Role of Reinforcement Breeding in a Natural Service Mating Program

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Mares receiving reinforcement breeding had a greater chance of getting pregnant than mares that were not reinforced; this resulted in an average increase of 12% per cycle pregnancy rate in 8 of 13 stallions in the study. Effect of reinforcement breeding was influenced by the number of sperm recovered: mares receiving more than an estimated 200,000,000 total sperm had a higher per cycle pregnancy rate. Results of this study show that considerable improvement in pregnancy rate can be achieved in some Thoroughbred stallions by using the reinforcement breeding technique. Authors’ addresses: Hill N Dale Farm, 4252 Spurr Road, Lexington, KY 40511 (Blanchard, Ramsey, O’Meara); and Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Texas A & M University, College Station, TX 77843 (Love, Thompson); e-mail: tblanchard@hillndalefarms.com (Blanchard). © 2006 AAEP.

1. Introduction

One management strategy sometimes employed with natural service mating programs is reinforcement breeding (sometimes termed impregnation). This process involves obtaining a dismount sample from the penis of the stallion immediately after mating (cover) and infusing that sample into the uterus of the mare just mated. Proponents for the process claim fertility is improved, perhaps because of an increased number of sperm accessing the uterus for transport to the oviducts. Others claim that the number of sperm recovered in dismount samples is too small and the quality of sperm too poor (e.g., because of cold, osmotic shock, and contamination with debris) to improve fertility of most matings. To better evaluate the potential benefits of reinforcement breeding to fertility of stallions used in a natural-service mating program, we performed the following study.

2. Materials and Methods

Records for 2005 were analyzed from a Thoroughbred breeding farm in central Kentucky. Mating results were available for 2171 estrous cycles of 1406 mares bred to 1 of 13 stallions. To confirm ejaculation, attempts were made to collect residual semen (drippings) from each stallion’s penis into a cup after dismount in each mating. When reinforcement breeding was planned, the dismount sample was mixed with 10–30 ml of warm (35°C) nonfat dried milk solids glucose extender containing gentamycin and poured through a nylon mesh filter into a clean warm cup to remove debris. A 10-μl drop of the
extended-dismount sample was placed on a warm slide, covered with a coverslip, and evaluated on a phase-contrast microscope with warming stage for estimation of progressive sperm motility and sperm concentration by one of two experienced observers. The filtered extended-dismount sample was aspirated into a sterile plastic syringe, and the volume was determined. The estimated total number of sperm in the syringe was calculated to be the product of volume in the syringe and estimated sperm concentration. The syringe was attached to a sterile insemination pipette and immediately taken to the mare. After breeding, the mare had been placed in a stock, and the perineal area was cleaned. With a sterile sleeve over the arm, a small amount of sterile non-spermicidal lubricant was placed on the fingers. The pipette was carried into the vagina with the fingers used to guard the pipette tip during dismount insertion through the cervix. The extended dismount sample was then infused into the body of the uterus of the mare.

An attempt was made to perform reinforcement breeding on all mares returning for mating on second or later estrous cycles. For two stallions in which pregnancy rates seemed to be dramatically improved by reinforcement breeding (determined after pregnancy reports had arrived on ~10 mares bred in this manner on a second cycle), attempts were made to use reinforcement breeding on all subsequent mares, including those presented for their first cycle of mating. In some cases, the mare or stallion inadvertently moved during mating (resulting in vaginal ejaculation without full penile insertion) or the penis was withdrawn from the vagina during the ejaculatory process, and reinforcement breeding was performed regardless of cycle. If an adequate dismount sample was obtained (e.g., insufficient volume, heavy urine contamination, all sperm were dead, etc.), reinforcement breeding was not performed.

Data tabulated for analysis included date of mating, stallion used for mating, session of the day for the stallion (1st, 2nd, 3rd, etc.), mare mated, mare age, beginning mare status (maiden, foaling, barren, not bred, slipped), cycle of breeding (1st, 2nd, 3rd, etc.), whether or not the mare was mated twice during the same estrus (double), whether or not the mating was on foal heat, whether or not the mare was reinforcement bred on that cycle, the estimated number of sperm infused into the uterus after cover if the mare was reinforcement bred, and pregnancy outcome for that cycle of mating.

To compare pregnancy rates between reinforcement and non-reinforcement bred mares, the statistical model used to adjust for potential confounding variables (stallion, mare, month, status, age, session, foal heat, and double) was relatively complex and employed recently developed estimation methods. Briefly, the model used Bayesian inference with vague prior beliefs and a Markov Chain Monte Carlo (MCMC) implementation. The MCMC implementation was performed by use of WinBUGS version 1.4.1. The initial 1000 iterations were discarded to allow for convergence, and the next 100,000 iterations were sampled for the posterior distribution. To evaluate whether estimated sperm number affected the change in pregnancy rate related to reinforcement breeding, the reinforcement dose was considered “high” if above the median for estimated sperm number (200,000,000 total sperm) and “low” if less than or equal to the median for estimated sperm number.

3. Results

Accounting for the effects of mare age, mare status, foal-heat mating, whether a mare was doubled, stallion, and month of the year, reinforcement breeding significantly improved pregnancy rates compared with those rates achieved without reinforcement breeding (mean odds ratio = 1.52, 95% CI = 1.15–2.02; p < 0.05). Compared with mares that were not reinforcement bred, pregnancy rate increased 11.7% (95% CI = 4.75–18.9%; p < 0.05) when the reinforcement dose contained at least an estimated 200,000,000 total sperm, but rates increased only 6.3% (95% CI = −2.2–13.1%; p < 0.1) when the reinforcement dose contained less than an estimated 200,000,000 total sperm.

4. Discussion

Reinforcement breeding (or “impregnation”) is a term used on central Kentucky Thoroughbred stud farms for a process where the dismount semen sample is collected after natural mating (cover) and is then infused into the uterus of the mare that was just mated (covered). Because debris from the mating process (mare, stallion) and breeding-shed floor can get into dismount samples, we believe it is wise to first filter the dismount semen sample to remove as much debris or dirt as possible. Before infusing the dismount sample into the uterus of the mare, it should also be mixed with a suitable volume of warm extender that contains a broad-spectrum antibiotic. The process has some potential advantages that might improve fertility: (1) it results in an increased number of sperm placed directly into the uterus; (2) the extender could provide some protection to sperm that may improve their livability within the uterus until they can access the oviduct where fertilization occurs; and (3) the antimicrobial present may help to control post-breeding endometritis. Our finding that reinforcement breedings with estimates of more total sperm resulted in higher pregnancy rates confirms that the number of sperm contained in the reinforcement breeding is important, and it also suggests that attempts should be made to collect as many sperm as possible in the dismount drippings (e.g., by using wide-mouth collection cups and quickly positioning the cup beneath the penis after the stallion dismounts the mare).

This study did not determine if extenders containing antibiotics protect sperm in the uterine environ-
ment or decrease post-breeding endometritis in mares. It is possible that the addition of extender containing antibiotics may improve livability of semen in a “hostile” environment such as occurs in mares with endometritis, fluid accumulation, and urine pooling. Infusion of semen extender containing antibiotics into the uterus of the mare immediately before cover has been proposed as a method to control and prevent endometritis, particularly when potentially pathogenic bacteria are being shed in the semen (dubbed the Minimum Contamination Technique). Though unproven, it is also possible that mixing the dismount sample with antibiotic-containing extender may be of value when “hostile” fluid accumulations are present within a mare’s genital tract. In this respect, recent research by Troedsson et al. showed that sperm are found bound to neutrophils in samples collected from mares that were bred while they had ongoing endometritis present.

References and Footnotes

aINRA-96, IMV, Maple Grove, MN 55369.
bDisposable Nylon Mesh Gel Filter, Animal Reproduction Systems, Chino, CA 91710.
cPriority Care Non-Spermicidal Lubricating Jelly, First Priority, Inc., Elgin, IL 60123.