

**Popular Article****Raj-Prathma: Country's First Marwari Horse Foal born through Embryo Transfer Technique****TR Talluri*, Sajjan Kumar, RK Dedar, Yash Pal, RA Legha and TK Bhattacharyja***Equine Production Campus, Regional Station, ICAR-NRC on Equines, Bikaner, Raajsthan-334001***Corresponding Author: raotalluri79@gmail.com**Received:20/12/2023**Published:07/01/2024***Introduction**

India is a unique amalgamation of varying climates zones and terrains where different species of the equine family are used for diversified roles. The population of our indigenous equine population are drastically declining as a faster pace. Among the all the registered breeds of horses, Marwari horse is the original desert horse breed of Marwari region of Rajasthan state of India, capable of covering long distances with good speed by virtue of its strong limbs and hooves. This breed can withstand intense heat and cold and adverse climatic conditions & is known for its elegance, beauty, vigour, endurance, intelligence, alertness, animated gait and their main feature is their unusual inward-curved ears. The 19th and 20th Livestock Census (2012-2019) data reveals that the total horse population in the country is 0.34 million, and they have gone down by 45.58%. Collectively, the total equine population was reduced by 52.71% during this period. High demand for genetic preservation of equids has led to developments and improvements in assisted reproduction and a rapidly growing area of equine medicine. Over the decades, NRCE has been working on the genetic conservation and propagation of indigenous horse and donkey breeds. For the faster multiplication of elite equine indigenous germplasm, NRCE has developed cryopreservation of semen technology and artificial insemination of frozen semen technologies through which NRCE is not only conserving but also propagating the indigenous equine germplasm. Live foals from Marwari, Kathiawari, Zanskari and Manipuri breeds have produced through artificially insemination of frozen semen^{2,3,4}. In this endeavour, scientific team at Equine production Campus, Regional Station, ICAR-national Research Centre on equines has taken up another challenge to cryopreserve the indigenous horse breed embryos and to produce foals through embryo transfer technology in equines.

What is Embryo transfer?

Embryo Transfer is the process in which a 6-8 day embryo will be flushed from the uterus of a donor mare (in which breeding done before 7-8 days) and transferring that embryo into the uterus of a recipient mare. This process requires that both recipient and donor mares are cycled in parallel so that hormonal levels are consistent in both mares for successful establishment of pregnancy. The stallion and donor mare contribute their genetics to the foal and not the recipient.

In simple terms, a donor mare is inseminated, but instead of carrying the pregnancy herself, the embryo is flushed from her uterus and implanted into a recipient mare, who then carries the pregnancy to term and delivers the foal.

Typically, *embryo transfer in Equines involves the following steps* or activities

- ✦ Identification of donor and recipient mares and their pre-breeding scanning
- ✦ Estrus synchronisation of donor and recipient mares
- ✦ Estrus monitoring in donor and recipient mares
- ✦ Ovulation monitoring in both donor and recipient mares
- ✦ Flushing of the donor mares (day 7 or 8) after ovulation
- ✦ Recovery of the embryos after flushing
- ✦ Transferring the embryo/freezing of the revered embryo
- ✦ Monitoring for the pregnancy in the recipient mare

Success with this procedure depends on several factors, including mare synchronization, availability of recipients, uterine or ovarian pathology, embryo quality, successful breeding management, and embryo flush and transfer techniques.

Recipient mare

The choice of recipient mare is important, as it plays a vital role in the ET process, to increase the embryo transfer success rate. The recipient should be as closely matched to your donor as possible in terms of height and build. It is important that she has a good temperament, is in good condition and is settled in her environment. A relaxed, happy recipient with no vices is essential. The recipient needs to have excellent reproductive health, with a known pregnancy rate and good maternal instincts or a young maiden (mare that has never been covered) must hence be chosen, preferably one that is larger than the donor mare to ensure optimal foal development. It is advisable to have at least three potential recipient mares available for a single donor mare. Recent experiments have shown that the uterine environment during pregnancy is important for development of the fetus and for growth and metabolic development of the foal after birth. The fetus is capable of adapting to any uterine environment, but the recipient should ideally be of the same breed as the donor to ensure that its metabolism is not modified.

Synchronization of the recipient mare

The recipient must be synchronized with the donor; i.e. they must both ovulate in the same period. This requires highly accurate gynecological monitoring, induction of ovulation and, in some cases, a synchronization treatment for the potential recipients. The best transfer rates (% of pregnancies 14 days after transfer of an embryo) are obtained when the recipient is on day 5, day 6 or day 7 with respect to the donor's ovulation (day 7). The recipient must hence have ovulated on the same day as the donor or 1 or 2 days later. The success rate is lower outside this 3-day window.

The procedure

The donor mare is restrained in travis/chute or stocks, where mare's perineal area is cleansed with a mild detergent, rinsed and dried. The operator introduces a sterile balloon-tipped catheter (two way), which enters the vagina and is passed through the cervix and into the uterine body. The balloon tip (approximately 30-50 ml of air) is inflated with air. The uterus is flushed three to four times with a warm embryo-flushing fluid. The flush fluid flows back out through the catheter and is passed through an embryo-capturing filter. The embryo capture filter cup is examined under a stereozoom microscope. When an embryo is identified, it is "washed," examined and graded for quality. The recovered embryo will be transferred to the estrus synchronized recipient/ surrogate as quickly as possible, as pregnancy rates drop if the embryo is out of the mare for more than one or two hours.

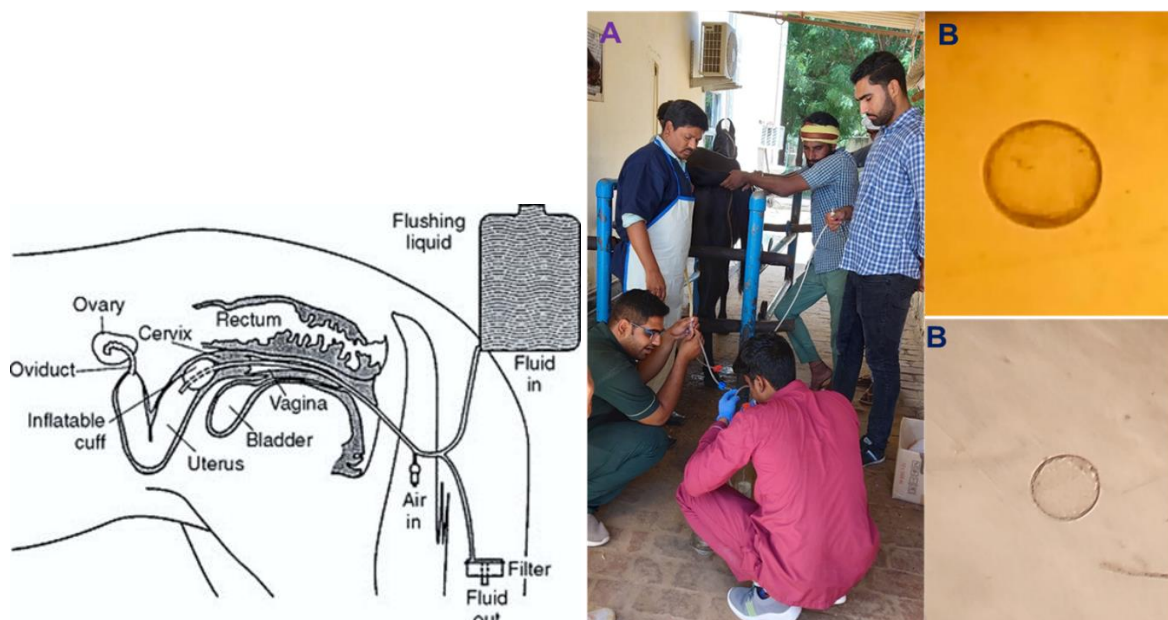


Fig. A. Flushing of Mare for recovery of embryo in process. B. Recovered Embryos of early blastocyst of 7.5d.

Transfer to the recipient is the most delicate stage, requiring the highest levels of experience. There are two transfer techniques:

❖ **Non-Surgical/ trans-cervical:** The recovered embryo is deposited in the recipient's uterus by being passing through the cervix. The embryo, packaged in a straw, is transferred using a special ET gun. This technique is currently the most widely used in the field. The technical difficulty lies in passing the tube through the recipient's cervix with minimal handling to avoid the release of prostaglandins by the uterus, which would endanger the future pregnancy.

❖ **Surgical:** The embryo is injected directly into a uterine horn after incision of the recipient's flank and exteriorization of the horn. The recipient then requires post-operative treatment. This technique is now hardly ever used in practice given the equipment, care and pharmacological treatments required during and after surgery. It could be recommended when the recipient's cervix is very difficult to pass through, but in such a case, it would be preferable to retire the recipient mare.

How the Raj Prathama was produced?

To produce the foals from embryo transfer, estrus synchronization of donor and recipient mares was carried out using CIDR (Controlled Intravaginal drug release). Estrus of the mares was closely monitored and at near ovulation the donor mares were inseminated either with fresh/frozen semen from proven fertile stallion and recipient mares are left without breeding. Donor mares were flushed at day 7 after ovulation for recovery of embryos and the recovered embryo was transferred in to synchronized surrogate recipient mare to get the conception. After eleven months, a filly through embryo transfer is born whose name was coined as Raj-Prathama (Fig 2).



Fig 2. Raj-Prathama- Country's First Marwari Horse Foal born through Embryo Transfer Technique

Embryo cryopreservation

Embryo cryopreservation is the best option to conserve the genetics of any species. This process enables long-term preservation of the elite germplasm. This will also help in transporting the embryos to the distant places or shipped across the globe. If the recipient mare is not ready, we can also proceed for cryopreservation of the embryos and later we can utilize them for transfer when surrogate is ready.

Equine embryos have several unique features that make them difficult to cryopreserve (preserve by cooling them to very low temperatures).

✓ The thick capsule (membrane) that covers the embryo when it arrives in the uterus prevents the cryoprotectants from fully penetrating the embryo, contrary to embryos of other species that do not have a capsule

✓ Their widely varying size, of up to 700µm at 7 days, is a further obstacle to cryopreservation due to the crystals that form in the fluid-filled sphere of the embryo as the temperature decreases.

To overcome these obstacles, the embryo is either punctured with sharp glass needles or emptied of most of its liquid by micro-aspiration before it is placed in contact with the cryoprotectants. It is then frozen by means of vitrification (an ultra-fast freezing process whereby the embryo is plunged directly into liquid nitrogen) at -196°C and kept in that state until transfer. ICAR-NRCE has vitrified nearly 20 Marwari horse embryo until date, that are ready for transfer in to recipients/surrogates.

Advantages of Embryo transfer programme

➤ **Multiplication of germ plasm/progeny:** Normally a healthy mare in her lifetime gives birth to 8-10 foals. However, through ET programme we can produce more than 20 foals from the same mare in her active healthy lifetime. We can flush the mare and recover embryos and transfer them successfully to establish pregnancy in donor mares.

A stallion has the potential to produce hundreds of foals each year, especially if stallion's semen is used for artificial insemination (each collection can be divided and inseminated into several mares). In contrast, the mare's pregnancy lasts around eleven months so mare can carry a maximum of one foal per year and in reality, this can be hard to achieve, as it requires her to become pregnant very soon after delivering her last foal. *Embryo transfer allows us to flush multiple embryos from one mare and transfer them into multiple recipients.* These embryos will all be genetic descendants of the donor mare and the *procedure allows a mare to produce multiple foals per year.* This allows a high quality mare to breed more foals throughout her lifetime than would be otherwise possible.

- **Genetics of valuable mares/stallion can be restored:** Elite and precious stallions'/ mares' germplasm can be restored. Embryo transfer is often used to produce foals from these mares and allow the genetic potential of the mares to be continued.
- *Race horses/show horses/Performance mares can continue competing* and transfers can be done around their show career.
- *Fertility of older mares may be enhanced,* increasing their chances of obtaining foals from them.
- *Mares unable to carry their own foals to term* (due to various conditions like lameness, cervical tears, systemic health conditions that preclude pregnancy) can also serve as efficient donor mares and can be selected and used as good candidates for embryo transfer. Embryos can be recovered from mares with uterine based infertility issues. ET also allows mares to produce foals if they are physically unable to carry a foal themselves. For example, if they have a gynaecological problem that makes them able to conceive but unable to carry a foal to term, or perhaps an orthopaedic problem such as a pelvic fracture, embryo transfer may be an option.
- Embryo transfer can be used on mares late in the season so the next year the mare can be bred earlier.
- Additionally, embryos can be frozen and shipped over-seas allowing horse owners to reach new markets around the world.