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CHARACTERISTICS OF REFRIGERATED STALLION EPIDIDYMAL SPERMATOZOA AT 24-H AND 48-H AFTER CASTRATION USING TWO COMMERCIAL EXTENDERS

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Abstract

Collection of spermatozoa from the cauda epididymis may be the last chance to preserve genetic material from valuable stallions in case of sudden death or emergency castration. In the present study we compared the characteristics of extended refrigerated stallion epidydimal spermatozoa at 24 and 48 hours after castration. Spermatozoa from 12 epididymides were recovered at 24 hours after the routine orhiectomy of 6 healthy stallions using the retrograde flush method and refrigerated for 24 hours. For refrigeration we used 2 commercial extenders - an egg yolk based extender (Triladyl®, Minitube) or a milk based extender (Gent®, Minitube). Concentration and motility parameters were assesed for each sample after collection and 24 hours later using computer assisted sperm anlaysis (SCA®, CASA). Viability was assessed using the eosin staining technique. Total motility, velocity, viability and percentage of progressive spermatozoa were similar among the two groups at 24-h post castration. However, at 48 hours the percentage of progressive spermatozoa was significantly higher in milk based extender. Therefore we concluded that stallion epididymal spermatozoa extended in milk protein based extenders can be successfully cryopreserved at 48 hours after routine orhiectomy.

Key words*: stallion; epididymal spermatozoa; refrigeration.*

INTRODUCTION

Collection of spermatozoa from the cauda epididymis may be the last chance to preserve genetic material from valuable stallions in case of sudden death or emergency castration. Given the fact that only a limited amount of sperm is available, efforts have been made to establish the best processing method. Extenders have a great influence on epidydimal sperm motility and milk protein based extenders seem to offer better results (Stefanie Neuhauser, 2018). Using modern protocols and new extenders pregnancy rates are increasingly higher than those reported in 2002 by Moris et. Al. (30%). Pregnancy rates in recent studies are comparable to those obtained with frozen ejaculated sperm when using pasteurised egg yolk based extenders (Stawicki, 2016). One study demonstrates 93% pregnancy rates using epididymal spermatozoa cryopreserved immediately after collection (Monteiro, 2011). Furthermore, epididymal spermatozoa stored for 24h at 5°C were used to obtain 60% one cycle pregnancy rates (Papa, 2008). Epididymal spermatozoa stored for 48h at 5°C in the epididymis seems to offer good results regarding pregnancy rates (Stawicki, 2016).
Collection of the spermatozoa from the cauda epididymis is routinely done in laboratory from refrigerated testicles at a certain amount of time post castration. Laboratories equipped for the technique might not be in range for fast shipment and often 48h are needed for transport. This has lead to the necessity to develop viable techniques to harvest and preserve epididymal stallion spermatozoa at 48h post castration.

MATERIALS AND METHODS

Animals

The testicles and epididymides were obtained from 6 healthy stallions after routine castration. Stallions were aged 4 to 8 years and all testicles were grossly normal. The breeding history of the stallions was unavailable.

Epididymal storage and collection

After routine castration the deferent duct was identified and a ligature was placed around the cut end of each deferent duct. Testicles were transported and then stored at 5°C for 24h. All epididymides were flushed using the retrograde flush technique firstly described by Monteiro et al. (2011). at 24h after castration and the first assessment was performed. From each stallion, one epididymis was flushed using 5 ml of Gent extender (Gent®, Minitube) and the second one was flushed using 5 ml of Triladyl extender (Triladyl®, Minitube). Samples were centrifuged at 1000 x g for 10 minutes at room temperature and the supernatant was removed. The sperm pellet was resuspended in 5 ml temperature-matched of the same extender. All spermatozoa were stored at 5°C for another 24 hours when the second assessment was performed.

Sperm motility and viability

Sperm motility was determined using computer assisted sperm analysis (SCA®, CASA). Viability was assessed using the eosin staining technique. Each sample was analysed at 20 minutes after collection and 24h later.

For motility assessment the samples were prewarmed at 37°C and placed on a prewarmed chamber (Leja Standard Count 4 Chamber Slide 20 micron, Leja Products B.V., Nieuw Vennep, the Netherlands) on a heated microcope stage. Using computer assisted sperm analysis we determined concentration (CONC M/mL) and motility parameters of each sample using the following parameters: total motility (TMOT %), total progressiveness (TPROG %), rapid progressiveness (RPROG %), medium progressiveness (MPROG), slow progressiveness (SPROG), velocity rapid (VELR %), velocity medium (VELM %), velocity slow (VELS %). Average values of speed determined were: curve speed (VCL), linear speed (VSL), average value (VAP), linearity index (LIN), straightness index (STR), oscillation index (WOB). Other parameters we assessed were amplitude lateral head (ALH), beat frequency (BCF), hyperactive and mucous penetration.

For viability samples were prewarmed at 37°C and equal parts semen and prewarmed eosin were mixed. A smear was prepared from each sample and analysed under the microscope at 40x. 100 spermatozoa per sample were assessed and divided into 2 groups – green viable and red non-viable and final results were expressed in percentages.

RESULTS AND DISCUSSIONS

Spermatozoa suspended in a commercial skim mik-based extender (Gent®, Minitube) had at 24h post castration a mean TMOT of 74,22% whereas at 48h TMOT was 77,59%. TPROG at 24h was 9,6% and at 48h 17,97%. Velocity was at 24h VELR 5,96%, VELM 15,35%, VELS 52,89% and at 48h VELR 14.02%, VELM 16,46%, VELS 52,89%. Viability at 24 h was 93,7% and at 48h 87,5%.

Spermatozoa suspended in a commercial egg yolk-based extender (Triladyl®, Minitube) had much lower mean parameters both at 24h and 48h post castration. At 24h TMOT was 44,93% and at 48h 23,94%. TPROG at 24h was 3.33% and at 48h 2.41%. Velocity was at 24h VELR 1,64%, VELM 5,3%, VELS 37,98% and at 48h VELR 1.27%, VELM 4,33%, VELS 18,34%. Viability was at 24h 85,3% and at 48h 78%.

The aim of this study was to evaluate motility parameters and viability of epididymal stallion spermatozoa at 24 h post castration and storage in the epididymis at 5°C and at another 24h after storage at 5°C post collection using two commercial extenders. Triladyl is an egg yolk based extender destined for use in bull but it has been successfully used for stallion ejaculated spermatozoa as well (Blottner, 2001).

The results of this study show better kinematic values of epididymal spermatozoa when using a milk-based extender. Prolonged cooled storage of epididymides prior to collection of spermatozoa (48h) do not seem to decrease the kinematic parameters (Neuhausser, 2018). In the current study, spermatozoa were stored for 24h post collection at 5°C in one of the two extenders and the kinematic parameters improved significantly in the milk base extender.

In conclusion, spermatozoa cooled stored for 24h in the epididymis and another 24h in cooled extender show adequate quality and fertilizing capacity and could successfully be used for cryopreservation.

In this study we only used young healthy stallions and two commercial extenders that were commercially available. However, new extenders should also be tested for accuracy of the method on both healthy and pharmacologically treated stallions.

CONCLUSIONS

The milk based extender used in this study improved substantially the kinematic values of epididymal spermatozoa at 48h post castration while the egg-yolk based extender showed poor results at 48h. Furthermore, parameters were much better at 24h post castration in milk based extender. Therefore, we recommend using milk based extenders for epididymal spermatozoa at 24h and 48h post castration of healthy stallions.

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