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The effect of the interval from PGF treatment to ovulation on embryo recovery and pregnancy rate in the mare

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ABSTRACT

The objective of this study was to determine the effect of the interval from induced luteolysis to ovulation on fertility of mares from two different farms. At farm 1, 215 mares were inseminated with frozen/thawed semen during 513 estrous cycles over seven consecutive breeding seasons. Estrus was induced with analogues of PGF2α in 179 cycles. At farm 2, 375 embryo flushings were performed in 65 donor mares inseminated with fresh semen; of which, 327 were performed following artificial insemination after PGF-induced luteolysis. In both farms, the intervals from PGF treatment to ovulation (ITO) data were divided into three interval groups: less than 6 days, 6 to 8 days, and greater than 8 days. A mixed regression model was created to determine the effect of different factors on the pregnancy rate (PR) and embryo recovery rate (ERR). Of all factors analyzed, the ITO was the only one that significantly influenced the PR and ERR ($P < 0.05$). In farm 1, the PR of mares with an ITO of less than 6 days, 6 to 8 days, and greater than 8 days was 26.6%, 39.4%, and 55.9%, respectively ($P = 0.01$). The PR for mares inseminated after spontaneous luteolysis (without PGF) was 42.5%. In farm 2, the ERR of donor mares for the same ITO groups was 55.0%, 62.6%, and 73.7%, respectively ($P = 0.02$). The ERR for mares flushed after a previous spontaneous estrus was 75.0%. In conclusion, the ITO had a significant effect on the PR and ERR in the mare. Fertility was reduced as the ITO became shorter.

1. Introduction

Spontaneous luteolysis occurs at a mean of 14.9 days after ovulation [1]. The length of spontaneous luteolysis has been shown to be 22.9 hours [2]. The interovulatory interval (IOI) in the mare varies from 16 to 25 days [3], with a mean of approximately 21 days. This large variation in IOI (16–25 days) appears to be because of the difference in the length of estrus rather than diestrus itself, which is rather constant [1]. Among others, this variation can be explained by breed and seasonal factors. Pony mares have, on average, a 2-day longer IOI than horses [1]. The IOI of mares earlier in the season is longer than during the more advanced ovulatory season (summer months). This is associated with a lower mean daily LH concentration [4]. However, the main factor responsible for such a large variation in the IOI appears to be related to the follicular dynamics. Follicular dynamics actively influence the length of estrus. Within an individual mare, the follicular diameter on the first day of estrus, the daily follicular growth rate, and the diameter of the preovulatory follicle influence the length of estrus [5]. Nevertheless, most mares have an IOI of 20 to 22 days, with an interval from the end of luteolysis to ovulation of 5 to 7 days [3].

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From the early 70s, PGF2α and its synthetic analogues (PGF) have been widely used in equine reproduction to induce luteolysis and so reduce the length of diestrus to bring the mare into estrus at the veterinarian's convenience. In mares with a mature CL (>5 days), PGF treatment induces a rapid fall in progesterone concentration (within 36 hours) to basal levels (<1 ng/mL) [6]. The interval from PGF treatment to ovulation (ITO) is also greatly variable: 2 to 16 days [7–9], regardless of the day of the estrous cycle in which treatment is administered [6]. As in spontaneous luteolysis, the main factor that influences the ITO is follicular dynamics. The diameter of the largest follicle at the time of treatment, its daily growth rate, and whether it progresses to ovulation or regresses influence the ITO.

Several reports have analyzed the effect of PGF treatment to induce estrus on pregnancy rates (PRs) [9–12]. However, only one study [9] correlated the ITO and fertility of PGF-treated mares. None of these studies found an overall negative effect of PGF on PRs compared with non-induced cycles. However, when the PR of PGF-induced cycles was analyzed according to the ITO, it was observed that the PR increased as the ITO became longer [9]. The authors speculated that the effect of ITO on PR could be due to differences in the oocyte quality and postmating uterine inflammation among mares with different ITO. However, these hypotheses were not tested critically.

The main objective of this study was to determine the effect of the ITO on the PR and embryo recovery rate (ERR) in two different clinical settings: a stud farm in which mares were inseminated with frozen semen to carry their own pregnancies (farm 1) and in an embryo transfer center (farm 2). The hypotheses tested were (1) the use of PGF to induce estrus would not affect the ERR and PR compared with mares with spontaneous luteolysis, (2) the ITO would be positively correlated with the ERR and PR, and (3) the reduced fertility of mares with a short ITO would be partly accounted for by a greater degree of postmating-induced inflammation.

Further objectives of this study were to (1) determine the effect of PGF and ITO on multiple ovulation (MO) rate and (2) describe the distribution of ITO length and correlate it with the follicle diameter at the time of PGF treatment and how it is influenced by the month of season.

2. Materials and methods

The effects of the ITO and several other factors on the PR and ERR were obtained from a retrospective analysis of data from a large stud farm in which the mares were inseminated with frozen/thawed semen (farm 1) and from an embryo transfer center in which the donor mares were inseminated with fresh semen and the embryos flushed 8 to 9 days after ovulation (farm 2).

For data analysis purposes, the ITO was classified into three groups: (1) intervals less than 6 days, (2) intervals between 6 and 8 days, and (3) intervals greater than 8 days. The group 2 was created to set a reference interval similar to control mares with spontaneous luteolysis because most mares have an IOI of 20 to 22 days, with a spontaneous luteolysis occurring at a mean of 14.9 days after ovulation [1]. Progesterone takes place between 24 and 36 hours to reach a concentration below 1 ng/mL after PGF treatment [6].

2.1. Farm 1

The stud farm was located in Denmark (northern hemisphere). During the breeding season (April to August) of 7 consecutive years (2007–2013), a total of 513 estrous cycles from 215 different mares (visiting and resident to the stud farm) were used in the analysis. The main breed of mare was Standardbred. The artificial inseminations (AI) were performed with frozen/thawed semen of 120 stallions. On arrival, new mares (of unknown reproductive history) were teased daily with a teaser stallion until they showed positive signs of estrus. Teasing behavior was classified as negative (−), positive (+), and highly positive (++) according to the intensity of estrous behavioral signs. If a mare did not tease positively for more than 4 to 5 days, she was examined with ultrasound. Mares with a CL were administered 7.5 mg of intramuscular luprostiol (1 mL Prosolvin; Virbac). Approximately, one third (34%) of estruses were induced with PGF. Once in estrus (positive teasing behavior, presence of endometrial edema or absence of a CL, and presence of a large follicle), mares were scanned with ultrasound three times a day at 8 hours, 16 hours, and 22 hours and teased once a day (in the morning at 8 hours) until ovulation. Mares in which the previous ovulation date were known were scanned after Day 17 (Day 0 = Day of ovulation). Mares not in estrus but not pregnant by Day 17 were treated with PGF. Ovulation was not induced routinely (only 66 of 513 cycles were induced with hCG). After the detection of ovulation, mares were inseminated with frozen/thawed semen of the desired stallion into the uterine body. Frozen semen straws were thawed during a minimum of 25 seconds in a water bath at 37.5 °C. After thawing, spermatozoa quality was assessed subjectively by a light microscope on a warm slide. According to sperm progressive motility (PM), semen was classified into "bad" (PM < 30%), "average" (PM = 30%–50%), and "excellent" (PM > 50%). Pregnancy diagnosis was performed 14 days after ovulation. Mares suspected of endometrial inflammation or infection were biopsied. The uterine biopsy was cultured directly. Positive mares were treated by the uterine route during the following estrus according to the bacteriologic culture results. Cycles with signs of endometrial inflammation (accumulation of fluid post-AI and or with a positive culture from the biopsy) were classified as "problem mares."

For each cycle, the following end points were obtained and taken into account for analysis:

- year, month, and number of AI within each season
- use of PGF to induce estrus
- the diameter of the largest follicle at the time of PGF treatment
- the interval in days from PGF treatment to the subsequent ovulation (ITO)
- number of ovulations at the same estrus
- teasing behavior on the day of AI (ovulation)
- postthawed semen quality
- number of embryonic vesicles at the first pregnancy diagnosis
- signs of endometrial inflammation (accumulation of fluid and positive culture from biopsy): "problem" or "normal" mare