ISSN 1068-3674, Russian Agricultural Sciences, 2011, Vol. 37, No. 2, pp. 175–178. © Allerton Press, Inc., 2011.
Original Russian Text © M.M. Atroshchenko, E.E. Bragina, 2011, published in Doklady Rossiiskoi Akademii Sel'skokhozyaistvennykh Nauk, 2011, No. 2, pp. 51–53.

ANIMAL HUSBANDRY

Change in the Ultrastructure of Stallion Spermatozoa under the Effect of Cryopreservation[1]

M. M. Atroshchenko[a] and E. E. Bragina[b]

[a]*All-Russian Horse Breeding Research Institute, Ryazan oblast, 391105 Russia*
[b]*Belozerskii Physicochemical Biology Research Institute, Moscow, 119199 Russia*
e-mail: atromiks-77@mail.ru

Received June 22, 2010

Abstract—The results of an electron microscopy study of the ultrastructural characteristics of stallion spermatozoa in native sperm and after freezing–thawing are given. Changes occurring in spermatozoa under the effect of cryopreservation are shown. Maximum damages are found in acrosomes.

Keywords: stallions, spermatozoa, ultrastructural characteristics, electron microscopy
DOI: 10.3103/S1068367411020029

An important component during artificial insemination with stallion cryopreserved sperm is its quality after thawing: activity, survivability, and number of abnormal forms of spermatozoa. But the correspondence of sperm to the established standards doesn't always characterize its quality. Disturbance of the functional properties of spermatozoa during investigation under the usual light microscope is impossible to detect in a number of cases. Therefore, conduction of electron microscopy investigations of spermatozoa for determining the ultrastructural characteristics of sex cells is an important stage in a comprehensive approach to a study of the reproduction process.

An electron microscopy study of sex cells allows evaluating their structural integrity [1, 2]. Under large electron microscope magnification 5000X–25000X, the structure of a spermatozoon and its organelles and their integrity can be examined in detail [3–8].

At present there are not enough data on the ultrastructure of stallion spermatozoa and on the effect of cryopreservation on their structure. In connection with this, the purpose of the present work was to study the ultrastructure of spermatozoa in native and frozen stallion sperm by the electron microscopy method.

METHOD

The experiments were conducted at the Physiology Department of the All-Russian Horse Breeding Research Institute (VNIIK), at horse breeding farms, and at the Belozerskii Physicochemical Biology Research Institute, Moscow State University in 2007–2009. Sperm of 17 stallions was investigated. During the investigations the conditions of feeding, housing, and utilizing the stallions corresponded to the zootechnical standards. Sperm was obtained, evaluated, and diluted according to the "Instructions on Artificial Insemination of Mares" (1969). A lactose–chelate–citrate–yolk medium in a 1 : 3 volume ratio was used for its dilution. Freezing was done according to VNIIK's technology in aluminum tubes of 18–20 ml each. The frozen sperm was stored in liquid nitrogen at –196°C.

For the electron microscopy investigations we used freshly obtained sperm and after freezing–thawing. Sperm was fixed with a 2.5% glutaraldehyde solution on a 0.1% cacodylate buffer (pH 7.2) and 1% osmic acid solution and embedded in an Epon–Araldite mixture. Ultrathin sections were obtained on a Reichert Ultra Cut III ultramicrotome, counterstained with lead citrate, and examined in a Hitachi 700 electron microscope. We studied spermatozoa at magnification 5000X (general examination) and 16000X–18000X (investigation of organelles). To determine anomalies of the axoneme, transverse thin sections of flagella were studied at magnification 20000X–25000X.

We counted spermatozoa with heads containing normal acrosomes (intact heads), acrosomes with electron-transparent content ("empty" acrosomes), and heads with degradation of the acrosome. We counted 100–150 cells in each specimen. The presence of anomalies of the axoneme of the flagellum (tail) of the spermatozoon was recorded.

[1] This work was supported by the Russian Foundation for Basic Research (project no. 09-04-13725 ofi-ts).

Table 1. Ultrastructural indices of stallion spermatozoa, %

Index	min	max	Average
Native sperm			
Intact heads	42.2	83.3	69.1 ± 3.0
Degradation of acrosome	1.2	27.3	7.9 ± 1.8
Acrosomes with electron-transparent content	0	22.6	9.9 ± 1.7
Abnormal shape of flagella	0	51.0	11.4 ± 3.1
Frozen–thawed sperm			
Intact heads	23.1	71.4	47.8 ± 3.4
Degradation of acrosome	3.3	43.1	16.5 ± 3.3
Acrosomes with electron-transparent content	9.6	63.5	32.4 ± 3.3
Abnormal shape of flagella	0	50.0	15.6 ± 2.7

Table 2. Effect of cryopreservation on acrosomes of spermatozoa, shape and structure of nucleus and chromatin, %

Index	Native sperm	Frozen–thawed sperm
Acrosome is present	92.0 ± 1.7	83.5 ± 3.2
Normal location of acrosome	96.7 ± 1.0	91.8 ± 2.3
Normal shape of acrosome	88.7 ± 2.3	81.8 ± 3.9
Normal shape of nucleus	94.1 ± 2.1	93.4 ± 1.9
Abnormal shape of nucleus	5.9 ± 2.0	6.3 ± 1.8
including		
spherical	0	0
elliptical	0	0
amorphous	5.9 ± 2.0	6.3 ± 1.8
Nuclear chromatin	99.9 ± 0.06	99.8 ± 0.20
matur vacuolated	1.07 ± 0.6	2.6 ± 0.7
destroyed chromatin	0.4 ± 0.2	1.1 ± 0.6

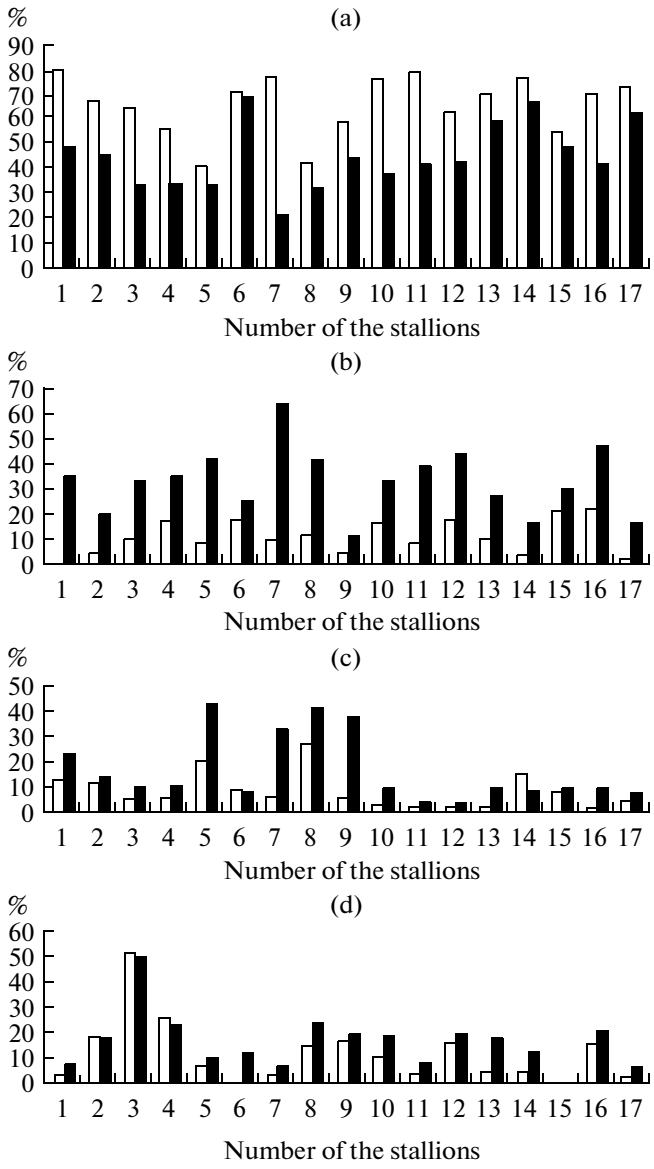
RESULTS AND DISCUSSION

In native and cryopreserved sperm of different stallions, we found large individual variations in the number of spermatozoa with intact heads and acrosomes with an electron-transparent content, degradation of acrosomes, and abnormal shapes of the flagella (Table 1). In the case of cryopreservation of sperm, the number of intact heads decreased on average by 21.3%, the number of acrosomes with degradation increased by 8.6%, with electron-transparent content by 22.5%, and spermatozoa with an abnormal shape of the flagellum by 4.2%.

The effect of low temperatures (–196°C) during cryopreservation of sperm led to changes in the structure of spermatozoa. The axoneme of the flagellum was damaged least of all and acrosomes considerably more. The total number of all pathologies of acrosomes of spermatozoa was 17.8% in native sperm and 48.9% in cryopreserved.

As was shown above, after cryopreservation the number of intact heads in the majority of stallions decreased. However, individual characteristics of cryoresistance of sperm were revealed. For example, then number of intact heads before and after freezing the sperm of stallion No. 6 (Fig. 1a) practically didn't change. The main problem of the sperm cryopreservation process with its subsequent thawing consists in the pronounced (by 2.3 times) increase in the number of acrosomes with an electron-transparent content. Thus, in stallion No. 1 (Fig. 1b) spermatozoa with empty acrosomes were not found in native sperm, but after freezing–thawing they amounted to 34.2%. The number of acrosomes with degradation on average doubled after cryopreservation. In this case, individual characteristics of the sperm of different stallions were clearly expressed (Fig. 1c). For example, stallion No. 9 had 4.9% acrosomes with degradation in native sperm and 38.0% after cryopreservation. The number of spermatozoa with an abnormal shape of the flagellum increased insignificantly after cryopreservation. Moreover, abnormal shapes of flagella either in native or in cryopreserved sperm were not noted in stallion No. 15 (Fig. 1d).

After cryopreservation the number of spermatozoa with no acrosomes increased 8.5% (Table 2). The number of spermatozoa with a normal location of the acrosome decreased by 4.9% (i.e., the number of spermatozoa with an acrosome located far from the nucleus increased as much) and with a normal shape of the acrosome by 6.9%. Damage and change of the shape and structure of the nucleus and chromatin were insignificant. The number of abnormal shapes of the nucleus increased by only 0.7%. Of the pathological shapes of the nucleus, only amorphous was found. Vacuolation of nuclear chromatin increased by 1.53%



Number of: (a) intact heads (with normal structure of chromatin and acrosome); (b) acrosomes with electron-transparent content; (c) acrosomes with degradation; (d) spermatozoa with abnormal shape of flagella; first column, native sperm; second column; thawed sperm.

and of spermatozoa with destroyed chromatin by 0.7%.

Thus, the acrosome is the most vulnerable during stallion sperm cryopreservation. The method of electron microscopy investigation of sperm is a valuable supplement to a comprehensive evaluation of the reproductive qualities of stallions, revealing disturbances in spermatozoa at the ultrastructural level.

REFERENCES

1. Bragina, E.E., Abdumalikov, R.A., Kurilo, L.F., and Shileiko, L.V., Electron Microscopy Study of Human Spermatozoa and Its Role in Diagnosing Male Sterility, *Probl. Reprod.*, 2000, no. 6, pp. 62–71.
2. Mashiach, R., Fish, B., Eltes, F., Tadir, Y., Ovadia, J., and Bartoov, B., The Relationship between Sperm Ultrastructural Features and Fertilizing Capacity in Vitro, *Fertil. Steril.*, 1992, vol. 57, pp. 1052–1057.
3. Green, G., Ballhorn, R., Poccia, D., and Hecht, N., Synthesis and Processing of Mammalian Protamines and Transition Proteins, *Mol. Reprod. Dev.*, 1994, vol. 37, pp. 255–263.
4. Yanagimachi, R., Mammalian Fertilization. In: Knobil, E and Neil, J., *The Physiology of Reproduction*, 2nd ed., New York: Raven Press, 1994, pp. 189–317.
5. Kurilo, L.F., Makaraova, P.P., Shileiko, L.V., System for Evaluating the State of Human and Mammalian

Spermatogenesis, *Androl. Genital. Khirug.*, 2005, no. 4, pp. 8–17.

6. Bielanski, W. and Kaczmarski, F., Morphology of Spermatozoa in Semen from Stallions of Normal Fertility, *J. Reprod. Fert.*, 1979, (Suppl. 27), pp. 39–45.
7. Fawcett, D.W., Ultrastructure and Function of the Sertoli Cell, *Handbook of Physiology: Section 7. Endocrinology, vol.5, Male Reproductive System*, Baltimore: 1975, pp. 21–55.
8. Zamboni, I., The Ultrastructural Pathology of the spermatozoon as a Cause of Infertility: The Role of Electron Microscopy in the Evaluation of Semen Quality, *Fertil. Steril.*, 1987, vol. 48, pp. 711–734.