Theriogenology 86 (2016) 1506–1515

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

The period of the follicular phase during which the uterus of mares shows estrus-like echotexture influences the subsequent pregnancy rate

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ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 2 May 2016
Accepted 12 May 2016

Keywords:
Mare
Endometrial edema
Estrus
Prostaglandin F2a
Fertility

ABSTRACT

The interval from both spontaneous and prostaglandin (PGF)-induced luteolysis to ovulation is greatly variable in mares. Several reports have shown a positive association between the length of the interval from PGF treatment to ovulation (ITO) and the subsequent pregnancy rate (PR). However, it is not known whether this association also occurs in estrous cycles with spontaneous luteolysis. The main objective of this study was to determine the effect of the duration of estrus-like echotexture of the uterus during the follicular phase on the subsequent PR in both spontaneous and PGF-induced cycles. A total of 768 estrous cycles from 325 thoroughbred mares were analyzed (401 estruses were induced with exogenous PGF and 367 cycles were not treated with PGF). The following factors were taken into account to determine the effect on PR: age of the mare, stallion, year of breeding, month of season, reproductive status of the mare, use of PGF treatment, duration of follicular phase with estrus-like echotexture, interovulatory interval (IOI; in spontaneous cycles), and ITO (in PGF-induced cycles). The age of the mare ($P = 0.017$), mare status ($P = 0.031$), the ITO ($P = 0.041$), and the duration of the follicular phase with estrus-like echotexture ($P < 0.001$) influenced the PR. The PR increased with the duration of estrus and of endometrial edema in both PGF-induced and spontaneous cycles. The correlation between the duration of endometrial edema and the IOI and ITO was positive ($r = 0.5$) and significant ($P < 0.05$).

1. Introduction

The interovulatory interval (IOI) in the mare varies from 16 to 28 days, with a mean of 21 [1] to 23.5 days [2], depending on the study reported. The estrous cycle extends between two ovulations and is frequently classified into two distinct phases, the luteal and follicular phases. The luteal phase begins with the rise in peripheral progesterone concentration more than 1 ng/mL after ovulation and ends at the time of luteolysis (structural and functional), when the corpus luteum (CL) regresses and the progesterone concentration first falls to less than 1 ng/mL [3]. This phase is also referred to as diestrus and has a relatively constant duration, with a reported mean of 14.9 days, and a range from 12 to 16 days [3]. In contrast, the follicular phase that extends from luteolysis to the subsequent ovulation has a reported mean duration of 7.3 [2] or 7.6 days [1], with a wider range from 2 to 13 or from 4 to 14 days, respectively.

In a field situation, the luteal and follicular phases can be diagnosed according to uterine echotexture and the

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http://dx.doi.org/10.1016/j.theriogenology.2016.05.009

ultrasonic observation of a CL [1,4]. The luteal phase, which is dominated by progesterone (>1 ng/mL), is characterized by an absence of endometrial edema and the presence of a visible CL, whereas most of the follicular phase is characterized by the presence of endometrial edema (an estrus-like echotexture) and the absence of a CL [4]. The period during which the endometrium shows an estrus-like echotexture is not precisely correlated with the duration of the follicular phase because some mares may show edema in none, one, few, or several days before ovulation, and others can even show it 1 to 2 days beyond ovulation [1]. Apparently, one of the main factors that regulates the presence and dissipation of endometrial edema is the balance between circulating concentrations of estradiol and progesterone [5,6]. During low circulating concentrations of progesterone, an ovariectomized mare can show prominent endometrial edema as soon as 10 hours after administration of 5 mg of estradiol and this lasts for as long as 7 days [6], whereas the administration of progesterone 1 day after estradiol treatment inhibits the estrus-like echotexture of the uterus. It was concluded that the threshold of circulating progesterone concentration necessary to inhibit endometrial edema is between 1 and 2 ng/mL [5]. However, this does not seem to be the only factor regulating the presence of endometrial edema because a small percentage of mares [1] show no edema during the follicular phase despite having basal concentrations of progesterone during a so-called "silent estrus."

In mares with a mature CL, treatment with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or its synthetic analogs (PGF) induces a rapid fall in progesterone concentration (within 36 hours) to basal levels (<1 ng/mL) [7]. The interval from PGF treatment to ovulation (ITO) is also greatly variable, ranging from 2 to 16 days [8–10], regardless of the day of the estrous cycle on which treatment is administered [7]. Several reports have analyzed the effect of PGF treatment to induce estrus on pregnancy rates (PRs), and although the overall PR of mares after PGF-induced cycles was not different to that of spontaneous cycles [9–13], when the PR of PGF-induced cycles were analyzed according to the ITO, it was observed that the PR increased as the ITO became longer [9,10,14]. It is likely that the ITO correlates well with the period in which the mare shows estrus-like echotexture. However, this has not been studied critically.

The objectives of this study were to determine (i) the effect of the duration of estrus-like echotexture of the uterus during the follicular phase on the subsequent PR and (ii) the degree of association between the ITO and days of endometrial edema in mares with PGF-induced cycles and between the ITO and the IOI in mares with spontaneous cycles. It was hypothesized that the longer the duration of the follicular phase with endometrial edema, the higher the PR would be. Second, we hypothesized that this association would occur equally in mares with both spontaneous and PGF-induced cycles.

2. Materials and methods

2.1. Animals and ultrasound examination

The data were obtained retrospectively from a thoroughbred stud farm located in England (52°N), during three breeding seasons (2012–2014). Overall, 768 cycles from 325 thoroughbred mares from February to July were analyzed. The mares were mated to 41 different stallions, some of them nonresident at the farm. However, in most cycles (83%, $n = 637$ cycles), mares were mated by only one of four resident stallions. The other nonresident stallions ($n = 37$) were mated on less than 10 cycles each. For analysis purposes, these 37 stallions were grouped as one. Mares were mated from one to six cycles each within a breeding season. However, of the 768 cycles analyzed, 498 (64.8%) were first-cycle matings. Some mares were mated in more than one season. The age of the mares ranged from 3 to 23 years (mean = 10.9 ± 0.1 years). The cycles were from foaling mares ($n = 434$) and from barren or maiden mares ($n = 334$).

Mares were routinely scanned every 48 hours by the same experienced practitioner using the same ultrasound scanner (Mindray DP-6600 vet) with a linear-array probe of 7.5 MHz. All follicles larger than 20 mm, ovulations, number of CL, and number of embryonic vesicles were recorded. The presence of endometrial edema was scored subjectively by the same operator (J.R.N.) on a scale of 0 to 3 (Fig. 1) at every visit. The duration of the follicular phase with estrus-like echotexture was considered to include the number of days during which the mare reported an endometrial score of 1 to 3.

An ovulation was diagnosed by the absence of the previously recorded preovulatory follicle and then confirmed by the presence of one or more CL in the same ovary. The day of ovulation was assigned to the day in which it was first detected when the postovulatory area appeared as an ill-defined hypoechoic area (Fig. 2) within the same ovary as the previous preovulatory follicle recorded 48 hours earlier. When a well-defined hyperechoic CL (Fig. 2) appeared within the ovary where a preovulatory follicle had been recorded 48 hours earlier, the day of ovulation was assigned to the day before the day of CL detection [15,16].

2.2. Post-mating management and pregnancy diagnosis

Mares were mated according to previous records of follicle growth, preovulatory size, and day of the cycle (if this was known). Ovulation inducing drugs were not used routinely, only to ensure that the interval from mating to ovulation was less than 5 days. Therefore, if a mare had not ovulated within 48 to 72 hours of mating, she was treated with 1500 IU of hCG subcutaneously (Chorulon; MSD Animal Health, Milton Keynes, UK) to ensure ovulation in the following 48 hours. Every mare on each cycle received an intrauterine instillation of 12 mL containing 1800 mg of procaine penicillin (6 mL of Depocillin 250 mL; MSD Animal Health) and 900 mg of framycetin (6 mL of Framomycin 15%; Novartis Animal Health, Cambridge, UK) between 4 and 48 hours after mating. In addition, mares were treated with 25 IU of oxytocin intravenously (2.5 mL of Oxytocin Leo; Leo Animal Health Laboratories, Aylesbury, UK) 24 hours after the intrauterine antibiotic treatment.

Pregnancy diagnosis was performed 12 to 14 days postovulation. Twin reduction was carried out manually, if possible at the first pregnancy diagnosis. The smaller of the