Theriogenology 86 (2016) 1399–1402

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

The effect of two levels of hemospermia on stallion fertility

C.E. Turner*, S.R. Walbornn, T.L. Blanchard, D.D. Varner, S.P. Brinsko, K.A. LaCaze, S.R. Teague, C.C. Love

Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA

ARTICLE INFO

Article history:
Received 26 February 2016
Received in revised form 29 April 2016
Accepted 29 April 2016

Keywords:
Hemospermia
Stallion
Fertility
Blood
Sperm

ABSTRACT

Hemospermia can occur consistently or intermittently in stallion ejaculates and may cause a reduction in the fertility of the affected ejaculate. It is unknown what amount of blood in an ejaculate leads to subfertility. This study investigated the effect of higher and lower levels of hemospermia (50% and 5%, respectively) on fertility using 24 reproductively normal mares inseminated over three consecutive estrous cycles with fresh extended semen. Mares inseminated with a 5% blood-contaminated ejaculate became pregnant at the same rate (75% per cycle; 18 of 24) as the mares inseminated with blood-free (control) semen (75% per cycle; 18 of 24). The ejaculates containing 50% blood were sterile (0% per cycle, 0 of 24). We concluded that it is the amount of blood, not the mere presence of blood, in an ejaculate that impacts fertility.

1. Introduction

Hemospermia, the presence of blood in semen, can occur consistently or intermittently and has been described as a factor associated with infertility or subfertility in stallions [1]. It has been observed in many breeds and has been reported to occur more commonly in stallions that are used heavily for breeding [2,3]. The causes of hemospermia in the stallion are varied and include penile and urethral lacerations [4], urethritis [4], neoplasia [5], blocked ampullae [6], epididymitis [7], inflammation of the accessory sex glands [8], urethral rent [2], coital exanthema [7], cysts of the uterus masculinus [9], and cutaneous habronemiasis [3]. These conditions can result in variable amounts of semen contamination with blood. Voss et al. [10] reported that the cause of subfertility may be more related to the presence of the erythrocytes than serum. Voss and Wotowey [1] stated that "stallions that have overt hemorrhage in each ejaculate are sterile, while the stallion that bleeds occasionally is infertile with the affected ejaculate being sterile"; however, our anecdotal experience suggests that it is the amount of blood (i.e., pink tinged to overt hemorrhage) that impacts fertility. The aim of this study was to evaluate the effects of two levels (low and high) of blood contamination (5% and 50% v:v, respectively) of stallion semen on fertility.

2. Materials and methods

2.1. Animals

The study was conducted in southeast Texas from August to November of 2015. Animal use and procedures were approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC 2015–0026).

Twenty-four light breed mares (5–18 years) and one light breed stallion (26 years), all in good body condition [11], were included in the study. Mares were maintained on pasture with *ad libitum* access to water and coastal bermudagrass hay. The stallion, with known normal fertility, was housed in a 3.25 × 3.88-m box stall with daily hand walking and a diet of coastal bermudagrass/alfalfa hay and concentrate with *ad libitum* access to water.

* Corresponding author. Tel.: (979) 845 3541; fax: (979) 458 4380.
E-mail address: ceturner@rocketmail.com (C.E. Turner).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.04.084

2.2. Semen collection

Gel-free semen was collected using a Missouri model artificial vagina (Nasco, Fort Atkinson, WI, USA) lightly lubricated with sterile, nonspermicidal gel (Therio-Gel; Agtech, Inc., KS, USA) and fitted with an inline nylon filter (Animal Reproduction Systems, Chino, CA, USA). A mare exhibiting behavioral estrus was used for sexual stimulation, and a breeding phantom was used as a mount source. Immediately before semen collection, the stallion's erect penis was cleansed with warm water and thoroughly dried.

2.3. Semen processing

Immediately after semen collection, the gel filter was removed from the collection bottle, and the gel-free semen was weighed for volume and placed in an incubator (37 °C). Sperm concentration was determined using the NucleoCounter SP-100 (ChemoMetec A/S, Allerød, Denmark) as previously described [12]. Prewarmed (37 °C) extender (INRA 96; IMV Technologies, L'Aigle, France) supplemented with ticarcillin/clavulanate (1 mg/mL; Timentin; GlaxoSmithKline, Research Triangle Park, NC, USA; INRA-T) was added to an aliquot of neat semen to obtain a sperm concentration of 30×10^6/mL. This aliquot was used to examine sperm motility (IVOS version 12.2 L; Hamilton Thorne Biosciences, Beverly, MA, USA) and viability [13] of each ejaculate to verify that semen quality was representative of what the stallion typically ejaculated (% total motility ≥41, average 59%; % viability ≥39, average 50%) for use in the experiment.

For the fertility trial, a portion of each ejaculate was extended 1:1 with INRA-T in a 50-mL conical tube and was subjected to cushioned centrifugation using MaxiFreeze cushion fluid (1 mL; IMV technologies, L'Aigle, France) as reported previously [14]. After centrifugation, the supernatant was immediately aspirated, and the majority of the cushion fluid was removed. The remaining sperm pellet was resuspended to 25×10^6 sperm/mL using each of the following diluents: (1) INRA-T (T0; control); (2) INRA-T containing 5% whole blood (T5); or (3) INRA-T containing 50% whole blood (T50). The final inseminate volume (10 mL) contained 250×10^6 sperm with 0%, 5%, or 50% (v:v) blood. All blood for the experiment was obtained from the project stallion by jugular venipuncture in red-top tubes. Blood was collected immediately before mixing with semen and within 5 minutes before insemination to avoid clotting.

2.4. Fertility study

Each mare was inseminated on three consecutive estrous cycles such that each mare randomly received each treatment. All mares were examined by transrectal palpation and B-mode ultrasonography (Edge; FUJIFILM SonoSite, Inc) of the genital tract for staging of the estrous cycle. These procedures were repeated as necessary for breeding management and pregnancy diagnosis for each mare for the duration study. All mares with active luteal tissue (based on palpation and ultrasound findings) were administered cloprostenol (Estrumate; Merck & Co., Inc., NJ, USA; 250 μg intramuscularly). Once mares developed a follicle with an average diameter ≥35 mm and were deemed in estrus because of the presence of uterine edema, a relaxed cervix, and/or behavioral signs of estrus, they were administered deslorelin (SucroMate; Thorn BioScience LLC, KY, USA; 1.8 mg intramuscularly) and inseminated once 24 hours later. Ovulation was confirmed by ultrasound and palpation at 24-hour intervals. All mares ovulated by 48 hours after insemination. Mares that accumulated fluid after breeding were examined daily until no detectable fluid remained. Intrauterine fluid was generally not detectable by 2 days after insemination. No mares were treated for intrauterine fluid. Pregnancy diagnosis was determined at 14 days after ovulation. All mares were administered cloprostenol (250 μg intramuscularly) on Day 14 after ovulation regardless of pregnancy status. Embryonic vesicles present were not reduced manually in an effort to minimize manipulation of the reproductive tract. Mares were inseminated on the next estrus for each of the remaining treatments using the same protocol.

2.5. Statistical analysis

Data were analyzed using a general linear model fitted with the method of linear least squares (PROC ORTHOREG; SAS Institute, Cary, NC, USA) to determine the effects of mare, treatment, cycle number (i.e., 1, 2, or 3), and carryover effect due to treatment. Carryover effect describes whether a previous treatment (i.e., T0, T5, T50) altered pregnancy outcome on the next cycle. The Fisher exact test (Statistix 9, Tallahassee, FL, USA) was used to evaluate the effect of previous pregnancy (positive or negative) on pregnancy rate during the next cycle. Differences were considered significant at $P < 0.05$.

3. Results

Pregnancy rate was not different between groups T0 and T5, and pregnancy rates in these groups were higher than that of group T50 (Table 1). No effect of mare on pregnancy outcome was detected. The carryover effect (i.e., the effect of the semen treatment during the previous cycle on

Table 1
Per-cycle pregnancy rate by treatment (T0, T5, T50) for cycles 1, 2, and 3 [number of mares pregnant/number of mares bred (percent pregnant per-cycle)].

Treatment	Cycle number			Total
	1	2	3	
T0	7/9 (78)	6/7 (86)	5/8 (63)	18/24 (75)[c]
T5	3/7 (43)	10/10 (100)	5/7 (71)	18/24 (75)[c]
T50	0/8(0)	0/7 (0)	0/9 (0)	0/24 (0)[d]
Total	10/24 (42)[a]	16/24 (67)[b]	10/24 (42)[a]	36/72 (50)

Data derived from 24 mares bred on three consecutive estrous periods with three different treatments (T0, T5, T50) for a total of 72 cycles.
1 = first estrous cycle; 2 = second estrous cycle; 3 = third estrous cycle; T0 = control, semen with 0% blood; T5 = semen with 5% blood v:v; T50 = semen with 50% blood v:v.
[ab]Within rows, values with different superscripts differ ($P < 0.05$).
[cd]Within columns, values with different superscripts differ ($P < 0.05$).