

## PROBLEMS AND SOLUTIONS IN EQUINE EMBRYO TRANSFER PROGRAMS IN BRAZIL

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### **Abstract**

Brazil is the second country on equine embryo world production. Problems related to the application of equine embryos transfer in Brazil are discussed in this article. The main factor related to low efficacy of Equine Embryos Transfer programs consists in the difficulty to induce multiple ovulations. Among the most common difficulties and limitations faced in our country, we emphasize the utilization aspect of a higher percentage of aged females which are used as donors. The interest for the continuity of their use is attached to the fact of slow genetic improvement on equine species. Other important aspect is related to recipient quality.

**Keywords:** embryos, equine, efficacy, ET, fertility.

### **Introduction**

According to the last survey of Embryos Transfer International Society Statistics Committee (IETS), Brazil is the second on equine embryo world production (12,000 recovered embryos) being overtaken only by the United States of America (USA), in which approximately 14,000 embryos were recovered at the stud farm in 2008-2009. The average growth of embryos produced in Brazil has been 20% per year in the last 5 years.

Among the breeds, Mangalarga Marchador is the breed with more products born from embryos transfer registered per year, followed by Quarter Horse breed. Quarter Horse is the breed that has grown more throughout the last years in number of embryos produced yearly. Regionally, the greatest centers of embryos production are the states of Minas Gerais and São Paulo, as they are the states with major concentration of Mangalarga Marchador and Quarter Horse, respectively.

In our country, there are approximately 40 Centers of Embryos Transfer in equine, being part of them Services provider for third parties and the other part for exclusive use in animals of a sole owner. Embryos Transfers in Quarter Horses and Sport Horses are rather taken place in Centers, while in other breeds, such as Mangalarga and Campolina, Embryos Transfer are mostly taken place in structures established at the breeder's own stud. The present publication aimed to show the most important factors that interfere on the success of ET programs in Brazil.

### **Particularities and limitations related to embryos transfer in equine superovulation induction limitations**

The main factor related to low efficacy of Equine Embryos Transfer programs consists in the difficulty to induce multiple ovulations. Multiple ovulations induction in mares' main objective is to increase the chances of embryonic recovery in a cycle and, consequently, to improve the efficacy and to diminish the costs involved in programs of Embryos Transfer (TE). Today, due to the difficulty to superovulate mares, TE programs efficacy in this species is very low, being needed an average of 2 to 4 cycles to attain a gestation of an embryos donor mare, therefore, the costs for production of a young horse deriving from TE are quite expensive. The adaptation of a superovulatory protocol which consents to at least guarantee one pregnancy for each donor mare cycle is highly desirable. A great difficulty in the superovulation of mares is related to the mare refractoriness to hormonal

preparations based on swine FSH commercially available for other species, such as bovines. The use of homologous FSH is being needed so that we have ovarian response to treatment. This way, Equine Pituitary Extract (EPE), purified equine FSH and most recently recombinant equine FSH are the indicated preparations to induce superovulation in female horse.

The response of cyclic mares to treatments with EPE or equine FSH allowed, according to several papers, the obtainment of a good superovulatory response, with a variation of 3 to 5 ovulations per cycle, although the embryonic recovery has been low, with an average of 30 to 40% recovery embryos per ovulation (Alvarenga *et al.*, 2008). Most recent papers have demonstrated that protocols where EPE lower doses are used, allow a better embryonic recovery. However, difficulty to prepare EPE and its commercial unavailability prevents its use in large scale. Recently, even equine FSH is no longer commercialized in USA. Recombinant hormones seem to be the most promissory perspective to the future, for it won't be necessary to extract FSH from equine pituitary anymore. Initial works using recombinant FSH in mares have shown very favorable results, although this product is still non available in the market.

On a paper published at the SBTE convention annals (Nagao *et al.* 2010) mention that the use of deslorelin acetate in a special carrier has allowed induction of double ovulation in most treated mares. On this protocol, deslorelin low doses applied twice a day, animals monitored daily through ultrasound and at the moment they have presented a follicle between 23-25mm and at least a second follicle  $\geq 20$ mm (diameter), the treatment with deslorelin acetate was initiated. It is worth to emphasize that the mares didn't always present this follicular dominance and co-dominance standard and consequently they were not treated. 85 % of treated mares presented double ovulation being their embryonic recovery per cycle almost duplicated (0,90 x 0,55 embryos per wash). This protocol using GnRH has two advantages: 1- it's synthetic, and 2- it has lower cost.

#### **The age factor**

Another particularity of TE in equine concerns the use of aged donor mares. In no other production animal species there is a percentage so high of aged procreators in reproductive activity as in equines. The interest for the continuity of their use is attached to the fact of slow genetic improvement observed in equines for their small quantity of descendents produced yearly, either by limitations imposed by breeders associations as well as by breeding and market profile. Stallions considered as their breeds' representative hardly produce more than 200 products per year. Similarly, mares are conserved in reproductive activity for a long time. Even mares in TE programs produce few young horses per year, an average of 2 to 4, and take a few years to show potential improvement. The age factor, in its own, is a low fertility determinant.

It is known that mares older than 18 years old present follicular maturation problems, and consequently oocyte maturation, which lead to future problems in their embryonic development capacity, increasing the precocious embryonic loss rate which is almost 60% from the fertilization to 30 days of pregnancy (Ball *et al.*, 1989).

#### **Difficulties with the use of frozen semen**

Difficulties faced for the application of frozen semen in large scale of embryos is another factor which restrains a faster improvement of the species. The application problem of frozen semen in large scale is related to mares long estrus period (5 to 7 days) associated to a large period of time until the ovulation happens during the estrus (last 48 hours), associated to limited time of survival in spermatozoon genital handling after frozenness and defrosting (12 hours), that obliges the accomplishment of artificial insemination at a moment very close to ovulation, which is only possible with control of intensive follicular development. On that basis, trying to trespass frozen semen limitations, the refrigerated semen is routinely used as it has survival time longer than the frozen semen (24 to 30 hours) which facilitates its use in large scale. It is thought that 80% of produced embryos are derived from insemination with refrigerated and transported semen. A bigger limitation to the application of refrigerated semen concerns the short storage period, being ideal not to exceed 24 hours. Some stallions have better fertility results with frozen semen than with refrigerated one. In situations where it's difficult to get to the mare which will be inseminated, the use of frozen semen is an alternative very appropriated.

#### **In vitro embryo production limitations**

Systems employed in other species to mature oocyte, to capacitate spermatozoon and to cultivate "in vitro" embryos do not adapt to equine species. In other species, such as bovines, the IVF application result in the birth of thousands of products yearly, being applied in large scale at a commercial level in Brazil where the number of bovine embryos derived from IVF is

higher than the ones produced in vivo, that is, recovered straight from uterus, on the other hand, up to this moment rare products are created through IVF in equines (Alvarenga *et al.*, 2009).

#### **Other particularities that interfere in the efficacy of equine the programs in Brazil receivers' scarceness**

The increase of interest for use of TE in equines led not only to a significant increase of receptors' market price as well as reduced the offer. The average prices adopted in the state of São Paulo have increased an average of 30 to 40% per year. In other words, a receptor that was worth 500 Brazilian Reais 3 years ago, at the last stud farm (2009-2010) was worth approximately 1200 Brazilian Reais. That led to two situations which certainly interfere negatively in the program: purchase of lower quality animals and reuse of receptors which would be dismissed for its bad fertility history. According to Central Bimbryo observation, located at São João da Boa Vista – SP, the chances of a receptor which gets pregnant after the loss of an embryo with more than 15 days old is lower than 25%.

The receptor is known to be the TE program success key point (Losinno *et al.*, 2006). In two moments accentuated losses in pregnancy rate and increase of precocious embryonic loss rate are observed in TE programs: the first during stud farm when new receptors are added to breeding stock (Pessoa *et al.*, 2009); and at the stud farm end when we start to reuse receptors which lost pregnancies throughout the stud farm (Riera *et al.*, 2008). In the first moment, when there is a need to replace receptors in the middle of stud farm, it is important to try to isolate those animals, because during the adaptation period they routinely develop strangles. Pessoa *et al.* (Pessoa *et al.*, 2009) mentioned decrease from 70% to 40% on pregnancy rates in a TE commercial program in equines when new receptors are added. However, Riera *et al.* (2008) mentioned decrease from 70% to 40% on pregnancy rates after TE when they compared the last two stud farm months (March and April) against the period from October to January.

The receptor's selection should be based in the reproductive system's age (< 12 years) and health. To minimize the receptors' scarceness problem at stud farm beginning and end, the use of progesterone in anoestrus mares has become a routine strategy. This way, we can increase the number of susceptible mares to be used, because either at stud farm beginning or at its end many receptors are not cycling different from donors which initiate the cycle early and finish it later at stud farm. So, 4 to 6 donors' cycles are lost throughout the year. An average of 2 to 3 more embryos are produced per year with the progesterone use. However, progestogens use may not be effective if appropriated drugs and protocols were not use. Another problem concerns the application responsibility. An application error may be an embryonic loss determinant factor as they are for weekly or biweekly use. We advise the establishment of a progesterone application day at the stud or central which should be applied, preferential, by the Veterinarian.

Most recently, protocols using cycle receptors treated with P4 in diestrus have also allowed a better improvement from receptors. In this protocol, published as recapitulation of this SBTE Meeting (Greco *et al.*, 2010), progesterone treatment was initiated in diestrus after one only dose of estradiol benzoate application. Pregnancy rates were close to 50% when using this protocol.

The mistakes most often observed in receptors' selection are related to the purchase moment, as they are usually purchased during the winter when mares are in anestrus. At the anestrus phase, it is known, that problems such as improper vulvar conformation and even uterine infections may not be clinically detected. A way out would be to apply estradiol benzoate exogenously on the day before when the receptors should be evaluated for purchase.

Another routine mistake concerns the exclusive use of ultrasound as uterine inflammatory processes diagnosis method, where mares with uterine fluid are rejected. The equivocation lies on the fact that, in some situations, mares with uterine infection don't have uterine fluid as well as in the estrus the presence of small quantities of fluid may be normal. The ideal would be to associated cytology exam to the ultrasound.

#### **Problems with aged mares improper treatments**

Every time we face an aged mare donor with fertility problems we should consider the situation in a different way than the one used for younger mares. It is important to have in mind that aged mares present, because of their advanced age, the following characteristics which may lead to fertility loss:

- Low LH serum levels at periovulatory period (Jacob, 2008)
- They show high percentage of atypical ovulations

- Have increased interovulatory interval because the follicular phase is longer
- In spite of a good fertilization rate, they show higher percentage of embryonic losses between 2 to 5 days after the fertilization
- They show embryonic development slower in the fallopian tube making, in several situations, the embryos to reach morula phase and migrate to the uterine environment only 8 days after the ovulation.
- What can we do to improve embryonic recovery rate of aged mares?
- To supplement with LH equine low doses during the follicular phase
- To induce ovulations associating it to hCG and Deslorelin
- To collect embryos between D 9 and D 10 after ovulation
- To use stallions of high fertility

It is always important to have in mind that aged mares need more care related to well being, such as differentiated feeding, to avoid environmental caloric stress and social stress as well as they should remain in pickets close to reproductive treatment center to avoid long courses.

Finally, other problems such as: non accomplishment of a proper andrologic evaluation of stallions to determinate the fertility capacity, improper treatment of semen to be refrigerated and transported, use of material improperly sterilized (catheters and others), manipulation inaccuracy during the uterine washes for embryos collection which after years of collections injures the cervix and excess of uterine treatments are usually observed in TE programs in Brazil and should be avoided.

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