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Characteristics of frozen epididymal spermatozoa from stallions that died 12 to 36 hours after colic surgery

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ABSTRACT

Equine spermatozoa from the cauda epididymis were previously collected and frozen, and the fertility was assessed. Most studies were performed on healthy stallions that had undergone routine castration or on the epididymis collected at the abattoir, but there are no studies on the quality of epididymal semen in subjects which have died from colic or which underwent intensive care. The present study was designed to verify whether a severe illness could affect epididymal semen quality and freezability in the stallion. Therefore, epididymal semen characteristics during the freezing process in stallions which had died from colic and in healthy stallions submitted to elective castration were compared. Five stallions that had died from colic (ill stallions [ISs]) and seven stallions that had undergone elective castration (healthy stallions) were castrated, and cauda epididymis spermatozoa were collected and processed. Sperm quality was tested after collection, after washing procedures, at the end of the equilibration (5 °C for 75 minutes), and after freezing/thawing. Sperm quality was measured by objective motility characteristics, membrane and acrosome integrity, and mitochondrial activity. After collection, sperm in ISs showed low kinetic parameters (total motility: 17.3 ± 3%, progressive motility: 6 ± 1%, average path velocity: 57.4 ± 35.4 μm/s, straightness: 74.2%) compared with healthy stallions (total motility: 90.8 ± 3.7%, progressive motility: 70 ± 4%, average path velocity: 118.1 ± 12.6 μm/s, straightness: 82.4%) but demonstrated similar membrane and acrosome integrity (85 ± 2.8% vs. 87.6 ± 3.1%). Sperm kinetic parameters increased after washing procedures and cooling in ISs, reaching comparable values after equilibration (5 °C for 75 minutes) and freezing/thawing. The data reported in this study suggest that the quality of the equine epididymal spermatozoa cryopreserved in stallions that had died from colic was similar to that reported in epididymal sperm after elective castration and was also similar to the data reported in literature for cryopreserved equine semen.

1. Introduction

Traumatic injuries or colic conditions can prematurely end the breeding career of a male. In such circumstances, owners may request a final semen collection to allow the propagation of valuable genetics. In specific cases, the epididymal sperm is the only available source of male gametes for use in assisted reproduction programs. Collection of epididymal sperm further offers the opportunity to acquire and use genetic material from elite males, even postmortem. Epididymal semen can be used fresh or cryopreserved and stored in a genetic resource bank [1]. Successful epididymal sperm collection with resulting pregnancies has been reported in a number of species, including goats, red deer, dogs, and humans [2–6]. Furthermore, frozen equine epididymal spermatozoa have been proven to be fertile [7,8]. The pregnancy rate after the use of frozen epididymal sperm ranges from 17% to 30% using hysteroscopic insemination [8].

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Recently, articles reported the collection of spermatozoa from the cauda epididymis in both horses and donkeys [9–13]. These studies were performed in experimental conditions, with normal and healthy males undergoing elective castration. However, no studies are available on the collection and handling of epididymal sperm in critical patients or in stallions which died after intense care, or the impact of these conditions on the quality of cryopreserved semen. If sperm recovery from the cauda epididymis means the last chance to obtain viable spermatozoa from a stallion, the success of this biotechnology in stallions which died after intensive care is an important area of study [10].

In this article, we reported the procedures for epididymal sperm collection and cryopreservation from stallions which died from colic complications, and we compared these characteristics with those recorded in stallions that underwent elective castration. Furthermore, unlike other studies [12,13], differences between sperm characteristics in epididymal samples from ill or healthy stallions (ISs and HSs, respectively) were evaluated using objective systems, such as the computer-assisted sperm analyzer (CASA) system and flow cytometry, which allow the detection of fine differences between samples [14].

2. Materials and methods

2.1. Animals and surgery

Twelve sexually mature stallions, Quarter Horse and Standardbred, aged between 5 and 8 years and weighing between 450 and 550 kg, were enrolled in this study. For all stallions, explicit informed consent for the owners was acquired.

Five stallions were referred to the Veterinary Teaching Hospital of Teramo (Italy) for severe colic (ISs), evaluated by clinical examination, abdominal distention and topography by transrectal palpation, heart rate, aspect of mucosae and capillary refilling time, decreased or absent gut sounds, liquid characteristics after abdominocentesis, and lack of response to analgesic–sedative treatment. The symptoms developed in an acute manner, and the diagnosis was performed within 12 hours. A surgical approach to confirm the suspected diagnosis and to treat the colic was attempted. Systemic preoperative antibiotics were administered (cefazolin: 10 mg/kg intravenous [IV]; gentamicin sulfate, 8 mg/kg IV). The horses were sedated with medetomidine hydrochloride (Domitor: 1 mg/mL; Pfizer Animal Health, Kirkland, Quebec, Canada), 0.007 mg/kg IV. Induction was performed using diazepam (5 mg/mL; Sandoz, Boucherville, Quebec, Canada), 0.02 mg/kg IV and ketamine hydrochloride (Vetalar; 100 mg/mL; Bioniche, Belleville, Ontario, Canada), 2.2 mg/kg IV. Anesthesia was maintained by 3% isoflurane inhalant in oxygen (10 L/min) and a constant-rate infusion of medetomidine (0.0035 mg/kg/h). Ringer lactate and colloids were infused, and dobutamine (maximum dose: 0.00125 mg/kg/min) was administered to maintain a mean arterial blood pressure of between 70 and 90 mmHg. After surgery, flunixin meglumine (0.25 mg/kg q6 hours) and DMSO infusion (10% solution in polyonic fluids q12 hours) were administered to prevent endotoxemia. Boluses of xylazine (0.4 mg/kg) and butorphanol tartrate (0.01 mg/kg) were administered when the horse showed acute abdominal pain. In all cases, the horses died 12 to 36 hours after recovery. All the stallions were orchidectomized soon after death.

Seven healthy stallions were submitted to the Veterinary Teaching Hospital of Teramo, Italy, for routine orchiectomy (HSs). In these cases, the surgery was performed following the same anesthetic protocol as described for the ISs, except that the postsurgical treatment consisted of only flunixin meglumine (1.1 mg/kg q12 hours). In both ISs and HSs, local anesthesia was not performed.

2.2. Collection and cryopreservation of epididymal sperm

The gonads were individually transferred to sterile beakers and transported to the laboratory in a Styrofoam box and processed within 30 minutes. The cauda epididymis of each testis was dissected [11], and spermatozoa from the cauda epididymis were collected by retrograde flushing via the deferent ductus. Briefly, each deferent ductus was cannulated and flushed using 20 mL of INRA96. Samples were collected in a 200-mL sterile glass beaker and transferred to a 50-mL centrifugation tube. Total sperm per sample was calculated as the product of the concentration, estimated by a Burker chamber, and the volume of the flushing. The samples were washed twice, with INRA96 and MAXI Freeze (IMV Technologies, L'Aigle, France) by centrifugation at $1000 \times g$ for 20 minutes, as reported by the manufacturer. After washing, the samples were diluted with INRA-Freeze (IMV Technologies) at the final concentration of 200×10^6 sperm/mL and equilibrated at 5 °C for 75 minutes. The samples were then packaged in 0.5-mL french straws, frozen using a nitrogen vapor programmable freezer as previously described (freeze rate of −60 °C/min from 5 °C to −140 °C) [15], and plunged into liquid nitrogen and stored (−196 °C). After 5 days of storage, ten straws for each stallion were thawed (37 °C for 1 minute in a water bath). Sperm evaluations were performed after collection (C), after the first (W1) and the second washing (W2), after equilibration (EQ), and after cryopreservation (FT).

2.3. Motility evaluation

Objective sperm motility evaluation was performed by the CASA system, IVOS 12.3 (Hamilton Thorne Biosciences, Beverly, MA, USA) as previously described [16]. Semen was diluted at 30×10^6 sperm/mL concentration using INRA96. Each sample was rewarmed at 38 °C for at least 10 minutes, a 6-μL drop was loaded into a Makler chamber (Sefi Medical Instruments, Haifa, Israel), and 12 nonconsecutive fields were analyzed [17]. The CASA settings were 60 frames/s (Hz) and 45 frames per field. In this study, the following parameters were considered: total motility (TM, %), progressive motility (PM, %), average path velocity (VAP, μm/s), straight line velocity (VSL, μm/s), curvilinear velocity (VCL, μm/s), amplitude of lateral head displacement (μm), beat cross frequency (Hz), straightness (STR, as VSL/VAP, %), and linearity (as VSL/VCL, %). On the basis of their VAP, spermatozoa were classified as rapid (VAP, >75 μm/s), medium (75 μm/s > VAP > 25 μm/s), slow (VAP, <25 μm/s), or static