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JSM Invitro Fertilization

Editorial

Preputial Washing, Addition of Antioxidants and Antimicrobial Peptides in Semen Extender- For Reducing Microbial Load during Cryopreservation

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Submitted: 15 February 2017

Accepted: 16 February 2017

Published: 17 February 2017

Keywords

- Microbial Load
- Antimicrobial peptides
- Antibiotics
- Male infertility

INTRODUCTION

For the artificial insemination in livestock and assisted reproduction for humans, the first step is to prepare a better semen extender for cryopreservation. For the better post thaw results and better success rate in ART centres before cryopreservation the sample need to be preputialy washed for the microbial load. Usually the semen samples will have more than 30% of microbial load and it needs to be cleared before going for cryopreservation. For this we suggest that 1. It needs to go for preputial wash, 2. Addition of antioxidants like catalase, vitamin c and vitamin e in the semen extender and 3. Addition of antimicrobial peptides instead of antibiotics in semen extender [1-3].

These three suggestions will lead to better semen extender preparation as well better post thaw results and success rate in assisted reproduction. The bacterial load should not be greater than 5000 Cfu/dose to use the semen sample for AI in getting the satisfactory results. Microbial contamination has effect on motility, morphology and various semen quality parameters [4], which may be due to direct effect or competition for nutrients [5], detected aerobic bacteria in almost all the collected semen samples but the various opportunistic pathogenic organisms in semen may cause reproductive disorders. Bacterial contamination in frozen semen first leads to the production of macrophages and polymorphonuclear granulocytes and these cells generate reactive oxygen species that in turn impair sperm function and reduces its fertilization capability [6]. This can be overcome by adding $KMnO_4$ as a preputial washing the semen [7]. This will remove 50% of the bacterial load present in the semen before cryopreservation. Preputial washing with $KMnO_4$ solution would facilitate quality semen production in terms of reduced microbial load, sperm abnormalities and higher sperm motility, livability, membrane integrity, acrosome intactness in the semen stored at refrigerator temperature (4°C) and in liquid nitrogen (−196°C) due to broad spectrum effect. Use of a higher amount of $KMnO_4$ for preputial washing is not advisable as it may act as an irritant, which may affect the quality semen production.

Antimicrobial agents play a major role in semen extender for increasing the success rate. Antibiotics in semen extender may be used abundantly in both human and veterinary medicine, but unfortunately all these steps were taken to prevent bacterial disease rather than to treat it [6]. One best suited example for using antibiotics in semen extender during the preparation of semen samples for AI, this will reduce the contamination of bacteria and other microbes during collection process. During collection process many healthy animal ejaculates also infected with microbes and bacteria to the small extent, for example it has been reported in ejaculates from stallions [8], boars and bulls. A very large volume of semen extenders were added with antibiotics and these semen extenders were used in animal breeding, mostly in pig and cattle, but the amount of antibiotics may differ with different species. Nevertheless, many antibiotics and antimicrobials have a determined consequence on spermatozoa; the choice of using the agents is limited to different semen extenders. So, there is a need for alternative for use of antibiotics that causes determined problems in spermatozoa. Currently many researchers were focusing on this alternative approach especially in European countries [9]. Even small amount of antibiotics in semen extender will result in antibiotic resistant species, for example tetracycline-resistant strains of Clostridium perfringens in Swedish broilers. During natural mating in humans, female reproductive tract is infected with many microbes as human male reproductive organ is capable of infection, but this can be neutralized in the female reproductive tract as physiological conditions is favouring the eradication of microbes. In case of ART, semen samples were injected either in cervix or in uterine, but in either case there is no natural mechanism in neutralizing the microbial infection, for this reason European society and many others were instructing to add antibiotics while preparing semen samples for ART procedures. So by using the antimicrobial peptides in semen extender we can easily overcome this antibiotics resistant problem in the species.

We, in our laboratory prepared a better cryoprotective medium named as E4 medium which have many antioxidants. This semen extender gave good post thaw results after three months of cryopreservation. Clearly these are the three suggestions we made for preparing the better semen extender without microbial load for cryopreservation

ACKNOWLEDGMENTS

The authors were very much thankful to Department of science and Technology, Govt. of India for providing funding with reference number YSS/2015/000040 on working with prostasomes and male infertility. The authors also thankful to the management of VIT University for providing with infrastructure.

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Cite this article

Vickram AS, Ramesh PM, Sridharan TB (2017) Preputial Washing, Addition of Antioxidants and Antimicrobial Peptides in Semen Extender- For Reducing Microbial Load during Cryopreservation. JSM Invitro Fertil 2(1): 1009.