Výroba, 21, 361-366.

Donadeu M. (2004): Advances in male swine artificial insemination (AI) techniques. The Pig Journal, 54, 110-122.

Johnson L.A., Weitze K.F., Fiser P., Maxwell W.M.C. (2000): Storage of boar semen. Animal Reproduction Science, 62,143-172.

Khan M.H., Naskar S., Anabrata Das, Bardoli R.K. (2006): Comparative efficacy of different diluents on liquid preservation of boar semen. Indian Journal of Animal Sciences, 76 (10), 780-783.

Kulkarni S.B., Sauna Z.E., Somlata V., Sitaramam (1997): Volume regulation of spermatozoa by quinine-sensitive channels. Molecular Reproduction and Development, 46, 535-550.

Pérez-Llano B., Yenes–Garcia P., García-Casado P. (2003): Four subpopulations of boar spermatozoa defined according to their response to the short hypoosmotic swelling test and acrosome status during incubation at 37°C. Theriogenology, 60, 1401-1407.

Vazquez J.M., Nartinez E.A., Martinez E.A., Garcia-Artiga C., Roca J. (1997): Hypoosmotic swelling of boar spermatozoa compared to other methods for analysing the sperm membrane. Theriogenology, 47, 913-922.

Yanagimachi R. (1994): Mammalian fertilization. In: Knobil E., Neil J.D., editors. The physiology of reproduction, 2nd ed., vol. 1. New York: Raven Press 189-317.

Zeng Y., Clark E.N., Florman H.M. (1995): Sperm membrane potential:Hyperpolarization during capacitation regulates zona pellucida-dependent acrosomal reaction. Developmental Biology, 171, 554-563.

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