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Application of seminal plasma in sex-sorting and sperm cryopreservation

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Abstract

Substantial dilution of boar semen during processing decreased the concentration of seminal plasma, perhaps contributing to the decline in sperm quality after cryopreservation and sex-sorting. Results of replacing seminal plasma in investigations from many laboratories have been contradictory. Results and discussion here suggest that whereas membrane status can be influenced by seminal plasma, the action of its various components, both positive and negative, is determined in part by the membrane status of the spermatozoa to which it is being exposed. Although progress has been made in identifying components of seminal plasma responsible for its protective effect (notably PSP-I/II spermadhesin for sex-sorted boar spermatozoa), little is known (in any species) regarding how external factors may influence their levels, and their functionality, in seminal plasma. It is noteworthy that seminal plasma is beneficial to post-thaw quality of sex-sorted ram spermatozoa only when added before freezing, not after thawing. Therefore, the action of seminal plasma and its components is dependent on sperm-related factors, in particular the type of processing to which they have been previously exposed. Further research is needed to unravel these biological complexities, and then characterise and synthesise useful proteins within seminal plasma.
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1. Introduction

Since reproductive technologies such as cryopreservation and sex-sorting were introduced, it has been recognised that these procedures often have a detrimental impact on sperm quality [1]. Many of these negative effects are attributed to the substantial dilution that occurs during processing, and the consequent decrease in the concentration of seminal plasma [2]. Therefore, re-introduction of seminal plasma at various points during sperm processing has been proposed as a method to slow, halt, or perhaps reverse some of the detrimental effects that the aforementioned technologies produce [3]. Unfortunately, examination of the literature reveals this hypothesis does not always hold true. Rather, the effect of seminal plasma on sperm function is highly variable, preventing its easy application in reproductive technologies. Therefore, research continues in an effort to identify the precise action and constituents of seminal plasma.

2. Addition of seminal plasma

The merits of adding seminal plasma to cryopreserved boar spermatozoa are, to say the least, equivocal.

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Initial research suggested that the removal of seminal plasma after a brief period of co-incubation delivered some protection from the trauma of cold shock [4], improving post-thaw motility [5]. However, others found any presence of seminal plasma during the freezing process was either detrimental or inconsequential to sperm quality [6,7]. Addition of seminal plasma post-thaw produced results no less confusing, with investigators reporting both improved membrane status [8] and diminished viability [9] of cryopreserved boar spermatozoa. The application of seminal plasma to sex-sorted boar spermatozoa has been somewhat more straightforward.

The hazardous journey of spermatozoa through the process of flow cytometric sorting has been well documented [10], as has its detrimental impact on their motility, viability and fertility [11]. Initial efforts to utilise seminal plasma, stimulated no doubt by its success in alleviating changes in other populations of highly diluted spermatozoa [2,12], met with reasonable success. Catt et al. [13] reported that the inclusion of 10% (v/v) seminal plasma in the staining medium of boar (or ram) spermatozoa improved both viability and motility. This finding was shared by Maxwell et al. [14], who also detected a beneficial effect of seminal plasma when included as a component of the collection medium into which boar or bull spermatozoa were sorted. Findings from these studies illustrated two distinct areas in which seminal plasma afforded protection to spermatozoa. Seminal plasma added prior to sorting was thought to reduce agglutination and in some way assist in reducing the impact of the dilution effect, whereas that provided to spermatozoa post-sort was considered to be acting independently of motility or viability. Like cryopreservation [15,16], the stressors of sex-sorting appeared to destabilise sperm membranes, allowing them to fertilise oocytes in vitro without further capacitation treatments. Moreover, the inclusion of seminal plasma in the collection medium was able to limit the effect of these stressors by reducing the percentage of sorted boar spermatozoa exhibiting capacitation-like changes [17]. While conducted on now superceded ‘standard’ speed flow cytometers and using relatively simple diluents (mainly phosphate-buffered-saline), these findings, at least for pigs, have been supported and extended by further work [18]. Indeed, seminal plasma is routinely included in the collection medium of most modern boar sperm sorting protocols [19]. Recent investigations have also begun to shed light on the components of boar seminal plasma that convey these positive effects, and their possible mode of action on sperm physiology. For highly diluted boar spermatozoa, the spermadhesin PSP-I/II heterodimer has been identified as the major contributor of protection [20]. Interestingly, when used to decapacitate sex-sorted spermatozoa, PSP-I/II spermadhesin appeared to afford a higher level of protection than whole seminal plasma [21], perhaps due to the variability in protein level found in whole seminal plasma as a result of differences among ejaculates, males etc. [22]. Regardless, the inclusion of seminal plasma or its beneficial protein constituents in the collection medium clearly improved the capacitation status [23] and fertilising capacity of sex-sorted boar spermatozoa [21].

The same cannot be said for the application of seminal plasma to sex-sorted spermatozoa of other species, most notably sheep. The beneficial effect of seminal plasma to the functional integrity and fertility of frozen–thawed ram spermatozoa is well documented [2,24]. However, attempts to translate these successes to sex-sorted, cryopreserved ram spermatozoa have thus far been equivocal. de Graaf et al. [1] directly compared the effect of whole seminal plasma on the post-thaw quality of both sex-sorted and non-sorted ram spermatozoa. Surprisingly, although non-sorted spermatozoa received the expected benefits of seminal plasma, the post-thaw function and quality of sex-sorted spermatozoa declined. Further investigation determined the effect to be dose-dependent, with an inverse relationship between traits of sperm quality and the percentage of seminal plasma to which both sex-sorted and non-sorted spermatozoa were exposed [1]. However, subsequent experiments by the same group have found seminal plasma, albeit prepared using a different method, to be beneficial to post-thaw quality of sex-sorted ram spermatozoa only when added before freezing (T. Leahy, J. Marti, W. M. C. Maxwell and G. Evans, unpublished observations; Fig. 1). Although at initial inspection these results appear contradictory, they highlight the simplicity of the historical view that the presence or absence of seminal plasma alone will determine capacitation status and hence longevity of the cell. Rather, it would appear that whereas membrane status can be influenced by seminal plasma, the action of its various components, both positive and negative, is determined in part by the membrane status of the spermatozoa to which it is being exposed. For example, sex-sorted ram spermatozoa may be considerably more susceptible than frozen–thawed controls to any negative components in seminal plasma, because the sorting process alters the level of membrane proteins (Fig. 2), possibly exposing ligands or binding sites on the sperm surface [3]. Consequently, when sex-sorted and non-

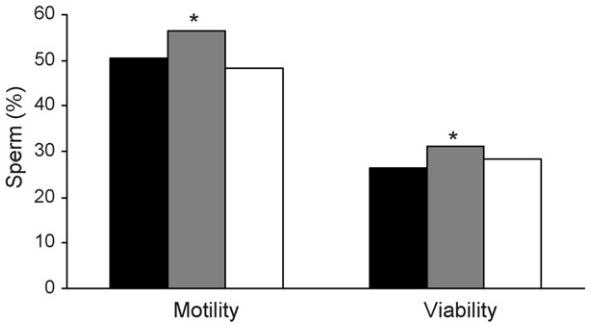

Fig. 1. Motility (determined by CASA) and viability (assessed by CFDA-PI staining) of sex-sorted, frozen–thawed ram spermatozoa using a standard protocol [■], or after the addition of seminal plasma proteins (4 mg/10^8 sperm), either before [▩] or after [□] cryopreservation. *Greater than standard ($P < 0.05$).

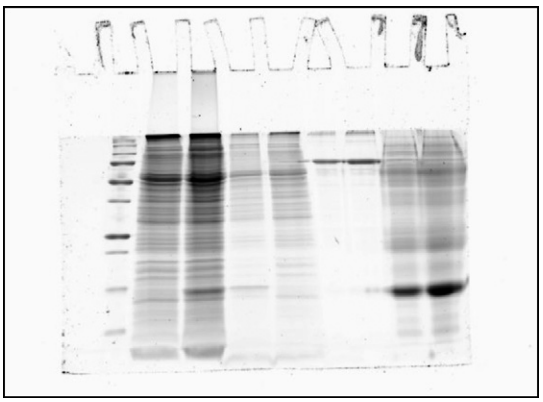

Fig. 2. SDS–PAGE patterns of sperm membrane proteins and whole seminal plasma. Lane 1: protein marker, Lane 2: sex-sorted (viable) sperm membrane proteins (58×10^6 sperm), Lane 3: non-sorted sperm membrane proteins (58×10^6 sperm), Lane 4: sex-sorted (non-viable) sperm membrane proteins (1.1×10^6 sperm), Lane 5: sex-sorted (viable) sperm membrane proteins (1.1×10^6 sperm), Lanes 6 and 7: BSA, Lane 8: (0.5 mg/mL) and Lane 9: (1.0 mg/mL) whole seminal plasma.

sorted ram spermatozoa are exposed to the same seminal plasma, its possible for an almost opposite response to be observed. When combined with evidence that the components of seminal plasma vary according to species, male, ejaculate, nutrition, stress, and stage of sperm maturity [3], it is not surprising that there is variation in the observed effects of seminal plasma, even within the same laboratories. With continued research to determine the precise component(s) of ram seminal plasma that elicit these positive or negative effects and how the aforementioned factors influence their levels, the fertility of sex-sorted, frozen–thawed ram spermatozoa, although already high [25], may be further improved.

3. Conclusion

To conclude, it is evident that seminal plasma has a role in reproductive technologies such as sex-sorting and cryopreservation. Although progress has been made in identifying the components of seminal plasma responsible for its protective effect (notably PSP-I/II spermadhesin for sex-sorted boar spermatozoa), little is known in any species about how external factors may influence their levels in seminal plasma and hence alter the observed effect. Furthermore, it is recognised that the action of seminal plasma and its components is dependent on sperm-related factors, in particular the type of processing to which they have been previously exposed e.g. flow cytometry. It is hoped that as these biological complexities continue to be unravelled, useful proteins within seminal plasma may be characterised and synthesised for their beneficial application to sex-sorting and cryopreservation.

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