

Current state of horse embryo transfer technology in Russia and factors influencing its effectiveness

Valery Kalashnikov, Elena Solodova*, and Lyudmila Lebedeva

The All-Russian Research Institute for Horse Breeding, 391105 Divovo, Russia

Abstract. The article presents data on activity in the field of embryo transfer in the Russian horse breeding system and analyzes of the factors influencing the success of the use of this technology. The results of Russian reproductive specialists work over the past 15 years have been collected (144 procedures of embryo recovery in 52 mares). The influence of age and degree of synchronization of ovulations between donors and recipients on the level of embryo recovery and survival rates was analyzed. It has been established that the highest embryo recovery rate is observed in mares of 2-10 years old (yo) - 84.6%. In mares of 11-16 yo, this figure decreased to 64.3% ($p < 0.05$), and for 17-24 yo mares - to 42.8% ($p < 0.001$). The survival rate of embryos in the uterus of recipient mares of 3-14 yo remains at 70-71%, in mares of 15-20 yo it sharply decreased to 42.8%. It has been shown that embryo transfer to a recipient whose ovulation occurred 1 day earlier or within a period of up to 5 days after the donor's ovulation provides a survival rate of 79% until the 35th day of pregnancy. When the recipient ovulation occurs 2-4 days earlier than the donor ovulation, the result of embryo transfer was dramatically reduced ($7,7 \pm 7,4\%$, $p < 0.001$).

1 Introduction

Embryo transplantation is one of the advanced methods of breeding the most valuable farm animals. Currently, abroad, thanks to the introduction of a non-surgical method of embryo recovery, a developed industry of consumables and media for embryo transfer, the presence of a large number of qualified specialists and, most importantly, high demand from horse owners to obtain foals from outstanding sports mares by embryo transfer technology, this method has become routine in the practice of horse reproduction. The leaders in the industrial application of this method are Italy, France, Brazil, Portugal, Argentina, and the USA. The number of embryos transplanted in these and other countries of North and South America, Europe, and Australia amounts to hundreds and thousands. Despite the fact that in Russia this method was developed by the Soviet scientist S.G. Lebedev at the end of the last century, it was not widely used in horse breeding farms in our country, due to the difficult

* Corresponding author: l.solodowa2012@yandex.ru

state of the horse industry and the lack of demand from horse owners after «perestroika». The process of introducing the method into horse breeding practice was significantly complicated by prohibitions and restrictions by horse breeding Associations on the registration of foals obtained with the embryo transfer method. The collected data suggests that total in Russia from 2009 to 2023 years, the production of transplant foals amounted only 1 to 10 animals per year. During this period, the Moscow Polo Club (Moscow region), Zavivalovsky stud farm (Penza region), Vorontsovsky stud farm (Yaroslavl region), LLC "Lokotskoy" stud farm (Bryansk region) made attempts to introduce this technology in horse reproduction practice. At the All-Russian Research Institute for Horse Breeding (Ryazan region), researches on cooling and freezing of horse embryos were continued and the first foals in Russia were obtained from the transplantation of cryopreserved embryos (L.F. Lebedeva, 2012), but this work also have not been introduce in horse breeding practice. During last 3 years, foals were obtained by fresh embryo transfer method in the Horse farm of the Kurganinsky Agrotechnological College (Krasnodar region, A.V. Nizhebovskaya), in the Horse reproduction center "Hartley Horse House" (Moscow region, D.I. Lazarev), Horse Farm «Leonov R.A.» (Moscow region, Solodova E.V.), Horse Sport Club "Vzlyot" (Tver region, Shmeleva I.V.), However, the embryo transfer services in such horse farms depends on highly qualified reproductive specialists, and as a rule, ends with their departure to another place of work. Currently the difficulties occur with the import of valuable breeding material from abroad, so we can predict an increase in demand for embryo transplantation services in Russia. Therefore, an analysis of the accumulated experience of domestic reproductive specialists will be relevant and interesting.

The purpose of our researches was to summarize the results of the work of domestic embryo transfer specialists at different stages of technology and to analyze the main factors influencing its result.

2 Material and methods

The results of embryo transfer practice usage at different stages of technology according the age of mares were studied. The following factors were analyzed: 1) The level of embryo recovery in groups of donor mares of different ages: I. 2-10 yo, II. 11-16 yo, III. 17-24 yo; 2) the rate of embryo survival in groups of recipient mares of different ages: I. 3-9 yo, II. 10-14 yo, III. 15-20 yo; 3) the rate of embryo survival depending on the degree of synchronization of the reproductive cycles of donors and recipients (when the recipient ovulates 1 day earlier than the donor, on the same day or within five days after the donor's ovulation (group I); when the recipient ovulates 2-4 days earlier than the donor (group II). The analysis was carried out using data from the documents of foaling and mating of mares in Vorontsovsky (2009), Lokotskoy stud farm (2013-2016), private horse farm of Leonov R.A. (2021-2023 years), the horse farm of the Kurganinsky Agrotechnological College (2020-2022), the Moscow Polo Club (2020-2022).

The initial indicators were processed by methods of mathematical statistics (Microsoft Excel 2010) using the Student's test to assess the reliability of the data.

3 Results and discussion

The first component of the success of the embryo transfer process is the level of embryo recovery from the uterus of the donor mares. The obtained data showed (Table 1) that the highest recovery rate is observed in young mares of 2 to 10 yo – 84.6%.

Table 1. Recovery rate of day 6.5-8.0 embryos from mares of different age groups.

Groups of mares	Age of mares, yo	n	Number of flushings of uterus, n	Embryos recovered	
				n	%
I	2-10 years	22	39	33	84,6±5,8 ¹
II	11-16 years	19	56	36	64,3±6,4 ²
III	17-24 years	11	49	21	42,8±7,1 ³
Total		52	144	90	62,5±4,0

p^{1,2; 2,3} < 0,05; p^{1,3} < 0,001

The decrease of the pregnancy rate in aged mares [1] is the reason for the very low embryo recovery rate in older mares (17-24 years) (42.8%, p>0.001). Table 1 demonstrates that the embryo recovery rate falls gradually between mares of groups I, II and III, decreasing by 20-21% between groups (p < 0.05, p < 0.001). This suggests that embryo transfer for high-value broodmares should be planned in young to mid-reproductive years when embryo recovery rates are predicted to be high or acceptable.

The procedure for uterine lavage in older mares is often accompanied by some difficulties. The uterus of multiparous mares is usually located very deep, so removing of all fluid volume during lavage is complicated [2]. However, for the high breeding value mares with inability to bear a foal due to health reasons, there is still a point in usage them as embryo donors.

The results of our analysis are consistent with the opinion of foreign researchers, that in mares younger than 10 yo, the recovery rate is much higher than in aged mares over 15 years [3]. In addition to the fact of decrease in the rate of embryo recovery in mares with progressive age, it was found that this rate is reduced when day 6 embryos are recovered (42%), compared to 7, 8, 9 day embryos (59-61%) [4]. There is no consensus on reducing the rate of embryo recovery with repeated uterine lavage procedures for this purpose. As the experience of some foreign reproductive specialists, as well as the experience of domestic specialists, shows, that multiple repeated attempts of uterus flushing for embryo recovery can lead to acute bacterial endometritis and chronic inflammation of the endometrium and, over time, a decrease in embryo recovery rates in a particular mare [5, 7].

Along with successful embryo recovery and transplantation, the main component of successful conception and pregnancy is the state of the reproductive system of the recipient and the degree of synchronization of its ovulation with the donor mare. Of course, the body and organs of the reproductive system of mares that regularly foaled gradually wear out. It has been proven that nulliparous mares with age progress experience the same degenerative changes in the endometrium as those who have foaled. In nulliparous mares over 10 years of age, there is a difficulty of uterine clearance due to a poorly dilating cervix [1,2].

An analysis of the age groups of recipients (Table 2) selected for embryo transfer by domestic reproductive specialists showed that the majority of recipients (74%) are selected from the group of 3-9 yo mares. The embryo survival rate in mares of this group is 70.6%.

Table 2. Results of embryo transfers depending on the age of recipient mares.

Groups of recipients	Age of mares, yo	Number of transfers, n	Pregnancy rate, n (%)	Embryo death, n (%)	Abortions, n (%)
I	3-9	51	36 (70,6±6,4)	-	6 (16,6±6,2)
II	10-14	11	8 (71,7±13,6)	1 (12,5±11,7)	2 (25,0±15,3)
III	15-20	7	3 (42,8±18,7)	-	-
Total		69	47 (68,1±5,6)	1 (2,1±2,0)	8 (17,0±5,5)

However, as can be seen from Table 2, the survival rate remains at the same level in mares of 10-14 yo - (71.7%). The smaller number of mares in this group suggests that at this age there are fewer mares capable of bearing a foal without problems and they undergo careful selection for reproductive qualities. The use of mares of 15-20 yo occurs only in extreme cases in the absence of suspicion of any gynecological diseases and with a good reproductive history. However, their survival rate, judging by the results of the work of domestic specialists, is still reduced.

The third factor responsible for the success of embryo transfer is the degree of synchronization of the reproductive cycles (ovulations) of the donor and recipient. As a rule, it is assumed that ovulation in the recipient can occur one day earlier or three days later than in the donor [7]. However, in practice, it is not always possible to synchronize mares in this way and it is necessary to transfer embryos to recipients who ovulated earlier than 1 day or later than 3 days. Therefore, we analyzed the level of embryo survival depending on the degree of synchronization of the reproductive cycles of the donor and recipient (Table 3).

Table 3. Pregnancy rate depending on degree of estrus cycles synchronization between donor and recipient mares.

Group of mares	Synchronization rate, day*	Number of transfers, n	Pregnancy rate, n (%)	Embryo death before day 35, n (%)
I including	(+1)-(-5)	54	46 (85,2±4,8) ¹	1 (2,1±2,1)
	-(4-5)	10	8 (80,0±12,6)	0 (0,0)
II	+(2-4)	13	1(7,7±7,4) ²	0 (0,0)

$p^{1,2}<0,001$; *ovulation of the recipient compared to the donor

The analysis showed that with the recommended synchronization scheme (Group I), including ovulation of the recipient 4-5 days later than the donor, the embryo survival rate is high (>85%). If ovulation in the recipient occurs 2-4 days earlier than in the donor (group II), the survival rate is significantly lower - 7.7% ($p<0.001$). According to foreign authors, acceptable pregnancy rates (61-71%) are obtained when synchronizing from +1 day to -5 days is provided. When the recipient ovulates 2 days earlier or 6 days later than the donor, the survival rate is significantly reduced (0-30%) [7, 8].

Due to the dependence of embryo survival on the degree of synchronization of the reproductive cycles between the donor and recipient, it is advisable to prepare 2-3 recipients for one donor in one cycle. This condition is not difficult to fulfill at a stud farm with the head of 50-60 mares, selecting less valuable recipients for valuable donors. In this case only hormonal treatment of recipients in estrus is needed for stimulation of ovulation on the day 0 or even 2-3 days after the donor's ovulation.

On farms with a small number of mares, it is often necessary to use hormonal treatment for donor mares. When synchronizing donors and recipients in more than three cycles in a row, the best option is to use GnRH analogues instead of hCG, since these drugs do not induce the formation of antibodies [9]. If it is necessary to interrupt the diestrus of the donor or recipient, it is impotent to take into account the size of the follicles at the moment of

injection of the PGF2a, since the presence of large follicles can cause premature ovulation or ovulation without pronounced signs of estrus [10]. It is important, when synchronizing of the ovulation between the donor and recipient not to allow a reduction in the duration of estrus, both in the donor and in the recipient. In a donor, if the duration of estrus is reduced to 3 days, the pregnancy rate may decrease. [11,12]. And in the recipient, a decrease in the duration of estrus will reduce the number of days with severe uterine edema. Estrogens are responsible for the presence of edema, as a result of which receptors for progesterone are formed in the endometrium [13, 14]. A decrease in their number will reduce the level of embryo survival after transplantation. The absence in small farms of a sufficient number of recipient mares that can be synchronized with donors, or the recovery of embryos outside the breeding season, dictates the need to introduce the embryo freezing method into practice. And although this technology is widely used abroad, it has not yet received proper distribution in Russia.

Undoubtedly, an important factor in the effectiveness of embryo transplantation in horses is the experience of a performer. This experience has been accumulated over the years, so the exchange of experience and knowledge between specialists is necessary to increase the effectiveness and further implementation of embryo transfer in Russian horse breeding practice.

4 Conclusion

An important factor influencing the effectiveness of horse embryo transplantation is the age of the mares. With progressing of mare age the level of embryo recovery and embryo survival rates are decreased. The extension of the synchronization window to day -5 (recipient ovulation occurs 5 days later than donor ovulation) does not reduce embryo survival. On the contrary, the embryo transfer in the interval of +2 +4 days (recipient ovulation occurs 2-4 days earlier than donor ovulation) provides significantly poor result of the procedure.

The research was carried out with the support of the Russian Science Foundation (project No. 23-16-00226).

References

1. E. V. Solodova, J.Horse breed. and equest. Sport **1**, 28 (2020)
2. E. V. Solodova, Pross., VNIIC, Divovo (2018)
3. J. Curvo-Arango, A. Claes, M. Ruijter-Villani, T. Stout, J. Eq. Vet. **50**, 386 (2018)
4. J. Jacoba, K. Haagb, G.Santosa, J. Oliveiraa, M. Gastalb, E.Gastalb, J. Theriog. **77**, 1159 (2012)
5. M. Campbell, J.Equine vet. Educ. **26(6)**, 322 (2014)
6. S. Brinsko, T.Blanchard, D.Varner, J. Schumacher, C.Love, K. Hinrichs, D.Hartman, *Manual of Equine Reproduction*, 276 (2011)
7. J. Cuervo-Arango, A. Claes, T. Stout J. Reprod. Fert. and Develop. **31(12)**, 1904 (2019)
8. S. Wilsher, A. Clutton-Brock, W. Allen, J. Reproduction **139**, 575 (2010)
9. V. Kalashnikov, L. Lebedeva E. Solodova, E. Krasilnikova. J. Horse breed. and equest. sport **3**, 7 (2023)
10. E. Solodova, L. Lebedeva, J. Horse breed. and equest. sport **4**, 9 (2023)
11. E. Solodowa, A. Kalinova, Siberian Journal of Life Sciences and Agriculture **14(2)**, 326 (2022)

12. E. Solodova, J. Horse breed. and equest. sport **1**, 20 (2019)
13. J. Cuervo-Arango A. Claes, M. Ruijter-Villani et al., Equine Vet. **50(3)**, 386 (2018)
14. S. Mateu-Sánchez, J. Newcombe, C. Garcés-Narro et al., Theriolog. **86(6)**,1506 (2016)