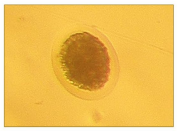
Horse Embryo Freezing in Russia: 2 Aspects of Technology

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

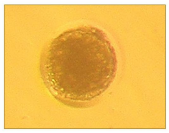
Lebedeva Lyudmila, Russia, e-mail: "Lebedeva-L18@yandex.ru"

Introduction. Embryo cryopreservation is a very important and perspective aspect of horse biotechnology work. There are two basic approaches in freezing technology: conventional slow cooling and vitrification. Different cryoprotective media for horse embryos are developed.

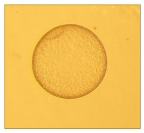
The aim of the work was to study the effect of two methods of freezing (conventional slow cooling and vitrification) and three cryoprotective media (Vit-Kit-set, medium I and medium II) on viability of 6.5-8 day horse embryos after thawing.



6.5 day horse embryo



7 day horse embryo



8 day horse embryo

programmable freezer "ЭМБИ-К" (Russia)

EquiPro® Vit-Kit™, ("Minitube", Germany)

Materials and Methods. 13 mares of the Russian Draft breed (9-13 years old) were used as embryo donors and recipients. Day 6.5-8 embryos of excellent and good quality were freezed in 0.25 plastic straws by one of two methods (Fig 1). A total of 27 embryos were used in the experiments.

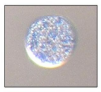
Experiment 1 (n=5). Embryo freezing by conventional slow cooling method in medium I.

Experiment 2 (n=9) Embryo freezing by vitrification in medium I.

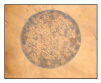
Experiment 3 (n=13). Freezing by vitrification in The Vit-Kit media (n=9) and in medium II (n=4).

Embryos (Exp.1 and Exp.2) were divided according to their diameter in 2 groups (Gr. 1 <350 μm and Gr. 2 >350 μm). Only small embryos (<300 μm, n=13) were used in Exp.3. Embryo quality after thawing was evaluated by the percent of dead cells in the embryos stained with Evans Blue (0,05% in PBS Dulbecco) (Exp.1, Exp.2), or by embryo transfer to recipient mares (Exp.3). The Vit-Kit media (ethylene glycol, glycerol, galactose) were commercially available (EquiPro® Vit-Kit™, "Minitube", Germany), but the medium I (ethylene glycol, DMSO, sucrose) and the medium II (ethylene glycol, glycerol, sucrose) were hand made.

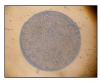
Results (Exp.1, Exp. 2). There is no significant difference in general damage effect on embryo cells by use of both (Exp.1 and Exp.2) methods of cryopreservation ($p > 0.1$) when using of medium I (37.4% (n=5) and 43.3% (n=6), respectively) (Table 1). The increase of dead cells rate from small (<350 μm) to large (>350 μm) embryos (Fig. 2) was noticed in both freezing methods (Exp.1 (n=5): 31.7% ±15.2% and 46.0%±9.0%;
Exp.2 (n=9): 45.0%±13.1% and 73.6%±0.18%, respectively) (Table 2).



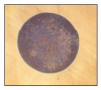
175 μm, 6.5 day



250 μm, day 7



480 μm, day 7



600 μm, day 8

Fig. 2. Dead cells (blue) in the embryos of different diameter after thawing and staining with Evans Blue

Table 1. Effect of two methods of freezing in media I on viability of 6.5-8 day horse embryos after thawing

Freezing method	n	% of Dead Cells
Slow-cooling	**5***	**37.4%**
Vitrification	**6**	**43.3%**

*3 of 5 embryos had fractured membrane

Table 2. Effect of cryopreservation in media I on horse embryos of different size

Freezing method	n	Diameter (μm)	% Dead Cells
Slow-cooling	**3**	**<350**	**31.7% ±15.2%**
	2	**>350**	**46.0%±9.0%**
Vitrification	**4**	**<350**	**45.0%±13.1**
	5	**>350**	**73.6%±0.18**

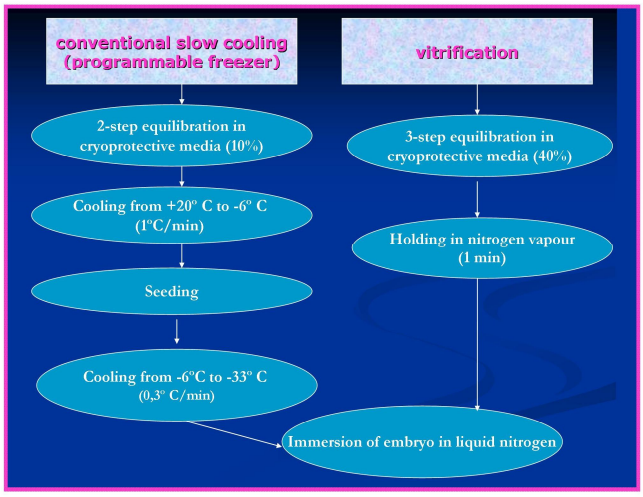


Fig. 1. The scheme of 2 freezing methods of horse embryos

Results (Exp.3). The vitrification procedure for both groups of embryos is shown at Fig. 3. The pregnancy rate after embryo transfer for "Vit-Kit group" and for "medium II group" was 11.1% (1/9) and 50% (2/4), respectively (Table 3). In 2011 the 1-st foal (Vit-Kit group) was born and died at the birth in the result of incident. The first two alive "frozen" foals (media II group) were born in Russia in 2012 (Fig. 4).

Table 3. Survival of 6.5-8 day horse embryos (<300 μm) after vitrification in two cryoprotective media

Media group	n	Pregnancy rate after embryo transfer, %
Vit-Kit group	**9**	**11.1 (1/9)**
media II group	**4**	**50.0 (2/4)**

Embryo recovery procedure

In conclusion:

1. Conventional slow cooling and vitrification methods of cryopreservation with using of medium I cause approximately the equal damage effect on equine embryos.
2. The rate of dead cells in the embryos after freezing-thawing procedure is rising together with the increasing of the embryo diameter.
3. The media II (ethylene glycol, glycerol, sucrose) can be used for horse embryo vitrification.

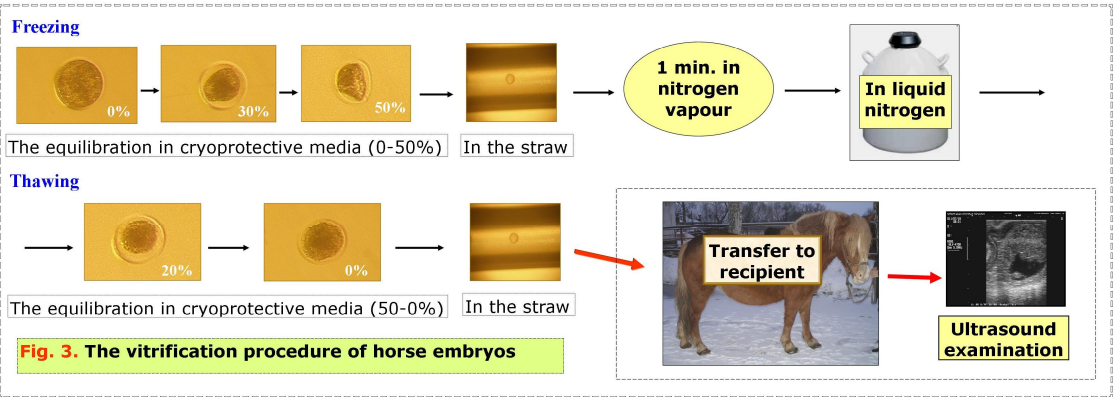


Fig. 3. The vitrification procedure of horse embryos

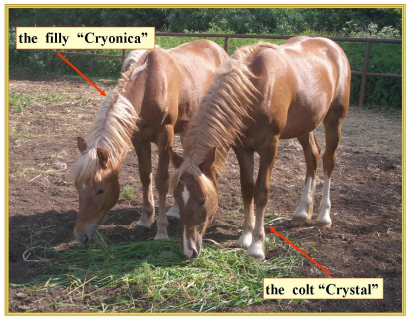


Fig. 4. The first "frozen" foals (media II group) were born in Russia in 2012