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Influence of storage time on functional capacity of flow cytometrically sex-sorted boar spermatozoa

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Abstract

Sex-sorting of boar spermatozoa is an emerging biotechnology, still considered suboptimal owing to the slowness of the process, which requires long sorting periods to obtain an adequate number of spermatozoa to perform a non-surgical insemination. This period involves storage of sorted cells that could impair their functional capacity. Here, we have studied how the storage of sex-sorted boar spermatozoa affects their functional capacity. Sorted spermatozoa were assessed at various times (0, 2, 5 h or 10 h) during storage after sorting and compared with diluted and unsorted spermatozoa for sperm motility patterns, plasma membrane and acrosomal integrity and their ability to penetrate homologous IVM oocytes. Sex-sorted sperm motility and membrane integrity only decreased significantly ($p < 0.05$) by the end of the storage period (10 h) compared to unsorted spermatozoa. Sperm velocity, ALH and Dance increased significantly ($p < 0.05$), immediately post-sorting, returning to unsorted sperm values during storage. Acrosome integrity was not seriously affected by the sorting process, but decreased ($p < 0.05$) during storage after sorting. Sorted spermatozoa stored 2 h after sorting did not differ from unsorted in penetration rates and numbers of spermatozoa per oocyte, reaching the highest ($p < 0.05$) penetration rates and sperm numbers per oocyte, when co-cultured for 6 or more hours. Non-storage or storage for 5 h or 10 h negatively ($p < 0.05$) affected sperm penetration ability. In conclusion, although flow cytometrically sex-sorted spermatozoa are able to maintain motility, viability and acrosomal integrity at optimal levels until 10 h of storage after sorting, fertilizing ability is maintained only over shorter storage times (<5 h).

Keywords: Flow cytometry; Sex-sorting; Seminal plasma; Sperm quality; Oocyte penetration; In vitro; Pig

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1. Introduction

The development of semen sexing is widely accepted as a major advance in reproductive technology. The preselection of the sex accelerates genetic progress as well as enhancing benefit the management and efficiency of pig production. The effectiveness of this technology for producing offspring of the preselected sex in swine has been proven in numerous trials where the semen was sorted and surgically inseminated [1] or used for in vitro fertilization [2–4] or intracytoplasmic sperm injection [5] and subsequent embryo transfer. Recently, offspring have been produced after non-surgical deep intrauterine insemination [6] of either fresh sorted [7,8] or sorted frozen–thawed (Maxwell WMC, personal communication) spermatozoa. This technology allows farrows to be obtained with doses as low as 50 million spermatozoa per insemination, although the success of these inseminations has been suboptimal.

Although, the current throughput of sperm sexing instruments is fast (5000–6000 sperm/s) [9], this yield is relatively slow and long sorting times are required to obtain the adequate number of sorted boar spermatozoa. Therefore, the final sperm population will be characterized by its heterogeneity, since it consists of sperm cells ranging from spermatozoa subjected to relatively long holding times after sorting to spermatozoa that have only just been sorted.

It is known that the sperm membrane is partially compromised during the flow cytometry and sorting process, which affects viability, storage capability and fertilization ability of spermatozoa [10]. Both, the physical effect of the sorting procedure and the high dilution rate by sheath fluid cause in the spermatozoa a short lifespan after sorting [11]. To improve post-sorting sperm survival, the impact that each sorting steps has on the sperm population, should be evaluated, and the interval during which the sperm cells preserve their fertilizing capability after sorting, needs to be defined. This would help to establish the potential fertilizing capability of the sex-sorted sperm population. Although, the effect of the Hoechst 33342 staining [12] and the sorting procedure [11,13,14] has been studied on boar spermatozoa, there are no reports monitoring the effects of liquid storage of flow cytometrically-sorted boar spermatozoa up 10 h on sperm functionality; this is a critical time for both the sorting and the transport of the spermatozoa from the cell sorter laboratory to the farm for performing a non-surgical insemination.

In the present study, we have monitored the changes in viability, motility, acrosomal exocytosis and ability to penetrate IVM oocytes of flow-sorted boar spermatozoa stored for 0, 2, 5 and 10 h. The main goal of this study was to know the variability in the sperm functionality of sex-sorted spermatozoa when a long holding time is required.

2. Material and methods

All chemicals used in this study were provided by Sigma–Aldrich Co. (Alcobendas, Madrid, Spain), unless otherwise stated.