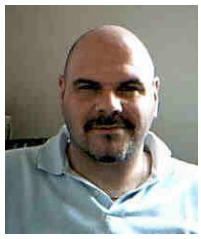
Barium alginate controlled release capsules for stallion spermatozoa delivery



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Introduction

Barium alginate controlled release capsules containing swine semen were developed to improve the preservation time of the spermatic cells and maximize the efficiency of a single artificial insemination procedure (Conte et al., 1999); controlled release capsules in sow breeding allows the same fecundity yields and higher parturition frequencies when compared to conventional artificial insemination procedures (Vigo et al., 2005a). In the mare, similar problems are common: if frequent veterinary scanning is not possible, fertility appears to be optimised by repeating AI on a daily basis (Sieme et al., 2003). The aim of this work is to verify the feasibility of the spermatozoa encapsulation technique in equine: a stallion semen controlled release formulation was evaluated *in vitro* in order to verify the encapsulated spermatozoa stability in terms of vitality and acrosome integrity.

Materials and methods

Spermatozoa encapsulation

Semen from two stallions with validated reproduction efficiency was employed. The ejaculate was split into two aliquots: one was diluted (free semen) 1:10 in an extender (aqueous medium containing: glucose 29 g/L; KCl 0.3 g/L; penicillin 106 I.U./L; streptomycin 1 g/L), and the other was processed for encapsulation. A saturated $BaCl_2$ solution was added to semen to obtain a Ba^{++} concentration of 5 mM; the resulting suspension was dropped into a 0.5 % w/v sodium alginate solution (Sodium alginate medium viscosity, Sigma-Aldrich, D) through a needle (25GX5/8") (Vigo et al., 2005b). Ions diffused out of the droplets and reacted with the alginate by ionic interaction when they reached the interface, leading to the formation of barium alginate around the semen droplet. Capsules were obtained, collected by filtration, rinsed twice and suspended in the abovementioned extender.

Capsule properties

The whole capsule diameter, core diameter and gel capsule thickness were measured by a digital video camera connected to an image analyser (CV 9000 Ver. 4.0 Image Analyzer, FKV Srl, Sorisole, BG, I); the weight of capsules was also measured using an analytical scale.

Spermatozoa storage and characterization

Both diluted and encapsulated semen samples were stored for 72 hours at 17°C in polypropylene vials. Sperm specimens, sampled at fixed times (0, 24, 48 and 72 hours) were stained with the double Trypan Blue/Giemsa staining technique; at least 150 spermatozoa/glass were counted. Stained spermatozoa can be subdivided in (1) live-non reacted; (2) live-reacted; (3) dead-non

reacted and (4) dead-reacted. The proportion in live-non reacted (LNR) cells for each sampling time was evaluated.

Statistical Analysis

For each time sampling, the difference between the proportion in LNR cells in free and encapsulated samples was evaluated with the Yates-corrected chi-square test. Significance level was set at p=0.05.

Results and Discussion

Capsule properties, reported in table 1, show limited ranges of dispersion, and these depend mainly on the extrusion method for droplet production; this is the critical point also for automated industrial encapsulation equipment.

Table 1: Capsule properties: mean values ± std dev. (sample size: 40)

	mean±std.dev.
Total diameter (mm)	**6.4±1.1**
Core diameter (mm)	**4.6±0.8**
Gel thickness (mm)	**0.9±0.2**
Weight (mg)	**107.0±23.3**

The percentage in LNR cells in encapsulated and diluted semen as a function of storage time is reported in figure 1: a lowering in LNR spermatozoa occurs between 24 and 48 hours. By this time, encapsulated semen maintains a significantly higher LNR proportion with respect to diluted semen. The encapsulation process appears to provide a better environment for sperm cells with respect to a classical extender: this encapsulation technique preserves the original sperm concentration within the core of the capsule, providing to only a ‘‘virtual’’ dilution and thus preventing the real dilution effect that adversely affects sperm structure.

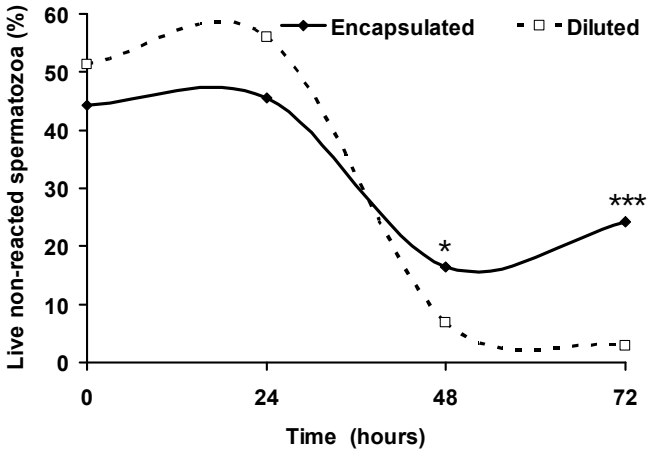

Figure 1 - Profiles of live-non reacted (LNR) encapsulated and diluted spermatozoa during the 72-hour storage time.
***- p<0.05 difference between treatment; ***- p<0.001 difference between treatment.**

Conclusions

The encapsulation process already developed for porcine semen was applied to equine spermatozoa: sperm cells are prone to severe membrane alterations when deprived of their physiological seminal plasma. Results previously indicate that capsule microenvironment creates a suitable milieu for spermatozoa (Faustini et al., 2004), since it does not alter the external spermatozoa environment. Commercial sperm extenders seem to alter the seminal plasma conditions since they dilute several molecules that contribute to maintain a suitable preservation medium.
The obtained results underline that the encapsulation process is versatile and allows, also in equine, capsules with suitable technological properties that permit their employ with the common AI catheters. These results represent the premise for the in-field evaluation of capsule performances not only in equine, but also in other livestock species, as well as to improve sperm storage in human assisted reproduction, as reported by Herrler et al.(2006).

References

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