Journal of Equine Veterinary Science 35 (2015) 956–959

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

The Effect of Time of Breeding Relative to Ovulation on Pregnancy Rate When Using Cooled Transported Semen or Natural Mating in the Mare

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ARTICLE INFO

Article history:
Received 30 May 2015
Received in revised form 1 September 2015
Accepted 4 September 2015
Available online 10 September 2015

Keywords:
Mare
Time relative to ovulation
Natural mating
Cooled semen
Pregnancy rate

ABSTRACT

This retrospective study analyzed data from 505 inseminations with cooled transported semen and 387 natural matings. The analysis shows that similar ($P > .05$) per cycle pregnancy rates (PRs) can be achieved with cooled transported semen equal to that with natural mating, provided that insemination is performed between 24 hours before and 12 hours after ovulation. Any earlier inseminations relative to ovulation yielded lower PRs ($P < .05$) than in mares bred by natural mating.

1. Introduction

Since the advent of artificial insemination (AI) originally designed to divide the ejaculate to inseminate more mares, in order to preserve the viability of semen during storage and during movement over long distances, it has been necessary to either cool or freeze it. Cooled transported equine semen is now used worldwide for most breeds except the Thoroughbred. Reported per cycle and seasonal pregnancy rates (PRs) after cooled semen inseminations varied from 48% and 74% [1], 59.4% and 74.7% [2] to 65% and 91% [3], respectively. The success rate has been considered to be dependent on the quality of the semen after cooling and transport. Metcalf [4] rated semen with a motility of >40% and morphology >50% as good to excellent and reported a PR of 71%. On the other hand, other factors such as the time of insemination relative to ovulation could play an even more important role in the final outcome of pregnancy [5–7].

Semen from most stallions survives slow cooling to 4°C and retains an acceptable level of motility (i.e., > 40%) for 48 to 72 hours if maintained at this temperature [8]. However, the process of cooling definitely diminishes the fertilizing capability of spermatozoa during its time within the mare's genital tract as compared with fresh spermatozoa deposited in the mare by natural mating [6,7]. This reduction in longevity is more marked in frozen/thawed semen and is thought to be due to premature acrosome reaction in frozen/thawed semen [9] or to membrane alteration in function and structure in cooled semen [10]. Most cooled semen appears to maintain its viability for 24 hours once inseminated. However, its viability decreased rapidly >24 hours after insemination [5–7]. Although there is enough evidence to show the shortened lifespan of cooled semen and how its viability is gradually reduced once deposited in the mare's genital tract [5–7], not every clinician makes the best arrangements to ensure

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http://dx.doi.org/10.1016/j.jevs.2015.09.004

the mare ovulates within 24 hours of insemination to maximize PR. This may occur because many still consider cooled semen as fresh semen/natural mating or simply because they forget to or do not routinely administer ovulation-inducing drugs 24 hours before the expected time of insemination.

The objective of this study was to determine the relationship between the interval from breeding (AI or natural mating) to ovulation and the outcome of pregnancy, to determine the ideal time window to breed a mare for highest PR with each type of breeding protocol. It was hypothesized that the PR of mares inseminated with cooled semen would be only comparable with that of mares bred by natural mating if the insemination occurred within 24 hours of ovulation.

2. Materials and Methods

The data from all mares inseminated with cooled transported semen at the clinic, regardless of quality, were used for a retrospective analysis of the effect of the interval from a single insemination to ovulation on PR during the breeding seasons of 2004 to 2014. All mares were examined at least twice daily (approximately every 12 hours), for diagnosis of ovulation. The time of every examination was recorded so that the interval between the last preovulatory and the first postovulatory examinations was known. The time of insemination/natural mating relative to ovulation in this study ranged from >48 hours (up to 72 hours for natural mating and up to 60 hours for insemination with cooled semen) before to 24 hours after ovulation.

Data from donor mares which were flushed for embryo transfer 8 to 9 days postovulation were also included. Recovery of at least one embryo was a positive result, whereas nonrecovery was a negative result. Overall, there were 505 inseminations with cooled transported semen and 387 natural matings available for analysis (837 for mares to carry their own pregnancy and 55 for embryo transfer). Pregnancy diagnosis was performed 12 to 20 days after ovulation. The number of mares for each type of breeding protocol and specific interval relative to ovulation is shown in Fig. 1.

All cooled semen samples were used unless the mare had already ovulated more than 24 hours previously. Inseminations with cooled transported semen were made by pipette, and the whole dose of semen supplied by the stud farm was deposited in the body or at the base of a uterine horn. No attempt was made to inseminate into the horn ipsilateral to the ovulating ovary or deeper than the horn base. At the next examination, the uterus of every mare was lavaged (in and out) with 2 L of sterile warm saline solution (0.9% NaCl). After recovery of all saline fluid, the mare was given an intrauterine instillation of a mix of 12 mL containing 6-mL (1800-mg procaine benzylpenicillin) of injectable Depocillin (300 mg/mL, MSD Animal Health, UK) and 6 mL (900 mg framomycin) of injectable Framycetin (15%) (150 mg/mL, Novartis Animal Health, Camberly, UK) as previously reported [6]. At subsequent examinations, when intrauterine fluid was visible on ultrasound, 25 IU of oxytocin (10 IU/mL Oxytocin Leo, Leo Animal Health, Aylesbury, UK) was given intravenously.

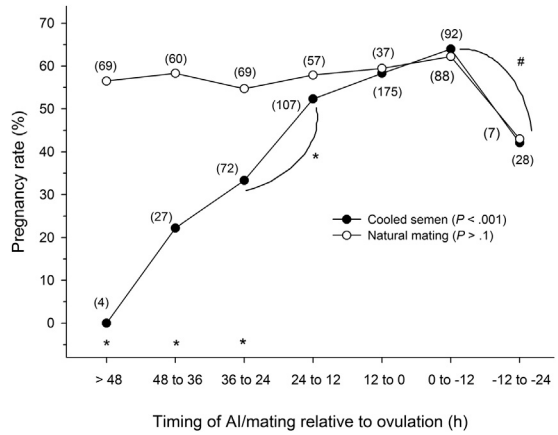

Fig. 1. Effect of time relative to ovulation on pregnancy rates (PR) of mares inseminated with cooled transported semen (closed circles) or bred by natural mating (open circles) between >48 hours before (up to 72 hours in mares bred by natural mating and up to 60 hours in mares inseminated with cooled semen) and 24 hours after ovulation. The number of mares in each interval is shown in brackets. The regression analysis showed a significant effect of the time of AI, but not of mating, relative to ovulation ($P < .001$) on PR. Asterisks (*) indicate a significant change ($P < .05$) or a tendency (#; $P < .1$) in PR between two consecutive intervals within a group and a difference ($P < .05$) in PR at a given interval between mares inseminated with cooled semen and bred by natural mating. AI, artificial insemination.

Although most inseminations were made in the most desirable period of 24 hours before ovulation, due to miscalculation, delayed ovulation, or delayed arrival of semen, this was not always possible. Some semen was therefore inseminated more than 24 hours before ovulation. Other semen samples which arrived after the mare had ovulated were also used, and therefore, considerable data on PR after postovulatory inseminations were generated. All mares were inseminated only once per estrus. For comparison, the results from natural mating during the same period and under the same management conditions were also analyzed.

The relationship between the interval from insemination/mating to ovulation and PR was determined by binary logistic regression analysis. Two separate models were created for inseminations with cooled semen and natural matings. The Fisher's exact test was used to test any difference in PR between two consecutive interval points within a group and between groups of different semen (cooled vs. natural mating) at a given interval point.

3. Results

The regression analysis showed a significant effect ($P < .001$) of the time of AI relative to ovulation on PR for mares inseminated with cooled semen (Fig. 1). As the interval from AI to ovulation became shorter, the PR increased. The first significant increase in PR occurred between the intervals of 36 to 24 hours and 24 to 12 hours before ovulation ($P < .05$; Fig. 1). The first decrease ($P = .08$) in PR occurred between the intervals 0 to 12 hours and 12 to 24 hours after ovulation. Therefore, the PR in these mares was highest in mares inseminated in the time window