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Conception rate of cyclic mares used as recipients of progesterone-treated embryos

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Summary

Reproductive biotechnologies applied in equine farming have allowed the optimization of reproductive rates by increasing the number of products from mares with high zootechnical value in a breeding season and also allowing them to reproduce without being absent from their sporting activities through techniques such as embryo transfer. The objective of this work was to demonstrate that the use of the hormone progesterone in embryo recipient mares produces significant beneficial effects regarding the conception rates of these animals, enhancing the advancement of the technique, reducing costs with the resynchronization of donors and recipients, semen logistics in addition to allowing better use of available receivers. In order to carry out this experiment, 80 female mongrel horses were divided into two groups, one without the use of injectable progesterone and the other with the use of injectable progesterone. The degree of uterine edema at the time of inovulations , and the conception rate at 13 and 40 days after inovulations were evaluated . There was an effect between the treatments for the evaluated parameters, with the treatment using progesterone presenting better results.

Keywords: Progesterone. Reproduction. Embryo transfer.

Abstract

The reproductive biotechnologies applied in equinoculture allowed the optimization of reproductive indexes, increasing the number of products of mares with high zootechnical value in a breeding season and still allowing them to reproduce without being absent from their sporting activities through techniques such as embryo transfer. The objective of this work was to demonstrate that the use of the hormone progesterone in embryo recipient mares produces significant beneficial effects regarding the conception rates of these animals, potentiating the advancement of the

technique, reducing costs with donor and recipient resynchronization, semen besides allowing logistics a better use of available receivers. In order to carry out this experiment, 80 female mixed breed horses were used, divided into two groups, one without the use of injectable progesterone and the other with the use of injectable progesterone. The degree of uterine edema at the time of the innovations and the conception rate at 13 and 40 days after the innovations were evaluated. There was an effect between treatments for the evaluated parameters, with treatment using progesterone showing better results.

Keywords: *Progesterone. reproduction. Embryo transfer.*

1. Introduction

Equine farming has a visible economic representation in the national territory, mainly due to the fact that the horse has a unique representation, since remote times in the development of the country, as in the era of Colonial Brazil, being used as a form of locomotion, means of traction, soil exploration and also in wars. Equines still in current times demonstrate notorious relevance, being still a means for work in the field, leisure and sport.

The equine species is seasonally polyestric , or positive photoperiodic , that is, mares have several heats at certain times of the year, which are manifested in periods of greater luminosity, where light, when penetrating the retina, inhibits the production of melatonin, the hormone responsible for by inhibiting the release of GnRH , which naturally leads to seasonal anestrus.

Equines are considered the least fertile species when compared to other species, since they have physiological peculiarities that negatively affect the conception rates of the species, considerably reducing the success of reproductive programs such as embryo transfer.

Research and various strategies have been implemented over the years in order to increase reproductive rates in horses, especially methods and techniques that aim to improve the biotechnology of embryo transfer in the species. Technologies such as hormonal protocols using exogenous progestogens have been a viable and satisfactory alternative, increasing the pregnancy rates of inovulated recipients .

Several exogenous and endogenous factors contribute to the deficit or instability of plasma concentrations of progesterone in embryo recipients, which can result in embryonic death caused by the maternal endogenous release of prostaglandin of the PGF2a type and by the momentary uterine inability to harbor the embryo and lead to term the pregnancy . In this context, the objective was to prove the effectiveness of the use of the hormone progesterone on the pregnancy rates of recipient mares in an embryo transfer program, having as a comparative method a control group in which the described hormone was not used.

2 . Theoretical Reference

2.1 Equine Market in Brazil

Brazil has the fourth largest equine herd in the world and the largest in all of Latin America, with around 5.8 million head, moving annually in the country 16 billion reais a year and generating something around 610 thousand direct and indirect jobs (

Anualpec , 2017). Based on economic, political and social aspects, the activity in the country has relevant visibility in livestock, recognized as the “Horse Agribusiness Complex” (Mapa, 2016).

According to the latest survey conducted in 2009 by *American HorsePublications* , the horse industry has an economic impact of 300 billion dollars worldwide. According to surveys of all countries mentioned by FAOSTAT (2007) and the largest world herds updated by FAO (2009), the world population is estimated at more than 59 million equines, with 5 countries having 54% of the total of these animals. The 5 countries mentioned with the largest herds are: United States (approximately 9,500,000 individuals); China (6,823,360); Mexico (6,350,000); Brazil (5,851,238 - IBGE, 2016); Argentina (3,680,000).

At the global level, equine farming has played an important role in the economy of nations, this factor is attributed not only to the attachment of people to working with these animals, but also to the fact that the horse industry presents extensive diversification, connecting areas and requiring professionals, different types of specialties, education, and skills. Thus, the equestrian agribusiness complex does not only cover the horse as a hobby, but also nutrition, advertising, production, veterinary care, fashion and several other fields that make the equine industry a multimillion-dollar enterprise (Dias, 2016).

Taking into account the fact that there are a series of chains linked to this production, agribusiness involving horses does not fit into the standard structure of an agribusiness. Pioneer studies on horse agribusiness carried out by the Confederation of Agriculture and Livestock of Brazil - CNA (2006) reported that equine farming should be classified as an agricultural complex .

The equine production chain supports countless activities and is visibly divided into a rural segment – breeding, training, maintenance, and riding – and an urban activity segment – sports, shows, presentations, sales and therapy. If combined, both segments represent the majority of the equine population, with micro-farms , mounted police departments, circuses, zoos and a few other smaller groups representing the remainder of the population, such flexibility has made the horse highly valuable to humans, these characteristics also have the ability to make the equine industry highly challenging when it comes to updating numbers, both for the population itself, as well as for involvement in the economy of the activity (Bluman , 2017).

2.2 Embryo transfer history

In current times, equideoculture has an important role in the global economy as a source of employment and income. In Brazil, the category is experiencing a moment of progressive growth. With the equine market visibly booming, the demand for animals with superior genetics and good performance in sports competitions has increased considerably (Cna , 2010).

The equine species was for some time considered the least fertile species when compared to the other domestic species, this factor being attributed to selection characteristics and problems related to reproductive management. However, the creation of innovative reproductive techniques allowed the best use of these animals, which makes it possible to quickly improve the breeds and their crosses, with the embryo transfer technique being the tool with the best effectiveness, generating satisfactory reproductive rates. Becoming an increasingly common method for obtaining pregnancies (Silva, 2014)

Thus, assisted reproduction biotechnologies stand out, which are of singular importance to meet the demand for superior animals, optimizing reproductive rates and improving the genetics of animals. Among several assisted reproduction techniques applied to horses, embryo transfer stands out, which is a reality all over the world and consists of removing an embryo from the uterus of a mare, this so-called donor, and then transferring it to the uterus of another mare, called the recipient. (Silva, 2014).

In 1969, the first studies involving the transfer of embryos in the equine species were carried out by a group of Japanese researchers. This same group later reported, in 1972, a rate of 45% of embryonic recovery, but without any confirmed pregnancy conception, after years these same researchers managed to reach a conception percentage of 40% of the transferred embryos, still continuing the line of research, where they used 20 donor mares, collecting 18 embryos, 15 of which were viable embryos transferred by the non-surgical transcervical method , to recipient mares in synchronism of –5 to +7 days, in relation to the donor mares (Silva, 2014).

In the world, the first embryo transfers performed in horses and classified as successful, occurred in the 1970s in Cambridge, England, between horses and mules, where the embryos were collected and transferred by surgery via laparotomy, through the flank or midline (Allen, 2005).

According to Silva (2015), the non-surgical transfer technique in mares occurred in Japan for the first time, since the techniques of inovulation until then were performed by surgical methods, through laparotomy being very invasive. From then on, this technique became widespread in several countries around the world, being considered one of the most used biotechniques in assisted reproduction in horses , as it is classified as less invasive and more practical, when compared to the surgical technique, showing statistically more representative indices.

In Brazil in 1987, the technique began in the equine species , where the main responsible were the Veterinary Doctor João Junqueira Fleury, and Cezinande Meira and Marc Henry, who used surgical and non-surgical methods, respectively, to carry out the transfers (Fleury *et al* ., 1991).

2.3 Reproductive Anatomic Approach

Teske (2017) the anatomical knowledge of the reproductive system of the mare is of great importance for the execution of the technique, in order to achieve success in reproductive management and reduce economic losses. The reproductive system of the mare is composed of two ovaries, two oviducts, two uterine horns, body of the uterus, cervix, vagina and vulva.

The oviducts run from the ends of the uterine horns until they reach the ovulation fossa, dividing into an ampulla, which covers the ovulation fossa, and an isthmus, which ends in a papilla at the end of the uterine horn. The uterine horns have a so-called “V” shape and diverge sharply from the ligament. In the non-pregnant mare, the uterus is approximately 20 cm in size ending at the cervix (Teske , 2010)

The cervix extends from the internal os of the uterus to the external os located in the vagina that extends to the vulva. It is recommended that the vulva has a vertical position without any deviation and the vulvar lips must be completely coapted, not allowing air to enter the vagina, preventing the occurrence of pneumovagina (Ley , 2013).

The study of the stallion's reproductive organs also becomes a relevant factor for equine reproduction as cited by Dittrich (2010) and this system consists of the testes, epididymis, vas deferens, urethra (pelvic and penile), penis and foreskin.

The stallion has three important accessory glands: the seminal vesicles, which have the function of producing most of the semen, serving to transport and nourish the spermatozoa ; the prostate, with the function of neutralizing the acidic pH of the vagina; and the bulbourethral glands, which clean the urethra and at the end of ejaculation secrete a kind of “plug”, which is spermicidal and has the function of reducing the chances of fertilization by other males (Dittrich, 2010).

2.4 Reproductive Physiology

The equine species is called seasonally polyestric , presenting a constant reproductive cycle at a certain time of the year, which is the period with the highest luminosity (Diniz, 2011). The natural selection process programmed the mare's central nervous system to recognize the length of the day, through the recognition of the luminosity that reaches the retina, and through this factor, they were selected to start their estrous cycle only when the days are longer. in spring and summer, so mares that are located further north or south in relation to the equator show later cyclicity within the season than those that are located closer to the equator. Mares that are very close to the equator show minimal seasonal variation in the length of the estrous cycle (Aristizábal , 2017).

In mares, the importance of increased luminosity in the reproductive period is due to the fact that when there is a greater presence of light, concomitantly there will be less production of the hormone melatonin by the pineal gland, which is regulated by stimuli of the optic nerve, through the reception or not of external light stimuli, with the lowest concentration of this hormone in the systemic circulation, there will be a greater production of GnRH (Gonadotropin Releasing Hormone) by the hypothalamus, since melatonin makes negative feedback for GnRH in the hypothalamus, inhibiting cyclicity in mares. The increase in luminosity occurs precisely in spring/summer and stimulates cyclicity in horses (Dittrich, 2010). The hypothalamus has, among its various attributions, the function of producing and secreting GnRH , which acts on the adenohypophysis , stimulating this part of the gland to secrete FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone), FSH has the role of stimulating follicular growth, the which will produce steroid hormones such as estrogen that allows the manifestations of heat, the follicles in the equine species grow about 2 to 3 mm per day (Lima, 2017). LH during diestrus has relatively low concentrations, however it has a progressive increase during the estrogen peaks that occur during estrus, LH also has its functions performed on the hemorrhagic body, stimulating the production of progesterone, a hormone with a fundamental role in the gestational maintenance (Teske , 2017).

The period understood as the estrous cycle is defined as the time in which several changes in the reproductive system of mares occur, repeating with averages close to 22 days (Frazão, 2017). The cycle is defined as the interval that occurs between two subsequent ovulations and is divided into the follicular and luteal phases (Aristizábal , 2017).

The reproductive cycle, also called the estrous cycle, can be classified as the follicular phase (estrus) in which there is a greater predominance of estrogen, a phase in which the mares are receptive to males and the reproductive tract is ready for copulation and also for the transport of sperm through the oviduct . (Diniz, 2011).

luteal phase (diestrus) in which there is the presence of the corpus luteum and the dominance of the hormone progesterone, in this phase the mare no longer accepts copulation and the uterus is prepared for embryonic implantation. The primary corpora lutea are those resulting from the ovulation of dominant follicles of primary waves at the end of estrus, on the other hand, the secondary corpora lutea are the result of ovulation of dominant follicles arising from secondary waves during diestrus or during pregnancy . Accessory corpora lutea arise from the ovulation of follicular wave follicles during pregnancy and/or from the luteinization of anovulatory follicles. Both secondary and accessory corpora lutea are referred to in the literature as supplementary corpora lutea. The diestrus phase ends when the regression of the corpus luteum, action also called luteolysis , with this will occur the beginning of a new follicular wave following the reproductive cycle (Diniz, 2011).

2.5 Hormone Therapy in Equine Gynecology

The increase in reproductive efficiency is a factor of great importance in horse breeding , with a view to better use and intensification of the pace of genetic improvement of the species. The incidence of ovulations vary significantly during the year due to equine reproductive seasonality, thus hormone therapy applied to equine gynecology plays an important role in reproduction biotechnologies (Faria & Gradela , 2010).

2.5.1 Prostaglandins (PGs)

A hormone present in all animal tissues, it has diverse functions and is part of the group of eicosanoids that derive from arachidonic acid , which is cleaved by cyclooxygenase enzymes and forms a pentane ring receiving various geometric arrangements. The by-products generated through the cleavage of arachidonic acid have several metabolic actions, physiological and pathological processes, ovulation, endocrine function, among others (Silva, 2012).

Among the various luteolytic agents , PGF2α is the primary agent in mares, as it has a high effectiveness of luteolysis in non-pregnant equine females that occurs after its release by endometrial cells between days 13 and 16 after ovulation. Prostaglandins can be used to perform luteolysis in persistent or anestrolactational corpora lutea , control ovulation timing, induce gonadotropin secretion, synchronize estrus, treat mares with endometritis, eliminate pseudopregnancy , stimulate uterine contraction, and promote miscarriages before formation of the endometrial calyces. Although several routes can be used for the administration of prostaglandins, the intramuscular route is preferred, as it combines practicality and minimal side effects. In the synchronization and induction of estrus, prostaglandin can be applied in any phase of the estrous cycle in two doses, with an interval of 14 days, or in a single dose, after the detection of a mature corpus luteum, or, still, associated with the administration of Progesterone (P4). The corpus luteum under normal conditions is responsive to prostaglandins after 4 days of ovulation, when it becomes mature (Neto, 2017).

2.5.2 Estrogens (E2)

Estrogens are steroid hormones which allow the signs of estrus to be manifested when they reach their peak and are mainly produced by the ovarian follicles and also by the fetoplacental unit , but on the other hand a small amount is also produced in other areas of the body (Grandela , 2010). Follicular estrogen

secretion peaks one or two days before ovulation and declines to baseline levels in diestrus . of LH (Silva, 2014).

Administration of a small dose of estradiol (0.5 to 1.0 mg) in mares in deep anestrus is capable of inducing estrus signs within 3 to 6 hours, whereas in mares with a functional corpus luteum, estrus signs are not. observed (Neto, 2017).

2.5.3 Progesterone (P4)

The hormone progesterone is a natural progestogen produced by the luteal cells of the corpus luteum, the placenta and the adrenal glands . Its secretion is stimulated by LH and its function is to promote the closure of estrus signals, keep the female non-receptive to the male, prepare the uterus to receive the embryo and maintain the initial pregnancy by increasing the secretory activity of the endometrial glands and uterine tone. . In addition, it has the ability to inhibit the episodic release of LH when at high levels, thus being an important regulator of the estrous cycle (Hafez, 2004).

Treatment with the use of progestogens , whether oral, injectable or through intravaginal devices impregnated with P4, can be used to control the estrous cycle in horses, aiming to suppress follicular growth and control the time of ovulation, in addition to performing heat synchronization. and ovulation in cyclical mares, P4 can be used in several functions applied to equine gynecology, such as: inducing cyclicity in anestrous recipients, enabling the use of anestrous mares and mules as embryo recipients, improving the tone of the embryo. uterus, favoring the maintenance of pregnancy, and suppressing the manifestation of heat (Frazão, 2017).

The need to create successful hormonal protocols that aim to stimulate ovarian cyclicity to overcome seasonal anestrus has become a target in the equine industry, especially in recent decades, since several biotechniques, including embryo transfer, have been expanding in several regions of the country (Frazão, 2017).

2.5.4 Human Chorionic Gonadotropin (hCG)

hCG has physiological activity with functions similar to those performed by LH, however it has a protein conformation different from LH, this hormone has been used effectively in inducing ovulation in mares, as it reduces the duration of estrus and the interval until ovulation, causing this to occur within 48 hours, which reduces the number of inseminations and the amount of coverage needed per estrus (Neto, 2017).

peptide hormone which is produced by the human placenta, able to stimulate luteal function and promote gestational maintenance in mares. The use of hCG in mares that have a preovulatory follicle of at least 35mm in diameter is effective in inducing ovulation within 48 hours in about 80% of the animals, presenting itself as an effective way of inducing ovulation in mares, the use of hCG in mares with more than one preovulatory follicle increases the possibility of occurrence of double ovulations (Frazão, 2017).

If, on the one hand, hCG has great effectiveness in inducing ovulation, when administered frequently in the same breeding season, it can develop and lead to the development of antibodies through the activation of the animal's immune system. Some authors mention that the intravenous route of administration of the hormone is less likely to lead to the development of antibodies, thus the intramuscular route has been described as the safest for administering the drug in view of the reduced chances of antibody formation (Frazão , 2017).

2.5.5 Gonadotropin Releasing Hormone (GnRH)

GnRH is a peptide produced and stored in the basal medium hypothalamus, it establishes a connection between the hypothalamic-pituitary-gonadal axis so that, in response to nerve stimulation, pulses of GnRH are released in the hypothalamic-pituitary portal system , inducing the anterior pituitary to release LH and FSH. It can be used to stimulate follicular development, induce FSH secretion in anestrus mares or mares that do not develop a preovulatory follicle during the breeding season, in addition to having the function of reducing the antigenicity caused by hCG (Hafez, 2004). .

Commercially there are 3 different types of GnRH analogues , these being buserelin acetate, deslorelin acetate and fertirelin acetate , the three of which are efficient in raising LH rates and promoting ovulation in cyclic mares, the difference between the three analogues is in the ovulation time that each one causes, with an average of 24 to 48 hours for buserelin acetate , 36 to 48 hours for deslorelin and 12 to 48 hours for fertirelin acetate . Among the analogues, deslorelin stands out , as it has the ability to reduce the number of matings, as well as the number of visits by the veterinarian to perform follicular control, as it has greater effectiveness of action and is also more accurate in the performance of the function , it becomes crucial to aid ovulation induction in embryo transfer programs and artificial insemination, especially for chilled and frozen semen (Samper et al., 2002).

2.6 Synchronization between embryo donors and recipients

The process of preparing mares that receive embryos in reproduction centers is the action that demands the most time and dedication, since the females need daily evaluation through transrectal palpation and ultrasound of the ovaries and uterus (Neto, 2017).

The synchronization between embryo donors and recipients is of great relevance for the establishment of pregnancy in embryo transfer programs. The absence of synchrony, or when it is performed incorrectly, affects embryonic mortality, especially in the first week of embryonic development. The use of hormones such as progestogens , estrogens, prostaglandin, hCG , GnRH and analogues has been used to control follicular development and also ovulation time (Neto, 2017).

It is imperative that donors and recipients are cycling normally for the synchronization of estrus and ovulation (Frazão, 2017). Synchronization between recipients and donors is a technique considered little complex in cyclic mares, previously knowing that recipients and donors are present between the sixth and fourteenth days of diestrus and the ultrasonographic examination of the ovaries reveals the absence of a large preovulatory follicle , it is routinely administered via intramuscular PGF2-α or analogue in the donor mare, and one to two days ahead, the same therapy in the recipient mare (Neto, 2017). The use of prostaglandin is a highly widespread method for synchronizing equine females, but the response to this type of luteolytic agent is totally dependent on the existence of a responsive corpus luteum, given that the luteal body becomes responsive to the action of the prostaglandin from the fifth day after ovulation (Frazão, 2017).

There are several forms of synchronization between donors and recipients, from monitoring to identify spontaneous ovulation , ovulation induction and hormone therapy, and it is important to have at least two recipient mares for each donor mare (Silva, 2014).

In synchronization techniques using ovulation induction, monitoring follicular dynamics by ultrasound and using hCG or GnRH for ovulation induction in recipient mares, 48 h after the donor is inseminated. It should be noted that successive applications of hCG induce the formation of antibodies, a factor that minimizes its effectiveness in the ovulatory response . The synchronization window between donors and recipients consists of the one in which the recipients are between the fourth and eighth day of ovulation (in relation to the donor's ovulation - D0), considering that the collection of the embryo is on the eighth day when using semen from fresh or chilled; and the recipient can ovulate from one day before (D+1) to 3 days after (D-3) the donor, who is considered capable of receiving embryos in this interval (Neto, 2017).

For the establishment of a hormonal protocol using progesterone in the synchronization of acyclic recipient mares, with the date of the uterine washing of the embryo donors, the hormonal protocols are based primarily on the administration of estrogen, in order to simulate the hormonal condition of estrus and stimulate the expression of uterine receptors for the P4 hormone, similar to what happens in the estrous cycle of cyclic mares prior to ovulation. 24 hours after estrogen application, the presence of uterine edema is checked through ultrasonography, and if this is satisfactory, P4 or 19-nortestosterone is administered , which can be injected or oral. Thus, the embryo transfer is performed with a window of 4 to 8 days after the application of the progestogen (Aristizábal , 2017).

2.7 Progesterone use in embryo recipient mares

According to Aristizálbal (2017) the equine species, similarly to other domestic species, has total production of progesterone during pregnancy due to the fact that it prolongs the functionality of the corpus luteum, presenting three different forms of P4 to maintain pregnancy: Corpus Luteum, Supplementary Corpora Lutea and the Placenta.

The term progestagens refers to natural steroid hormones such as 'progesterone (P4) or also synthetic ones such as 19-nortestosterone that have the ability to bind to progesterone receptors, exercising its attributions (Aristizábal , 2017). There are currently formulations of injectable 19-nortestosterone and short- and long-acting progesterone, but due to greater ease and less handling of the animals, long-acting P4 (P4 LA) is more used (Rocha Filho et al., 2004)

Progesterone has a role in gestational maintenance, the intense production of this hormone and its by-products, such as 5α- dihydroprogesterone (5αDHP), are necessary to maintain an adequate uterine environment and provide embryonic development. After ovulation and until approximately day 40 of gestation, the corpus luteum is responsible for P4 synthesis. In pregnant mares, the corpus luteum tends to persist for more than 14 to 16 days, as a result of gestational recognition, a period in which the embryo, through uterine migration, sensitizes the uterus, preventing the release of prostaglandins (Aristizábal , 2017) .

The allantochorion is in a period of expansion between the 40th and 120th of gestation, in this period the trophoblast cells have the capacity to synthesize several hormones, especially progesterone, being observed around the 70th of gestation. Between days 70 and 150 of gestation, the progesterone found in the plasma represents a mixture of P4 secreted by the declining corpora lutea and placental progestogens secreted by the growing allantochorion . Approximately after 180 days of gestation, the production of P4 by the mare's corpora lutea no longer occurs and

the fetus-placental unit synthesizes this hormone until the end of gestation (Allen, 2001).

Early embryonic loss is related to low concentrations of systemic progesterone in early pregnancy. Concentrations below 2.5 ng / mL on day 12 were used as a critical value for identifying mares with impaired endogenous progesterone synthesis via the corpus luteum. Experiments demonstrate that ovariectomized mares treated with 300 mg/day of progesterone maintained pregnancy after embryo transfer (Ginther , 1985).

The treatment of recipients with progesterone increases the proportion of recipients classified as excellent or good on the day of embryo transfer , in addition to extending the useful period for transferring these recipients. Protocols using P4, in addition to increasing the rate of use of recipients, also increase the rates of confirmed embryos after inovulation , optimizing reproductive rates (CAIADO, 2012).

Protocols involving the use of progesterone in recipient mares in anestrus or in the transition phase have been described by several authors, with numerous functions, such as, for example, the improvement in uterine tonus, a factor that reduces uterine contractility and prevents the expulsion of the embryo. (Tridapali , 2018).

3 Material and Methods

The experiment was carried out in the municipality of Unaí-MG between the months of December 2018 to March 2019, on the Agropecuária Galiléia property , located in the Palmeiras microregion on the banks of the Roncador stream, with an altitude of approximately 565 meters, and a tropical climate with seasonal dry. The period selected for carrying out the experiment comprised the 2018/2019 breeding season, being conducted in months with higher incidence of sunlight, comprising the period of reproductive cyclicity, since the equine species is seasonally polyestrous , as described by Dittich (2001).

were used in the experiment, mixed breed, with an average weight of 400 kg, aged between 5 and 8 years, which underwent a previous ultrasound examination for evaluation of the reproductive system and ability to receive embryos in addition to carrying out vaccine prophylaxis against: rabies, herpes virus and leptospirosis. In all recipients, tests were carried out for the diagnosis of equine infectious anemia and glanders, in addition to vermifugation based on oral ivermectin , and control of arthropods in infested animals was carried out using drugs based on Calmafós and Propoxur . The animals were fed on mixed pasture composed of tifton and mombasa in an area of approximately 50 hectares and supplemented with specific mineral salt for horses.

In the study, 20 embryo donor mares were used, of the breeds: Manga Larga Marchador, Quarter Horse, PaintHorse and PSI (English Thoroughbred), with an average age of 11 years and an average weight of 500 kg of live weight, originating from various Haras and breeders in the region.

Recipient mares were divided into two experimental groups: Group Without (P4) containing 40 mares that will not receive treatment with progesterone, using natural heats and inducing ovulation, to be used as embryo recipients and group with (P4) with 40 mares which received treatment. All mares underwent regular ultrasound examination, which evaluated uterine contractility and edema, as well as follicular dynamics.

Mares belonging to Group With (P4) were evaluated mainly regarding uterine edema and follicular dynamics, which when in conditions of absence of heat, that is,

without uterine edema and absence of corpus luteum, received application estradiol debenzoate at a dose of 2 mg on the first day and 0.5 mg on the second day, and these animals were evaluated daily. Cases in which the response was satisfactory in terms of estrogen responsiveness, manifested in an increase in uterine edema, the first dose of progesterone was administered using Altrenogest ® (19 - nortestosterone) 300 mg.

The mares in the group Without (P4) were evaluated mainly regarding follicular diameter and uterine edema, as well as in the group treated with progesterone, mares when in conditions of absence of heat, that is, without uterine edema and absence of corpus luteum, received application of estradiol benzoate at a dose of 2 mg on the first day and 0.5 mg on the second day, and these animals were evaluated daily. Cases in which the response was satisfactory in terms of estrogen responsiveness , in which, when in conditions of a pre-ovulatory follicle , measuring over 36 mm and in the uterine evaluation, they presented grade II or III uterine edema, ovulation induction was performed with 0.25 mg/kg GnRH analogue deslorelin (gonadotropin-releasing hormone) . From 36 to 48 h, the time mentioned by Faria (2016), ovulation was checked using ultrasound to synchronize the recipient with the donor, as well as in the group treated with progesterone the mares in the control group when in conditions of absence of heat, that is , without uterine edema and absence of corpus luteum received administration of estradiol benzoate at a dose of 2 mg on the first day and 0.5 mg on the second day, Cases in which estrogen responsiveness was confirmed , manifested in increased uterine edema and presenting dominant follicles from 36 mm, ovulation induction was performed.

Embryos were recovered from donors previously inseminated with fresh, cooled or frozen semen, and after 8 days of ovulation in the case of cooled or fresh semen and 9 days when inseminated with frozen semen, collections were made by the non-surgical method by uterine lavage medium with lactated Ringer's solution, using the Teske technique (2017) through transcervical probing . After recovery, the embryos were washed with holding media suitable for washing embryos (Botuembryo - Botupharma) and subsequently inoculated in recipient mares previously synchronized groups With and Without (P4). Only embryos with grade I and II quality classification were used for inoculation in this experiment (CAIADO et al., 2005).

Inovulations were performed in suitable recipients between day 4 and 8 after ovulation in the case of recipients belonging to the Without group (P4), and between 2 and 8 days after the administration of 19-nortestosterone in the With group (P4). After the inovulations, the group (P4) received an additional dose of 180 mg of 19-nortestosterone and after 13 days of embryonic development, early gestational diagnoses were performed. long-acting progesterone (P4 LA) at a dose of 1,500 mg, this dose being repeated every 7 days until 120 days of gestation, when the placenta will start to produce endogenous progesterone.

In the Without group (P4) the early gestational diagnosis was also carried out with 13 days of embryonic development and in negative cases, the recipients of this group received 5.0 mg of Dinoprost (Lutalyse) to carry out the luteolysis .

randomized design was used , with 2 treatments (recipients treated and not treated with progesterone) and 40 repetitions (animals). The data were submitted to analysis of variance, with comparison of means through the Tukey Test at 5% probability, through the statistical program (SISVAR).

4 ANALYSIS AND DISCUSSION OF THE RESULTS

For the evaluation of the uterine echotexture , gestational diagnosis at 13 days and 40 days of gestation, there was a significant effect of the treatments on all evaluated parameters, at the 5% significance level of the Tukey test (Table 1).

Table 1. Degree of Uterine Edema at the moment of inovulation (GEUI), Diagnosis of Pregnancy at 13 Days (DG13) and Diagnosis of Pregnancy at 40 Days (DG40) of mares according to treatments.

Treatments	(GEUI)	(DG13)	(DG40)
No progesterone	0.25b	0.55b	0.55b
with progesterone	0.65 to	0.85 to	0.85 to

Means followed by the same letter in the column do not differ by Tukey 's Test at the 5% probability level.
Source: Authors of the work, 2019.

As assumed, the treatment with the use of progesterone showed positive results related to the conception rate of the animals treated with (P4) in relation to the animals in the control group, so that in the group with (P4) the pregnancy rate was (85 %) on days 13 and 40 of gestation. On the other hand, untreated mares presented a gestational index of (55%) at 13 and 40 days of embryonic development in the second evaluation .

Regarding the parameter degree of uterine edema at the time of inovulation evaluated in the experiment, the groups showed a significant discrepancy for this variable, so that the mares in the control group in which the ovulation induction protocol was performed, had an average of uterine edema of (0.25), while the group tested with progesterone had a mean value of uterine edema of (0.65) .

Silva et al. (2012) used 64 cyclic recipients, ovulated and classified as excellent, who were treated with 1500 mg of long-acting progesterone on the day of ovulation, which were injected on the second day after ovulation and achieved 71% pregnancy. Similar results were found by Bartolomeu (2014), when he treated 17 acyclic mares with 2000 mg of long-acting progesterone with previous application of 10 mg of estradiol benzoate 48 hours before the administration of progesterone, being injected between the fourth and eighth day of treatment in which they presented 76% of pregnancy, rates close to those found in this study.

In a study Aristizábal (2014), suggests that the use of the hormone 19-nortestosterone manages to maintain the plasma concentrations of progesterone in the luteal phase at a level of (3.41 to 3.33 ng / dl) standard found in pregnant mares without sources of endogenous progesterone, in the same study the author evaluated the pregnancy rate at 15 days in a study with 81 acyclic mares treated with 19-nortestosterone and a control group with 74 cyclic mares, obtaining a result of 46% and 59% of pregnancy respectively, no statistical difference was obtained between the groups, possibly the lower pregnancy rate in the 19-nortestosterone group may have been influenced by the fact that they were acyclic mares.

Lower pregnancy rates, when compared to those found in this study, are reported by Tridapali (2018), when, through an experiment with 37 recipients using long-acting progesterone application, these being inovulated between the second and seventh day after treatment, conception was obtained of 56%, in the same study using progesterone in recipients, but ovulated and with a small corpus luteum and unable to produce enough progesterone to maintain a pregnancy and inovulated between the second and seventh day after ovulation, demonstrated conception of

61%. Rates considered high when compared to the control group of the same study without the use of progesterone, in which this group presented 31% of conception.

Additionally, Júnior (2017) reports that protocols involving the use of estrogen and subsequent applications of short- or long-acting progesterone or the use of 19-nortestosterone in acyclic recipient mares, show satisfactory results in embryo transfer programs, increasing the number of of recipients capable of inovulation and raising the incidence of conception.

According to Rocha and Filho et al . (2004) demonstrate that the use of short- or long-acting progesterone, in studies with cyclic and acyclic embryo recipient mares, did not present statistical difference. Thus, stating that the initial use of the hormone 19-nortestosterone with subsequent applications of long-acting progesterone does not interfere with pregnancy rates.

Progesterone plays a fundamental role with regard to embryonic maintenance (Monteiro, 2016) studies carried out by Hinrichs et al. (1987) who used ovariectomized recipients treated with 300 mg of oily long-acting progesterone daily for 20 days, following the treatment for 100 days of gestation, demonstrated that these mares were able to maintain the pregnancy even without the presence of ovaries and still demonstrates in the work that the plasmatic levels of progesterone of the mares treated with progesterone were similar to those of the control group.

Starting from the justification that long-acting progesterone, when compared to short-acting progesterone; 19-nortestosterone has a prolonged period of activity at the reproductive level, as it remains in high plasma concentrations for a longer period, reflecting with the demand for a longer time to return to estrus (SILVA, 2015), culminating in an increase in the period of waiting for reuse of these recipients, in case they reabsorb the inovulated embryo . Thus, the protocol adopted with the use of long-acting progesterone was conducted with prior use of 19-nortestosterone , and positive gestational diagnosis at 13 days of gestation.

The previous use of estradiol benzoate in certain recipients with an unsatisfactory degree of uterine edema performed in the experiment is justified by the need to temporarily maintain a high plasma concentration of estrogen (Allen, 2001), and this concentration of estrogen is manifested by the appearance of uterine edema characteristic in that circulating estrogen stimulates the expression of progesterone receptors and prepares the endometrium to carry the pregnancy to term (Hughes et al., 1977).

Regarding the assessment of uterine tone, the group treated with progesterone showed a higher degree of uterine edema compared to the control group. Fontes et al. (2007) presents a study in which just over 25% of recipients treated with progesterone had a low degree of uterine edema, while those belonging to the control group without the use of P4, more than 71% had a low degree of uterine edema, both groups evaluated on the second day after ovulation. Based on the results presented, it is suggested that the lower degree of uterine edema presented by the animals in the control group of this experiment is due to the fact that these recipients were physiologically in diestrus, the phase that precedes ovulation, for this reason the levels of estrogen already showed the onset of decline around two days before ovulation, with low concentrations on the day of inovulation , culminating in low degrees of uterine edema (Mckinnon & Carnevale , 1993).

5. Conclusion

Thus, the use of progesterone in mares that receive embryos is an effective alternative for raising conception rates, since treatment with this hormone makes it

possible to maintain a constant controlled plasma concentration of the hormone, preparing the uterine environment for gestational maintenance and increasing pregnancy. availability of recipients capable of inovulation in an embryo transfer program.

6. References

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