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Note

Boar semen controlled delivery system: analysis of batch seasonal variability

M.L. Torre [a,*], M. Faustini [b], R. Norberti [a], L. Maggi [a], G. Maffeo [b], U. Conte [a], D. Vigo [b]

[a] *Department of Pharmaceutical Chemistry, Via Taramelli 12, 27100 Pavia, Italy*
[b] *Department of Veterinary Science and Technology for Food Safety, Via Celoria 10, 20133 Milan, Italy*

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Abstract

A new encapsulation technology of swine semen is proposed to improve the quality of stored spermatozoa and to obtain the controlled release of viable cells, reducing the number of instrumental inseminations. This technology has been employed to produce barium alginate gel capsules in different seasons; an analysis of batch reproducibility was performed, and total capsule diameter, gel thickness and weight of capsules were determined as indices of batch properties. A seasonal variability was found but each batch was substantially homogeneous. The variability could be related to the biological variability of the semen employed as raw material as well as to the technological process.

Keywords: Swine spermatozoa; Encapsulation; Alginate; Batch reproducibility

During the past decade, considerable attention has been paid to the development of new reproductive technologies: artificial insemination is a widely used technology in swine breeding. In this field, research has brought about the development of methodologies with the purpose of satisfying some essential requirements, such as low prices, the optimization of storage, high efficiency in terms of fertility and ease of handling by operators (Johnson et al., 2000). To improve artificial insemination technique in bovine species, spermatozoa encapsulation was developed by Nebel et al. (1985) to obtain cell controlled release. The use of a poly-L-lysine or protamine sulphate membrane may give good results in terms of in vivo fertility: starting from Nebel's idea, a swine spermatozoa delivery system was designed: a one-step reverse phase encapsulation method (Klein et al., 1983) was employed (Conte et al., 1998). An in vitro fertilization test on encapsulated semen demonstrated that the encapsulation process does not compromise fecundation ability even though a significant connection between oocyte cleavage probability and seasonal variation was found (Torre et al., 2000).

In this work the seasonal variability of the production batches was evaluated: weight, total

* Corresponding author. Tel.: +39-0382-507-779; fax: +39-0382-527-151
E-mail address: marina.torre@unipv.it (M.L. Torre).

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diameter and gel thickness of capsules were determined as indices of batch reproducibility: the former two variables were chosen since capsule size conditions the passage of the capsules through usual catheters, and gel thickness could influence the release rate of viable spermatozoa.

A saturated $BaCl_2$ solution was added to boar semen to obtain a Ba^{2+} concentration of 5 mmol/l; the resulting suspension was dropped into a 0.5% w/v sodium alginate solution (sodium alginate medium viscosity, Sigma–Aldrich, D) by a needle (1 mm diameter), using a peristaltic pump. Gel capsules were obtained (G caps), collected by filtration, rinsed twice and suspended in an extender for swine sperm (aqueous medium containing: 29 g/l glucose; 0.3 g/l KCl; 10^6 IU/l penicillin; 1 g/l streptomycin); a second kind of capsule (Cr-link caps) was obtained by the cross-linking of G caps in a 0.05% w/v aqueous solution of protamine sulphate (Sigma–Aldrich, D) for 20 min, then were collected, rinsed twice and transferred to the extender. Ten batches were produced in different seasons of the year, from March to December. The capsule diameter and gel capsule thickness were measured by a digital video camera connected to an image analyzer (CV 9000 ver. 4.0 image analyser, FKV Srl, Sorisole, BG, I). Control charts for process mean (X-chart) and standard deviation charts (S-chart) were performed for each parameter in order to proceed to an in line analysis. The upper and lower limits of the process were calculated according to Walpole and Myers (1993). Twenty capsules constituted each sample.

Results concerning the parameters are depicted in Figs. 1–3: variations under and over the tolerance limits (± 3 s) are present for total diameter (Fig. 1a), gel thickness (Fig. 2a) and weight (Fig. 3a). The batch production in different seasons of the year shows deviations from the process mean for considered parameters, in particular for capsule total diameter. Whereas S-charts for batch homogeneity show limited dispersion around the mean as reported in Figs. 1b, 2b, 3b for diameter, gel thickness and weight, respectively.

These results show that the batches are substantially homogeneous, especially for the gel thickness; nevertheless a variability of the indices chosen as parameters of batch reproducibility is evident. This variability could be related to the droplet production method; in fact this is the critical point also for automated industrial encapsulation equipment. Nevertheless, mean sizes are suitable to permit the flux through French catheters. On the other hand, the biological variability of semen employed as raw material could be the reason for the high data dispersion; the season can modify some physico-chemical characteristics of the boar semen, such as changes in sperm pH, a possible expression of the metabolic activity, with variable production of lactic acid (Xue et al., 1994; Paulenz et al., 2000); these changes in semen properties could influence several technological properties of the capsules and their fertilization potency as well as accounting for batch variability.

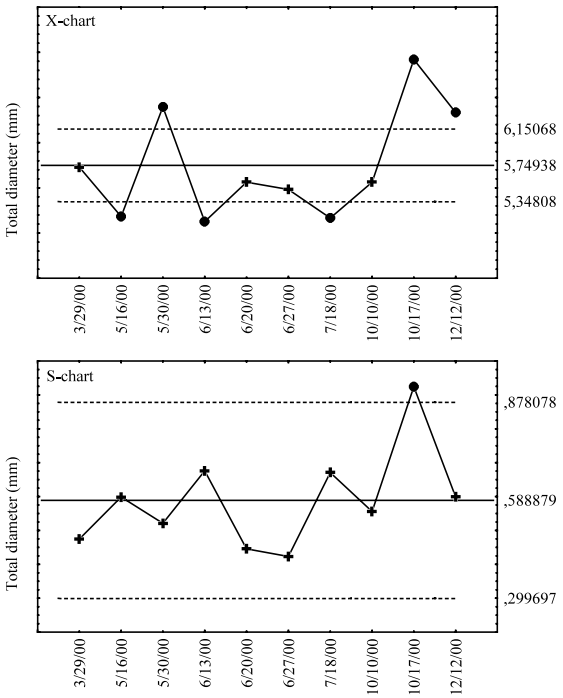

Fig. 1. X-chart (a) and S-chart (b) of capsule total diameter.

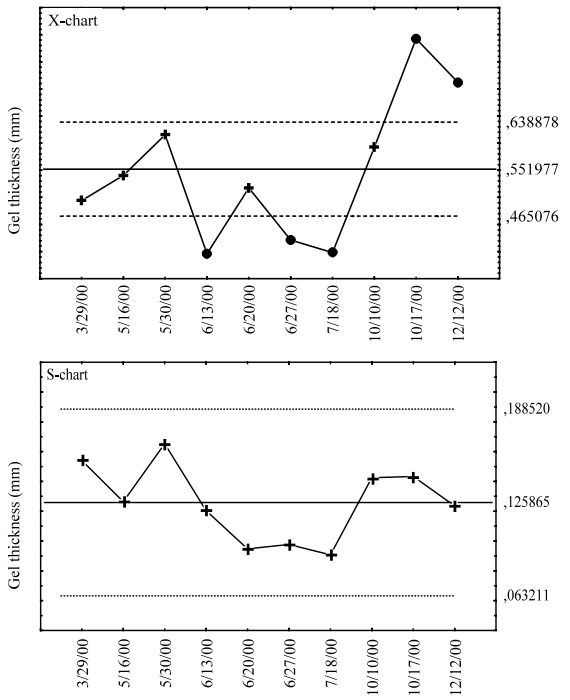

Fig. 2. X-chart (a) and S-chart (b) of capsule gel thickness.

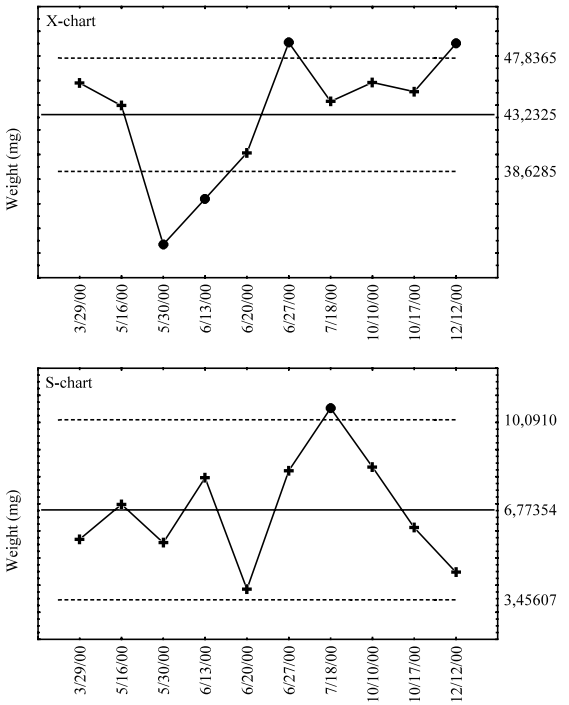

Fig. 3. X-chart (a) and S-chart (b) of capsule weight.

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