Theriogenology 97 (2017) 34–40

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

The influence of age, antral follicle count and diestrous ovulations on estrous cycle characteristics of mares

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ARTICLE INFO

Article history:
Received 14 October 2016
Received in revised form
10 April 2017
Accepted 11 April 2017
Available online 13 April 2017

Keywords:
Equine
Age
Antral follicle count
Diestrous ovulation
Primordial follicle
Estrous cycle

ABSTRACT

Reproductive aging must be well understood to optimize the reproductive management of older mares and to predict their reproductive life-span. The objectives of this study were to: 1) examine age-related differences in follicular dynamics, endocrine profiles, and primordial follicle counts, 2) evaluate the influence of antral follicle count (AFC) on age-related changes in follicular parameters, and 3) determine the influence of diestrous ovulations on the estrous cycle. Young (3–8yr; n = 10), middle-aged (9–18 yr; n = 16), and old (>18 yr; n = 19) light horse mares were examined with transrectal ultrasonography to monitor follicular growth over two consecutive estrous cycles. Jugular blood samples were taken and plasma progesterone and FSH concentrations were determined by an enzyme immunoassay and radioimmunoassay, respectively. Both interovulatory intervals and follicular phases were longer and the day of follicle deviation occurred later in aged mares. Furthermore, older mares had a tendency to ovulate smaller follicles. Neither follicular growth rate, the number of ovulations nor the length of luteal phase was influenced by mare age. Interestingly, as mare age increased, mares with low AFC had longer interovulatory intervals and follicular phases than mares with medium or high AFC. In addition, the number of primordial follicles declined with an increase in mare age but varied considerably between mares of the same age. Progesterone concentrations were positively influenced by age, whereas FSH concentrations were not, despite that FSH concentrations appeared higher in aged mares during the follicular phase. Estrous cycles with a diestrous ovulation had a longer interovulatory interval as well as a longer follicular and luteal phase while day of deviation occurred later. Progesterone concentrations were significantly higher on day 14 and 16 in estrous cycles with a diestrous ovulation than without a diestrous ovulation. In conclusion, aging in mares is associated with changes in follicular parameters which in turn are closely linked to differences in antral follicle count suggesting a relationship with ovarian reserve. Therefore, determination of antral follicle counts in aged mares can provide valuable information about the reproductive aging process. Finally, diestrous ovulations have a significant influence on different estrous cycle parameters.

1. Introduction

Aging is an important physiological process that has a negative impact on reproductive success [1]. Reproductive aging in mares is associated with a decreased fertility which is the result of a variety of factors including a delayed uterine clearance [2], reduced oocyte quality [3], and a higher rate of early embryonic loss [4]. Nevertheless, older mares are often bred because of their genetic value

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http://dx.doi.org/10.1016/j.theriogenology.2017.04.019
0093-691X/

and/or performance and not for their fertility per se. Therefore, aged broodmares are frequently encountered in equine practice. To optimize the reproductive management of these older mares, veterinarians should understand which, how, and when reproductive parameters change with age. Reproductive aging in pony mares is characterized by prolonged follicular phases, longer interovulatory intervals and increased circulating FSH concentrations [5–7]. Nevertheless, there are distinct differences in estrous cycle parameters between pony and light horse mares. Compared to light horse mares, pony mares have longer interovulatory intervals [8], fewer multiple ovulations, and fewer diestrous ovulations [9]. Because of these differences in reproductive parameters between pony and light horse mares, extrapolation of findings related to effects of reproductive aging from the existing studies in ponies to horse mares may not always be possible.

Aging in mares is also associated with a decrease in the population of antral follicles [7,10], but the number of antral follicles varies widely between aged mares [11]. As large individual differences in follicular populations exist within the same age group, it is important to distinguish the mare's reproductive and chronological age [12,13]. Therefore, it is possible that some of the age-related changes in follicular parameters are linked to differences in antral follicle count (AFC). In addition, the number of primordial follicles, representing ovarian reserve, decreases with age due to a high turnover of follicles during each follicular wave. The follicular pool eventually becomes depleted which results in ovulation failure and cessation of estrous cycles [14]; however, the age at which ovarian senescence occurs varies widely between older mares [15] suggesting that there are inherent differences in the number of primordial follicles between mares of the same age.

Mare age is not the only factor that has an impact on estrous cycle parameters. The occurrence of a major secondary follicular wave is also associated with changes in the estrous cycle such as a longer interovulatory interval and a delay in the emergence of the primary follicular wave [16,17]. A major secondary follicular wave is characterized by the development of a large diestrous follicle which either ovulates (diestrous ovulation), luteinizes, or regresses [18]. The incidence of diestrous ovulations is extremely low in pony mares [9] but can be as high as 21% in Thoroughbred mares [19]. It is generally accepted that when a diestrous ovulation precedes luteolysis it can result in a prolonged luteal phase (>21 days) as the immature corpus luteum has a reduced sensitivity to PGF2α released from endometrium. However, the majority of diestrous ovulations occur within the first 10 days after ovulation [20], and therefore, are not associated with prolonged luteal phases. To the authors' knowledge, relatively little work has been published concerning the influence of early diestrous ovulations on estrous cycle parameters in light horse mares. Therefore, the objectives of this study were to: (i) determine age-related differences in follicular dynamics, endocrine profiles, and the number of primordial follicles, (ii) examine the influence of AFC on age-related changes in follicular parameters, and (iii) examine the influence of diestrous ovulations on follicular parameters and progesterone concentrations.

2. Materials and methods

2.1. Animal procedures

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Kentucky (#2010-067). Light horse mares of mixed breeds with unknown reproductive history, weighing approximately 450–650 kg, were kept in pastures with ad libitum access to water and hay. All mares were healthy and in good condition. Mares were assigned to one of the following groups: young (n = 10, 3–8 y, mean age: 5.2 y), middle-aged (n = 16, 9–18 y, mean age: 14.3 y), or aged (n = 19, 19–27 y: mean age: 23 y) mares. This study was part of a larger experiment that examined the relationship between anti-Müllerian hormone, antral follicle count, and age in mares [11]. Briefly, mares were examined with transrectal ultrasonography over two consecutive estrous cycles during the physiological breeding season (June, July and August). Ultrasound examinations were conducted every-other day for 14 days after ovulation and then daily until the subsequent ovulation. Follicular growth was monitored by recording two dimensions (width and height) of the six largest follicles and the location of all six follicles was recorded on an ovarian map. The six largest follicles were used for tracking follicular waves based upon previously published observations indicating that the diameter of the six largest follicles were associated with FSH surges [21]. In addition, the presence of uterine edema and intra-uterine fluid was recorded during each examination primarily to identify mares with uterine abnormalities. Mares with intra-uterine fluid during the luteal phase and a premature decline in progesterone concentration (due to premature luteolysis) were excluded from the analysis. On day 12, (Day 0 = day of detected ovulation) of each estrous cycle, AFC (e.g. total number of antral follicles) was determined as previously described [11]. Mares were classified as low, medium, and high AFC based upon the interquartile ranges (low AFC (lower 25th percentile): ≤16 follicles, medium AFC (middle 50th percentile): 17–33 follicles, and high AFC (upper 25th percentile): >33 follicles) for AFC. Mares with a detectable corpus luteum and turgid uterine tone that did not return to estrus by day 22 post ovulation were given prostaglandin F2α (Lutalyse®, dinoprost tromethamine, 5 mg intramuscularly), and data from those estrous cycles were excluded from the analysis. Daily blood samples were collected into heparinized tubes by jugular venipuncture between 8 and 9 a.m. Plasma samples obtained after centrifugation (1200×*g*, 10 min) were stored at −20 °C until analysis for progesterone and follicle stimulating hormone (FSH). After completion of the study, both ovaries were removed from five young mares (age range: 3–8 y, mean age: 4 y) and four aged (age range: 19–26 y, mean age: 22 y) mares for determination of the number of primordial follicles. The ovary without the corpus luteum was separated from the oviduct and mesovarium and sectioned into 0.8-cm transverse slices starting at the cranial pole of the ovary. All ovarian slices were stored in 10% buffered formalin until further processing.

2.2. Follicular parameters and dynamics

The following reproductive parameters were determined: interovulatory interval, length of the luteal and follicular phase, day of luteolysis, day of follicle deviation, diameter of the dominant follicle at deviation, growth rate of the emerging and dominant follicle, diameter of the pre-ovulatory follicle two days (d-2) and one day (d-1) prior to ovulation, number of major follicular waves per estrous cycle, number of diestrous ovulations and number of estrous ovulations. The interovulatory interval was the number of days between two consecutive ovulations each associated with a different estrous period [18]. The length of the luteal and follicular phase was determined by calculating the number of days from ovulation to luteolysis, and between luteolysis and subsequent ovulation, respectively. The last day of the estrous cycle with a progesterone concentration greater than 1 ng/mL was assigned as the day when luteolysis occurred [22]. The day of deviation was determined as described by Gastal et al. [23]. The growth rate of the dominant follicle was determined for two different time periods: from deviation until two days prior to ovulation (deviation to d-2), and from two days prior to ovulation to one day prior to ovulation (d-2 to d-1). The following two formulas were used to calculate the growth rate of the dominant and emerging follicle: