

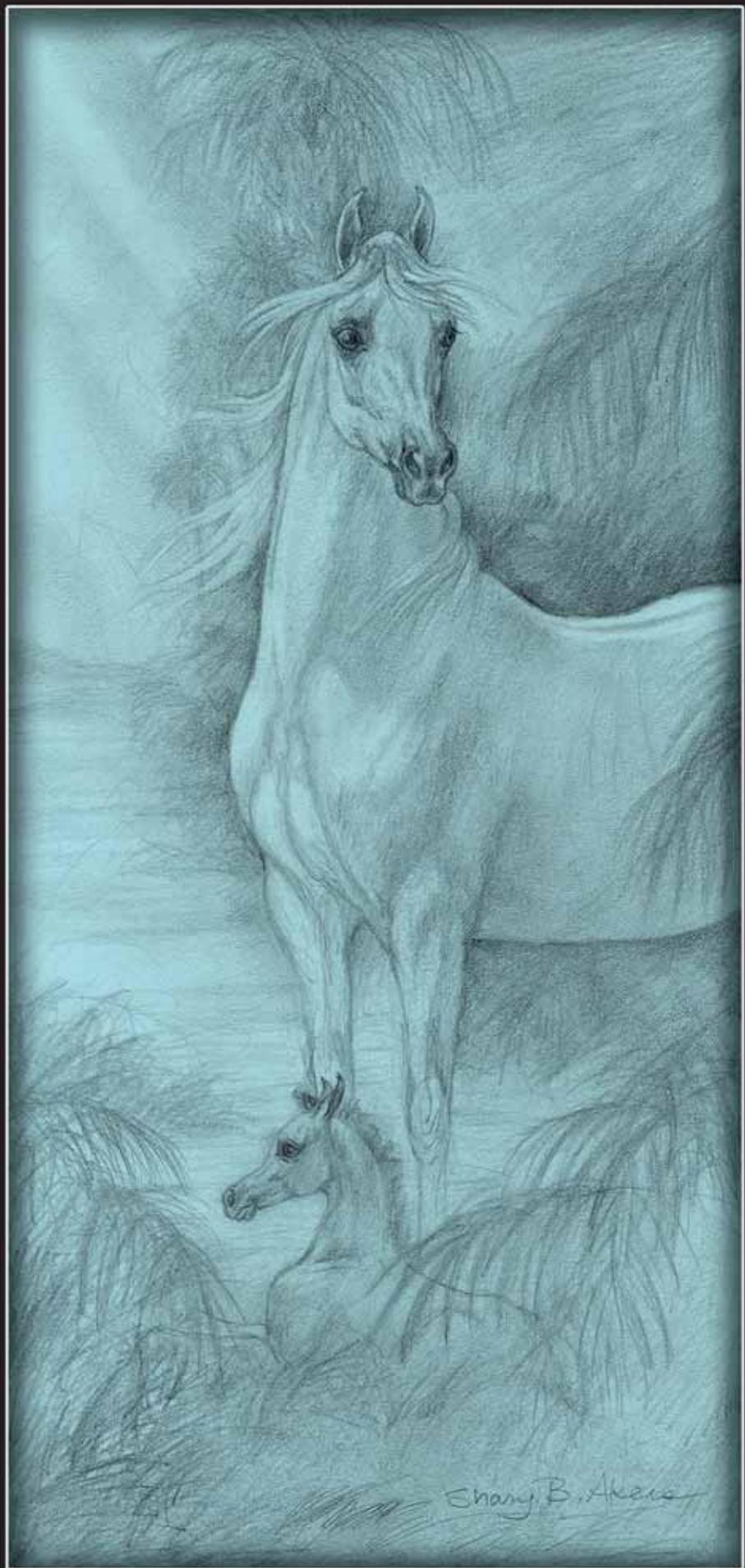
# Equine Embryo Transfer

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photos by Shary B. Akers

## Introduction

The embryo transfer (ET= embryo transfer) is a breeding method or reproductive technology, where an embryo is flushed from a donor mare and implanted into another (recipient) mare for development and foaling. The liquid containing the embryo collected from the donor mare can be transferred into a younger or genetically lower mare (recipient), healthy in terms of reproduction. ET is considered successful when the pregnancy can be established and maintained, resulting in the birth of a live offspring having the donor's genetic background of the stallion's sperms and mare's ovum.

For the equine field, the all story had begun in Cambridge with the participation of 'Twink' Allen in the early 1970s. However, the first successful reported foal produced by ET had been observed in 1974 in Japan, surgically, by Oguri and Tsutsumi. The first foal resulting from the transfer of a frozen-thawed embryo was also born in Japan in 1982 under the care of Yamamoto and his team. Then, a series of conferences on equine ET was initiated in 1985 and has been held every 4 years since, with proceedings published as supplements to the *Equine Veterinary Journal* and, more recently, by the Havemeyer Foundation. Since the last three decades, the developing of ET and associated technologies as super ovulation, embryo freezing, in vitro fertilization (IVF), oocyte transfer, gamete intrafallopian tube transfer (GIFT), and oocyte freezing had never stop growing. Today, the collection and transfer of fresh and cooled embryos are permitted in most breeds. As countries strongly involved in equine embryo transfer we can mention: United States (~1500 foals/years), Argentina, and Brazil and some others with less extend like Australia, Canada, Italy, Germany, and France.



## Développement

*Many arguments can explain the attraction in using equine ET. For example:*

*Show and performance mares can still compete;*

*Increasing ecological fitness (foals/mare/lifetime);*

*Older mares with or without poor reproductive histories (unable to foal) can now foal;*

*Represent an alternative solutions for high genetically mares unable bring a foal to term or passing through the parturition's process;*

*Plan for optimal intra (genetic profile) -and extra-uterine (maximum milk production) environment;*

*Not as stressful (some mares get stressed when pregnant);*

*Conserving genetic diversity and quality for the future by freezing the embryos;*

*Help for the breed of exotic equids;*

*Diminish the breeding process cost considering the significant lower price of transporting embryos than live mares overseas;*

*Decrease international concerns linked with disease control.*

*What is involved in ET*

*From a practical point of view, to obtain positive results in a horse's program for ET, many all-fundamental steps need to be accomplished. Then, while considering those steps, important questions need to be answered.*

*Choose, evaluate and ensure the reproductive health of both donor and recipient mares.*

***What kind of mare makes a good embryo donor?***

*The ideal embryo donor is a mare with a high genetic potential, young, healthy, in good physical shape, normally cycling and free of reproductive abnormalities. If a negative answer is given for one of these conditions, examination must be performed and any abnormalities treated before starting the process. So, to attempt embryo recovery from old, barren mares or mares with reproductive disorders, we have to be aware that probability of success will be decreased. The decision to proceed in this situation should depend on the potential value of any foal that may result.*

*What kind of mare makes a good embryo recipient?*

*The choice of the recipient mares is probably the most critical factor affecting pregnancy rates. Recipients mares should*

*have a good general health, be young (five to ten years of age), have a breeding record, have no history of uterine infection and ideally be larger than the donor to provide a largest uterus and maximize foal birth weight.*

*In a recent study, Carnevale and her team discussed that the most important factors in selecting the recipient are the tone of the uterus and tone of the cervix prior to embryo transfer. Mares that have excellent uterine and cervical tone have higher pregnancy rates than recipients with marginal uterine and cervical tone. The same Carnevale team in 2003 also reported that the transfer of in vivo-matured oocytes from young, healthy mares into a recipient's oviduct results in a 70-80% pregnancy rate compared with a 30-40% pregnancy rate when the oocytes are from older, sub fertile mares.*

*Synchronize the ovulation stage of the uterus into which the embryo is transferred (recipient mare) with the uterus from which it was collected (donor mare).*

***Why synchronize ovulation stage?***

*One of the main reasons to synchronize ovulation stage is that the uterine environment and the levels of hormones of the recipient uterus must be very similar to the one of the donor. To achieve this, hormone therapy (PGF2 ?) is normally used for both, donor and recipient. They are generally treated the same and the organization is generally draft this way:*

*Day 0 PGF2 ?*

*Day 2 Oestrus may start*

*Day 3 Oestrus*

*Day 4 hCG (human chorionic gonadotropin), Oestrus*

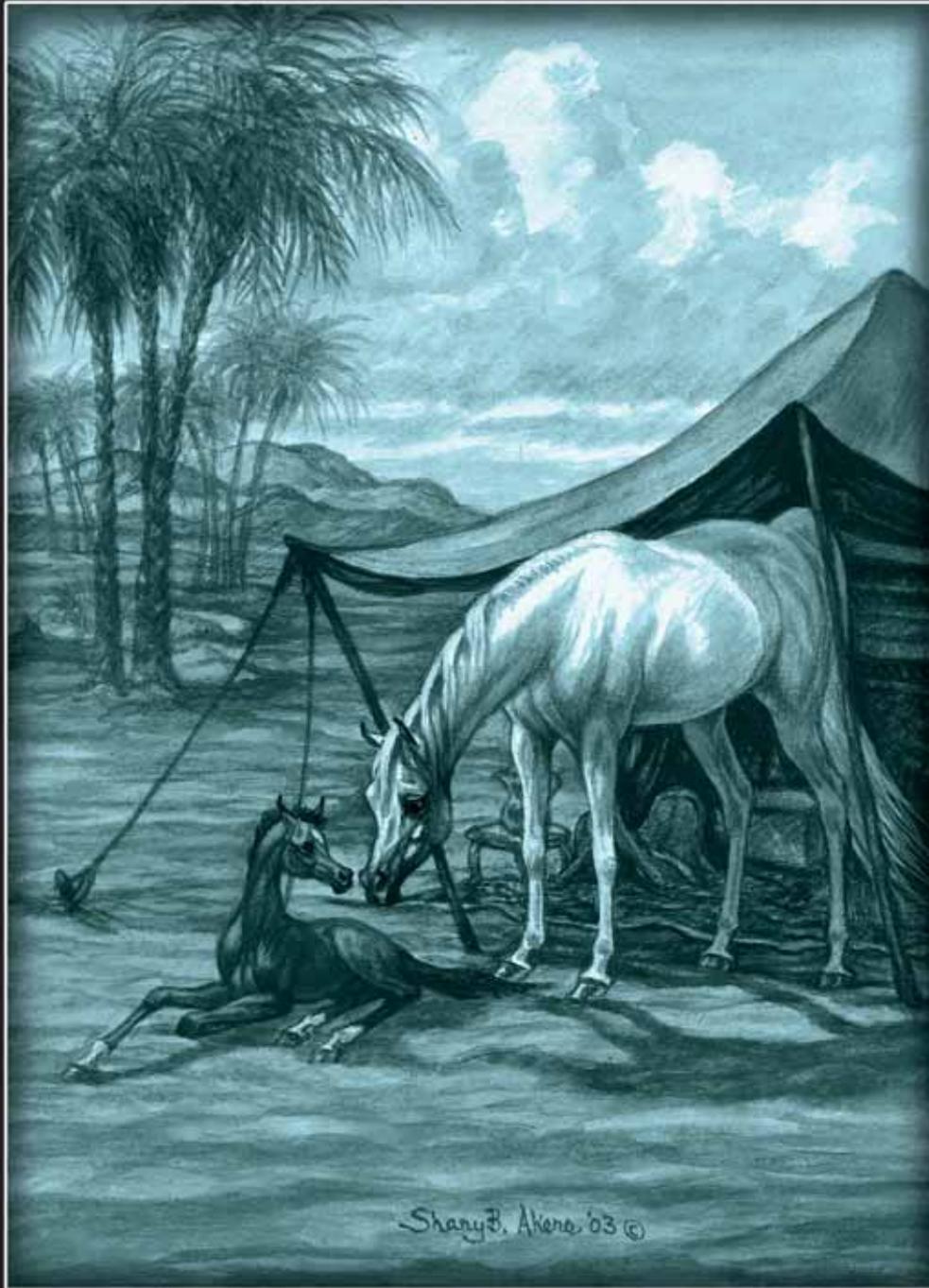
*Day 5 Ovulation may occur*

*Day 6 Ovulation may occur*

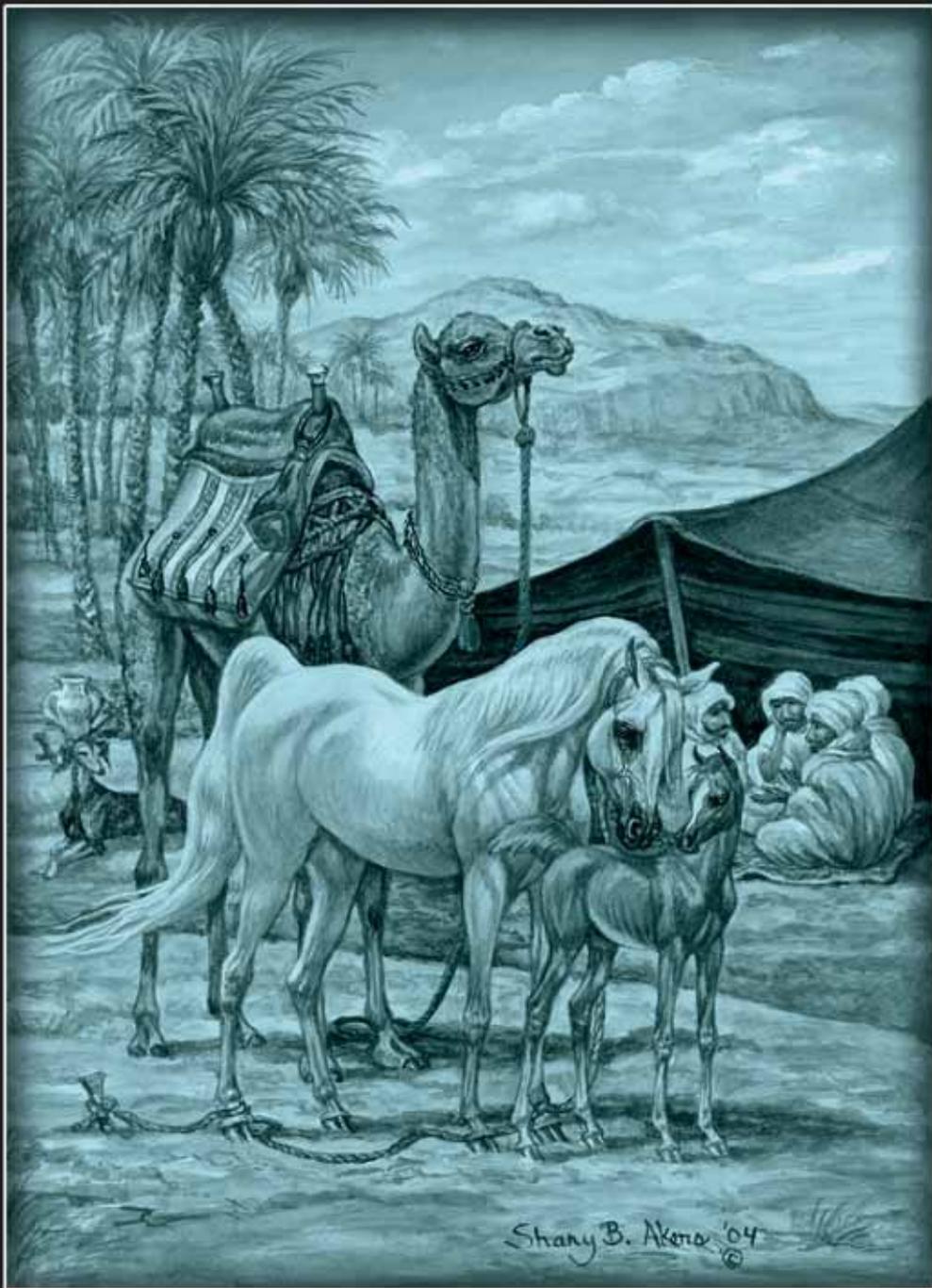
*Day 7 Ovulation may occur*

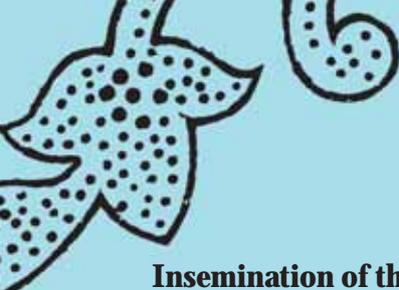
*Day 12 Embryo collection and transfer*

*Unlike ET in other species, the majority of equine embryos, as for Arabians, are collected from single ovulating mares, as there is no commercially available product for super ovulation. This method involving induction of multiple ovulations could be useful in future for example to diminish the cost of the operation by increasing the number of embryos recovered per donor. However, like Squires and co-workers stated in 2003, pituitary extract rich in FSH, can be used today to increase embryo recovery three- to four-fold.*



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## **Insemination of the donor**

### *How is an embryo produced?*

Through the breeding season, the mare's reproductive cycle has a predictable length of approximately 21 days. A follicle is developed on the mare's ovaries. During the mate period, follicle (sometime two) grows, matures and releases an ovum in the uterine tube which can be fertilized by the stallion sperm, naturally or artificially, resulting in a zygote. Then, the zygote becomes step by step an embryo by undergoing cells division on his road for the uterus. Usually, only viable embryos are transported into the uterus. Twenty-five days after ovulation, the embryo stops to move freely and get attached to the uterine lining.

### *Use artificial (IA) or natural insemination?*

First of all, both the donor and recipient should be teased, rectally examined and scanned by echography to ensure that they have reacted to the synchronisation programme and ovulated. No evidence in research had showed till today significative differences in term of pregnancy rate between the two methods. Actually, only practical and logistical concerns are involved in the decision. But, in either case, it is important that the time of ovulation is known within 12 hours for the program efficiency.

## **Donor and embryo's collection**

### *When are embryos collected?*

Embryo recovery is usually attempted between days 4 and 8 in the uterus of the donor mare, where day 0 is the day of ovulation. According to the idea, Carnevale's team made in 2000 a study on 638 equine embryos transferred. They reported that 7 or 8 days after ovulation, for embryos (fresh or cooled/transported) transferred by surgical or nonsurgical techniques into recipients ovulating from 5 to 9 days before transfer, pregnancy rates was 65.7% after 12 days of gestation and 55.5% after 50. They also noted that embryo loss rates were significantly higher in recipients used 7 or 9 days versus 5 or 6 days after ovulation. McKinnon and his team in 1988 also reported highest pregnancy rates when the recipient ovulates either the day before the donor, or up to 3 days after the donor mare.

### *How are embryos collected surgically?*

Surgical ET has to go through abdominal surgery. Mare receives general anesthesia and is laid with her ventral side up. Uterus is exteriorized through ventral midline incision in the area between the mammary gland and position of

umbilical chord attachment. Uterine horn is ligated at uterine body end and a fluid is flushed from oviduct towards horn where the collection can be performed.

### *How are embryos collected non-surgically?*

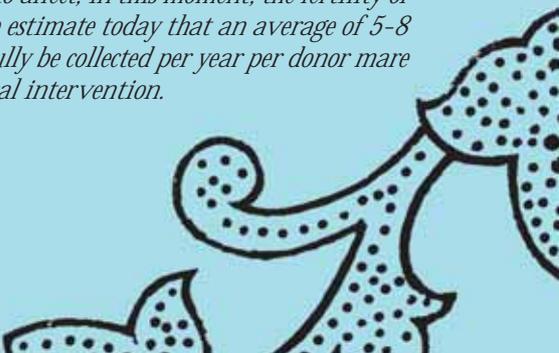
Non-surgically ET consists in a uterus wash of a pregnant mare that gives the embryo (donors). A catheter is introduced through the cervix of the donor mare and passed high up into the uterine horn. Fluid is flushed in through the entry catheter, up into the top of the uterine horn, and then returns, along with any embryos present, via an opening into a tube for collection.

### *What should be considered?*

The technique that had been used in the first stage of the embryo transfer was performed surgically. Today, most of the embryo collection and transfer are done in a non-surgical way (uterine wash) as it involves less danger for the donor mare; reduction of aftercare, lower operating cost and allows multiple recoveries. In an other case, this choice should also consider the age of the embryo. As a matter of fact, younger embryos should be collected surgically (less than 5 days) and older ones non-surgically (more than 5 days) to increase the chances of success in both cases. Another essential point to take in account is the mare's reproductive history. As mentioned by Camillo, about only 30% embryos can be recovered from donor's mares that have uterine or oviductal pathology and 80% in the contrary. Moreover, mares inseminated with fresh and high quality semen are more likely to produce an embryo than those inseminated with cooled or frozen-thawed semen. For those reasons, a veterinarian should always verify sperm cell concentration, viability (number of live cells) mobility, and directionality of the donor stallion.

### *How often can embryo recoveries be performed in a season?*

Embryo recoveries can be repeated on consecutive heats. To shorten the interval between collection attempts, or to concentrate collections within a particular time frame, mares can be 'short cycled' using prostaglandins. An injection of prostaglandin after the attempt of an embryo recovery will clean the mare's uterus of residual fluid, which should bring back a normal oestrus. In case of infection, the program must be stop for investigation or treatment. The numbers of recoveries not seems to affect, in this moment, the fertility of the donor's mare. We estimate today that an average of 5-8 embryos can successfully be collected per year per donor mare in case of non-surgical intervention.



## Embryo evaluation and manipulation

### *How to evaluate the embryo?*

After collection, equine embryos can be held at room temperature (35–38 °C) for 2–3 hours. At this right moment, they have to be examined and graded on a value scale from 1 to 5 (1 being excellent and 5 dead) according to the system used by the International Embryo Transfer Society. Embryos graded under 3 are rejected. They should ideally be transferred into a recipient mare or frozen as soon as possible after this manipulation.

### *What means an embryo of quality?*

The attribution of grade is determined by embryonic factors that significantly affect pregnancy rates like: morphology grade, diameter and stage of development. So, in order, an embryo classified grade 1 with no morphological abnormalities will present less death probabilities than a grade 2 embryos with minor morphological changes or smaller than normal for his age according to Carnevale and her team. Based on data collected in the laboratory of Squires and co-workers in 2002, a 70–75% pregnancy rate can be anticipated with nonsurgical transfer of Grade 1 embryos at the initial gestation examination (Day 12) and a 65% pregnancy rate at Day 50. It is also important to remember that the recipient selection (age, day after ovulation, quality on Day 5) significantly affected pregnancy and embryo loss rates, according to Carnevale.

### *Can equine embryos be stored short term?*

If the embryo cannot be transfer immediately in the recipient mares, he can be conserved with either short or long terms storage methods. In short term, equine embryos can be stored for up to 24 hours at 42 °C with careful regulation of temperatures and if holding in a special solution. Alternatively they can be cooled and stored successfully for 36 hours at 4–5 °C. In this case, embryos can be cooled, stored and transported in a receptacle (as used for equine semen). This method gives the advantage to ship the embryos somewhere else for transfer into recipient mares. For example, breeders and practitioners have the option, at least in the United States; to ship embryos in large stations specialized for the management of recipient mares. Carnevale and co-workers first described procedures for cooling and transporting equine embryos in 1987.

### *Can equine embryos be stored for long term?*

Longer term storage is requires 36 hours after embryo recovery. Cryopreservation is the path used to freeze embryos. This necessitates specific control programs and storage in liquid nitrogen. It allows in fact the conservation of the embryos for many years and further their transfer into recipients

mares at the appropriate stage of her hormonal cycle. Of course, this technique add flexibility to equine embryos transfer programs as it could: allow long distance movement, help for preservation of maternal germplasm and genetic materials, increase selection pressure within an entire animal group, increase breeding line regeneration or proliferation and give tools for genetic rescue. But in the meantime, cryopreservation is young and not easy for horses. In fact, the first successful birth of a foal from a frozen embryo was achieved in 1982 by Yamamoto's team, and still need today implications in many directions. First, the majority of equine breed disapprove the registration of foals from transfer of frozen-thawed embryos. Moreover, the difficulties found with super ovulation in horse result in very few extra-embryos available to be cryopreserved which diminish the possibilities in term of development. Then biologically, as reported by Legrand and others in 2000, the equine embryo is protected by a unique protein membrane called the capsule, which obstructs penetration of the cryoprotectant and makes the freezing of blastocysts and expanded blastocysts quite difficult. Most of the researches implicated in this field suggested better results for smaller embryos (days 6 after ovulation) than the larger embryos (days 7 after ovulation). Additional studies are needed to refine the techniques for freezing embryos collected from mares 7 or 8 d after ovulation. Non-toxic cryopreservation agent is also yet to be found. In term of actual practice, the pregnancy rate with frozen embryos is obviously lower compare with fresh transferred embryos.

## Transfers of the embryo

### *How are embryos transferred in the recipient mare?*

The transfer of embryos to another equine uterus for pregnancy can be done either surgically (USA, Brazil) or non-surgically (only this one is used in Europe), which is recognizing to be the easiest and fastest method limited to embryos older than five days. Surgery, that requires a general anaesthetic, is generally used only in the transfer of young frozen embryos. In both cases, to aseptic is strictly asked to protect the mares from infections.

## Requirements for ET

Requirements for ET could be different from a country to an other, and can vary from time to time. For this reason, having contact with WAHO (World Arabian Horse Organization) for further informations could be necessary. Generally, an ET permit is required for each purebred Arabian mare used as a donor for embryo transfer. The required fee has to be paid. Donor mare's blood type has been placed on permanent record with the Registry. A horse that is the result of an embryo transfer may be registered. The blood type

of the horse has been placed on permanent record. Often, the horse must qualify as an offspring of the stated sire and dam through blood testing. An embryo must be transferred to a recipient mare within three days of the collection from the donor mare. Only one horse registration per year is often permitted from the donor mare.

If the recorded owner of the donor mare sells the embryo prior to the birth of the horse, the Certificate of Registration will be issued in the recorded ownership of the purchaser in accordance with the terms of the Embryo Sale form.

## Conclusion

The need for new assisted reproductive techniques for the horse will be growing in the future for a large number of species and breeds, including Arabian horses. This will step by step change both the market and the "ethical" perception about these techniques. Some items are in fact still questionable, and need further detailed studies, for example the impact of repeated hormonal treatments on donor mares. From this point of view, equine ET has a large potential,

still mostly unemployed, and it is easy to say that it will be more and more used for the different purposes listed in the first part of this article.

Some knowledge is however still lacking, in particular in the cryopreservation and super ovulation areas, that will be the key point for successful commercial application of this technique.

Embryo sexing could also be an important point for further large scale application of equine ET.

Several factors can also play a pivotal role in the further use of ET for Arabian horses, including the value of the horses used, the performance of the foals produced by embryo transfer; the cost of the procedures, the refinement of the techniques and the attitude of the equine industry to its application.

Just a few words about breed registration restrictions: they are still present in the rules of most breeding society. We really think that these rules will undergo a revision in the next years. The key point is to take into due account the scientific backg. □

