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Maximilian Mitteneder

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University of Veterinary Medicine
Institute of Animal Breeding, Nutrition and Laboratory Animal Science
Department for Animal Breeding and Genetics

Application of a modern reproductive technology in horse breeding: a careful analysis of a successful embryo transfer

by Maximilian Mitteneder

Supervisor:
Dr. András Gáspárdy
Assoc. Prof., Head of Department

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1 List of abbreviations

ET	Embryo transfer
DM	Donor mare
OPU	Ovum pick up
ICSI	Intracytoplasmic sperm injection
AI	Artificial insemination
DSO	Daily sperm output
PGF2a	Prostaglandin F2 Alpha
hCG	Human chorionic gonadotropin

2 Introduction

This paper describes a successful ET in horses, since it became an important and common field in veterinary medicine nowadays.

We live in a time of specific scientific work and acceleration. Modern horse breeding has to enclose multiple factors, like the timing of ovulation and insemination, ancestry, the usage of the horse and also what type of breed is used. To achieve higher efficiency, all these factors were considered and included in a successful embryo transfer, which is a precise, short time and overall, not too expensive reproductive procedure. The success rate of an ET depends on the way of insemination and quality of the semen, as well as the age and fertility of the mare and recipient. This is actively demonstrated by using fresh semen. The recovery rate covers 60-77%, in chilled semen 44-52% and in frozen semen 33-46%. Using young and fertile mares provides a 70-75% success rate while using mares older than 15 years results in 40% and less (Sieme, Tönißen, Köhne 2019)[i]. In total the success rate is a value of 35 to 80 %.

One important factor considering embryo transfer is a horse's versatile use. For instance, their value in breeding due to their good ancestors or their character, as well as their behavior and talent in special directions of sports (dressage riding, show jumping, carriage driving, eventing, polo et cetera). Science and medical expertise enable success in the mentioned fields, even though the mare isn't carrying the foal herself which would result in missing important sporting events for years while being used for breeding.

Proper selection is another important detail in fertile mares and recipients as well as high hygienic standards during the procedure. The inseminated mare and the recipient have to be synchronized in cycle and daily ovulation controls must take place. After a successful insemination of the mare the uterus is flushed, whereby a light microscope is used to detect the embryo afterwards. If the flushed embryo has to be transported (packed in Equitainer), a cooled (+5°C) or a deep-frozen transport can take place.

After the transfer of the embryo into the selected recipient, good management is key to a high success rate which lays between 35 to 59% in total (Carnevale, Ramirez, Squires, Alvarenga, McCue 2000)[ii].

3 Aims

The aim of this paper is to reflect the procedure of a successful embryo transfer in horses. Including the precise selection of the mare and recipient (fertility and age), the collection of semen of the sire as well as materials needed. To increase the success rate of an embryonic transfer, a very accurate timing of synchronization, insemination and uterine flushing is necessary, which are analyzed and evaluated. For this purpose, with the help of Dr. Thomas Pfefferle (Insemination Center PBM GmbH, Mengen, Germany) the embryonic transfer was performed and evaluated.

Above all of that, however, the main goal is to give an overview of the already existing scientific work in the field and newly won impressions to improve upon horse breeding since its first steps in embryonic transfer from the early 1970´s (McCue, Squires 2015)[iii].

4 Horse as a special breed for embryo transfer

4.1 Purpose of an embryo transfer

Choosing an embryo transfer in horses can have several reasons, the most important one being their multifactorial use (Embryotransfer, FU Berlin)[iv]. To give a little overview they are listed on the below (I-VI):

I. A main goal is to use a mare for competition and breeding due to its good behavior and talent, as well as her excellent ancestry. Due to the fact that her embryo is flushed and inseminated into a recipient, the breeding season for the mare is still useable even though she is not carrying the foal herself. The mare is still able to be a part of the competition season but will also have at least one offspring a year.

II. Young mares (> 2 years) often do not have the anatomical precondition and development to carry a foal already. With the help of an embryo transfer it is possible to use a filly for breeding without harming a young mare.

III. Some mares are also not able to give birth to a foal themselves or are unable to carry a pregnancy. Among other reasons, this could be due to endometrial cysts, pelvic fractures that cause a narrowed birth canal or a cervical laceration which causes the cervix to not seal properly, which can cause an abort.

IV. Another significant point to mention is to obtain several foals per year, to achieve optimal utilization of fertility in high value mares.

V. Producing embryos for freezing and longtime storage is also a good opportunity preserve a bloodline via embryo transfer. Embryo sexing or the detection of diseases is also realizable but uncommon.

VI. In addition to this, nursing mares that are late in season are still able to have offspring without being delayed for the upcoming season. Also, ET provides an opportunity to let the mare's body rest without having a year off from breeding.

4.2 Famous breeds

When the first ET took place back in the early 1970`s the procedure was set as a test to revolutionize the horse breeding field (Samper 2007)[v]. After implementing the first success a new medical and scientific field was created. When comparing breeds used for ETs, distinct countries should be differentiated.

Figure 1 shows the famous Argentine Polo Pony, which was used for ET from 1980 on in Argentina. A successful embryo transfer in these sports horses enabled a versatile use for both, their breeding and sports career (https://animals.mom.com/breed-horse-used-polo-11421.html Accessed: 03.03.2022).

Figure 1- Argentine Polo Pony

Figure 2 illustrates a Hannoveraner horse, a so-called warmblood, which is used in many sporting directions like dressage riding, showjumping and carriage riding mainly in Europe. Warmblood mares cover a high percentage of horses used for an ET in Europe, especially in Germany (https://www.zooplus.de/magazin/pferd/pferderassen/warmblut Accessed: 03.03.2022).

Figure 2 - Hannoveraner Horse (Warmblood)

Figure 3 indicates an American Quarter Horse, which is mostly common in America, Brazil and Europe. It's a very athletic horse that is used for western riding competitions, which leads to its high use for ET (https://www.britannica.com/animal/American-Quarter-Horse Accessed: 03.03.2022).

Figure 3 - American Quarter Horse

4.3 Breeding selection and development

Selection of mares for ET follows modern economic parameters such as efficiency, affordability, success rates and monetary value. A lot of money flows into horse breeding, as well as into the sporting career of a horse. Therefore, it is extremely difficult to balance income and expense in order to ultimately make profit out of a foal established via ET. Therefore, when choosing the recipient mares, a precise analysis of the anatomy, breeding experience, age and previous illnesses is taken into account. A healthy cycle in the recipient mare, which is synchronized with the donor mare, paves the way to a successful ET. Since the costs are borne by the buyer, an initial positive result of the flushed embryo after the transfer is required to obtain pregnancy. This can be established by good stabling (nutrition, society, activity), above all, hygienic conditions and a psychologically healthy environment must be created. Particular attention must be paid to continuous medical monitoring, which is also of economic value (embryo monitoring, ultrasound, vaccinations, deworming, birth control).

Important facts of the donor mare are key data of its pedigree and career (both in sports and breeding), as well as behavior and character to justify an embryo transfer. Genetics and reproductive experience provide a good basis for ET; however, some donor mares are already too old to carry a pregnancy, show physical deficiencies or achieving great successes in their sporting carriers currently (Campbell 2014)[vi].

The focus here is on the timing of fertilization in order to detect ovulation early and thus generate a high pregnancy success rate. This also requires good medical care and patience.

If you combine all these details for the selection of the donor and recipient mare and meet the financial requirements (**Chapter 7**) for an ET, great success can be achieved.

5 Reproduction

Reproduction has the highest priority in horse breeding and management. Due to a horse's high economic value, the mares and stallions must undergo specific investigations to qualify them for a breeding career. Especially the semen quality of a stallion is important when it comes to an artificial insemination, which is one option to impregnate a mare before proceeding with an ET.

All in all, favorable anatomy and genetics of a horse is needed to achieve success in horse reproduction. Other notable factors influencing the success rate are, for example, nutrition, general health and social behavior (Little, Holyoak 1992)[vii].

5.1 Stallion reproduction

A stallion is a male horse that is able to be used in reproduction. From the age of 1 year, some males are already able to produce progeny. This chapter gives an overview of a stallion's most important anatomical structures and shows some important key data that influence and/or enable a successful embryo transfer (sperm quality, amount, age) (Pickett, Voss 1972)[viii].

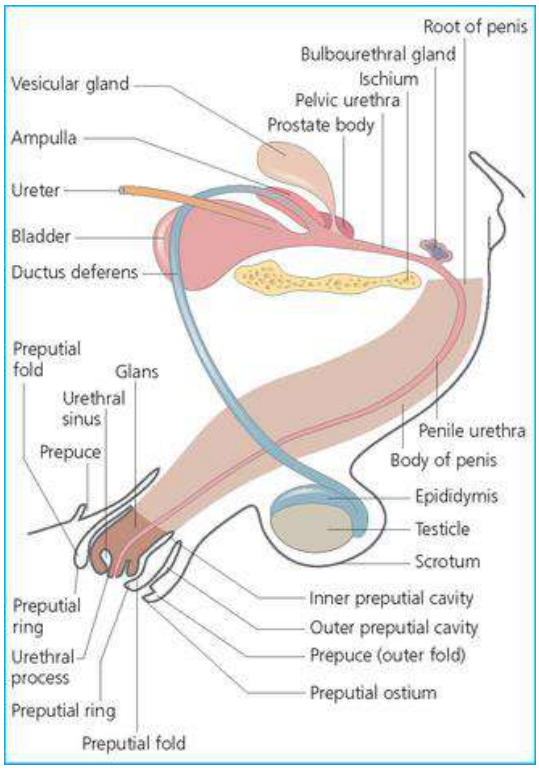

Figure 4 - Reproductive anatomy of the male horse (Lopate et al. 2016)

The illustration (Figure 4) depicts the anatomical structures of a male horse in a lateral view. It includes two testicles and their associated ducts, which can be discovered in the scrotum, two epididymides and their spermatic cords as well as two vas deferens with their ampullae.

Testicles produce the hormones testosterone and dihydrotestosterone and are the location for spermatogenesis, the production of sperm. Covered by connective tissue, the two testes are loosely inside the scrotum, which can be divided into 2 sacs, one separating septum and four layers: the skin, tunica dartos, the scrotal fascia and the vaginal tunic (SynlabVet 2018)[ix].

The epididymis can be fractionated into head (caput epididymis), body (corpus epididymis) and tail (cauda epididymis). Overall, the duct has 70 meters in length and functions in storing sperm in the terminal cauda epididymis till the ejaculation occurs, produce important secretions for the maturing of the spermatozoa and resorbs fluids into the caput (Lopate, LeBlanc, Knottenbelt 2016)[x].

Having a look at the accessory sex glands, we have to mention the outer bulbourethral gland, the prostate gland in front of the seminal vesicle, which then leads to the ampulla (Figure 5).

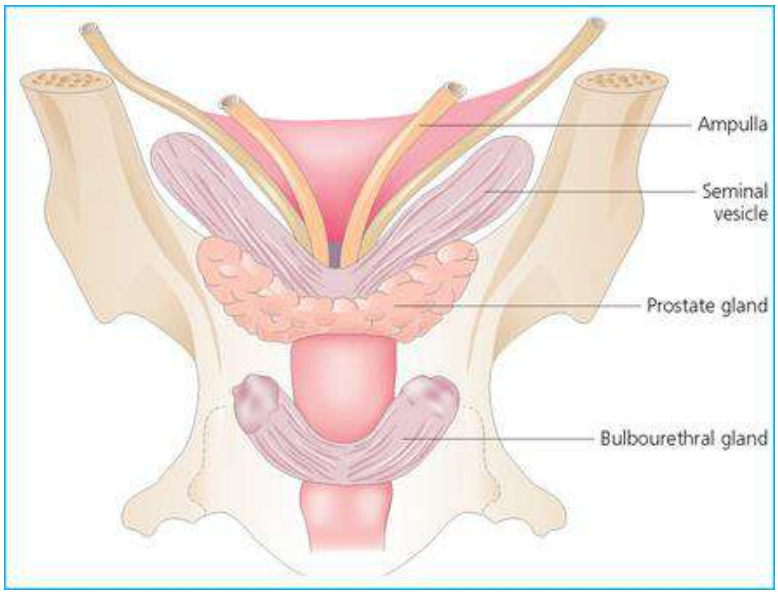

Figure 4 - The accessory sex glands (Lopate et al. 2016)

Male horses are seasonal breeders, which leads to an increase of Leydig - (production of testosterone) and Sertoli cells (blood-testes barrier) during breeding season, as well as more spermatozoa/g of testis. By getting older, a stallion loses its fertility due to a decline in Sertoli cells/g of testis, called a testicular degeneration (Lopate, LeBlanc, Knottenbelt 2016).

Analyzing the sperm quality and quantity (via light microscopy) before choosing a stallion for an ET is a minimum requirement. This is also affected by the stallion's age, which is preferably between 6 to 9 years (highest fertility in this age range). Younger than 6 years of age, the maturation of a stallion isn't fully completed yet.

Collecting daily semen in stallion, the sperm output varies from 3.2 to 6.6 billion spermatozoa per ejaculate. The frequency of a male's ejaculation, also his age, testicular size and the season influences his daily output of sperm. Nutrition, environmental conditions and the hormonal status of the stallion should also be taken into account, as these can secondarily influence the spermatic output.

To calculate the daily sperm output (DSO), the following formulae can be used:

$$DSO = (3.36 \times 10_9) + (0.066 \times 10_9 X)$$

X: scrotal width in mm

By using supportive diagnostic tests (radiography, ultrasound, blood sampling), the normal physical examination can be supported to prevent some unseen findings that could influence the quality of a stallion in breeding.

Stallions that qualify for a breeding career are so called "satisfactory prospective breeder", which means they are expected to impregnate 75% of mares naturally, or 120 artificially inseminated mares in one single breeding season.

5.2 Mare reproduction

A female horse is a polyestrous seasonal breeder, their sexual cycle is controlled by the amount of light a day, causing their estrous cycle to normalize in the end of April to the beginning of May until mid-August. Mares are sexually mature at the age of 18 months. At this age, they produce wavy-like follicles with a diameter of around 20 mm which are not viable ovulation follicles yet (selection of a follicle for AI with a diameter of < 35 mm). From then on, their anatomical structures are still improving until the age of 3 years, when they become mature and grow to their genetic potential (Aurich 2009)[xi]. Figure 6 shows a schematic drawing of the female horse's reproductive system in sagittal view.

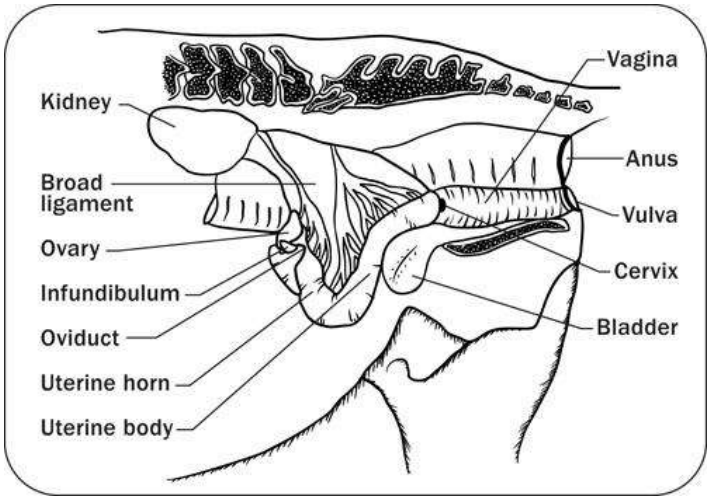

Figure 5 - Sagittal view of the mares' anatomical reproductive structures

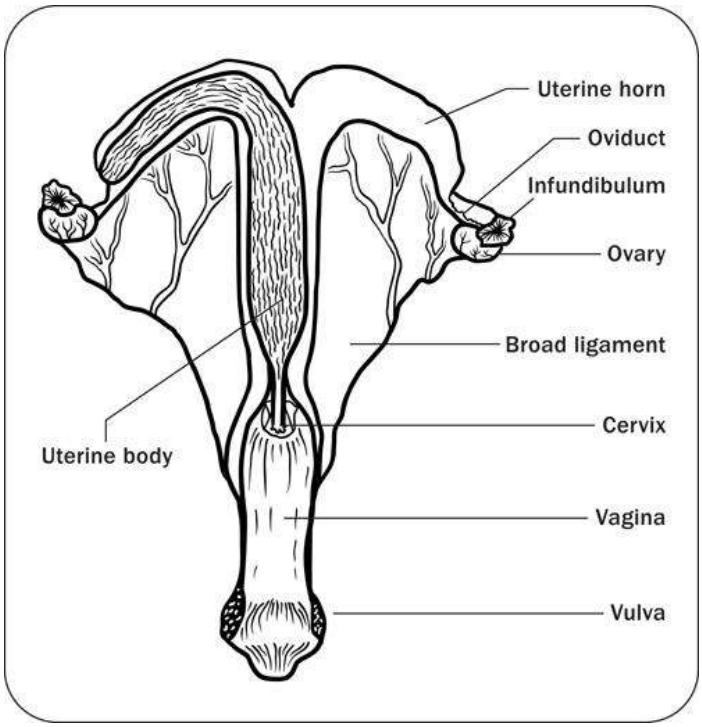

Figure 6 - Frontal view of the mare's reproductive tract

Figure 7 shows the external opening (vulva) of the urogenital tract of a female horse, which enters the birth canal in between the uterine body and the vulva. The uterus divides into two uterine horns that finish in the oviduct (sperm transport), connected in position with the ovaries (production of the ovum, estrogen and progesterone). Most of the embryonic development and supply takes place in the uterine body. The embryo moves through the whole uterus in the beginning of the pregnancy and is flushed out of the donor mare in an ET from here. The cervix has a length of around 10 cm and provides a sterile environment in the uterus by opening (in heat) and closing (not in heat or pregnancy) (http://omafra.gov.on.ca/english/livestock/horses/facts/10-099.htm Accessed: 08.09.2022).

A mare's estrous cycle has a length of 21 days (± 3 days), divided into an estrous- and diestrous phase. The estrous phase normally lasts for 6 days (4-10 days), while the diestrous phase persists for 15 days (12-18 days).

During estrous, the follicle matures until ovulation takes place, also the production of estrogen can be documented. In diestrous, the corpus luteum is formed and progesterone is produced. It can be established via ultrasound or rectal palpation. If the mare was not fertilized, the corpus luteum regresses from the endometrium under the influence of PGF2a and the mare comes into heat again (Samper 2007).

Even though a mare's estrous cycle and timing of ovulation is perfectly calculated and documented for fertilization and we are assuming a normal stallion fertility, sporadic subfertility in breeding mares happens. This can be linked to unseen obstetric injuries, bacterial or viral infections, fluid accumulations, nutritional and hormonal problems as well as age-related diseases and changes in genital organs. Furthermore, a failure of ovulation of follicles can be seen due to normal physiological processes or pathological incidents. For that reason, a detailed and well-structured reproduction management, physical examination before and after a breeding season, vaccinations and general health check-ups are necessary to increase the success rate for maximal breeding efficiency.

5.3 Artificial fertilization

Artificial fertilization is a method in reproductive medicine, in which the previously obtained sperm of a fertile stallion is introduced into the uterus of a recipient mare with instrumental support. This procedure is very common in horse breeding and offers various advantages, for instance the safety for the mare, a hygienic insemination of the semen and an exact data acquisition of the mating date (Walter, Weisbeck, Bleul 2017)[xii].

The semen of the breeding stallion gets collected at an insemination station. Further, the procedure is dependent on the use of the semen. There are possibilities of using the fresh semen for insemination immediately, chilled semen that can be cooled at 4°C for 3-4 days and frozen semen, which will be analyzed in the next sub-item (5.4).

A basic insemination kit for frozen semen includes a stainless-steel AI gun, a scissor, tweezers, non-spermicidal lubricant, a thermometer and a thaw unit (water bath).

Via rectal palpation, the mares' reproductive organs can get examined by the vet. It is important to control the uterus, the uterus horns and the ovaries. Follicle and/or corpus luteum can be detected, for safety reasons an ultrasound is used. The best timing for an insemination is 36 hours prior to until 6 hours after the ovulation. It is possible to induce ovulation by administering PGF2a (or Regu-mate equine) 24 hours in advance of the actual AI to increase the success rate of impregnating the mare.

Procedure:

a. Emptying the rectum to fulfill a rectal palpation and to guide the insemination pipette
b. Ultrasound of the reproductive organs to confirm ovulation of the mare
c. Insemination pipette, like shown in Figure 8, is inserted vaginally, goes through the cervix into the uterus. The syringe on the outer part of the pipette is constricted to insert the semen into the uterus (fresh and chilled semen)
d. Frozen semen is mainly implanted into the uterus horns right in front of the oviduct to secure a higher chance of success
e. Fertilization occurs around 4 hours after the insemination

Figure 7 - Insemination pipette (Picture by Maximilian Mitteneder)

5.4 Sperm freezing

Cryopreservation enables the protection and storage of horse semen for short and long periods of time. After the collection of the semen, a short cool-down is needed before storing it at minus 196 °C in liquid nitrogen. It is very important not to interrupt the freezing process unless the semen is used, otherwise it may be damaged and rendered unusable.

Sperm freezing brings a lot of benefits with it e.g., long storage, shipment of special and valuable semen all around the world, saving good genetics and the collection of semen is possible at any time.

On the negative side, frozen semen has a low success rate (33-46%) in embryo transfer. After a cryopreservation, the semen has a limited survival of max. 48 hours, with the highest chances of fertilization being 8 hours before and 6 hours after ovulation (Sieme, Tönißen, Köhne 2019).

5.5 Embryo freezing

After the rinsing of an embryo, a morphological examination is done to check the number of vital cells. Then the freezing process takes place:

The embryo is placed in a freezing medium with the addition of some cryoprotectants (such as glycerol, sucrose or propanediol). Before the embryos are completely frozen, they are packed in straws with an additional volume of 0.2-0.5 ml. An automatic freezing control then starts the cooling process from 22 °C to minus 3 °C within a few minutes. Ice crystallization (seeding) takes place automatically from minus 6 °C on. After the embryo has gotten used to the low temperature for 10 minutes, further freezing takes place at minus 0.3-0.5 °C per minute until a final temperature of minus 30 °C is reached. After this process, the freezing turns into liquid nitrogen at minus 196 °C, (like normal sperm freezing). When the frozen embryo is needed for an ET, it is warmed to 37°C in a water bath, which dilutes out the cryoprotectants (Samper 2007).

Basic preservation of embryos with a diameter ≤ 300 µm has proven to be successful. Embryos of this size are flushed and recovered on day 6.5 post ovulation. It must also be considered that the percentage of dead cells increases with the use of cryoprotectants, since they affect metabolic processes in the embryos. An exact number of successful frozen and implanted embryos cannot be given, since the success rates range between 0 to 60 % in total (Carney, Squires, Cook, Seidel, Jasko 2003)[xiii].

6 Embryonic transfer (ET)

6.1 Definition

Embryo transfer as a reproductive technique includes the extraction of embryos from the uterus of donor mares, the assessment of the embryos and the accompanying transfer of the embryos to recipient animals. Various associated techniques (e.g., preservation of embryos, in vitro production of embryos, cloning, intracytoplasmic sperm injection) may be integrated into the embryo transfer procedure (Aurich 2009).

6.2 Selection of recipient

A recipient mare is a mare selected through clinical pre-testing, into which a flushed embryo is implanted. The recipient must meet some important criteria e.g., high fertility, good general health and nutrition, good lactation and mothering ability and if possible, same size or greater than the donor mare (prevention of birth complications).

It is important for a recipient to be relatively young (3-10 years of age) and to indicate a normal reproductive cyclicity (Ball, Little, Weber, Woods 1989)[xiv].

A fact that is often forgotten is that the recipient takes on the entire part of raising the foal. Her behavior and character form the foal's attitude, only good heredity and thus the genetic components are not sufficient to obtain a strong character and an above average foal.

The synchronization of the donor and the recipient mare in ovulation, which should be in between -1 to +3 days maximally, are the most important steps in ET (Imel, Squires, Elsden, Shideler 1982)[xv]. In order to create a suitable synchronization of the recipient mare with the donor mare, at least 2-3 mares should be available at that time. If there is no possibility of having several recipients at one time, a synchronization can be achieved by hormonal therapy (6.3).

6.3 Synchronization of cycles

Four basic methods are mainly used for estrous synchronization: luteal phase termination, the length of it, triggering of ovulation and inhibition of the follicular phase. This can be achieved by using the following pharmacological agents: progestins, prostaglandins, deslorelin acetate and estradiol-17 beta. The best success rates can be document by using a combination out of all of them.

A recipient mare is brought into heat by the administration of PGF2a. If a clear estrus can be recognized in both mares, daily follicle checks must be carried out. Follicles > 35 mm are treated with human chorionic gonadotropin (Intervet, Ovogest) to trigger ovulation within the next 36-48 hours post injection. Whenever the ovulation of the donor mare can be documented, the recipient gets induced immediately so that her ovulation takes place latest 2 days after (Samper 2007).

6.4 Donor mare management

The mare needs to conceive to have an embryo, that's why a strict management for the donor mare is needed. A thorough physical examination, vital parameters and bacterial swabs taken from the uterus are essential (McKinnon, Squires, Harrison, Blach, Shideler 1988)[xvi]. Especially the uterus of the mare has to be investigated in-depth due to possible bacterial contaminations, which can be caused by many sources e.g., contaminations during the breeding process or contaminated semen, endometritis due to prior reproductive procedures (inflammations of the uterus). Diagnosed diseases must be treated before the mare can be inseminated (McKinnon, Squires, Vaala, Varner 2011)[xvii].

Good monitoring by the veterinarian is necessary to determine the exact timing of ovulation. In older mares the embryo arrives later in the uterine body (around day 7-8). The embryo flush should therefore take place on day 6,5 to 7 to achieve an embryo survival for the common transfer, this is mainly related to the size of the embryo (This is also illustrated in Table 1).

Table 1 - Timetable of the perfect embryo flush (Sussex Equine Hospital, K. Ducheyne)

Interval from ovulation	Embryos/collections	Positive collections (%)
144h = 6d	0/20	0%
156h = 6,5d	9/17	53%
168h = 7d	12/23	52%

The day of ovulation is called **day 0**. Timing of the embryo flush has high priority due to the embryo's size. Large embryos (> 1mm) are more fragile than smaller ones and it is more difficult to transfer them from one medium onto another, as is the likelihood to damage the embryo.

Table 2 demonstrates the normal growth of embryos after leaving the oviduct and arriving in the corpus uteri, where they receive all the nutrients and start growing exponentially.

Table 2 - Diameter of equine embryos recovered from the uterine lumen (Vanderwall, 2000)

		Embryo Diameter (mm)	
Day post ovulation	**Number of embryos**	**Mean**	**Range**
6	121	0,208	O,132-0,756
7	144	0,406	0,136-1,460
8	142	1,132	0,120-3,980
9	41	2,220	0,730-4,520

6.5 Embryo flush

The embryo flushing takes place 6,5 to 8 days post ovulation. At first, the mare is placed in a stand, where its tail is wrapped and elevated. After cleaning and preparation, a balloon-tipped silicone catheter is placed into the uterus (passing through the cervical opening into the uterine body), leading to a Y-shaped junction on the outer part of the vagina that combines the flush medium and the filter (Figure 9). The balloon is filled with 50-70 cc of air to secure a good seal of the catheter at the junction of the uterus (McKinnon, Squires, Vaala, Varner 2011).

Figure 8 - Placement of the uterine catheter for the embryo flush

(Pictures by Maximilian Mitteneder)

The type of flush medium is a matter of personal preference, hereby the use of 750-1500 ml of Ringer`s lactate solution (isotonic, crystalloid fluid) at a temperature of 30-35 °C is advised.

The procedure of flushing the uterus has to be repeated 3 times, before starting to examine the filter, which is shown in Figure 10, for potential embryos. By manipulating the uterus via rectal palpation, the complete filling of the uterine horns can be achieved. Measuring the returning fluid is important to guarantee the retrieve of at least 90% of the infused fluids to prevent infections with subsequent inflammations.

Figure 10 - 70-75 µm pore size embryo filter (Picture by Maximilian Mitteneder)

Figure 11 demonstrates the remaining fluid of the filter, which is transferred to a sterile Petri dish to be examined under a light microscopy, to identify any embryo. Via rinsing, the contents around the embryo can be washed away to secure a clean embryo. The identified embryos are aspired into a sterile 0.25 or 0.5 cc French straw to transfer it into a wash dish containing filter-sterilized flush medium, where it is washed 4-6 times.

The flushed embryo is now ready for a transfer into the recipient mare or can be stored at room temperature (20-23 °C) for 3-6 hours. Another option is embryo freezing (5.5) or cooling of the embryo (5°C) for transport and transfer within 24 hours (Klewitz, Heer, Behrendt, Probst, Martinsson, Siemen 2010)[xviii]. The cooled embryo should be transported in a small plastic tube filled with 4.5 ml of pre-warmed holding solution, placed in a Equitainer®.

Figure 9 - Embryo filter under the light microscopy (Pictures by Maximilian Mitteneder)

6.6 Embryo transfer

For the embryo transfer, two transfer options are possible: a surgical and a non-surgical transfer. A surgical transfer, in where a standing flank laparotomy is performed, is uncommon nowadays. The transcervical transfer of the embryo into the recipient follows the same scheme as mentioned in point 5.3 (Artificial fertilization). The only difference, that has to be mention, is the embryo being placed in a 0.25 or 0.5 cc French straw implantation cannula with a detachable tip.

After receiving the embryo, the recipient has to be treated with antibiotics and Regumate for 5 days. Monitoring the embryo is very important, especially in the beginning of the gestation. 5-6 days after the transfer, her pregnancy can be confirmed via ultrasound (day 12-13 in pregnancy).

The recipient will be reexamined on days 20, 30, 45 and 60 of the ongoing pregnancy. The highest mortality of the embryo is between the 15th and 60th day of pregnancy.

7 Success rates and costs

Taking all the factors mentioned into account, an overall success rate of 35 to 59 % of an embryo transfer can be achieved. This still depends on the type of mares being used as well as the expertise and experience of the veterinarian.

It can be calculated by the following:

$$Pregnancy\ rate\ per\ cycle = (50 - 65\%) \times (70 - 90\%) = 35 - 59\%$$

50-65 % have been calculated from the success quotes of flushed embryos, while 70-90% cover the pregnancies achieved after an ET.

The cost of a foal produced by embryo transfer is significantly higher than if a foal is produced conventionally. In addition to the financial expenses for the veterinary work (embryo flushing and transfer, synchronization of the recipient mares, ultrasound examinations, insemination of the donor mare), the costs for keeping the donor, the recipient mare and the stud fee must also be considered. Another major cost factor is the acquisition and keeping (including vaccinations, deworming, farrier) of recipient mares.

Ideally, if the donor mare produces an embryo after the first insemination and the recipient mare successfully adopts this embryo, the costs for producing an ET foal, excluding the stud fee but including the rental fee for the recipient mare, amount to around 4,000 euros. In the case of problematic mares, however, these costs can increase drastically, depending on the number of inseminations and flushes.

8 Discussion

Many detailed factors such as the housing conditions of the donor and recipient mare, their physical and psychological well-being, as well as their prior injuries and diseases influence a pregnancy. The implantation of a foreign embryo in particular requires maximum physical and medical performance. Medical expertise and experience are necessary to achieve success in transferring embryos. The lighting of the topic embryo transfer shows many options for modern horse breeders, which offers scope for multifunctional (mainly sports career) uses of the horse. At the same time, an above-average number of medical examinations and implantations in mares are carried out, which in the worst case do not show in pregnancy.
From a financial point of view, by proceeding an ET a loss must always be expected, whereby the selection of donor and recipient mares and the consideration of structured processes promise high success rates. The author's experience shows, that the sperm quality and collection, the timing of the ovulation, the flushing of the uterus of the donor mare (due to the size of the embryo obtained), as well as a continuous follow-up care of the horses involved, speaks in favor of the choice of an embryo transfer.
Insufficient literature confirms a strict process of the procedures, which leaves no room for experimentation. A calculated success rate of 35-59% (including insemination, flushing and transfer) indicates risks and lack of continued pregnancy success (Chapter 7). This must be taken into account when choosing an embryo transfer for horse breeding.
The storage and transport of embryos has taken a big step forward in recent years, whereby here, too old medical processes are benefiting and further innovations are faltering.

In conclusion regarding the literature and the authors experience it can be stated that, overall, the procedure of a successful embryo transfer is an increasing field in horse breeding, which receives more attention nowadays. Despite the financial risks, with good management and precise execution, there are high chances of success to carry out an ET.
It is recommended to consider the influence of the recipient mare on the bred foal in further studies, since changes in behavior can be seen despite having good genetic ancestry. The cost-benefit ratio is questioned here.

9 Abstract

Horse breeding is a multifaceted and constantly evolving field, which is being introduced more and more deeply into veterinary medicine and science. In order to attain insight into modern biotechnologies concerning embryo transfer in horses and to enable their versatile use for breeding and an ongoing sports career, relevant literature was reviewed and compared. Furthermore, an ET station in Germany was visited by the author and the procedures were documented. Since the first embryonic transfer took place in the early 1970`s, the field of horse reproduction/obstetrics has changed completely. The key to good breeding management lies in focusing on a mare and stallion's reproductive quality, which covers their general health, age, nutrition, vaccination, deworming and social behavior. The quality of semen used for insemination also influences the results of a successful procedure (varying from 30-80%), that is why we have to differentiate between fresh, chilled and frozen semen being used. After the mare is inseminated in a precise and hygienic procedure and provides a positive result, her embryo gets flushed out of the uterus 7-8 days post ovulating. The flushed embryo has to be controlled and examined via light microscopy and can be planted directly into a chosen recipient, which is synchronized in estrus and ovulation like the donor mare. Otherwise, it can be cooled (+5°C) and transported within 24 hours, as well as being stored longtime as a frozen embryo. 12 days after the transfer of the embryo into the recipient, the pregnancy can be confirmed via ultrasound.

The collected literature data show, that a detailed and continuous management is needed to achieve a successful embryo transfer in horse breeding to improve the success rates constantly, which also facilitate a mare's varied utilization without being overloaded.

Összefoglalás

Modern reprodukciós technológia alkalmazása a lótenyésztésben: a sikeres embriótranszfer mélyreható elemzése

A ló szaporítása sokoldalú és folytonosan változó terület, ami egyre nagyobb teret hódít az állatorvoslásban. Annak érdekében, hogy naprakész betekintést kapjunk a lovakban a folyamatos sportpályafutásuk lehetővé tétele érdekében megvalósított embrióátültetés – mint a modern biotechnológia egyik eleme – helyzetéről a vonatkozó szakirodalmak áttekintésére és összehasonlítására került sor. Továbbá, a szerző felkeresett egy németországi ET állomást, és dokumentálta az eljárásokat. Az 1970-es évek elején történt első embrionális átültetés óta a lószaporítás/szülészet területe teljesen megváltozott. A jó tenyésztési irányítás kulcsa a kanca és mén szaporodási minőségére való összpontosításban rejlik, amely kiterjed általános egészségi állapotukra, életkorukra, táplálékukra, oltásukra, féregtelenítésükre és szociális viselkedésükre. A termékenyítéshez felhasznált ondó minősége is befolyásolja a sikeres eljárás eredményét (30-80%), ezért különbséget kell tenni friss, hűtött és fagyasztott sperma között. Miután a kanca precíz és higiénikus eljárást követően eredményesen termékenyült, embriója az ovulációt követő 7-8 napon kimosásra kerül a méhből. A kinyert embriót fénymikroszkóppal ellenőrizni és vizsgálni kell, és közvetlenül a kiválasztott recipiensbe lehet ültetni, amely a donor kancával szinkronban van az ivarzás és az ovuláció tekintetében. Másik lehetőség szerint lehűthető (+5°C) és 24 órán belül szállítható, illetőleg mélyfagyasztott embrióként sokáig tárolható. Az embrió recipiensbe ültetése után 12 nappal a terhesség ultrahanggal igazolható.
Az összegyűjtött adatok azt mutatják, hogy a lótenyésztésben a sikeres embriótranszferhez részletes és folyamatos eljárás szükséges a sikerességi arányok folyamatos javítása érdekében, ami egyben lehetővé teszi a kanca változatos, túlterhelés nélküli hasznosítását.

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University of Veterinary Medicine

Department for Animal Breeding, Nutrition and Laboratory Animal Science

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Mr. Maximilian Mitteneder, born 20.12.1995, took part on the process of an embryonic transfer on the 20th and on the 23rd of march 2021.
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Értesítési cím / Address: Dohány utca 39., 1078 Budapest

Telefonszám otthoni, mobil / phone nr.: +49 1732427847

E-mail: mittenedesmax@t-online.de

Szak / Major: **Veterinary**

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