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EMBRYO PRODUCTION IN MARES BY ICSI AND CONVENTIONAL ET AFTER GRADE III PERINEAL RUPTURE: A CASE REPORT

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Abstract

This study reports the case of a high genetic merit Quarter Horse mare that suffered a Grade III perineal laceration, which was a dystocia, at her first foaling. Five surgeries were performed over a three-year period to restore anatomy and reproductive function. A follicular aspiration protocol followed by cytoplasmic sperm injection (ICSI) was then performed, resulting in five embryos that were cryopreserved by vitrification. Two embryos were transferred and one of them resulted in pregnancy and the birth of a healthy foal. The animal was then subjected to a conventional embryo collection program in which a viable embryo was collected and transferred. Pregnancy was confirmed by ultrasound and the foal was born in July 2023. It can be concluded that the ability to produce offspring was restored in this mare, using the ICSI and TE biotechniques.

Keywords: dystocia; ICSI; embryo transfer; Quarter Horse; perineal reconstruction.

PRODUÇÃO DE EMBRIÃO EM ÉGUA POR ICSI E TE CONVENCIONAL APÓS LACERAÇÃO DE PERÍNEO GRAU III: RELATO DE CASO

Resumo

Este estudo relata o caso de uma égua de alto valor genético, da raça Quarto de Milha, que sofreu laceração de períneo grau III durante seu primeiro parto, o qual foi distócico. Para restaurar a anatomia e função reprodutiva foram realizadas cinco cirurgias num período de três anos, a seguir foi feito um protocolo de aspiração folicular seguido de injeção citoplasmática de espermatozoide (ICSI), gerando cinco embriões que foram criopreservados por vitrificação. Dois embriões foram transferidos e um resultou em gestação e nascimento de uma potra saudável. Em seguida, o animal foi submetido a um programa convencional de coleta de embriões, cujo embrião viável coletado e transferido, a gestação confirmada por ultrassonografia e o parto ocorreu em julho de 2023. É possível concluir que a capacidade de gerar descendentes desta égua foi recuperada, utilizando as biotécnicas ICSI e TE.

Palavras-chave: distocia; ICSI**;** transferência de embrião; Quarto de Milha; reconstrução perineal.

Introduction

Equine breeding has increasingly improved and the field of reproduction has gained importance in the training of veterinarians for the treatment of reproductive diseases in female animals, such as perineal lesions, which are very common because they affect fertility and can occur regardless of breed (Schöniger; Schoon, 2020; Frietman *et al.*, 2019). These lesions are caused by large fetuses, dystocia or mares without assistance at birth (Nagel; Aurich, 2022). Dystocic deliveries can cause a tear in the vaginal wall leading to a junction between the perineum, rectum and anus (Sitters, 2021), as the perineum is a fibrillar segment of skin located between the anus and vulva (König; Liebich, 2021).

According to Lanci *et al.* (2022), it is possible to classify the degree of perineal injury: Grade I are injuries to the dorsal mucosa of the vaginal vestibule and the upper part of the vulva, including the skin with minor muscle injury; Grade II are lacerations involving tearing of the vulvovestibular muscles, mainly the perineal body, but not affecting the integrity of the rectal floor and anal sphincter; Grade III includes traumatic rupture of the dorsal vaginal wall, including the rectal floor, anal sphincter and perineal body with tissue loss. During estrus, the cervix is dilated and the uterus is relaxed, causing feces to passively enter the vaginal space and migrate to the floor of the vagina and into the uterus (Prestes; Souza, 2019). Another pathology that a perineal tear can cause is urovagina, in which urine accumulates in the vaginal canal, causing irritation and contributing to inflammatory and infectious processes in the uterus and vagina (Arnold; Stout, 2022). Mares that suffer this type of rupture are impaired in their natural reproduction.

One technique commonly used in mares with reproductive problems is ultrasound-guided follicular aspiration (OPU), followed by fertilization using the intracytoplasmic sperm injection (ICSI) technique. The technique consists of retrieving mature oocytes without a cumulus, selecting those with the first visible polar body (Agnieszka *et al.*, 2021). ICSI requires a mature oocyte (in metaphase II), which can be retrieved from the mare's preovulatory follicle after stimulation with gonadotropin. ICSI is performed using a micromanipulator and in vitro development to the blastocyst stage, where it is finally transferred into the recipient mare's uterus (Squires, 2020). This technique has shown promising results, Jacobson *et al.* (2010) achieved a rate of 41% blastocysts per injected egg in ICSI and the pregnancy rate was between 55% and 80% after the transfer of these embryos into the recipients (Stout, 2020). In another study, production was 1.7 to 2 embryos per OPU-ICSI procedure, with high pregnancy (70%) and calving rates (Lazzari *et al.*, 2020).

Another biotechnique traditionally used in mares is equine embryo transfer (ET), which is an important tool for increasing the number of offspring from mares with high genetic value (Segabinazzi *et al.*, 2021) and also allows the production of foals from donor mares with reproductive disorders or various pathological situations (Mccue; Squires, 2015). In a review, Flores *et al.* (2022) report that the pregnancy rate with the conventional embryo transfer (ET) technique is between 40.3% and 84.6%. In this biotechnique, the embryo is created in vivo after ovulation is induced in the donor, followed by artificial insemination, embryo retrieval eight to nine days after artificial insemination and transfer to a recipient.

Development of the case report

In 2017, a four-year-old Quarter Horse mare born on March 19, 2013 was treated. At her first calving, she experienced dystocia resulting in a severe Grade III laceration in which the roof and floor of the vagina and rectum were torn, with communication between the rectum and vagina. Pneumovagina, urovagina with involvement of the urinary tract and retention of feces in the vaginal canal were observed. One of the immediate measures is protection against tetanus; treatment with broad-spectrum antibiotics (penicillin G procaine 20,000 IU/kg by deep intramuscular injection) and anti-inflammatories (flunixin meglumine at a dose of 1.1 mg/kg BID intravenously) is essential (Queiroz *et al.*, 2019).

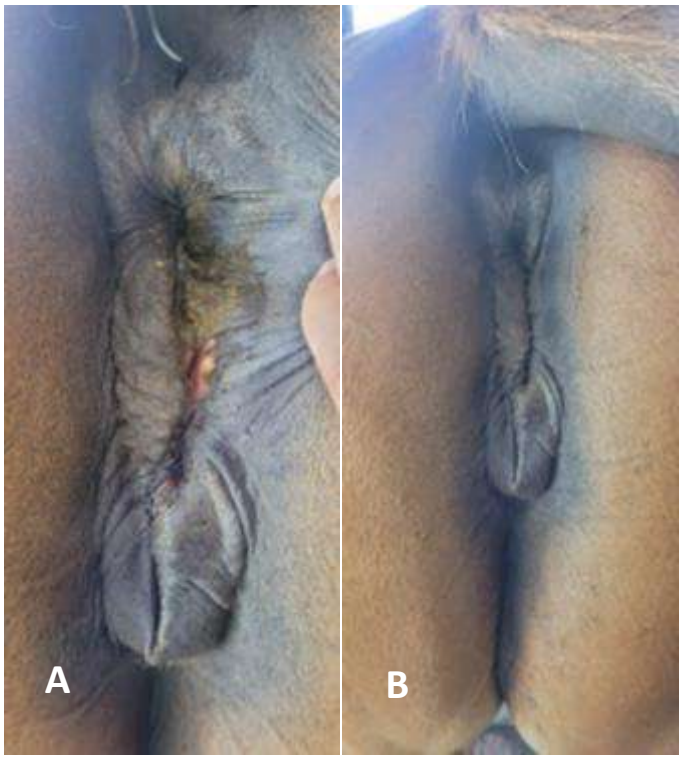
Five perineal reconstruction surgeries were performed with the aim of restoring the anatomy and function of the reproductive system as much as possible while maintaining a partial reconstruction of the perineum (Figure 1). In the preoperative phase, laboratory examinations of the uterus (bacterial culture and cytology) are required, fasting is necessary, the animal is restrained to the rump, and infection prevention measures such as bandaging the tail, washing the area with water and mild soap, and antisepsis with iodine-based alcohol must be performed in preparation for surgery (Auer; Stick, 2018).

The perineum is corrected using the Aanes technique (Aanes, 1988), which is performed after the preoperative phase, immobilization of the animal and an appropriate anesthetic protocol (low caudal epidural anesthesia with 8 ml of 2% lidocaine without vasoconstrictor). Local anesthesia was performed with 30 ml of 2% lidocaine without vasoconstrictor). The operation is performed in two phases: In the first phase, the vaginal roof and rectal floor are reconstructed; suturing was performed with a continuous mattress pattern with 2-0 catgut suture immediately after an interrupted suture; after a period of 15 days, the perineal body and anal sphincter were reconstructed with simple interrupted sutures with Vicryl 1-0. The final closure was performed with 2-0 nylon sutures (Rodriguez *et al.*, 2015). In the postoperative period, antibiotic therapy with enrofloxacin 10% was administered intravenously. The first dose, 7.5 mg/kg, was administered

immediately after surgery, followed by a second dose 72 h later. The anti-inflammatory flunixin meglumine 5% IV was also administered, at a dose of 1.1 mg/kg, once daily for three days.

After the surgeries, the mare was closely monitored for three years, from 2017 to 2020, and no reproductive intervention was performed for her recovery. In 2021, two ultrasound-guided transvaginal follicular aspirations (Ovum Pick Up - OPU) and one ICSI were performed over a period of 60 days, resulting in five embryos that underwent vitrification. Two of these embryos were transferred to previously synchronized healthy recipients, one of which (50%) became pregnant, resulting in the birth of a healthy foal. In 2022, after the reconstructive surgeries, protocols for conventional embryo retrieval were performed. This consisted of ovulation induction when a dominant follicle with a diameter of ≥ 35 mm and grade III uterine edema was detected by ultrasound. Ovulation induction was performed with 1 mg of the GnRH analog Sincrorrelin® (deslorelin, Ouro Fino, Cravinhos-SP, Brazil) and 1000 IU of Vetecor® (hCG, human chorionic gonadotropin, Hertape Calier Saúde Animal, Juatuba-MG, Brazil). The average ovulation according to this protocol is 24 hours if artificial insemination was performed with fresh sperm. Eight days after ovulation, a uterine flush was performed to retrieve the embryo, which was then transferred to the recipient, whose pregnancy was effective with delivery expected in July 2023. This protocol was performed three times, with only one embryo retrieved, resulting in pregnancy.

Figure 1. (A) and (B) demonstrate restored coaptation after partial perineal reconstruction surgery of the perineum resulting from Grade III perineal laceration. Source: Veterinarian MSc. Wilson Rigoletto Jr.



Discussion

In this report, the mare regained her esthetics but not her reproductive functionality, and she only received products after the use of biotechnologies, as she had a severe grade III perineal tear that required multiple surgeries to reconstruct the perineum. On the other hand, Frietman *et al.* (2019) describe a case of grade III perineal tear in which a single corrective surgery was performed and the mare was able to become pregnant and give birth. The surgical site is always contaminated and the success of the repair depends on perfect coaptation of the suture in conjunction with the mitotic regenerative capacity of the rectal and vaginal mucosa (Prestes *et al.*, 2019).

The first biotechnique used after recovery from surgery was OPU, followed by ICSI, which resulted in 50% pregnancy of transferred embryos. Advanced assisted reproductive techniques such as ICSI is indicated to overcome infertility problems. There are a number of mares that are unable to produce embryos due to various pathological conditions of the reproductive tract, so in vitro and *in vivo* techniques can be used to create embryos. Currently, ICSI is an effective method for creating embryos in horses in certain clinical cases, e.g. mares with perineal tears that maintain the follicle population in the ovaries. Once the oocytes have matured, they are fertilized and the embryos produced using this technique can either be transferred fresh to the recipient mare or cryopreserved for later use.

However, it is an expensive technique, but one that is becoming increasingly attractive, so much so that it is already being used on normal mares and stallions in some countries (Cuervo-Arango *et al.*, 2019). In this sense, as the efficiency of the ICSI technique has improved, the interest of horse breeders in combining these two techniques (OPU-ICSI) has also increased (Briski; Salamone, 2022). After several reconstructive surgeries and the creation of in vitro embryos by ICSI, a conventional oocyte retrieval was performed in this mare, in which an embryo was obtained in three retrievals, resulting in pregnancy after transfer. ET in horses is an old technique of assisted reproduction with pregnancy rates of 73.5% in healthy mares, which can also be used in females with reproductive diseases (Rigoleto Júnior *et al.*, 2021).

The esthetic and partial restoration of reproductive functionality in this mare through several perineal surgeries, followed by the application of advanced biotechniques such as ICSI and ET, highlights the potential of these interventions for the reproductive rehabilitation of mares with severe injuries. Although the natural functionality of the reproductive tract could not be fully restored, the production of offspring was made possible.

Conclusion

This case report demonstrates the effectiveness of perineal reconstruction techniques as well as advanced reproductive biotechniques in restoring reproductive function to mares of high genetic value that have suffered severe reproductive system injuries.

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