Equine Sperm Selection

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Abstract

Stallions become sires primarily based on pedigree, athletic prowess and phenotype, with little consideration of reproductive soundness. Inseminating only high-quality spermatozoa selected prior to insemination should improve pregnancy rates. The sperm selection goal is to have a measurable improvement of quality and quantity of spermatozoa when compared to the original ejaculate, while making sure that the process does not impact the integrity of the sample. While several sperm selection techniques are available, the only technique regularly used in commercial equine reproduction programs is the colloid centrifugation technique. Migration methods utilize sperm motility as the main selection factor, where the swim-up method is the most used in humans. Glass wool and Sephadex gel are examples of filter techniques related to frozen-thawed stallion semen. Recently an approach combining the use of a porous membrane and the migration capability was suggested for fresh semen. To this date, none of the techniques described were able to accomplish the goals of an ideal sperm selection, suggesting that further investigation, development and research of new procedures must continue in order to have the best methods to select the fittest spermatozoa.

Keywords: Centrifugation; Porous membrane; Artificial insemination; Fertility; Filters

Introduction

Stallions become sires primarily based on pedigree, athletic prowess and phenotype, with little consideration of reproductive soundness. The equine reproductive industry tends to rely on sires whose fertility is at best, a secondary characteristic [1,2]. Although the minimum number of spermatozoa in an insemination dose is likely to suffer individual variation [3], the reference for mare fertilization was established at 500million sperm with progressive motility (PMS). In a typical artificial insemination (AI) program this dose ranges from 250-500 million PMS, but recent studies have indicated that doses below 100million PMS do not reduce fertility [4,5]. Several methods have been suggested to mimic the selection of superior spermatozoa in the mare reproductive tract: the filtration of the spermatozoa (active or passive), from the seminal plasma; mimicking the migration of spermatozoa to the uterine horns; or the *in vivo* process that occurs in the uterus-tubal junction that selects the fittest sperm from the remnant of the ejaculate [6]. In assisted human reproduction the colloid centrifugation and swim-up are laboratory techniques routinely used for the exclusion of immobile and abnormal ejaculate sperm. This processing is performed to obtain high quality inseminating doses [7]. A device using filtration and migration techniques combined was developed recently [8], dispensing deleterious factors involved in centrifugation. The sperm selection goal is to have a measurable improvement of quality and quantity of spermatozoa when compared to the original ejaculate, while making sure that the process does not impact the integrity of the sample [9]. Inseminating only high-quality spermatozoa selected prior to insemination should improve pregnancy rates [6,10]. The objective of this paper is to review what has been published on this interesting subject called sperm selection in stallions, raising some questions and answering others about the techniques described below.

Sperm Selection Methods

Colloid centrifugation

While several sperm selection techniques are available, the only technique regularly used in commercial equine reproduction programs is the colloid centrifugation technique [11]. This technique centrifuges extended semen through layers of a varied-density colloid, which promotes separation of the plasma and the viable sperm cells [6]. During this process, the cells will move to the isopycnic point, or the equilibrium position. At this point, the cells can be separated from the plasma [12], not being a physiological method of separation [13]. A

disadvantage of this technique is that sperm cells are fragile when you look at the effect's centrifugation can have on the integrity of spermatozoa, as described in previous publications [14,15]. Another problem detected is the number of samples that must be prepared for commercial use [6]. Two examples of discontinuous density gradient are: RediGrad [16] and EquiPure [17]. An alternative on this method is the single layer centrifugation, which obtained results like those obtained in centrifugation of different layers. This method allows the processing of a larger volume of the ejaculate at a time [18]. A commercial example of this technique is Androcoll-E [19].

Migration

The migration method utilizes sperm motility as the main selection factor, where the swim-up method is the most used in humans [20]. This technique consists of centrifuging semen until it generates a cell pellet, and the motile sperm migrate from this pellet into supernatant medium, from which they are then collected [20]. The motility of the ejaculate samples is greater when using the swim-up technique than when using the centrifuge gradient technique [13]. The main disadvantage of migration methods is the low sperm retrieval [21], making it unfeasible to use this technique in the preparation of inseminating doses in most animal species [6].

Filters

Different techniques of selection of viable spermatozoa were developed in order to retain low quality sperm cells. Glass wool filter, Sephadex gel, and porous membranes are examples of filtering techniques in different species [22]. In pony stallions, a significant increase of seminal parameters is shown when using the glass wool filter [23]. This filter bonds the corpuscular content (proteins, immobile spermatozoa, and rounded cells of other origins) to surface fibers of the glass wool filter. If the fiber mesh is too dense, the cells will still adhere to the surface, but will not properly permeate the glass wool fibers [9]. The combination of glass-wool and Sephadex filtering for frozen stallion semen has proven to have a high correlation with fertility, because stallion membrane proteins give capacitated stallion sperm the ability to bind to Sephadex [24,25]. The higher correlation of glass-wool and Sephadex with fertility is due to the ability of the filters to remove cells that show capacitation-like changes or membrane impairment from the freeze-thaw process [26]. Porous membrane filters were not exactly a sperm selection technique. They were developed to avoid the centrifugation process and increase sperm concentration through the passage of the seminal fluid, retaining the spermatozoa [27,28].

Migration/filtration

Recently an approach combining the use of a porous membrane and the migration capability was suggested for fresh semen [8]. This technique consists of a device divided in half by a porous membrane, and the motile spermatozoa actively cross this membrane (Figure 1). Because the selection method needs no centrifuge or laboratory equipment, it has proven to be an effective and feasible option for sperm selection [8]. Spermatic membrane integrity and functionality, as well as enhanced kinetics were observed after using the selection device [8]. AI is the basis for other biotechnologies ranging from embryo transfer to cloning [29]. To achieve successful pregnancy using AI, enough viable spermatozoa capable of reaching the fertilization site are required [18]. Selecting the healthiest spermatozoa from a raw semen sample improves the quality of the AI dose, and is therefore more likely to achieve fertilization [6]. The ideal sperm separation technique was postulated to

a. be quick, easy and cost-effective,

b. isolate as many motile spermatozoa as possible,

c. not cause damage to the sperm, or non-physiological changes to the separated sperm cells,

d. eliminate dead spermatozoa and other cells, including leukocytes,

e. eliminate toxic or bioactive substances like decapacitation factors or reactive oxygen species (ROS), and
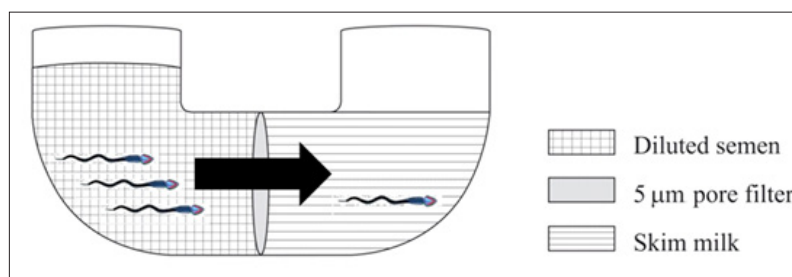
f. allow processing large volumes of ejaculates [30].

Figure 1: Schematic drawing of filtration/migration device.

To this date, none of these techniques described were able to accomplish these goals (Table 1). In the selection procedures mentioned above, one or more centrifugation stages are required to enrich the spermatozoa in a desired sample [9]. A major problem of centrifugation is the loss of approximately 25% of spermatozoa in the supernatant liquid [31]. Adverse effects in stallion semen after centrifugation have also been described [14,15]. Damaged and abnormal spermatozoa produce larger quantities of ROS which can contribute to reduced fertility, or problems associated with semen preservation [32]. Sperm membranes and DNA damage can be caused by oxidative stress, eventually converting the sperm into a non-viable spermatozoon. Damaged sperm has been shown to

alter embryo development in assisted reproductive technology in humans [33]. Filters have been developed for the removal of seminal plasma, and the concentration of spermatozoa, in an attempt to avoid the issue of plasma membrane damage that centrifugation causes [28].

Table 1: Comparison of the main techniques of the four principles described.

	Migration	**Filtration**	**Colloid Centrifugation**	**Filtration/Migration**
Laboratory equipment	Yes	May be required	Yes	No
Special material	Special tubes	Glass wool, Sephadex, filters	Colloids, special tubes	Filtration device
Commercial use	No	No	Yes	No
Final number of PMS in one single use	Data not available	Data not available	Data not available	200-280x106 [8]

Conclusion

Further investigation and development of the methods presented in this review, and the idealization of new procedures must continue in order to have a better method of selecting the fittest spermatozoa.

Acknowledgement

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