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Use of immobilized cryopreserved bovine semen in a blind artificial insemination trial

Fride Berg Standerholen [a,b], Karin Elisabeth Waterhouse [c], Anne Guro Larsgard [d], Randi Therese Garmo [c,1], Frøydis Deinboll Myromslien [b], Jan Sunde [d,2], Erik Ropstad [a], Geir Klinkenberg [e], Elisabeth Kommisrud [b,c,*]

[a] Department of Production Animal Clinical Sciences, Norwegian University of Life Sciences, Oslo, Norway
[b] Department of Natural Sciences and Technology, Hedmark University College, Hamar, Norway
[c] SpermVital AS, Hamar, Norway
[d] Geno SA, Hamar, Norway
[e] SINTEF Materials and Chemistry, Department of Biotechnology, Trondheim, Norway

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ABSTRACT

To make timing of artificial insemination (AI) relative to ovulation less critical, methods for prolonging shelf life of spermatozoa *in vivo* after AI have been attempted to be developed. Encapsulation of sperm cells is a documented technology, and recently, a technology in which sperm cells are embedded in alginate gel has been introduced and commercialized. In this study, standard processed semen with the Biladyl extender (control) was compared with semen processed by sperm immobilization technology developed by SpermVital AS in a blind field trial. Moreover, *in vitro* acrosome and plasma membrane integrity was assessed and compared with AI fertility data for possible correlation. Semen from 16 Norwegian Red young bulls with unknown fertility was collected and processed after splitting the semen in two aliquots. These aliquots were processed with the standard Biladyl extender or the SpermVital extender to a final number of 12×10^6 and 25×10^6 spermatozoa/dose, respectively. In total, 2000 semen doses were produced from each bull, divided equally by treatment. Artificial insemination doses were set up to design a blinded AI regime; 5 + 5 straws from each extender within ejaculates in ten-straw goblets were distributed to AI technicians and veterinarians all over Norway. Outcomes of the inseminations were measured as 56-day nonreturn rate (NRR). Postthaw sperm quality was assessed by flow cytometry using propidium iodide and Alexa 488–conjugated peanut agglutinin to assess the proportion of plasma membrane and acrosome-intact sperm cells, respectively. In total, data from 14,125 first inseminations performed over a 12-month period, 7081 with Biladyl and 7044 with SpermVital semen, were used in the statistical analyses. There was no significant difference in 56-day NRR for the two semen categories, overall NRR being 72.5% and 72.7% for Biladyl and SpermVital, respectively. The flow cytometric results revealed a significant higher level of acrosome-intact live spermatozoa in Biladyl-processed semen compared to SpermVital semen. The results indicate that the level of acrosome-intact live spermatozoa in the AI dose did not affect the 56-day NRR for the two semen processing methods. In conclusion, this study has showed that immobilized spermatozoa provide equal fertility results as standard processed semen when AI is performed in a blinded field trial, although the immobilization

* Corresponding author. Tel.: +47 62517247; fax: +47 62517601.
E-mail address: elisabeth.kommisrud@hihm.no (E. Kommisrud).
[1] Present address: TINE SA, Ås, Norway.
[2] Present address: Cryogenetics AS, Hamar, Norway.

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procedure caused increased sperm damage evaluated *in vitro* compared to standard semen processing procedure.

1. Introduction

Artificial insemination (AI) is commonly used for a range of domestic animals and in aquaculture. The technique can be applied with fresh, liquid, and cryopreserved semen, depending on species, and is the most valuable tool for animal breeding programs and genetic improvements [1]. In commercial animal production, fertility results are crucial to the total economic outcome. In the cattle breeding industry, AI is mainly applied with cryopreserved spermatozoa, a technology developed around 1950 on the basis of the discovery of glycerol being protective to sperm cells during freezing [2]. To obtain good fertility results, AI must be performed within a restricted number of hours before ovulation. Therefore, new technologies that prolong the lifetime of spermatozoa *in vivo* after AI will be beneficial to the industry because timing of AI relative to ovulation will be less critical.

Alginate gels are formed by interactions between divalent ions such as Ca^{2+} and block structures of the guluronic acid in the alginate polymer chain. Therefore, the formation of alginate gels can be conducted under very mild conditions, and have thus been commonly used for immobilization of various types of cells [3,4]. However, immobilization in alginate has been most commonly used as a starting point for later formation of various types of capsules with a liquid core. Several studies of encapsulation of spermatozoa within microcapsules are published [5–9]. These studies have used methods resulting in particles where the spermatozoa are located within a liquid core surrounded by a membrane.

Nebel et al. [5] reported that encapsulation was compatible with bovine sperm survival and that negligible sperm injury was observed during storage of encapsulated spermatozoa at 37 °C. Later, several studies have reported that encapsulated spermatozoa maintain their fertilizing capacity after AI [6–8], also when AIs were performed in proestrus, i.e., early relative to ovulation [9]. The technique has been applied with semen from other species such as ram [10] and boar [11], and finally, novel systems for encapsulation and release of bovine spermatozoa in capsules with a liquid core have been described [12,13].

The SpermVital technology for immobilization and cryoconservation of bull spermatozoa for AI has been developed during the past decade [14] and is implemented in large-scale production. This technology uses a fundamentally different approach to immobilize spermatozoa than previously published methods as spermatozoa are immobilized within a solid gel network made of calcium alginate gel. Immobilization within a solid gel network may have advantages compared to encapsulation within capsules. Using this method, movement of the immobilized spermatozoa might be restricted because of constraints of the gel network. This might resemble the situation in cauda epididymis where physical limitation of movement due to high concentrations of cells and presence of high viscosity polymers may be one of the factors that influence survival and maintenance of functionality of spermatozoa over long periods of time [15].

The success of bovine AI programs, regardless of semen processing techniques, is largely dependent on the use of good-quality semen, and it is reported that sperm characteristics are correlated to differences in fertility [16]. Simultaneously evaluating postthaw viability and acrosome integrity of spermatozoa by flow cytometry is a valuable testing tool in both research and routine work [17].

The SpermVital technology has been shown to be applicable for AIs performed early relative to ovulation (unpublished data); however, it is also important to know how the semen performs when used at normal timing of AI. The dissolving of the gel and thereby release of sperm cells is designed to last for at least 24 hours. Insemination performed at conventional timing may therefore be suboptimal for SpermVital-processed semen because fewer sperm cells are assumed to be available at the time of ovulation. The question to be raised in this investigation was whether enough sperm cells are released and capable of fertilization at conventional timing of AI.

The aim of this study was to compare the fertilizing capacity of semen processed with two different methods: conventional (Biladyl) and immobilization processing (the first-generation SpermVital technology) [14] in a blinded field trial. Furthermore, the semen samples were analyzed to reveal differences in sperm quality between the processing techniques by assessing acrosome and plasma membrane integrity. Moreover, the association between these sperm quality parameters and field fertility was determined.

2. Materials and methods

2.1. Semen samples for insemination trial and in vitro studies

All procedures for semen processing, being in compliance with European Union Directive 88/407, were approved by the Norwegian Food Safety Authority. The insemination trial was not in violation of any ethical guidelines or legislation.

During the June–August 2010 period, semen from 16 Norwegian Red (NRF) young bulls with unknown fertility was collected and processed by the breeding company Geno SA (Geno Breeding and AI Association, Hamar, Norway). The bulls had a mean age of 469 days (range, 432–497) on the first day of semen collection. Collection was performed once a week, with two separate ejaculates collected with approximately 15 minutes of interval before pooling. Collection continued until a minimum of 2000 doses were produced from each bull, resulting in processing of 4 to 9 ejaculates per bull, in total 85 ejaculates. Sperm concentration was estimated by spectrophotometer, and motility was evaluated by phase-contrast microscopy before and