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Assessment of the efficacy of Sephadex G-15 filtration of bovine spermatozoa for cryopreservation

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Abstract

Semen from five dairy AI bulls was split-filtered through a Sephadex G-15 filter and frozen in a Tris–citric acid buffer egg yolk-based extender. The effect of filtration was studied morphologically for individual sperm abnormalities. Computer-assisted sperm analysis (CASA) was used for motility and sperm motion assessment. Flow cytometry was used to disclose sperm viability (SYBR-14/PI), mitochondrial membrane potential (Mitotracker Deep Red/SYBR 14), acrosome integrity (SYBR 14/PE-PNA/PI), plasma membrane stability (Merocyanine 540/YO-PRO 1/Hoechst 333342), and chromatin stability (acridine orange staining). Filtration significantly reduced the concentration of recovered spermatozoa ($P < 0.01$), but improved semen quality, reducing the number of spermatozoa with various forms of morphological defects. Filtration also affected percentages of sperm motility after equilibration and after freezing/thawing. Sperm motion characteristics were, however, not significantly affected by filtration at any stage of the cryopreservation protocol, including post-extension, equilibration, or freezing/thawing. Filtration enhanced sperm viability after thawing ($P < 0.05$), but had no significant effect ($P > 0.05$) on recovery of spermatozoa with high mitochondrial potential, intact acrosomes, or preserved sperm chromatin structure. Sperm plasma membrane stability was also not affected by the filtration method used ($P > 0.05$). It can be concluded that filtration effectively separates weaken or abnormal spermatozoa in pre-freezing semen samples and therefore the procedure could be recommended to improve post-thaw sperm viability of selected, fertile sires.
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1. Introduction

Semen production centers are sometimes confronted with the need to use semen from ejaculates of fertile bulls of high genetic merit that for reasons of age or incidents during their active life show a lower semen quality than previously. The negative influence of dead and abnormal spermatozoa on the remaining sperm population [1], as well as on fertility, has long been known [2]. Semen from bulls with uncompensable semen quality traits must be discarded while ejaculates from bulls with compensable sperm defects seem to be significantly improved by filtration of extended semen [3]. Separation of dead and morphologically abnormal spermatozoa is performed in the female [4]. Various centrifugation gradients [5,6], filtration columns [7,8], or methods based on active sperm movement, that is, swim-up [9], have been used to separate motile from immotile cells, and to enhance the quality of ejaculates. Graham and Graham [7] were the first to report a significant improvement in fertility (as non-return rates) for low fertility bulls after removal of dead and abnormal spermatozoa from extended ejaculates using Sephadex filtration. In previous studies, semen quality assessment after filtration was usually based on subjective estimation of sperm motility, viability, appearance of the acrosome, and in some cases, morphology.

The objective of the present study was, by applying a battery of tests in bull semen considered of acceptable or sub-standard normality, namely conventional assessment of morphology, computer-assisted motion analysis, fluorometric evaluation of sperm viability, mitochondrial membrane potential, acrosomal integrity, chromatin stability, and plasma membrane destabilization, to determine which sperm attributes are positively affected by Sephadex gel filtration applied for cryopreservation.

2. Materials and methods

2.1. Semen collection and preparation

Semen was collected by means of artificial vagina from five dairy AI bulls, culled from the AI enterprise Svensk Avel ek för (Skara, Sweden) and stanchioned at the Department of the Obstetrics and Gynaecology, SLU. Two ejaculates, were collected 10 min apart twice weekly, assessed for subjective motility analysis, pooled, and further processed accordingly. Three animals were considered to have spermiograms within acceptable limits (e.g. acceptable), and had a total sperm concentration of $>2 \times 10^9$, initial motility of >70%, and more than 80% morphologically normal spermatozoa. The other two animals were regarded as ‘‘sub-standard’’ and had initial sperm characteristics below these figures. They had therefore been excluded from the national recruitment AI program due to low spermiogram values. In any case, ejaculates with extremely poor motility values ($\leq$40%) were discarded from further processing within the trial. Each bull was, at least, collected on five occasions thus represented by five replicates of pooled semen. The semen was extended with Tris–fructose–citric acid egg yolk-based extender, as previously described in [3], but without antibiotics. The extender was stored at -20 °C before it was used for semen extension.

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