The Scientific Development in Horse Reproduction in Russia

The All – Russian Research Institute for Horse Breeding , Rybnoe , Ryazan region, Russia

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Cryopreservation of stallion's sperm and Artificial insemination

In the 1900-th in Russia artificial insemination (AI) was worked out, thoroughly improved and widely introduced in farm animal breeding practice. In 1954 the first in the world foal after AI with frozen semen was born in the USSR. In the 1970-th the cryobank of stallion semen was organized in the Institute for Horse Breeding. At present there are about 3000 doses of semen of outstanding stallions of 15 stud horse breeds at the Sperm Bank (Fig. 1, 2).

Fig. 1. The cryobank of stallion semen in the Institute for Horse Breeding (the beginning of 1970-th).

Fig. 2. Tubes for stallion sperm doses by Russian technology of stallions sperm freezing.

Fig. 5. The stallion **Khalif** , Akhal-Teke breed, was born in 1969 (**Fakirpel-Khanum**).

Fig 3.The stallion **Samotsvet 10**, Tersk breed, was born in 1959 (**Simvol-Tsema**).

Fig. 4. The stallion **Sobol**, obtained by artificial insemination with Samotsvet's sperm stored for 35 years in liquid nitrogen.

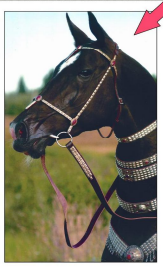


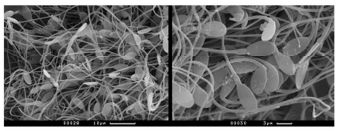
Fig. 6. The stallion **Tokhtamysh**, (**Khalif-Tiazegul**),1999, the world champion of Akhal-Teke breed in 2005.

In 2009 foals by the stallion Samotsvet of Tersk breed were received after artificial insemination with frozen sperm stored **for 35 years** (pregnancy rate per cycle - 55% (5/9)). It is very important for restoration of Tersk horse breed (Fig.3, 4).

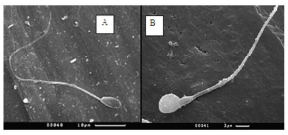
20 years after the freezing of semen and 11 years after the death of the Akhal-Teke stallion Khalif (Fakirpelvan - Khanum) the colt Tokhtamysh was born that became the best among the descendants of the stallion, including those obtained in the result of natural mating during the life of the stallion (Fig. 5,6).

Effect of freezing on the ultrastructure of stallion spermatozoa

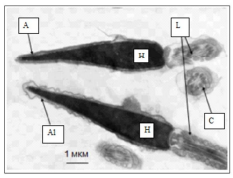
Scanning electron microscopy and transmission electron microscopy of stallion's sperm.



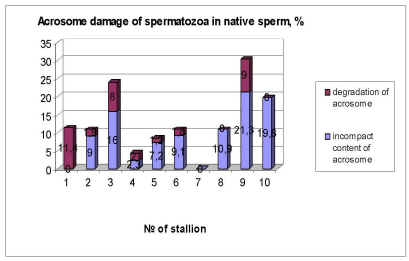
Stallion's spermatozoa



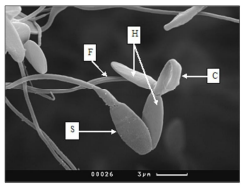
Normal stallion's spermatozoon (A), abnormal spermatozoon with rounded head



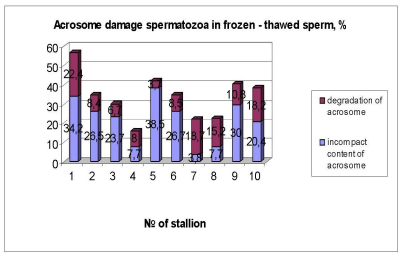
A -The head of the spermatozoon with intact acrosome,
A1 -The head of the spermatozoon with "empty" acrosome with electron-transparent content
H – chromatin of nucleus of the head,
L – a longitudinal section through a flagellum of a spermatozoon,
C –cross-section through a flagellum of a spermatozoon



Among stallions with sperm of **high quality and cryoresistance** large individual differences in cryoconservation influence on ultra structural organelle integrity are represented.



Spermatozoon of normal morphology (S); Spermatozoon with two heads and two flagellums: H – two heads of the two headed spermatozoon, F – double flagellum, C– curved section of the middle part of the flagellum.



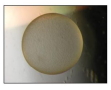
Each stallion dominates one or another pathology of acrosomal region with approximately equal quantity of sperm cells with intact heads.

Horse embryo transplantation

From 1974 the embryo transfer technology started to develop in the Institute for Horse Breeding and in 1982 the first foal after embryo transfer was born in the USSR (Fig.1). The author of Russian transfer method, Dr. S.Lebedev, obtained about 40 foals by transfer of fresh horse embryos between different breeds. After him his students continued this work in three main directions: culture, cooling and freezing of horse embryos.

Fig.1. Dr. S.Lebedev with his 1-st foal, obtained by embryo transfer.

Fig. 2. The catheter for recovery of 6-12 day horse embryos.



Embryo cooling

Immersion of the 8 day embryo to the holding media.

Storage of the embryo at (+5)°C during 24 hours.

Transfer to recipient on the 7-th day after ovulation.

Embryo culture

Special culture media (egg yolk, PBS Dulbecco and/or mare milk) was worked out in the Institute for Horse Breeding (**Certificate of Authorship N1497215, 1.04.1989**) for 8 day horse embryos.

The foil obtained in 1989 by transfer of embryo cultured in author's medium during 24 hours at 37°C .

Embryo freezing

The first "frozen " foals in Russia (2012).

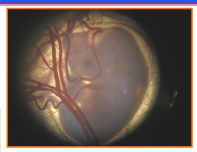
Conclusions:

1. Firstly developed in Russia the technology of artificial insemination and cryopreservation of sperm in horses is widely used today for improvement, preservation and restoration of horse breeds in Russian Federation.
2. At present, Russia successfully develops technologies of transplantation of fresh, cooled and frozen embryos in horses.
3. The All-Russian Institute for Horse Breeding carries out research work in the field of the horse physiology reproduction, embryonic development and stallions sperm cryobiology.

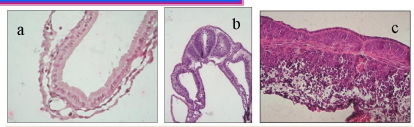
Horse embryo development

Morphological, histological, ultrasound characteristics.

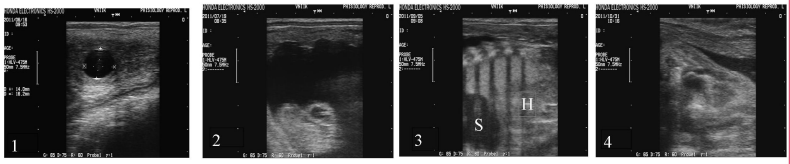
The catheter for non-surgery recovery of large (day 12-36) horse embryos.



Day 34 horse embryo



Histological samples of different parts of horse embryos: a) day 16, the wall of egg yolk sack; b) day 18, the neural tube and somites; c) day 22, renal tubules.



Ultrasound monitoring of horse embryo development:
1) day 14, 2) day 48, 3) day 163 (S - stomach, H - heart) 4) day 220 (head)