GAVIN PUBLISHERS

Archives on Veterinary Science and Technology

Derbala MK and Abdou MSS. Arch Vet Sci Technol: AVST-135.

DOI:10.29011/AVST-135/100035

Research Article

Transfer of Large Equine Embryos in Arabian Mares

Mohamed K Derbala[1*], Mohamed Samy Sayed Abdou[2]

[1]Animal Reproduction Research Institute-ARC-Egypt

[2]Theriogenology Department, Faculty of Veterinary Medicine, Cairo University, Egypt

[*]**Corresponding author:** Mohamed K Derbala, Animal Reproduction Research Institute-ARC-Egypt. Tel: +20 1003337013; Email: mohamed_equine@yahoo.com

Citation: Derbala MK, Abdou MSS (2017) Transfer of Large Equine Embryos in Arabian Mares. Arch Vet Sci Technol: AVST-135. DOI:10.29011/AVST-135/100035

Received Date: 21 September, 2017; **Accepted Date:** 29 September, 2017; **Published Date:** 9 October, 2017

Abstract

In the vast majority of equine embryo transfer programs, flushing takes place on days 6, 7 or 8 post ovulation. In the present study, embryos could, instead, be obtained on days 10-11 after ovulation. For this purpose, 36 Arabian mares (7-24 years old) were used as donors for embryos and 6 mares were kept as control. Of the 36 donor animals, 2 mares died suddenly and flushing was carried out after excision of the uterus. Recipient mares (N=70) aged 5-10 years, and were kept in embryo transfer facility. The degree of synchronization was -4 to -6 days. The procedure used depended on flushing of the donor mares after detection of embryonic sac using ultrasonography. Large pore AI catheters and external sheath of double guarded uterine swabs were used in the process of embryo transfer. A controllable manual pipette was used in the control process of loading, washing and transfer. This method overcame the problem of burst of large embryos. A high recovery (94.4%) and pregnancy (73.5%) rates could be obtained.

Results have also shown that higher pregnancy rate was obtained with recipient mares on day 4 post ovulation, whereas lower pregnancy rates was found in recipient mares on day 6 post ovulation. In conclusion, this study demonstrated that there was a possibility of embryo transfer on day 10-11 post ovulation i.e. after embryo detection with ultrasound scanning. This method permits flushing of mare's uterus after death on 10-11 days of pregnancy for maximum exploitation of the donor mare. Furthermore, concerning mares with a history of low embryo recovery flushing did not take place until the embryo was detected with ultrasound so as to save flushing media and number of flushes.

Keywords: Embryo transfer; Equine; Large embryos; Mare; Synchrony

Introduction

The use of embryo transfers and development of embryo technology for the horse have increased steadily over the past two decades [1]. Embryo Transfer (ET) is now generally accepted as a valuable tool for increasing the number of progeny from genetically valuable mares, for producing foals from competing mares without interrupting their sporting careers, and for obtaining foals from mares incapable of carrying a pregnancy to term [2]. Furthermore, ET can be performed on yearling or 2 years old mares as a method to get them into production 1 or 2 years earlier than traditional method [3]. Embryos are usually collected on day 6, 7 or 8 (day 0 is day of ovulation) from naturally single or occasionally multiple ovulations. Embryos are collected 1 day later if the mare has been bred to frozen semen rather than fresh or cooled semen [4]. Recovery rates for days 7, 8 and 9 post ovulation are similar, but recovery of embryos on day 6 slightly lower which attributed to failure to identify the embryo in recovery medium, loss of embryo during recovery procedure due to its small size, failure to obtain the embryo in the uterine flush because of its greater specific gravity or failure of some embryos to enter the uterus by day 6 [5].

The major factor affecting embryo recovery is the mare's reproductive history. Older mares with poor reproductive histories produce fewer embryos. Causes of reduced embryo recovery from these older mares include uterine and oviductal pathology and increases early embryonic death [6]. The majority of embryo transfer has been performed 6 to 8 days' post ovulation. Limited studies

are available addressing the effect of embryo age on pregnancy rate [5,7-9]. But in recent years there has been a tendency by some veterinarians to flush mares on day 8 or 9 so that the embryo may be visualized in the filter, enabling the flush to be stopped after just one flush rather than three that are recommended. This practice can easily leave a twin embryo in the uterus [10]. However, the team in Goulburn Valley Equine Hospital (GVEH) produced a foal that transferred at 10 days old. Once they identify the mare pregnant using ultrasonography, they then harvest and transfer the embryo. The catheter used to flush the mare is necessary large [4,11] reported no pregnancies after transfer of day 9 and 10 embryos, whereas pregnancy rates of 61%, 55%, and 25% were observed after of days 5, 7, and 8 embryos, respectively.

Conflicting results were found by Fleury et al (1989) [12] who achieved 69% pregnancy rate after transfer of 16 embryos collected 9 days' post ovulation. It is noteworthy that these two studies were performed while the procedure of equine embryo transfer was still under development and had not been in practice for a long time, which could have contributed to the divergent results. In recent study that carried out by Jacob et al (2012) [13], they demonstrated that embryo recovery rates between Days 7 and 10 were similar and acceptable (e.g., 63%), the degree of synchrony between donor and recipient mares does not need to be as restricted as previously reported in horses. Acceptable pregnancy rates (e.g., 70%) were obtained even when recipient mares ovulated 4 to 5 days after the donors. Similar pregnancy rates were obtained when recipient mares received embryos within a large range of days' post ovulation (Days 3 to 8), and Day 7 embryos produced higher pregnancy rates when compared with Days 8 and 9 embryos. There are few studies about transfer of 10 days' embryo like that carried out by Wilsher et al (2010) [14] who mentioned that transfer of day 10 embryos to asynchronous recipient mares could benefit the management of commercial equine transfer programs and may provide a useful model for investigation of maternal influences on early embryogenesis.

Although they mentioned that the problematic aspect of the transfer of large day 10 embryos is the use of transfer pipettes with an internal diameter ≥ 5 mm; the capillary forces required to hold the embryo in its transfer medium in set columns within the pipette do not come into play in these larger bore pipettes. Furthermore [14], couldn't recover embryos > 5 mm despite repeated times of flushing. Nearly all embryos are transferred nonsurgically into synchronized recipients. For nonsurgical transfer, embryos are loaded into 0.25- 0.5 ml straws and inserted into the body of the uterus using an embryo transfer gun. Alternatively, the embryo is loaded into a flexible insemination rod, which is covered with a protective sheath and placed through cervix into the body of the uterus [1].

Materials and methods

Embryo Donor Mares

This study was carried out during (September, 2015- October 2016). The donor mares were sub fertile mares with a history of low embryo recovery rates. The donor mares (N= 36) were straight Egyptian mares weighing between 350-400 kg and between 7-24 years old. In addition to 6 mares were kept as a control. The mares were fed green fodders mixed with hay, and were given barely, access to mineral salt and fresh water was provided ad libitum.

Ultra Sonographic Scan

Transrectal ultrasonography was applied for donor mares for determination of proper time of insemination as well as for detection of ovulation time (using 7.5 MHz linear endorectal probe, MYLAB 30, Italy and Sonoscape; China)

Insemination and Breeding Management

Natural covering has been done for some mares and others have been freshly inseminated from the stallions kept in the same ET facility. These stallions were examined periodically for assessment of semen quality. All mares were treated for infertility problems (using of ecbolics, uterine lavage, intrauterine antibiotics and corticosteroids) for increasing embryo recovery rates.

Embryo Recipient Mares

Recipient mares (N= 70) were kept in the ET facility as a recipient population for our facility and for shipped embryos. They were between 5- 10 years old and weighing between 350-450 kg. All mares were fed green fodders, hay and concentrate. Estrous cycles were monitored using transrectal palpation and ultrasonography to synchronize ovulation between the donor and recipient mares. The degree of synchronization was-4 to-6 days (recipient ovulated 4-6 days after donor). The degree of synchrony was achieved using Cloprostenol (Estrumate; MSD, Animal Health) as a luteolytic agent, and 1500 IU of hCG (Epifasi; EPICO, Egypt) as an ovulatory inducing agent.

Collection and Evaluation of Embryos

Beginning from day 9 post ovulation, the uterus of donor mare was scanned daily using 7.5 MHz trans rectal ultrasound probe (MYLAB 30, Italy and Sonoscape A5v, China). The uterine flushes were performed with detection of embryonic sac by ultrasound scan (Figure 1-A, 1-B). After washing the perineal area with mild soap and povidone iodine solution, a 36 Foley catheter (Bioniche Animal Health, Belleville, ON, Canada), was inserted into the vagina and through cervix. Passage of the catheter through the cervix was performed manually by guiding it through using the operators finger. Once into the uterine body, a cuff on the end of the catheter was inflated with 30 ml of air then pulled caudally to ensure a tight seal against the internal so of the cervix. Lactated Ringer's solution (1 L) was infused through the catheter and into the uterus then drained into a 65 µm EmSafe filter for embryo recovery with integrated petri dish with grid (Minitüb, Germany).

The process of flush continued until the embryo recovered in the filter and was seen by naked eyes. Gross evaluation of embryonic shape was carried out as well as microscopic evaluation of embryo capsule and trophoblast cells.

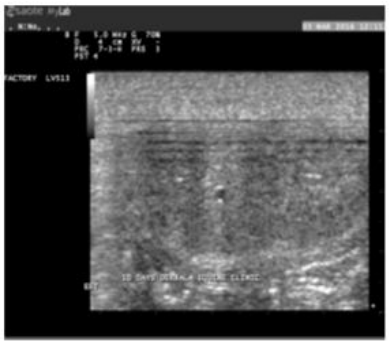


Figure 1-A: Showing detection of embryo with ultrasound scanning in a donor mare for ET on day 10 post ovulation.

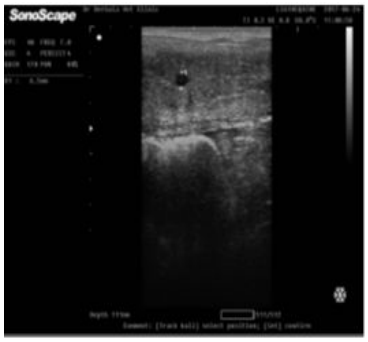


Figure 1-B: Detection of an embryo by ultrasound on day 11 post ovulation.

Transfer of Embryos

After detection of the embryo in the EmSafe filter, it has been picked up and washed in a holding media. The embryo was loaded in a catheter using a special kind of pipette filler (A manual propipetter adjusted by turning the wheel with the thumb) to control a suction and release to avoid rupture of the embryo (Figure 2-A). A.I catheters, modified A.I, ET catheters and external protecting sheath of double guarded uterine swab have been used respectively for embryo transfer according to embryo size (Figure 2-B). Contamination was minimized by enclosing the AI catheter in a sterile sheath. Passage of the pipette through the cervix was mainly performed only transvaginally. The embryo was deposited into the uterus and the catheter and sheath were withdrawn from the mare.

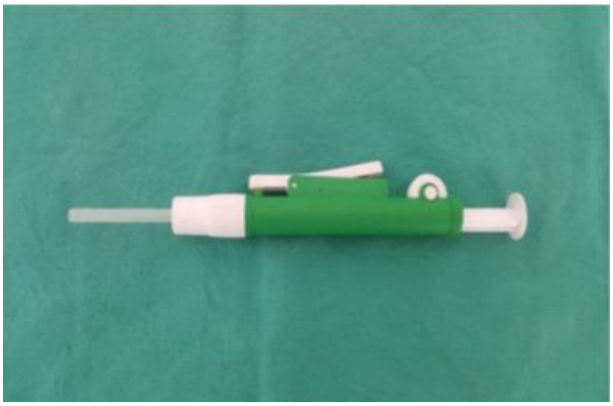


Figure 2-A: A manual propipetter used for loading of embryo into a catheter and release of the embryo into mare's uterus.

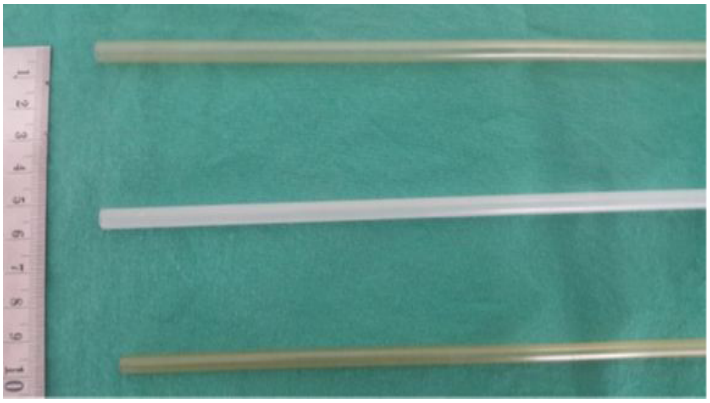


Figure 2-B: Different sizes catheters used for large equine embryo; 1- external sheath of double guarded uterine swab, 2- AI and ET catheter, 3- catheter used for AI.

Pregnancy Diagnosis in Recipient Mares

Pregnancy diagnosis in recipient mares was carried out using transrectal ultrasound scanning. Examinations were done 15, 30, 45, 60 and 90 days' post ovulation.

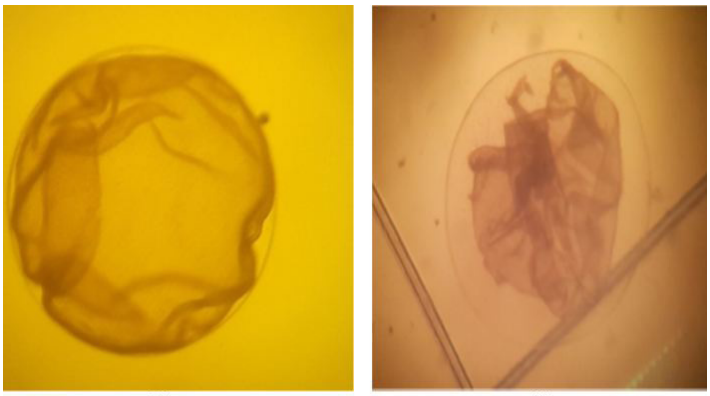
Results

During this study, 36 donor mares were flushed after detection of embryos using transrectal ultrasound scanning. No flushing was carried to donor mares unless embryonic sac was recorded by ultrasound. Control mares (6 mares) were scanned as well for detection of embryonic sacs and minoring pregnancy. All recovered embryos were large expanded blastocysts with diameters ranged from 4 -6.5 mm (Figure 3-A, B).

(A) (B)

Figure 3(A-B): Large expanded blastocyst seen by naked eye on day 10 (A) and on day 11 (B).

The recovery rate was 94.4% (34/36) as there were 2 recovered intact capsules enclosing collapsed trophoblasts of a day 10 and 11 embryos damaged during flushing (Figure 4-A, B). There were 2 embryos recovered from uteri of mares that have been died suddenly at 11 days of pregnancy, only one of them succeeded post-transfer (Table 1). The success rate post transfer was 73.5% for embryos transferred at 10 and 11 days' post-ovulation. There were 12% (3/25) embryonic deaths have been observed at 20, 35 and 45 days of pregnancy in recipient mares; so that the conception rate was ultimately 64.7%.



(A) (B)

Figure 4(A-B): collapse of the trophoblast within an intact capsule of a day 10 (A) and day 11 (B) Embryos.

Day of Flush	Number of Donor Mares	Recovery Rate	Pregnancy Rate	Embryonic Deaths
10	16	15/16	12/15	2
11	18	17/18	12/17	1
11	2 (After death)	2/2	1/2	-
		94.4% (34/36)	73.5% (25/34)	12% (3/25)

Table 1: Recovery rate, pregnancy rate after transfer and embryonic deaths after success of transfer and formation of embryo proper.

The recovery rate was similar for 10 and 11 day old embryos. The effect of day of ovulation in recipient mare on pregnancy rate post transfer was studied.

Days Post Ovulation	Pregnancy Rate (%)	No.
4	92.3	12/13
5	71.4	10/14
6	42.8	3/7

Table 2: Shows that the pregnancy rate was higher in 4-day post ovulation and lower at 6 days' post ovulation.

Discussion

In the vast majority of equine embryo transfer programs, the embryo recovery usually carried out on days 6, 7 and 8 post ovulation and transferred into recipient mares that ovulated 1 day before to 3 days after the donor mare. The current study was greatly different, as it was depending on flushing of donor mares when embryos were detected by ultrasound scanning (on day 10-11 post ovulation) followed by transfer into a recipient mare depending on using a large pore catheter to fit the embryo size. The current embryo transfer programs do not depend on ultrasographic detection of embryo in the donor mare, so that there are many flushes should be carried out to get the embryo from the donor mare that means loss of large volumes of flushing media and stress on mare's uterus through applying several washes of the uterus particularly, in mares with a history reduced embryo recovery due to subfertility. Furthermore, in old mares there was a delay in descend of the embryo into the uterus and thus the determination of day of flush is not easy, but with using of the modified method once the embryo detected by ultrasound, the flushing could be performed. So the operator could safe flushing media and times of flushing until he could be sure that is a formed embryonic sac.

David Hartman (2011) [10] mentioned that veterinarians tend to flush on day 8 or 9 to visualize embryo in the filter to stop flushing once they detect embryo, but in this practice twins may be lost. Comparatively, in the current study twins can be detected with ultrasonography and flushing continues until obtaining the twins and this result make this study is more practical and precise in recovery of twins.

The studies that was carried out concerning transfer of old age embryos were few as mentioned by [5,7-9] but there were trials achieved by Vogelsang et al. (1985) [11] who reported no pregnancy post transfer of day 9 and 10 embryos while Fleury et al. (1989) [12] who achieved 69% pregnancy rate for embryos collected 9 days' post ovulation, these contrastive results might be

due to recipient factors or method of transfer. In the current study, the success rate after transfer was 73.5% although the embryo size was larger (10-11 days) than the previous studies. Based on the present findings, there was a high degree of success in embryo recovery (94.4%) and pregnancy rate 73.5% compared to the results obtained by Jacob et al (2012) [13], who got recovery rate (63%) and pregnancy rate (70%). Wilsher et al. (2010) [14] mentioned a success in transfer in 10 days' embryo and the problematic aspect of the method of transfer as well as failure of recovery of embryo > 5 mm after detection with ultrasonography. The current study succeeded recovery of embryos 4- 6.5 mm in diameters, furthermore using different sizes catheters according to the embryo size and using special kind of manual controllable pipettes to manage embryo loading, amount of holding media as well as slow release of the embryo during transfer to avoid embryo burst.

Based on the current results, there is a new use of embryo transfer for large equine embryos over 10 days that can work for mares that died suddenly or might be subjected to euthanasia for urgent reasons and they were pregnant 9-11 days. In conclusion, all previous studies concerning transfer of large equine embryo ranged from 9 to 10 days and the results were contrastive. The current study emphasized on transfer of large embryo that could be detected by ultrasound scanning. This method can be used for mares with a history of low embryo recovery to save times of flushing and avoiding loss of flushing media. As well as it can be applied for mares died at 10-11 days' pregnancy or have been subjected to euthanasia.

Furthermore, the technique can be used for mares when the operator missed the day of flushing for any reason or has to delay the time of flushing 1-3 days for availability of a recipient.

Acknowledgements

Thanks are kept for all my team of assistants and labors in my lab. And owners of the farms that helped me to achieve this work.

References

1. Squires EL. Carnevale EM, McCue PM, Bruemmer JE (2003) Embryo technologies in the horse. Theriogenol 59: 151-170.
2. Stout TAE (2006) Equine embryo transfer: review of developing potential. Equine vet J 38: 467-478.
3. Camillo F, Vannozzi I, Rota A (2002) Age at puberty, cyclicity, clinical response to PGF2 alpha, hCG and GnRH and embryo recovery rate in yearling mares. Theriogenol 58: 627-630.
4. Angus MO, Edward SL (2007) Embryo transfer and related technologies, current therapy in equine reproduction textbook Chapter 51: 319-334.
5. Iuliano MF, Squires EL, Cook VM (1985) Effect of age of equine embryos and method of transfer on pregnancy rate. J Anima Sci 60: 258-263.
6. Ball BA, Little TV, Weber JA, Wood GL (1989) Survival of Day-4 embryos from young, normal and aged sub fertile mares after transfer to normal recipient mares. J Reprod Fertil 85: 187-209.
7. Allen WR, Stewart F, Trounson AO (1976) Variability of horse embryos after storage and long distance transport in the rabbit. J Reprod Fertil 47: 387-390.
8. Steffenhagen WP, Pineda MH, Ginther OJ (1972) Retention of unfertilized ova in uterine tubes of mares. Am J Vet Res 33: 2391-2398.
9. Iuliano MF (1983) The effect of age of equine embryo and method of transfer on pregnancy rate, Clorado State University.
10. David Hartman (2011) Embryo transfer, Equine reproduction textbook, second edition, Blackwell's publishing, USA, Chapter 303: 2871-2879.
11. Vogelsang SG, Bondioli KR, Massey JM (1985) Commercial application of equine embryo transfer. Equine Vet J Suppl 3: 89-91.
12. Fleury JJ, Costa Neto JBF, Alvarenga MA (1989) Results from an embryo transfer programme with Mangalara mares in Brazil. Equine Vet J Suppl 8: 73-74.
13. Jacob JCF, Haag KT, Santos GO, Oliveira JP, Gastal MO, et al. (2012) Effect of embryo age and recipient asynchrony on pregnancy rates in a commercial equine embryo transfer program. Theriogenol 77: 1159-1166.
14. Wilsher S, Amber CB, Allen WR (2010) Successful transfer of day 10 horse embryos: influence of donor-recipient asynchrony on embryo development. Reprod Res 139: 575-585.