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Storage of sexed boar spermatozoa: Limits and perspectives

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ABSTRACT

Despite the great potential application of sex-sorted spermatozoa in swine, the technology is not practiced in the pig industry because of technical factors and species-specific issues. The susceptibility of boar spermatozoa to stresses induced by the sorting procedure, the relative slowness of the sex-sorting process together with the high sperm numbers required for routine artificial insemination in pig are some of the main factors limiting the commercial application of this technology in pigs. This review briefly describes the damage to spermatozoa during sex sorting, focusing on an additional limiting factor: increased susceptibility of sexed boar spermatozoa to injuries induced by liquid storage and cryopreservation that, in turn, impairs sperm quality leading to unsatisfactory results *in vivo*. Strategies to extend the lifespan of sex-sorted boar spermatozoa and to improve their fertilizing ability after liquid storage or cryopreservation need to be implemented before this technology can be used in pig farms. In this regard, encapsulation in barium alginate membranes could be a promising technique to optimize the *in vivo* use of sexed boar spermatozoa, by protecting, targeting, and controlling the release of sperm into the female genital tract.

1. Introduction

Flow cytometric sperm sorting based on X and Y sperm DNA difference is currently the only accurate method to predetermine the sex of offspring before fertilization [1].

Even if the sexing technique has already reached a commercial level in the bovine species [2], the use of sexed semen in the swine industry is far from being a routine procedure. Reproductive management in pig production would benefit from sex preselection by accelerating genetic progress and allowing the production of preselected female livestock [3]. Moreover, female production through use of sexed semen may be an alternative to the castration of male piglets to prevent the distasteful "boar taint" [4]. Castration is regarded as an infringement of animal welfare, and in response to these growing concerns, several leading players within the pig and pork industry have agreed to a plan to voluntarily end the practice of surgically castrating pigs in the European Union by January 1, 2018.

In the research field, sex sorting in association with sperm-mediated gene transfer could be strategically useful to shorten the time for producing homozygous transgenic pigs [5] as organ donors for xenotransplantation, as valuable models for biomedical studies, and in the use of transgenic swine as bioreactors [6–8].

2. Factors limiting the large-scale use of sexed boar spermatozoa

Even if flow cytometric sorting of pig spermatozoa could have great potential for application, the technique is currently under research and it is still unknown whether

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the use of sex-sorted semen through routine pig artificial insemination (AI) will be economically feasible [3].

As indicated by the flow cytometric sorting index elaborated by Garner et al. [2], boar spermatozoa are approximately as easy to sort as ram and bull sperm due to both the relatively high difference in the DNA content between X- and Y-chromosome–bearing sperm (3.6%) and the flattened oval heads that tend to be readily oriented in a sperm sorter using hydrodynamics. Therefore, the effectiveness of the sexing technology in this species is not an issue [9]. However, the efficiency of the sex sorting procedure in the porcine species can be influenced by interboar and intraboar variability in the sortability of spermatozoa due to differences in the ability of ejaculates to exhibit well-defined X and Y peaks in the split on a flow cytometry histogram [10,11]. Alkmin et al. [10], analyzing 67 ejaculates from different boars, found that around 15% failed to exhibit a well-defined split in the first ejaculate (bad sperm sorters; interboar variability). Analyzing five ejaculates from three of the bad sperm sorter boars, the percentage of the ejaculates not exhibiting a well-defined split ranged between 20% and 70% (intraboar variability) [10]. Such variability in pigs, unlike other species (dogs, horses) [12,13], is not influenced by the percentage of non-viable spermatozoa in the semen samples but is closely related to ejaculate sperm concentration. Ejaculates are diluted to achieve the optimal sperm concentration for Hoechst 33342 (Ho) staining; during the staining step, samples from ejaculates with low sperm concentration would have a high proportion of seminal plasma that may alter Ho entrance into the sperm cell thereby affecting the effectiveness of DNA staining [10,11].

2.1. Sperm sorter output

Although there has been significant progress in the throughput of sperm sorters (about 20 million sperm/h), one of the major limiting factors for the broad use of sexed semen in pig farms is the unreasonable sorting time (about 100 hours) necessary to obtain an adequate number of sexed spermatozoa for conventional AI (2–3 billion spermatozoa/insemination dose). In an attempt to overcome this problem, offspring were produced by a combination of reproductive technologies such as surgical insemination, deep intrauterine insemination (DIUI), IVF-intracytoplasmic sperm injection, and embryo transfer (ET) (Table 1). Fresh sexed boar spermatozoa have been successfully used for low-dose insemination protocols by nonsurgical DIUI depositing as few as 70 to 140 $\times$ 10^6 bulk-sorted [19] or 50 $\times$ 10^6 sex-sorted spermatozoa in the anterior third of the uterine horn of sows [20,23]. However, the relatively high number of sexed spermatozoa needed and the reduced fertility rates limit the use of sexed semen in DIUI on a large routine scale [25]. Laparoscopic insemination with a very low number of sex-sorted sperm (3–6 $\times$ 10^6 spermatozoa) has been reported to produce satisfactory fertility at the farm level with a farrowing rate of around 80% [24]. Owing to its high cost, routine use of this insemination technique is not feasible in the pig industry but could at best be confined to niche situations such as elite breeding units or nucleus herds [24,25]. In addition, the fertility outcome using these techniques is strictly dependent on proper timing of semen deposition and hormonal treatments for accurate prediction of ovulation [25].

In vitro techniques such as IVF or intracytoplasmic sperm injection, which greatly reduce the number of sperm required, combined with surgical or nonsurgical ET offer a more efficient use of fresh sorted sperm and have proved a feasible, albeit expensive, alternative to using sexed sperm in pigs [15–17,21].

2.2. Damage to boar spermatozoa during sorting

Another factor limiting the application of sperm-sorting technology in the pig is the susceptibility of boar spermatozoa to stress induced by the sorting procedure that seems to be more severe than that in the bull and ram [26,27]. Sex-sorting–induced damage has been extensively documented and reviewed; thus, it is only described briefly focusing on the increased susceptibility to storage for pig spermatozoa sorted by flow cytometry.

Chemical, physical, and electrical insults during the sex-sorting process (Ho staining, variations in temperature, high pressure, exposure to the ultraviolet laser beam, electrical charging of droplets containing spermatozoa, projection into the collection tube, high dilution, centrifugation) can induce the death of some sperm cells. However, those sperm that survive such processing can undergo sublethal modifications that, in turn, can shorten sperm lifespan after sorting and reduce their fertilizing ability [28–35]. The stressors associated with the sex-sorting procedure seem to primarily affect the sperm surface. After sorting, heat shock protein 70 (Hsp70) has been reported to be relocated, without consumption of the protein as evidenced by Western blotting, from the equatorial subsegment toward an equatorial line, and this lateral movement suggests the beginning of a capacitation-like process [33,36]. Likewise, changes in chlortetracycline (CTC) labeling patterns in boar spermatozoa after sex sorting suggest a destabilization of the sperm surface and reflect a capacitation-like state of the sperm membrane [28,37]. This is not surprising as processing steps for sorting (dilution, promotion of protein release from the sperm surface by mechanical forces, presence of BSA in media) can mimic the condition used *in vitro* to induce sperm capacitation [38]. The induction of a capacitation-like process due to the sorting procedure is confirmed by the data on sperm motility patterns obtained immediately after sorting [32] and by the need to reduce the number of spermatozoa for IVF to avoid polyspermic fertilization [17]. However non-membrane parameters considered to be markers of the capacitation processes, such as actin cytoskeleton polymerization and protein tyrosine phosphorylation, seem to be less affected by the sex-sorting process, and sexed sperm do not completely reflect the changes detected during capacitation *in vitro*. This suggests that the evolution of capacitation-like changes in sexed spermatozoa probably follows a different pathway to that of true capacitation [37].

3. Storage of sex-sorted boar spermatozoa

Storage of sexed boar semen is necessary to ship it from sorting facilities to recipient females for use on a wider