University of Veterinary Medicine Budapest
Department of Obstetrics and Food Animal Medicine
Clinic

Control of Foaling after Embryo Transfer

By

Aoife Hayes

Supervisor: Prof. Dr. Szenci Ottó

Budapest, Hungary

2023

Abstract

Equine embryo transfer is used globally to obtain an increased number of high-quality foals from mares without interfering with their competitive career. However, there are various factors that can influence the production of a healthy foal, the control of foaling after embryo transfer will be explored throughout this review. Those investigated throughout include the quality, size, and day of recovery of the embryo, the donor mares age and whether she is competing, and the parity and size of the recipient. Intrauterine growth retardation and its effects on the development of the foal are reviewed through between breed transfers, such as thoroughbred in pony. Deepening our understanding of such areas will ultimately produce top foals.

Contents

1. Introduction

In this thesis I will be using current literature to investigate various aspects of foaling in embryo transfer mares. The aim of this thesis is to analyse the current information available and discuss the process of embryo transfer and how numerous elements regarding the embryo, donor and recipient mare can affect the foal born. I will explore through current and relevant literature how intrauterine growth retardation (IUGR) impacts foetal growth, physiological processes, and possible athletic capacity.

Embryo transfer in the equine industry allows owners to choose top mares and stallions to produce exceptional offspring with minimum interference to either sporting careers. This method has been highly successful in obtaining numerous foals in the one year and has grown in popularity in recent years.

When owners enter embryo transfer programs their main interest is obtaining a strong and healthy foal. Therefore, analysing literature that explores factors such as day of

embryo recovery, embryo size, stage and quality, donor age and reproductive category and recipient reproductive career, gives us insight regarding recipients' pregnancy losses and foaling rates.

The idea of IUGR is explored through studies involving between breed transfers, for instance, pony into thoroughbred and so on. According to Peugnent et al. [27], research within human medicine have linked early life events with a range of pathologies in adulthood and within production animal medicine the Developmental Origins of Health and Disease are of interest for their role in obtaining beneficial characteristics such as growth rate, body composition and milk and meat qualities. Therefore, the in-utero environment is crucial for the development of a healthy foal.

2. Literature review

2.1 History of embryo transfer

Embryo transfer was first performed and recorded by Walter Heape in 1890, he transferred two Angora rabbit embryos into a gestating Belgium doe [1].

The first records of embryo transfer in the twentieth century were in Vienna in 1922, rabbits being the experimental animals. The success rate in that study was not only low (1 pregnancy from 70 experiments) but also doubtful, because the young were born at night and had been eaten by the next morning [2]. The research continued into areas such as superovulation, synchronisation of oestrus, artificial insemination and early transfers which were mainly carried out in farm animals [1]. Embryo transfer in food animals began in the 1930s with sheep and goats, but it was not until the 1950s that successful embryo transfers were reported in cattle and pigs by Jim Rowson at Cambridge, England [1].

From this point early embryo transfer in equids was undertaken simultaneously in the early 1970s in

Cambridge, England and Kyoto, Japan [3]. In Cambridge, surgical methods of embryo recovery and transfer were developed in cattle and horses [3]. Simultaneously, a group of workers in Japan were using nonsurgical methods to both recover and transfer horse embryos between donor and recipient mares, however, they failed to achieve any pregnancies from nonsurgical transfer in their first attempts [3]. Although, they subsequently achieved a very encouraging 40% pregnancy rate after transferring 15 day 6 morulae/early blastocysts via a long needle passed through the anterior fornix of the vagina and thence through the wall of the uterus, thereby avoiding passage through the cervix which they assumed had been the cause of their failure to achieve any pregnancies from their first attempts [3].

Adaptions of embryo transfer and the various related technologies have generally been more rapid in equine than in cattle, with the exceptions of superovulation, in vitro fertilisation, and cryopreservation [4]. During the early development of equine embryo transfer, there were two major hurdles in comparison to other farm animal species. Firstly, the registration authorities for most pure

“breeds” (rather than “types”) of horses worldwide were led by the national Jockey Clubs representing the Thoroughbred racehorse, would not permit entry into their respective stud books of progeny conceived by either artificial insemination or embryo transfer [3]. Second, there did not then, and nor does there still today, exist a practical and efficient method for inducing superovulation in donor mares, as existed for the female donors of the other farm species [3].

Equine embryo transfer grew in popularity in the early 1990s in Argentina, where it was used to produce offspring from the best polo pony mares without terminating their competitive careers [5].

However, today equine embryo transfer has proved a commercial success and an extremely beneficial tool within the sport horse industry. During the last decade, embryo transfer (ET) has become increasingly accepted as a valuable tool for increasing the number of progenies from genetically valuable mares, for producing foals from competing mares without interrupting their sporting careers, and for obtaining foals from mares’ incapable of carrying a pregnancy to term [6].

2.2 Method of equine embryo transfer

2.2.1 The recipient mare

Correct selection of the recipient mare is one of the most crucial steps in a successful embryo transfer program according to most veterinarians. Ideal recipient mares are usually between 3 and 10 years of age, in good physical condition, easy to handle and reproductively sound [7].

Prior to transfer, a veterinarian must perform a thorough breeding soundness exam to determine if the recipient mare can adequately sustain a pregnancy. This includes ultrasound and uterine culture, biopsy, and cytology. The purpose of the ultrasound is to visualize the cervix, uterus, and ovaries. A mare with multiple uterine cysts, for example, is not considered a good candidate. The culture, cytology, biopsy results are used to determine the health of the uterine lining and presence of inflammation or infection [8].

2.2.2 The donor mare

The donor mare is usually valuable and more often in competition. In selecting a mare as a donor for an embryo transfer, the cost of the procedure, reproductive history of the mare, breed registry restrictions, and potential value of the foal are all important considerations [9]. As with recipient mares, donor mares should be healthy, and a breeding soundness exam should be carried out as described above. Embryo recovery rate is correlated with age and reproductive status of the donor mare. A higher percentage of embryos are recovered from mares aged <10 years than from mares aged >15 years [10].

Any reproductive abnormality of the donor mare reduces embryo recovery rates. Endometritis of the donor mare, although it does not necessarily preclude embryo recovery, reduces the chances of the embryo surviving in the recipient mare after transfer [11]. As age increases embryo recovery rates decrease. It is said that decreased oocyte quality appears to be the most age-related factor driving this [12].

Progressive tilting forward of the upper part of the vulva over the pelvic brim with advanced age may significantly affect the ability of a mare to become pregnant, this can be

even more dramatic if the older mare is in poor body condition [13]. Therefore, correct nutrition and reduced stress is important when considering older mares.

2.2.3 Oestrus synchronisation

Oestrus synchronisation plays a key part in successful embryo transfer programs. The aim is to get the recipient and donor mare to ovulate at the same time or it can be up to 3 days afterwards. This is essential as it ensures that the uterus receiving the embryo will be at a similar stage to the one in which it is being removed. This involves the use of hormones and regular ultrasound scanning of both the recipient and the donor.

Prostaglandin ($PGF_{2\alpha}$) is commonly used. When administered IM to a mare in dioestrus it causes luteolysis and allows a follicle to mature and ovulate, the corpus luteum must be 5-14 days old to respond to $PGF_{2\alpha}$ [14]. After administration the mare usually comes into oestrus 2-5 days later. Time to ovulation is variable (3–10 days) and depends on the stage of the mare's current follicular wave and on the size and character of follicles at the time

of $PGF_{2\alpha}$ administration [14]. It is recommended that the mare's ovaries be examined by palpation and ultrasonography just before $PGF_{2\alpha}$ administration to optimize the prediction of ovulation [14]. The most common drugs used are Cloprostenol which is a synthetic form and Dinoprost which is naturally occurring.

Human chorionic gonadotrophin has been widely and extensively used as an ovulation-inducing agent because of its luteinizing hormone (LH)-like activity, the hormone is produced after being extracted from urine of pregnant woman [15]. Repeated use of hCG over a long period is associated with antibody formation and may decrease response to treatment; this has not been seen with deslorelin [16].

Gonadotrophin releasing hormone (GnRH) synthetic analogues such as Deslorelin have a predictable effect in inducing ovulation within 48 hours when administered as intramuscular injections or by means of subcutaneous implants [15]. Through administration of a deslorelin implant, 2.2 mg, SC; or by administration of deslorelin, 1–2 mg, IM, in a biorelease vehicle, ovulation is seen in 85% of mares within 48 hours, typically 36–42 hours

after hCG or injectable deslorelin treatment or 40–44 hours after treatment with a deslorelin implant [16].

2.2.4 Insemination

Artificial insemination is the process of using fresh or frozen semen shipped from the stallion the same day if needed. It is important to choose a stallion of known high fertility, a breeding soundness exam should also have been carried out on the stallion. Factors that influence the success of AI in mares include the following: whether fresh, chilled, or frozen semen is used, the quality of the semen, the number of spermatozoa used per insemination, the preparation (extending, chilling or freezing) of the semen, the number of inseminations, the timing of inseminations and the anatomic placement of the semen [17].

Fresh semen by natural cover or collected at the farm shows the best results with 65% pregnancy rates [18]. Sometimes only frozen semen will be available, and it has a lower viable sperm count and lower pregnancy rates. The optimal time for AI using chilled semen is within 24

hours before expected ovulation. The insemination should be repeated after 48 hours if the mare has not ovulated. When frozen semen is used, every attempt should be made to inseminate the mare within 12 hours before ovulation or within 6 hours after ovulation [17].

2.2.5 Flushing and transferring the embryo

The flushing procedure for embryo recovery in horses is straightforward and easy to perform. To flush out the embryo(s), the uterus is repeatedly irrigated with 1 to 2 L of medium, which is passed through an embryo filter, up to 8L of flushing medium is required [17].

Embryo collection is usually performed on day 7 or 8 with day 0 being ovulation, it is performed via a routine transcervical uterine lavage procedure [19]. A sterile silicone catheter, typically of 8.0-mm internal diameter with an inflatable cuff, is used to facilitate transcervical lavage [10]. Once the uterus is filled, it can be gently manipulated per rectum to aid the recovery of fluid [19]. The media used for embryo collection can be a commercially prepared flush media that contains a buffer

system, antibiotic, and surfactant, or it can be as simple as lactated Ringer's solution with the optional addition of either calf serum or purified bovine serum albumen (BSA) as a surfactant to prevent adherence of the embryo to the tubing, filter, or search dish [10]. Administration of oxytocin can be used to reduce fluid retention. If fluid retention is suspected or if the outflow of media is interrupted, transrectal ultrasonography may be used to assess the presence of intraluminal fluid [19].

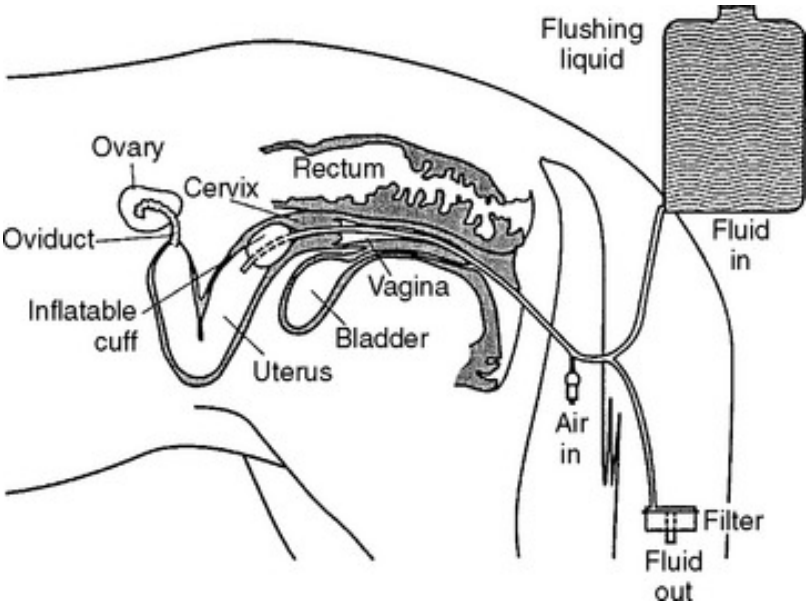

Figure 1. Diagram of the flushing system for recovering uterine embryos [7].

Embryo-recovery rate is influenced by many factors, such as age and fertility of the donor mare, quality of the sire's semen, day of recovery, number of ovulations, and the level of expertise of the staff. Embryo-recovery rate is correlated with age and reproductive status of the donor mare [10].

The search for the embryo then begins. In an equine embryo transfer program, morphologic assessment of the

embryo is important for two reasons: (1) final pregnancy rates are depressed after transfer of abnormal embryos; and (2) differentiation of unfertilized oocytes from abnormal embryos may save unnecessary time and use of recipients [9].

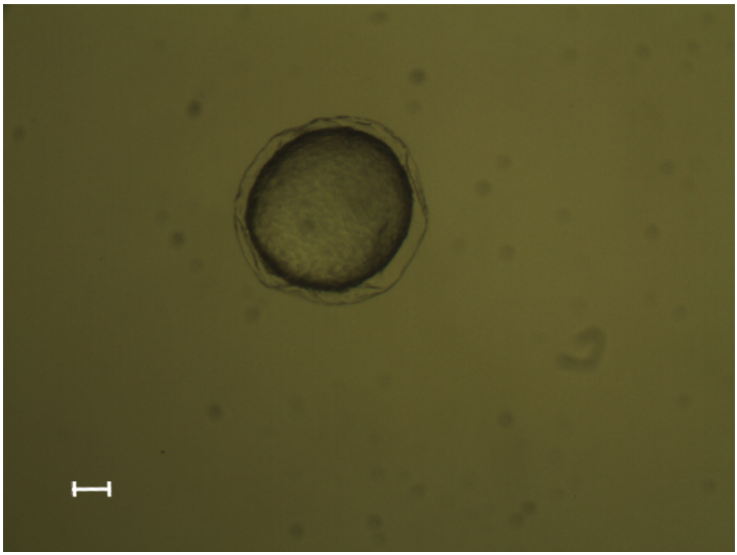

Figure 2. An equine embryo 7.5 days after ovulation (Bar = 100 μm) [17].

When examining the embryos, the morphological parameters evaluated are compactness of blastomeres,

extruded and damaged blastomeres, colour of embryo, embryo shape, size of the perivitelline space, damage to the zona pellucida, and developmental stage compared to embryo age [9].

Once the embryo has been found and evaluated, the process of packaging can begin. As explained by Vanderwall and Woods [7], to package an embryo, the transport medium is filter-sterilized into “snap-cap” tube, leaving a small air gap at the top of the tube. The embryo is then carefully transferred into the medium, the cap is securely snapped onto the tube, and the tube is wrapped with Parafilm [7]. A 50-ml centrifuge tube is then filled with transport medium (unfiltered), and the 5-ml tube containing the embryo is placed into the 50-ml centrifuge tube [7]. The cap of the 50-ml centrifuge tube is closed, eliminating as much air as possible, and it is wrapped with parafilm. The packaged embryo is then placed into an Equitainer, which passively cools the embryo to 5° C. Under those conditions, embryos can remain viable for at least 24 hours, during which time they can be transported [7].

2.2.6 Embryo transfer

At this point the actual transfer of the embryo to the recipient mare is carried out. Mares can either be sedated or not depending on the veterinarian and the mare. Surgical transfer of the embryos was once preferred but now the non-surgical method is usually advised. The non-surgical method is done using transcervical catheterisation and the washed embryo should be transferred as soon as possible.

Plastic straws (0.25 or 0.5 mL) are used to load the embryo; the column of medium containing the embryo should be surrounded by two small columns of air, which are in turn surrounded by two columns of medium [19]. The straw loaded with the embryo is fitted into an embryo transfer gun [19]. The rectum is evacuated from manure, the tail wrapped, perineum washed with povidone iodine soap and rinsed three times and, finally, dried with clean paper towels. The operator inserts the guarded gun protected by a sanitary sheath through the vagina and the embryos are released in the body of the uterus [20].

It is important that the recipient mare is in good health and as discussed earlier, have gone through a thorough examination, and synced with the donor mare. Good pregnancy rates result from using recipients that have ovulated from 1 day before to 3 days after the donor [19]. The recipient mare should be scanned approximately 7 to 9 days later to ensure she is pregnant. If she is, further scans at days 28 and 45 following ovulation should then be performed to ensure that the pregnancy is healthy and developing normally [21]. Mares may be placed on Regumate afterwards for a period.

2.3 What effects the foaling rate and pregnancy loss in equine embryo transfer

2.3.1 Day of embryo recovery

Embryo recovery is generally scheduled 7 to 8 days after ovulation, with Day 0 being designated the day of ovulation [22]. The process of embryo recovery has been explained above. According to a recent study by Panzani et al. [20] day of embryo recovery (embryo age) affected

recipient's pregnancy losses, but not recipient's pregnancy or foaling rates. In particular, day 9 and day 10 embryos resulted in a pregnancy loss rate after 40 days significantly higher than day 8 embryos [20]. Day 10 embryos resulted also in a significantly higher overall pregnancy loss rate than day 8 embryos rates [20].

2.3.2 Embryo stage, quality, and diameter

When equine embryos enter the uterus after ovulation they are at the late morula or early blastocyst stage of development and are still surrounded by the zona pellucida [23]. From the results of Panzani et al. [20], embryos in the blastocyst stage demonstrated the highest incidence of pregnancy and foals born compared to those in the early blastocyst or morula stage.

Each embryo should be measured and assigned a quality score upon initial identification [22]. Squires et al. [22] reported that 70% pregnancy rate can be anticipated with Grade 1 embryos at the initial pregnancy examination (Day 12) and a 65% pregnancy rate at 50 d.

In the study carried out by Panzani et al. [20], a higher pregnancy loss rate was recorded after the transfer of smaller embryos. In particular, embryos <400 mm resulted in significantly higher pregnancy loss rates compared with larger embryos (between 400 and 1,199 mm) [20]. These smaller embryos may imply a delay in embryo development therefore being a negative prognosis for a normal pregnancy, it is commonly accepted that the evidence of an underdeveloped embryo at ultrasonographic pregnancy diagnosis 14 days post ovulation is a negative prognostic factor for the prosecution of a normal pregnancy [20].

2.3.3 Donors age, reproductive category, and sport activity

The donors age has a huge effect of the success of the embryo transfer programme. Transfer of embryos recovered from donor mares >20 years resulted in a significantly higher overall pregnancy loss, this is consistent with the high pregnancy loss rate affecting old mares, both if carrying their own pregnancies or after transfer of their embryos in recipients [20]. Advanced

mare age is associated with a decline in fertility, the causes of this is multifactorial. Nevertheless, intrinsic oocyte defects are thought to be a major contributor because transferring oocytes from old mares into the oviduct of younger recipients does not improve the likelihood of fertilisation or reduce the risk of pregnancy loss [24].

According to Panzani et al. [20], a significantly higher pregnancy loss between days 14 and 40 was observed in recipients receiving embryos from donors affected by reproductive pathologies compared with those receiving embryos from healthy donors performing sport activity [20]. The impact of exercise on early pregnancy is still an area that needs further research and can be controversial. Although within the study mentioned above embryo recovery rate was not affected by sport activity, and the results of the present study on pregnancies after transfer seem to indicate that mares performing sport activity should not be discriminated as embryo donors [20].

2.3.4 Recipient reproductive career

Within the study discussed throughout this section there were no differences between pregnancy and foaling rates between nulliparous and pluriparous recipients which may be due to specific selection before inclusion into the programme [20]. Primiparous mares are known to produce smaller foals than multiparous mares, this difference seems to be partly explained by the reduced exchange surface and volume of the placental villi in primiparous compared to multiparous placentas [25]. In a study carried out by Robles et al. [25], foals born to primiparous mares were also lighter until 360 days of age and smaller until 540 days of age compared to foals born to multiparous mares.

2.4 Enhanced or reduced foetal growth based on transfer into smaller or larger breeds

2.4.1 Intrauterine growth retardation

Intrauterine growth retardation (IUGR) is defined as the impaired growth and development of the mammalian embryo or its organs during pregnancy [26]. In horses, placentation is epitheliochorial and occurs over the entire surface of the endometrium, thus, the nutritional supply to the foetus, which depends on the contact surface between the placenta and the endometrium, is governed by the size of the uterus and therefore the mare's size [27]. Foetal growth within the uterus is a complex biological event influenced by genetic, epigenetic, and environmental factors, as well as maternal maturity [26]. These factors impact on the size and functional capacity of the placenta, uteroplacental blood flows, transfer of nutrients and oxygen from mother to foetus, conceptus nutrient availability, the endocrine milieu, and metabolic pathways [26]. IUGR in equids has been reported to induce various detrimental effects in newborn foals, affecting the

pulmonary microstructure balance, the respiratory function efficiency, the development of neuropathies or hyperlipidaemia, as well as muscle and skeleton development and function [28].

Allen et al. [29], demonstrated that the foal birth weight is determined primarily by the growth of the allantochorion, thoroughbred in pony foals were severely growth retarded and had a mean birth weight that was only just over half that of the thoroughbred in thoroughbred foals [29].

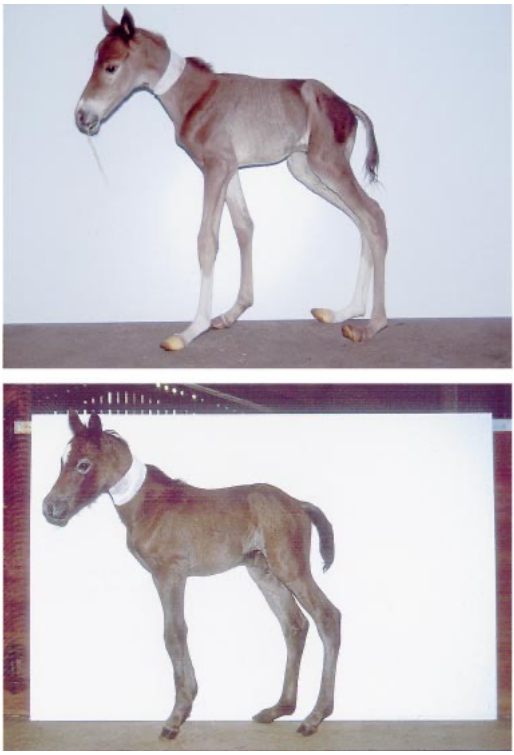

Figure 3. Foals at day 1 after birth. (a) A Thoroughbred-in-Pony (Tb- in-P) foal showing the classical signs of intrauterine growth retardation, including muscle wastage on the back and upper limbs, overextension of the fetlock joints and ill-formed hooves. (b) A Pony-in-Thoroughbred (P-in-Tb) foal, which is clearly larger, better muscled and generally more robust, and has upright pasterns and well formed, hardened hooves [29].

In horses, glycaemic control variations have been linked to the development of laminitis, obesity, equine metabolic syndrome, and osteochondrosis [30]. In a study carried out by Peugnet et al. [30], they showed that glucose homeostasis in growing foals is massively impacted by the maternal environment even post weaning. From days 180 to 540, overgrown pony in draft foals had lower glycaemia than control pony foals, together with higher plasma concentrations of NEFA, while on the opposite, IUGR saddlebred in pony foals showed catch up growth from day 180, together with higher glycaemia than control saddlebred foals and a transition from increased insulin sensitivity and lower plasma concentrations of NEFA on day 200 toward normal values of both parameters on day 540 [30].

2.4.2 Effect of intrauterine growth on skeletal health and possible athletic capacity

In a study carried out by Peugnet et al. [31], the role of antenatal events on growth and predisposition to osteochondrosis (OC) was investigated in foals born to between breed embryo transfer, pony, saddlebred and draft horses were used, body measurements were recorded from birth to 18 months and OC status was evaluated after weaning and at age 18 months. OC is one of the most important and prevalent developmental orthopaedic disease of horses, it is considered to arise from a focal disturbance in endochondral ossification, with subsequent trauma or physiologic loading resulting in lesion formation [32]. Foetal growth was enhanced in P-D foals (abbreviations: see in Figure 4) with overgrowth of most body segments until age 18 months while it was restricted in S-P foals compared with S-D foals [31]. The relative risk of developing OC was increased in restricted S-P foals compared with S-S and S-D foals shortly after weaning where all S-P foals were OC positive, only two S-P foals were still OC positive at age 18 months [31].

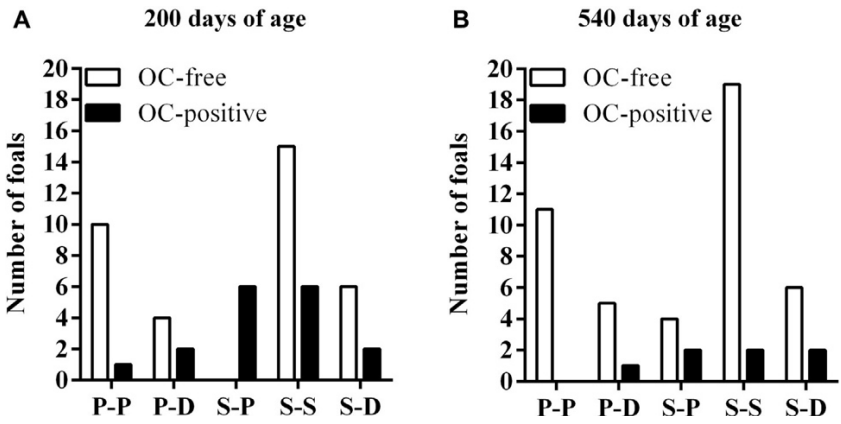

Figure 4. Incidence of osteochondrosis in foals of the five groups. OC: osteochondrosis; P-D: pony-in-draft; P-P: pony-in-pony; S-D: saddlebred in draft; S-P: saddlebred-in-pony; S-S: saddlebreed in saddlebred [31].

To summarise this study demonstrates that modifications of fetal growth in equids through the transfer of embryos of large or small breeds into recipient mares of large or small breed mares alter durably the postnatal growth, with some catch-up growth in case of intrauterine restriction, body conformation is affected in both IUGR and

overgrown groups, and this induces effects on skeletal development [31].

3. Conclusion

There are various factors influencing the control of foaling in equine embryo transfer. First and foremost, choosing the appropriate recipient and donor mare is crucial. There are numerous factors that contribute to the prevalence of pregnancy loss such as older donor mares or those with reproductive pathologies. Primiparous recipient mares are associated with lower foaling birth weights. The impact of exercise on the success of the donor is somewhat a controversial area and would definitely benefit from further research. Some say that ceasing exercise is essential for successful embryo recovery, but some believe the opposite. This is certainly an area worth researching further. A high-quality embryo is vital for the success of an embryo transfer program. Embryos in the blastocyst stage have the highest incidence of pregnancy while smaller embryos are associated with pregnancy loss. The preferred day for embryo recovery is day 7 or 8. IUGR has led to the production of below average foals. Exploring this through transfer between breeds, for instance with thoroughbred in pony, it was evident with the production

of underdeveloped foals with fetlock overextension, poorly formed hooves, and muscle wastage. The in-utero environment also impacts skeletal health with restricted foals being OC positive. This can go on to impact the athletic capacity of the foal down the line, perhaps another area where further investigation is needed. I think as equine embryo transfer continues to develop and grow in popularity so will areas of research regarding the control of foaling as people will continue to want high quality, healthy foals.

4. Acknowledgements

I would like to thank my supervisor Professor Szenci Ottó for his advice and support throughout the writing of this thesis. I would also like to thank my friends and family, particularly my Dad; Vincent Hayes, without whom the pursuit of this career would not have been possible.

5. References

1. Troxel, T.R. (2007): *Embryo transfer in cattle*. Cooperative Extension Service, University of Arkansas Division of Agriculture, US Department of Agriculture, and county governments cooperating.
2. Betteridge, K.J. (1981): An historical look at embryo transfer. *Reproduction* 62:1–13. doi:10.1530/ jrf.0.0620001.
3. Allen, W.T. and Wilsher, S., 2020. Historical aspects of equine embryo transfer. *Journal of Equine Veterinary Science* 89:102987. doi:10.1016/ j.jevs.2020.102987.
4. Kraemer, D.C. (2013): A history of equine embryo transfer and related technologies. *Journal of Equine Veterinary Science* 33:305–308. doi:10.1016/ j.jevs.2013.03.007.
5. Riera, F.L., McDonough, J. (1993): Commercial embryo transfer in polo ponies in Argentina. *Equine Veterinary Journal* 25(S15):116–118. doi:10.1111/j.2042-3306. 1993.tb04843.x.
6. Stout, T.A.E. (2006): Equine embryo transfer: review of developing potential. *Equine Veterinary Journal* 38:467-478.
7. Vanderwall, D.K, Woods, G.L. (2007): Embryo transfer and newer assisted reproductive techniques for horses. *Current therapy in large animal theriogenology* volume:211-218.
8. Abraham, A. (2022): *Broodmare care: Nutritional support of the recipient mare*, *Kentucky Equine Research*. Available at: https://ker.com/equinews/broodmare-care-nutritional-support-of-the-recipient-mare/ (Accessed: 01 October 2023).

9. McKinnon, A.O. and Squires, E.L. (1988): Equine embryo transfer. *Veterinary Clinics of North America: Equine Practice* 4:305–333. doi:10.1016/s0749-0739(17)30643-0.
10. McCue, P.M., Ferris, R.A., Lindholm, A.R., DeLuca, C.A. (2010): Embryo recovery procedures and collection success: results of 492 embryo-flush attempts. In *Proc. Am. Ass. Equine Practnrs* 56:318-321.
11. Hartman DL. Embryo transfer. In: McKinnon AO, Squires El, Vaala WE, Varner DD, editors. *Equine Reproduction.* 2nd edn. Vol. 2. Oxford: Blackwell Publishing Ltd; 2011. pp. 2871–2879.
12. Vanderwall, D.K. (2008): Early embryonic loss in the mare. *Journal of Equine Veterinary Science* 28:691–702. doi:10.1016/j.jevs.2008.10.001.
13. McCue, P.M. (1991): Breeding the older mare. *Journal of Equine Veterinary Science* 11:316–318. doi:10.1016/ s0737-0806(06)81259-1.
14. Sertich, P.L. (2023): *The reproductive cycle of horses - management and Nutrition*, *Merck Veterinary Manual*. Available at: https://www.merckvetmanual.com/ management-and-nutrition/management-of-reproduction-horses/the-reproductive-cycle-of-horses (Accessed: 03 October 2023).
15. Pinto, C.R. (2013): Hormones and breeding. *Proceedings of American Association on Equine Practitioners* 59:331-336.
16. Romano, J.E. (2023): *Hormonal control of estrus in horses - management and Nutrition*, *Merck Veterinary Manual*. Available at: https://www.merckvetmanual.com/ management-and-nutrition/hormonal-control-of-

estrus/hormonal-control-of-estrus-in-horses (Accessed: 03 October 2023).
17. Scherzer, J., (2011): Artificial insemination and embryo transfer in mares. *Compendium (Yardley, PA)*, *33*: 1-5.
18. Squires, E.L. (2009): 'Changes in equine reproduction: Have they been good or bad for the horse industry?', *Journal of Equine Veterinary Science*, 29:268–273. doi:10.1016/j.jevs.2009.04.184.
19. Pinto, C.R. (2023): *Embryo transfer in horses - management and nutrition*, *MSD Veterinary Manual*. Available at: https://www.msdvetmanual.com/management-and-nutrition/embryo-transfer-in-farm-animals/embryo-transfer-in-horses (Accessed: 04 October 2023).
20. Panzani, D., Vannozzi, I., Marmorini, P., Rota, A. and Camillo, F., (2016): Factors affecting recipients' pregnancy, pregnancy loss, and foaling rates in a commercial equine embryo transfer program. *Journal of Equine Veterinary Science*, *37*:17-23. doi:10.1016/j.jevs.2015.10.011.
21. *Embryo transfer (ET)* (2023a): *Rossdales Veterinary Surgeons*. Available at: https://www.rossdales.com/services/sport-and-leisure-horses/breeding-services/embryo-transfer (Accessed: 05 October 2023).
22. Squires, E.L., McCue, P.M. and Vanderwall, D. (1999): 'The current status of Equine Embryo Transfer', *Theriogenology*, 51:91–104. doi:10.1016/s0093-691x(98)00234-9.
23. Betteridge, K.J., Eaglesome, M.D., Mitchell, D., Flood, P.F. and Beriault, R., (1982): Development of horse embryos up to twenty two days after ovulation:

observations on fresh specimens. *Journal of anatomy*, *135*(Pt 1):191.
24. Rizzo, M., Ducheyne, K.D., Deelen, C., Beitsma, M., Cristarella, S., Quartuccio, M., Stout, T.A.E. and de Ruijter-Villani, M., (2019): Advanced mare age impairs the ability of in vitro-matured oocytes to correctly align chromosomes on the metaphase plate. *Equine veterinary journal*, *51*:252-257.
25. Robles, M., Dubois, C., Gautier, C., Dahirel, M., Guenon, I., Bouraima-Lelong, H., Viguié, C., Wimel, L., Couturier-Tarrade, A. and Chavatte-Palmer, P., (2018): Maternal parity affects placental development, growth and metabolism of foals until 1 year and a half. *Theriogenology*, *108*:321-330.
26. Wu, G., Bazer, F.W., Wallace, J.M. and Spencer, T.E., (2006): Board-invited review: intrauterine growth retardation: implications for the animal sciences. *Journal of animal science*, *84*:2316-2337.
27. Peugnet, P., Wimel, L., Duchamp, G., Sandersen, C., Camous, S., Guillaume, D., Dahirel, M., Dubois, C., Jouneau, L., Reigner, F. and Berthelot, V., (2014): Enhanced or reduced fetal growth induced by embryo transfer into smaller or larger breeds alters post-natal growth and metabolism in pre-weaning horses. *PloS one*, *9*:102044.
28. Ansari, T., Beech, D., Sibbons, P.D., Rossdale, P.D., Ousey, J.C. and Chavatte, P., (1998): Pilot investigations into microanatomical defects associated with IUGR [intrauterine growth retardation] in the horse and other domestic animals. *Equine Veterinary Journal (United Kingdom)*.

29. Allen, W.R., Wilsher, S., Turnbull, C., Stewart, F., Ousey, J., Rossdale, P.D. and Fowden, A.L., (2002): Influence of maternal size on placental, fetal and postnatal growth in the horse. I. Development in utero. *REPRODUCTION-CAMBRIDGE-*, *123*:445-453.
30. Peugnet, P., Wimel, L., Duchamp, G., Sandersen, C., Camous, S., Guillaume, D., Dahirel, M., Dubois, C., Reigner, F., Berthelot, V. and Chaffaux, S., (2017): Enhanced or reduced fetal growth induced by embryo transfer into smaller or larger breeds alters postnatal growth and metabolism in weaned horses. *Journal of Equine Veterinary Science*, *48*:143-153.
31. Peugnet, P., Wimel, L., Duchamp, G., Sandersen, C., Camous, S., Guillaume, D., Dahirel, M., Dubois, C., Jouneau, L., Reigner, F. and Berthelot, V., (2014): Enhanced or reduced fetal growth induced by embryo transfer into smaller or larger breeds alters post-natal growth and metabolism in pre-weaning horses. *PloS one*, *9*:102044.
32. Whitton, C. (2023): *Osteochondrosis in horses - musculoskeletal system*, *MSD Veterinary Manual*. Available at: https://www.msdvetmanual.com/musculoskeletal-system/lameness-in-horses/osteochondrosis-in-horses (Accessed: 19 October 2023).