Semen sexing techniques and their applicability to cuniculture

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Introduction & Objectives

AI (artificial insemination) has been practiced widely in rabbit production. Thus, a great interest in developing a method for sexing rabbit sperm has grown. The aim of this review is to summarize the different techniques of sex mammalian sperm and revise how to better investigate the application in cuniculture.

Sexing techniques

• **Density gradient centrifugation:** This techniques takes advantage of the difference in weight and density between X- and Y- sperm. Using different substances it creates a medium with a gradual density and by adding semen in this medium, followed by a centrifugation, its possible to separate the medium into two fractions: a top layer with a majority of male sperm and a bottom layer with more female. Substances used are:

- Percoll: This substance is a commercial preparation composed by silica particles surrounded by polivinilpirrolidona. It is one of the more commonly used.
- Polysaccharides: Some examples of polysaccharides are sucrose, glucose or Ficoll.
- Albumin: Most commonly BSA (Bovine Serum Albumin) or other mammalian albumin.

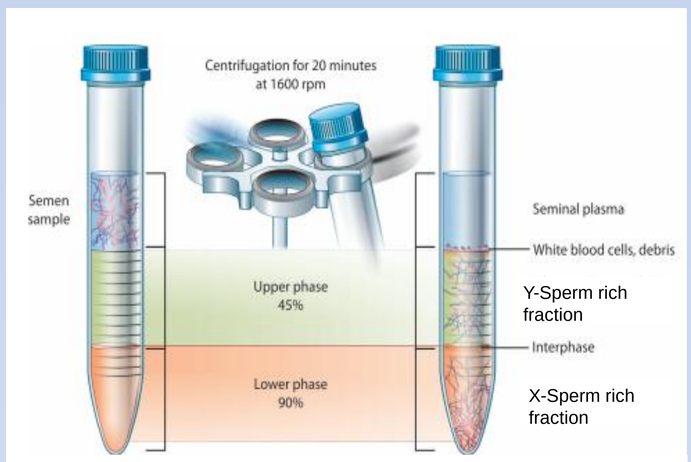

Figure 1: Scheme of density gradient centrifugation. [1]

• **Swim-up:** Semen is centrifuged and the supernatant discarded. The semen is then incubated in a medium, decanted 45º, during 30-45 minutes at 37ºC.

The smallest, most mobile semen will migrate to the top fraction and larger, less mobile and dead semen will rest at the bottom of the tube.

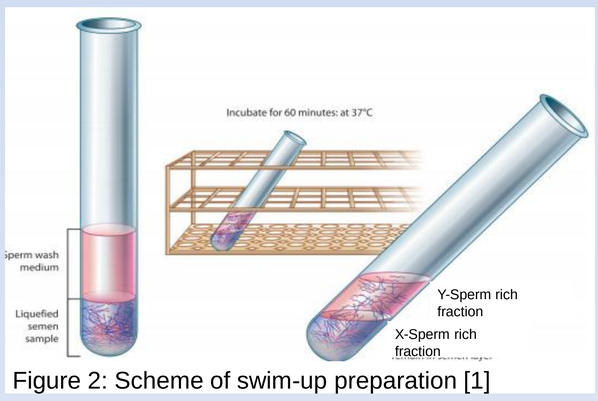

Figure 2: Scheme of swim-up preparation [1]

• **Immunology:** Less investigated than others, the aim of this technique is to produce antibodies to male sperm antigens to precipitate them and recover female ones.

• **Flow cytometry:** This technique is the most effective in sorting sperm and the most used in other mammals . It consist of separating male and female sperm using their difference of DNA quantity (3-4%). DNA of the sperm is stained with Hoechtst 33342, this stain is illuminated with a laser. When sperm enter the flow chamber one at a time they are evaluated individually. X-sperm will have a higher light intensity and Y less so. The computer recognizes this light and can assign the sperm as either X or Y, or uncertain.

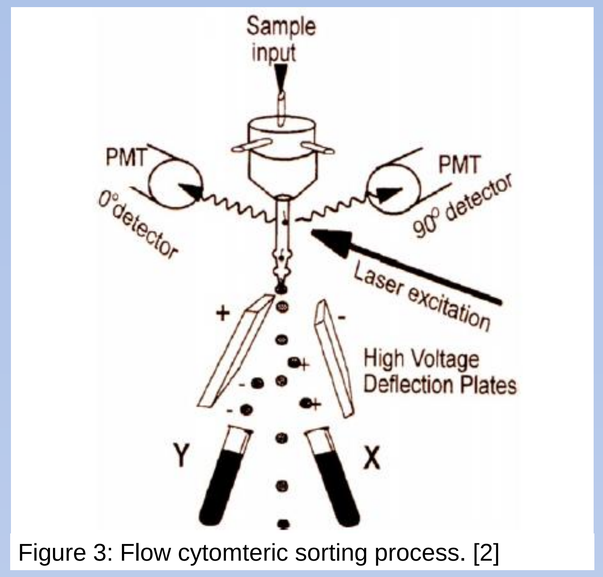

Figure 3: Flow cytomteric sorting process. [2]

Applicability to cuniculture

Since investigators first began investigating the optimal way to sex semen, rabbits have been used for various things:Sometimes, rabbit semen have been used for sexing studies but hardly ever with the aim of producing comercial doses.

The first time someone used the flow cytometric sorting technique was in 1989 (*Johnson et al.)* They reached a 90% of female bunnies. However it is an expensive and complicated technique and for this reason its not applicable to the rabbit semen industry. Other techniques have been studied and someone have shown interesting results as can be seen in Table 1.

Article	Mètode	% fem/masc	Significatiu (p-value)
Zavos 1983	Immuno, anti H-Y	74% fem	Si (p<0,01)
Zavos 1995	Separació gradients de densitat (albúmina)	55% mas	No (p<0,05)
Johnson et al 1989	Citometria de flux	81% masc 94% fem	Si (p<0,004) (p<0,0003)
Hernández et al 2008	Separació gradients de densitat (albúmina)	72,7% masc	Si (p<0,05)
Copello 2011	Swim up Percoll	63,7% masc 67,5% fem	Si (p<0,01)

Table 1: Results of different studies about sexing rabbit semen

Conclusions

• Semen sexing techniques are very interesting for farm production, and should be investigated further.
• For rabbit production techniques like density gradient centrifugation and swim-up are the most interesting due to their easier and cheaper application.

Bibliography
• Jafar SI, Flint APF. Sex selection in mammals: A review. *Theriogenology* , Vol 46, p191-200, 1996.
• [1]Beidola T, Sharma RK, Agarwal A, et al. Sperm preparation and selection techniques. *American Center for reproductive medicine*, Chapter 29, p244-251, 2010.
• [2]Seidel GE. Overview of sexing sperm. *Theriogenology*, Vol 68, p443-446, 2007.