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Original Research

Reproductive Characteristics in Old and Young Subfertile Mares: Are They Really Different?

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ABSTRACT

Physiological and pathological mechanisms that determine subfertility (pregnancy failure, irregular cycles, and abnormalities in reproductive tract) in old mares (OM) have being studied by many authors. However, some young mares also share this reproduction condition although no previous reports have being published. We decided to investigate reproductive parameters of young subfertile mares (YSM) in order to understand the basis of their reproductive behavior. Forty-nine subfertile mares were classified and separated into 2 groups: YSM (3–10 years old; n = 28) and OM (13–23 years old; n = 21). Different number of cycles (1–8) was used for data analysis on the embryo recovery rate (ERR), interovulatory interval (IOI), multiple ovulation rate (MOR) and plasmatic progesterone. Embryo quality was evaluated by gene expression through RNAm analysis. Effluent samples were taken for bacteriological and cytological evaluation and endometrial biopsies were performed to evaluate the presence of inflammatory cells and endometrial progesterone receptors (PR). There was no significant differences in ERR ($P = .1230$) on the percentage of each embryonic stage found on the different days of flushing ($P > .05$); on embryo gene expression ($P > .05$); on MOR ($P = .1218$); and on plasmatic progesterone at day 8 PO ($P = .1230$). However, differences were found on the percentage of positive cytologies ($P = .0122$) and bacteriological cultures ($P = .0023$); the amount of mononuclear cells in biopsies ($P < .05$) and distribution of PR on endometrial localization. In conclusion, YSM share some physiopathological mechanisms with OM that could explain their reproductive performance's similarities.

Animal care and welfare: All procedures were approved by the Committee for the Use and Care of the Veterinary Sciences of the University of the Center of Buenos Aires Province (UNCPBA).

Ethical approval statement: All procedures performed with animals were in accordance with the ethical standards of the Facultad de Ciencias Veterinarias, UNCPBA (Universidad Nacional de Centro de la Provincia de Buenos Aires) where the studies were conducted.

Conflict of interest statement: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the present paper.

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1. Introduction

Horses are selected by their reproductive competence but mainly as a result of their athletic performance. This leads to aged animals remaining in reproductive commercial programs due to the high genetic value of their offspring. It has been estimated that 10%–25% of the overall population of broodmares are older than 16 years old (y.o.) [1]. Advanced maternal age is an important predisposing factor on the reduction of reproductive efficiency [2,3,4,5]. It is widely accepted that mare fertility begins to decline from 13 to 17 y.o. leading this category of animals to be referred to as subfertile [6,7]. A subfertile mare can be defined as a mare that has been inseminated or covered by a stallion of proven fertility more than three opportunities during the breeding season and did not get pregnant; has abnormalities in the reproductive tract; shows irregular cycles during the breeding season; or does not get pregnant during several reproductive seasons [8]. As well as in other mammalian females, reduced pregnancy and foaling rates can be originated from ovulatory failure, poor oocyte quality, early embryonic loss, and fetal death, among others [9,2,10].

As a result of a longer follicular phase [9], older mares have been associated with an increased interovulatory interval (IOI) and also with lower circulating estrogen and inhibin concentrations, if compared to younger mares [11].

Multiple ovulation rate (MO) is also influenced by the age of the mare [12]. The MO can be defined as the percentage of cycles with double or triple ovulation in relation to the total number of cycles. Older mares have a higher MO rate than younger ones [5,13]. The effect of age on MO may be driven by a gradual increase in the IOI and differences in concentrations of gonadotropins as the mare becomes older [9].

It is known that the main cause of reduced pregnancy and foaling rates in old mare (OM) is the poor quality of embryos and the resultant high embryonic death between fertilization and the first 2 weeks of pregnancy [14]. The presence of intrinsic defects in oocytes and/or embryos significantly contributes to a reduction in embryonic survival related to maternal age [15]. Possible causes of structural and/or functional alterations in oocyte competence that could impact on embryo development are, for example, the decrease in mitochondrial activity and an increased incidence of chromosome abnormalities in aged females [1,16]. Frequently, OM are considered as the unique subfertile category, but they are not the only ones with reproductive problems. In fact, some young mares have also low reproductive efficiency, although no previous experiments have reported reproductive parameters responsible for this performance. Moreover, most research works have described reproductive disorders in old mares compared with fertile young mares.

Taking into account the information regarding physiological and pathologic mechanisms by which OMs are considered subfertile [14,17,18,2,3,4,15,19], we decided to investigate reproductive parameters of young subfertile mares (YSM) in order to understand the basis of their reproductive behavior. We hypothesize that YSM share reproductive characteristics with old subfertile ones such us IOI duration, plasmatic progesterone concentrations, MO, effluent contamination, embryo recovery rate (ERR), and embryo quality. The main objective of this study was to compare these reproductive parameters between both categories.

2. Materials and Methods

This work was performed during three consecutive breeding seasons at a farm located in Argentina (latitude 37° 25′ 28″S; longitude 59° 14′ 37″W). Forty-nine subfertile mares of Silla Argentino breed between 3 and 23 y.o. were used as donors. All mares used in this study belonged to the farm and were diagnosed as subfertile based on their individual history. These animals were classified and separated into two groups according to their ages and reproductive history: YSMs (3–10 y.o; n = 28) and OMs (13–23 y.o; n = 21). The older group included mares that had normal reproductive parameters when they were young, but for some reason, they become subfertile and were excluded from the farm breeding program. Subfertile mares were defined as those with a previous history of pregnancy failure, endometritis susceptibility, abnormal vulvar conformation, irregular cycles, hemorrhagic anovulatory follicles, embryo loss after ET, or no previous history of gestation. Mares from 11 to 12 y.o. were not included in the analysis in order to generate a clear age gap between both experimental groups. Different number of cycles (1–8) were used for data analysis on the ERR, IOI, and MO rate. Stallions younger than 13 y.o (n = 6) of proven fertility were used as semen donors.

Mares were routinely examined by transrectal palpation and ultrasonography to assess ovarian follicular activity. Once follicles of 32–35 mm in diameter and uterine edema were detected, and mares were artificially inseminated with fresh semen. Ovulations were evaluated by ultrasonography at 24-hour intervals, and their number was recorded in each estrus cycle. Mares in which ovulation had not been detected were reinseminated 24 hours after ultrasonography. The IOI was defined as the days between two consecutive ovulations. The MO rate was calculated by dividing the number of cycles with two or more ovulation by the total number of estrous cycles.

2.1. Embryo Recovery and Quality Assessment by Gene Expression Evaluation

The mare's uterus was flushed between days 7 and 9 after ovulation with 2 L of sterile Ringer Lactate solution at 35°C, using an equine lavage catheter CH 32 (Minitube International). Prior to the flushing, effective cleanness was performed to the vulva and perineal zone of each mare; sterile gloves were used to perform the flushing, with the objective of reducing possible external contamination or iatrogenic endometritis. The effluent was collected through a sterile plastic closed circuit, connected to an embryo filter. The collected fluid was rinsed into a sterile petri dish, where embryos were searched using a stereomicroscope at 20×. The number of recovered embryos was recorded for each flush. A uterine flushing was considered positive when one or more embryos were found.