THE INTERNATIONAL SYMPOSIUM ON ANIMAL SCIENCE (ISAS) 2015
&
19th INTERNATIONAL CONGRESS ON BIOTECHNOLOGY IN ANIMAL REPRODUCTION (ICBAR)

09-11.09.2015. Novi Sad, Serbia

Co-Organizers

Slovak University of Agriculture in Nitra
Faculty of Biotechnology and Food Sciences – Slovakia

National Agricultural and Food Centre
Research Institute for Animal Production in Nitra - Slovakia

University of Josip Juraj Strossmayer
Faculty of Agriculture Osijek – Croatia

Banat University of Agricultural Sciences and Veterinary Medicine Timisoara
Faculty of Animal Science and Biotechnology – Romania

University of Zagreb
Faculty of Agriculture - Croatia

Co-Sponsorship

European Society of Agricultural Engineers

Revised edition
EQUINE EMBRYO TRANSFER: APPLICATION ON HORSE PRODUCTION PROGRAMS, EXPERIENCE FROM SOUTH AMERICA

Losinno L.¹, Urosevic I.M.²

Abstract: Embryo transfer (ET) is a biotechnological process that on equines species involves scheduled artificial insemination (AI) of a donor mare, recovery of embryos by transvaginal uterine lavage 7 to 9 days after ovulation, and nonsurgical embryo transfer to a synchronized recipient mare(s). The objective of this review is to update some recent information and field experiences on equine embryo transfer programs in horses with special reference on applied aspects of the technique and procedures. For general description, we use the conventional in vivo embryo production model and direct transfer, besides mentioning the variations due to peculiarities of application for other purposes. The technique is simple and easy to learn but immersed in a complex system with multiple variables acting simultaneously. To maximize the success of embryo transfer programs should be used fertile mares and stallions, but that usually is not a Veterinarian’s decision. Under ideal conditions (i.e., with donor, recipient, fertile stallions, and trained personnel), 70% to 85% recovery rates and 75% to 85% pregnancy rates can be expected, resulting in overall ET efficiency of 45% to 65%. Both rates are lower (20% to 45%) in older subfertile mares and subfertile stallions. This is why weekly or monthly analysis of records must be performed and categorized by donor age, stallion, and month. In a standard breeding season and continuous program, it is possible to obtain, on average, 4 to 6 annual pregnancies from the same donor, but an Argentinian polo mare has provided 14 pregnancies without additional stimulation of ovulation.

Keywords: stallion, mare, biotechnological process, Argentina

Introduction

Embryo transfer (ET) is a biotechnological process that on equines species involves scheduled artificial insemination (AI) of a donor mare, recovery of embryos by transvaginal uterine lavage 7 to 9 days after ovulation, and nonsurgical embryo transfer to a synchronized recipient mare(s). Some commercial programs have transfer efficiency higher than 65%, but a 50% rate is considered acceptable, as this value is based on a 70% per-cycle recovery rate (RR) and a 70% pregnancy rate (PR) after transfer (RR × PR = 0.7 × 0.7 = 0.49). In practical terms, this means that two flushings (cycles) are needed to achieve one pregnancy. According to the International Embryo Transfer Society (IETS), in 2009/2010 breeding season, 24.515 equine embryos were transferred worldwide with 90%...
of the reported activity in America. Today, with some important exception (Thoroughbreds), due to the partner’s pressure, most associations decided to incorporate it into their statutes. On the first step, regulating the number of offspring registered per donor, per season; just for qualified females; using only recipients from the same breed, and a series of requirements that usually disappear after pressure from the market and members. Initial resistance to ET by some breed organizations has largely been resolved (with the exception of Thoroughbreds until now, all over the world), and a significant percentage of embryos in America are transferred (Brazil, 43%; Argentina, 29%; and United States, 18%) according to IETS-International Embryo Transfer Society-data. The objective of this review is to update some recent information and field experiences on equine embryo transfer programs in horses with special reference on applied aspects of the technique and procedures.

**Indications And Applications**

ET has the potential to increase reproductive efficiency, but it also has limitations that can lead to unfounded expectations. The main indications include:

- **Increase the number of foals per year in selected mares.** On average, a broodmare can produce between 5 and 10 foals naturally in their reproductive lives. In continuous programs, ET can increase production as much as 10-fold. There are records of more than 80 offspring produced by ET from the same Polo Argentino mare.

- **Producing foals from mares in competition programs.** On most valuable sport mares, intensive competition time (polo, jumping, dressage, shows) occurs during the breeding season and reduce the time for embryo donation on classical ET programs on clinics and centers.

- **Producing foals from mares that cannot gestate for specific reproductive (embryonic death and/or recurrent abortions, cervical incompetence, etc.) or non-reproductive problems (musculoskeletal injuries, abdominal hernias, etc.).**

- **Reduce generation interval using one or two year’s old fillies as donors or before taming / training.** This is a common practice in commercial systems where the select fillies produce embryos for transfer or cryopreservation before beginning his sport life or sale.

- **Eliminate the risks of pregnancy and parturition in valuable and particularly old mares.** In many breeds this technique allows "rescue" elderly females (> 20 years) with high breeding values.

- **An exceptional tool in genetic programs evaluating certain crossings scheduled (i.e. 5-10 pregnancies from the same donor to the same or all different stallions in the same season).**

**Characteristics of equine embryo transfer programs**

These programs can be categorized in relation to different variables: a) Closed (only one owner / company for their own production); Open (commercially, as a reproductive service) or Mixed; b) Small (1-50), Medium (60-200) and Large (> 200) scale in relation to the number of pregnancies obtained per season. The scale determines the degree of logistical complexity. There are commercial programs that produce more than 1,500
pregnancies / season; c) Fixed and Ambulatory. Fixed programs (ET Centers), control important variables (stallions, donors, recipients, facilities, food, health, assistants). Results are more consistent and efficient but higher fixed costs. Ambulatory programs represent a lower operating cost for the service provider, but with less control of variables determining success. However, it is a growing practice and the results of many programs are comparable to fixed; d) Permanent (refers to exclusive ET programs that only additionally provide other services off-season as freezing semen) and sporadic (when ET is only part of the services offered in addition other related to clinical medicine); e) Complete (when the programs involve the complete process, i.e. donors, recipients and stallions/semen management, and partial, when reproductive management of donors and AI occur in different places and time and use cryopreservation (cooled, frozen) systems, sending the embryo to a recipient herd when they were transferred.

**Embryo transfer procedures and techniques**

For general description, we use the conventional *in vivo* embryo production model and direct transfer. The technique is simple and easy to learn but immersed in a complex system with multiple variables acting simultaneously. However, what we recommend is to make an individual report from exhaustive donor’s breeding soundness examination, estimating potential reproductive ability and determine: a) if the animal (mare or stallion) is suitable (and to what extent) to enter a ET program (or not) and; b) its potential output in the program, based on statistical data consider race, age, health status and our own records of efficiency.

**Donor Mare Management**

The timing and number of ovulatory cycles per year will vary with breed, age, body score and geographic region, but in subequatorial countries (i.e. Argentina), a young donor should have 5 to 8 months of regular cyclic activity. Regular reproductive examination by palpation and ultrasonography is imperative to monitor follicular waves and development (“mapping” ovarian activity) and ovulation that are critical points. The day on which ovulation is detected considered day 0. Every donor in an ET program should be artificially inseminated, except in exceptional cases in which controlled natural mating may be recommended. Examples would include stallions with low *in vitro* sperm survival, in which natural breeding, for reasons unknown, improves embryo recovery rates and stallions that are refractory to semen collection. Artificial insemination allows control of semen quality and that, in an assisted reproduction program, should not be resigned. Natural mating is a suboptimal procedure because there is no control of either the concentration or progressive motility of sperm cells or the presence of urine, blood, or pus in the ejaculate. A donor can be flushed, on average, every 14 days during the physiologic breeding season. Depending on a mare’s follicular status and the veterinarian’s clinical judgment, ovulation inducers such as administration of 1500 IU human chorionic gonadotropin (hCG) intravenously or 1 mg of gonadotropin-releasing hormone controlled
release analog\(^3\) given intramuscularly can be used to increase the number of ovulations per cycle. Results from one of the author’s laboratory (Losinno et al., 2008) indicate that in commercial embryo production, ovulation, and embryo recovery rates differ significantly with donor age and type of induction (Table 1).

Table 1. Number of cycles and ovulation rate in young and old Polo argentino donor mares treated with human chorionic gonadotropin (hCG) and long-acting deslorelin (LA-DES)

| Treatment | Young mares | | Old mares | |
|-----------|-------------|-----------|-----------| |
|           | Cycles (n)  | Ovulation rate (%) | Cycles (n) | Ovulation rate (%) |
| hCG\(^c\) | 171         | 63.2\(^a\) | 36.8\(^d\) | 48         | 58.3\(^a\) | 41.7\(^d\) |
| LAD\(^a\) | 390         | 56.9\(^b\) | 43.1\(^c\) | 266        | 50.7\(^b\) | 49.2\(^e\) |
| Control   | 849         | 76.1\(^c\) | 23.9\(^f\) | 327        | 68.8\(^c\) | 31.2\(^f\) |

Values with superscripts a and b are not significantly \((P < .001)\) different from each other but are significantly different from values with superscript c. Values with superscripts d and e are not significantly \((P < .001)\) different from each other but are significantly different from values with superscript f. (Losinno et al., 2008).

Immediately post flushing, should be done an ultrasound examination for intrauterine fluid that increase the risk of inflammation and one dose of luteolytic prostaglandin F\(_2\)\(\alpha\) analog (sometimes two doses separated by 12 or 24 hours). On continuous programs, donors are inseminated and flushed every 2 weeks during at least 6 months. Practical observations and published reports (Carnevale, 2005) shows in some mares an increased incidence of bacterial endometritis and chronic endometrial inflammation, starting in some cases a vicious circle of unspieic antibiotic therapy on every cycle and consequently increments of mycotic endometritis.

**Recipient’s selection and management**

Recipients represent the bottleneck and one of the greatest economic impacts on commercial ET programs. In many programs, the ET center owned the recipients and rent them temporarily until foal’s weaning. Some current variants are using early weaning (4 months) and / or post partum mares. Recent studies shows that pregnancy rates on recipients after second post partum cycles were not significantly different compared with non-foaling mares. There are some basic and unavoidable guidelines in the process of selecting a recipient.

\(^3\)LAD Deslorelin, BET Pharmacy, Lexington, KY, USA
Type and size: The closest to the donor. Never smaller or heavy type mares to light horses breeds. Often used mares of the same breed, less valuable genetic (and in some pure breeds is a condition of accepting the ET). It’s usually a good choice because they are generally well known and adapted to farm management.

Age: Between 4 and 14, ideally 5-9 years. Age determination should be made by trained and experienced professional.

Breeding soundness examination: In our programs every recipient candidate must be biopsied, joined the rectal palpation, ultrasound, forming and integrity of external genital and cervical competence examination. Sexually transmitted diseases (i.e. Equine Herpes Virus 3; Contagious Equine Metritis and STBI-Sexually transmitted bacterial infections-, must be cheked carefully. Results from our laboratory have shown a significantly embryonic loss rates difference in mares selected using biopsy score or not in addition to the above parameters (Table 2).

Table 2. Pregnancy rates and embryonic loss in recipient mares selected with or without endometrial biopsy

<table>
<thead>
<tr>
<th>Recipient mares</th>
<th>Pregnancy rate (%)</th>
<th>Embryonic loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Day 14 after transfer</td>
</tr>
<tr>
<td>With biopsy</td>
<td>1194</td>
<td>72.5</td>
</tr>
<tr>
<td>Without biopsy</td>
<td>926</td>
<td>71.8</td>
</tr>
<tr>
<td>Total</td>
<td>2129</td>
<td>72.2</td>
</tr>
</tbody>
</table>

Values are significantly different between a and b; between c and d; and between e and f (P < .001). (Castaneira et al., 2008)

Tameness: ET program is not a taming program and cannot operate simultaneously. A recipient must be palpated and ultrasound at least 5-10 times before transfer, vaccinate, dewormed, gestate and then raise a foal.

Maternal ability: Should be assessed objectively and independently of the fertility. If negative, is exclusionary. It’s important the specific examination of structural and functional integrity of the mammary gland.

Health: Despite serological tests for Equine Infectious Anemia, must include at least Babesiosis, Leptospirosis and other specific diseases according to countries. Strangles is the most prevalent infectious disease associated with recipient’s management in large scale ET programs, even using controlled immunization programs. We recommend using an isolation sanitary area in any open program and quarantine for recent arrival and clinically affected mares.

Tolerance: How many negative post-transfer pregnancy checks can tolerate in a season? If we assume a consistent and standardized program and we can "rule out" management factors and integrity of the embryo, no more than 2 negative scans on a recipient.
**Synchronicity:** No significant differences were reported in terms of pregnancy rates when recipient mares ovulated between days -1 to +5 days relative to donor’s ovulation and optimum of 0 and +2. However, Wilsher et al. (2006) shows that it’s possible to extend to +9 and +10 days suppressing normal luteolytic effect using Meclofenamic acid (1gr PO daily) from day 9 post ovulation to day 7 after transfer. Jacob et al. (2012) on commercial ET programs reported not different pregnancy rates in recipients ovulated +4 or +5 compared with recipients ovulated in a “classical” window of -1 to +3 (70% versus 66% respectively).

**Post transfer management:** We try to make pregnancy checks on fixed dates to transferred groups to avoid unnecessary movements and, if possible, with portable ultrasound, on the field. The first control between 5-7 days post transfer (PT) (12-14 days post-ovulation-PO-), the second on days 12-15 PT, the third 30 days PT and if it is a commercial program, the last one before deliver to the client, on days 45-50 PT.

**Artificial Insemination**

Although it is one of the critical points of the system and has a great impact on embryo recovery rates, semen handling and AI are beyond the scope of this review. For complete and updated information see Brinsko, 2011.

**Uterine flushing-embryo recovery procedures**

Immediately before flushing, the donor should be palpated and undergo an ultrasound examination to evaluate uterine tone, the number of corpora lutea, and the presence of uterine fluid or edema. The perineal area should be washed at least three times with clean water and nonirritant liquid skin soap without antiseptics and dried with paper towels. For direct transfer, embryos should be recovered on days 7 or 8 after ovulation. On our laboratory the procedure for collecting embryos for vitrification include an intensive ovulation monitoring of donors every 6 hours in a fixed schedule (6am-12am-6pm-12pm) in order to establish the flushing time between 150-160 hours post-ovulation. On day 8 or 9, the embryo is usually 0.8 to 2 mm in diameter, allowing it to be seen macroscopically on the filter. Embryo collection is performed by transcervical uterine lavage, with the operator using an 80-cm long silicon Foley-type catheter (28 to 34 French). The flushing solution used in most commercial programs in the countries that produced close to 70% of the equine embryos in the world (Brazil and Argentina) is sterile lactated Ringer’s solution without bovine fetal serum (BFS) or any other compounds. The uterus is filled with 0.5 to 1 L per flush, up to a total volume of 1 to 3 L, and the effluent recovered in a closed two-way system with an online filter. With the operator’s arm covered by a sterile lubricated sleeve, the front end of the catheter is passed smoothly through the closed cervix into the uterine body, and the catheter balloon is inflated to prevent backflow.

**Embryo Management**

For processing embryos, the author recommends the use of clean overalls, masks, and nitrile gloves. Ideally, embryos should be processed in a specialized laboratory within a...
HEPA-filtered, horizontal laminar flow hood under indirect ambient light. At the clean working area, the filter residual volume is slowly poured into a sterile Petri dish; the filter walls and cap are also washed with flushing media. Embryo searching must begin at lower magnification on a stereo microscope. After the embryo has been identified, it is categorized by size, development, and morphology on a scale of 1 (excellent) to 4 (poor).

**Transfer Technique**

Perineal hygiene must be the same as that described for the donor. Additional treatments should include hCG (1500 IU, IM) on the day of transfer, flunixin meglumine (500 mg, IV or PO) on the day of transfer and 2 to 3 days after transfer, a single dose of long-acting progesterone (1.5 g, IM), and oral antibiotics (trimethoprim-sulfamethoxazole, 25 mg/kg, PO, once daily) from the day of transfer until the first pregnancy check. Manual transfer is most widely used, and the technique is similar to rectally guided deep artificial insemination. Alternatively, a vaginal speculum and special atraumatic cervical forceps described by Wilsher (2004) can be used to pull the cervix close to the vestibule so that the pipette can be visually guided into the uterus. This method is quicker and easier, carries less risk for contamination, and gives comparable or better results than manual transfer.

**Expected results**

Under ideal conditions (i.e., with donor, recipient, fertile stallions, and trained personnel), 70% to 85% recovery rates and 75% to 85% pregnancy rates can be expected, resulting in overall ET efficiency of 45% to 65%. Both rates are lower (20% to 45%) in older subfertile mares and subfertile stallions. This is why weekly or monthly analysis of records must be performed and categorized by donor age, stallion, and month.

Acknowledgments: The presented work is part of the research done in scientific projects „TR-31084“ and „III-46005“ granted by the Serbian Ministry of Education and Science.

**References**


