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Original Research

Utilization of One-Dose Postovulation Breeding With Frozen-Thawed Semen at a Commercial Artificial Insemination Center: Pregnancy Rates and Postbreeding Uterine Fluid Accumulation in Comparison to Insemination With Chilled or Fresh Semen

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ABSTRACT

Insemination with frozen-thawed (FZ) semen has been associated with an increased incidence of postbreeding intrauterine fluid (IUF) accumulation and decreased pregnancy rates (PRs) per cycle compared with fresh (F) and chilled (CH) semen. This retrospective study was undertaken to determine the PR and incidence of postbreeding IUF associated with insemination of FZ semen. Records for 1,023 insemination cycles (578 mares) using F, CH, or FZ semen from 240 stallions over a 3-year period from a single artificial insemination center were reviewed. Clinical data were collated for univariable and multivariable analyses. Over all semen types, PR was 52.2%. Frozen-thawed semen achieved a similar PR to CH semen (48.6% vs. 43%, respectively), and both were lower than F semen (63%). The use of FZ or CH semen also resulted in similar PR in mares of advanced age and old maiden mares (>11 years). The use of prophylactic uterine lavage (4–8 hours after insemination) in mares inseminated with FZ semen decreased over the study, being 40%, 22%, and 8% in years 1, 2, and 3, respectively. There was no difference in FZ semen PR over this time nor was there a difference in the incidence of >2 cm IUF 1-day after insemination in FZ cycles (5.5%, 8.3%, and 5.6%, respectively), and this incidence was less than that recorded for F (16.8%) and CH (17.8%) cycles over this time. These data indicate that FZ semen may be used in a commercial setting with no difference in PR or uterine sequelae to that for CH semen.

1. Introduction

The use of frozen-thawed (FZ) semen offers horse breeders an option to breed to stallions regardless of their current availability or geographic location. However, studies on FZ semen have reported that its use is associated with lower pregnancy rates (PRs) [1,2], increased intrauterine fluid (IUF) accumulation postbreeding [3], and increased risk of persistent postbreeding endometritis (PPBE) [4]. The greater risk and severity of PPBE with FZ semen have been attributed to both the relative absence of seminal plasma and the relatively increased concentration of spermatozoa in the inseminate compared with fresh (F) or chilled (CH) semen [4–6]. For these reasons, avoidance of

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FZ semen has been advised for the insemination of "problem mares" [1]. A "problem mare" can be defined as one that is susceptible to PPBE, has delayed uterine clearance, has reproductive anatomical abnormalities, or is simply of advanced age [7,8]. This can cause an issue for owners of such mares, as it greatly narrows the range of potential stallions that may be used as sires.

Pregnancy rates of over 50% per cycle have been reported with the use of FZ semen from selected stallions from which semen was frozen by the authors [9–12], but it is unclear how applicable these results are to commercial practice. To best understand the impact of the use of FZ semen, as compared with F or CH semen, on breeding efficiency under commercial practice conditions, a comparison of PRs using a wide variety of commercially marketed semen under standard practice conditions should be performed. Crowe et al [13] compared PRs between FZ and CH semen under commercial conditions, using a two-dose fixed-time FZ semen insemination protocol. The study evaluated records from 251 mares, managed on three stud farms, and reported a 59.1% PR per cycle for FZ semen compared with 44% for CH. However, this study had several factors that made interpretation difficult: All mares were treated with oxytocin and uterine irrigation with antibiotics after insemination, and the population of mares inseminated with the two semen types differed, but the effect of mare type was not analyzed statistically. Additionally, although the use of two-dose fixed-time insemination with FZ semen has been associated with greater PRs [14], it may be resisted by some owners due to the value of the FZ semen, which is often sold by the dose or even by the straw. Similarly, the use of a standard postinsemination treatment increases client cost and contributes to overuse of antibiotics. However, in light of the reported increase in postbreeding IUF accumulation associated with FZ semen, the set postbreeding treatment may have been a factor in the high PRs obtained with FZ semen in that study.

This retrospective analysis was performed to determine whether (1) single-dose postovulation insemination of FZ semen resulted in similar PRs to those for CH semen across a population of mares with mixed reproductive histories in a commercial setting; (2) to evaluate if the use of FZ semen under these conditions resulted in an increased incidence of postbreeding IUF accumulation, and (3) to evaluate the impact of prophylactic postinsemination lavage on these parameters.

2. Materials and Methods

2.1. Animals

Data were collected from a single commercial artificial insemination (AI) center in Northern England during the breeding seasons of 2012, 2013, and 2014 (years 1, 2, and 3, respectively). Clinical records for 578 mares during 1,023 cycles were evaluated. Mares were predominantly of sport horse breeds, aged 2 to 27 (12.3 ± 4.62, mean ± standard deviation) years, and had varied reproductive histories. Maiden mares were defined as those that had not foaled before, foaling mares were those that had a foal at foot at the time of breeding, and barren mares were those that had previously had a foal but were open for unknown reasons (either due to subfertility in the previous season or owner preference).

Stallion choice was determined by owner preference. Semen used in the recorded breeding cases originated from 240 different commercially available stallions. Fresh semen was collected on site, diluted 1:1 with INRA 96 (IMV Technologies, France), and portioned so that a minimum insemination dose of 500 million progressively motile sperm was achieved for each mare (n = 322 cycles). Chilled, transported semen was from various studs in the UK and Europe, arriving in volumes ranging from 10 to 40 mL at unknown concentrations (n = 242 cycles). Frozen-thawed semen was processed at various centers worldwide, packaged in 0.5-mL straws at an unknown concentration. Insemination doses for FZ semen were recommended by the processor and varied from 1 to 10 straws (n = 459 cycles).

2.2. Breeding Protocol

All veterinary work was carried out by one of four clinicians (M.M. 85%, N.L. 13%, and two others 2%) in accordance with clinical protocols established by the lead veterinarian. On arrival, mares were examined by transrectal palpation and ultrasonography to determine ovarian cycle stage. If mares were in diestrus, cloprostenol (Estrumate, MSD Animal Health, Buckinghamshire, UK) was administered to induce luteolysis when deemed appropriate to best suit semen availability. Mares were examined daily when in estrus, and if indicated (e.g., history of subfertility, detectable IUF), an endometrial swab sample was taken for bacteriological culture and sensitivity. When a follicle >35 mm diameter was observed in the presence of uterine edema and a relaxed cervix, an ovulation agent, either 1,500 IU human chorionic gonadotropin (hCG, Chorulon, MSD Animal Health) or 2.1 mg deslorelin (Ovuplant, Dechra, Northwich, UK), was administered. Deslorelin was used preferentially if there was history of nonresponse to hCG in individual mares or if CH semen availability meant ovulation was induced on a smaller follicle (30–35 mm). For F and CH semen, insemination was performed into the uterine body approximately 24 hours after administration of an ovulation agent and confirmation of the continued presence of the preovulatory follicle (i.e., preovulation breeding). If ovulation had not occurred by 24 hours after insemination, the mare was reinseminated if semen was available. Only cycles in which ovulation occurred within 24 hours of insemination were included for analysis.

For FZ semen, mares were treated with an ovulation-induction agent at 21.00 hours. The following day, mares were examined at 08.00 hours and 16.00 hours. If the mare had signs of impending ovulation (decreasing edema or follicular serration) at 16.00 hours, an additional examination was performed at 22.00 hours; otherwise, the next examination was at 07.00 hours the next day (34 hours from administration of the ovulation agent) and was repeated at 2 to 6 hour intervals until ovulation was detected. When ovulation was detected, straws comprising one insemination dose of FZ semen, as defined by the