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Treatment of human sperm with serine protease during density gradient centrifugation to reduce pathogenicity and to enhance fertilization potential

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INTRODUCTION:

Seminal pathogens can bind specifically or non-specifically to spermatozoa, rendering semen decontamination procedures ineffective, whereby vertical or horizontal transmission of the infection could occur. Serine proteases have been demonstrated to effectively inactivate viruses and to break pathogen-sperm bonds. However, the addition of a protease to density gradient layers during semen processing could negatively impact on sperm parameters.

AIM:

This study investigated the effect of the addition of a recombinant, human-sequence protease (rhProtease) on sperm parameters during density gradient centrifugation.

MATERIALS AND METHODS:

Prior to experimentation, the proteolytic activity of rhProtease (Nidacon International, Sweden) was confirmed by the effective trypsinization of an adherent cervical carcinoma cell line. (i) Pooled semen samples (n=9) were split and processed by density gradient centrifugation, with the top density layers supplemented, or non-supplemented with rhProtease at three different concentrations (diluted 2, 10 and 20 times). Sperm parameters were then analysed by flow cytometry and computer-assisted semen analyses. (ii) Semen samples (n=5) were split and similarly processed using PureSperm® Pro, with rhProtease in the 40 % density gradient layer, or standard PureSperm® not supplemented with rhProtease (Nidacon International). Hemizona assay was then utilized to compare sperm-zona binding post processing.

RESULTS:

Evaluation of sperm parameters indicated that rhProtease did not, at any of the tested concentrations, have a negative impact on mitochondrial membrane potential, apoptotic/necrotic status or sperm motility. Processing of semen using the PureSperm® Prof did cause a reduction in the number of sperm bound to the hemizonae when compared to processing with standard unsupplemented PureSperm® (p=0.732).

CONCLUSION/DISCUSSION:

The addition of rhProtease to density gradients is a non-detrimental approach, possibly due to the low concentrations of the protease added to the layers, together with the washing step after exposure to the enzyme and silane-coated silica particles. The usage of rhProtease could improve (i) sperm yield of hyper-visocous semen samples, (ii) the effectiveness of semen processing for the elimination of seminal pathogens, and potentially (iii) the prevention of sperm-antibody formation, all of which could enhance the outcome of assisted reproduction.