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SHORT COMMUNICATION

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Studies of gel with immobilized semen by intrauterine endoscopy post-artificial insemination

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Abstract

An extended lifespan of spermatozoa following artificial insemination (AI) can make the timing of insemination less critical, as previously demonstrated with immobilized spermatozoa that are gradually released from an alginate gel. The purpose was to examine the in vivo dissolution of SpermVital (SV) alginate gel over time by endoscopy and secondly to assess spermatozoa quality after incubation of the gel. In vivo endoscopy showed SV gel in the uterus 3, 6, 20 and 24 hr after AI, demonstrating the potential release of spermatozoa to the uterus during this period. In utero ex vivo incubation of the semen demonstrated that high motility and viability of sperm cells was sustained following overnight incubation.

KEYWORDS

artificial insemination, cattle, endoscopy, immobilized spermatozoa

1 | INTRODUCTION

During standard AI in cattle, semen is deposited in the uterine body. To enable fertilization, the spermatozoa must travel through the female genital tract to the site of fertilization at the ampullaristhmic junction of the oviduct (Rath, Schuberth, Coy, & Taylor, 2008). Following AI, the selection and survival of spermatozoa, is affected by timing of AI, individual variation in the genital tract environment (Rath et al., 2008) and the choice of semen processing technology (Layek, Mohanty, Kumaresan, & Parks, 2016).

By the patented SpermVital (SV) technology, spermatozoa are immobilized in an alginate gel to enable their gradual release following insemination (Kommisrud, Hofmo, & Klinkenberg, 2008). Timing of AI relative to oestrous signs is assumed more flexible applying SV semen compared with standard semen. Alm-Kristiansen et al. (2017) reported similar pregnancy rates between AIs using a single AI dose with immobilized SV semen early in heat and AIs using standard processed semen inseminated on two consecutive days. Further, in vitro evaluation of SV spermatozoa quality post-thaw and following incubation has demonstrated prolonged viability compared with standard processed semen (Alm-Kristiansen et al., 2018). Multiple physiological and biochemical interactions take place between spermatozoa and the female genital tract which result in the selection of specific sperm subpopulations after AI (Suarez, 2016). Therefore, it is interesting to follow the in vivo dissolution of SV gel by genital tract endoscopy (GTE).

The main objective of this case study was to examine the in vivo dissolution of SV gel by intrauterine endoscopy and to assess spermatozoa motility and viability over time by incubating SV semen in utero ex vivo.

Data material can be acquired on request by email: halldor.felde.berg@nmbu.no

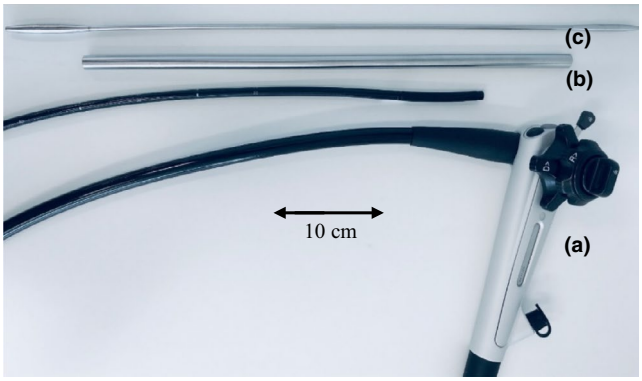

FIGURE 1 Endoscopy instruments: (a) endoscope with a controllable apex: external diameter = 7.9 mm, length = 140.0 cm, working channel = 2.8 mm (Karl Storz Silver Scope) (b) introducer, external diameter = 10.0 mm, internal diameter 9.0 mm, length = 42.0 cm (c) dilator, maximum diameter = 7.0 mm, length = 55.0 cm

2 | MATERIALS AND METHODS

2.1 | Ethical statement

The study was performed following criteria approved by the Animals in Science Regulation Unit of the Norwegian Food Safety Authority (FOTS No.: 20207).

2.2 | Semen processing

Two ejaculates were collected from one Norwegian Red bull (Geno Breeding and AI Association) within 15 min and pooled before split in two aliquots and processed using the SV technology in an alginate-based extender. One aliquot was added 0.23% Blue Dextran (D4772, SigmaAldrich) to enable identification of SV gel by GTE, the other served as control. Semen samples were cryopreserved as described by Alm-Kristiansen et al. (2017). Blue Dextran staining did not compromise spermatozoa post-thaw quality.

2.3 | Animals

Three Norwegian Red cows (A, B and C: parity 3, 2 and 3, respectively) held in free-stalls, fed grass silage and grain concentrate with mineral supplement were included in the study. The animals had no record of disease within 2 months before the study.

2.4 | Oestrous synchronization

Oestrus was synchronized with cloprostenol (500 μg i.m. Estrumate vet., Intervet International B.V.). In accordance with the manufacturer's recommendation for cloprostenol used for synchronization, the animals were treated with one dose on day 0 and another on day 11. Transrectal ultrasonography (BCF Easi-Scan with 7.5 MHz broadband straight linear rectal probe, BCF Technology Ltd) of the ovaries was used to confirm presence of a mature corpus luteum on day 11, identifying animals potentially responsive to oestrous synchronization.

2.5 | Endoscopy

Directly after GnRH analogue (buserelin acetate, 10.5 μg i.m. Receptal vet., Intervet International B.V.) treatment on day 13 at the time defined as 0 hr the cows were inseminated with SV semen. To facilitate retrieval, 5 semen doses were consistently deposited at

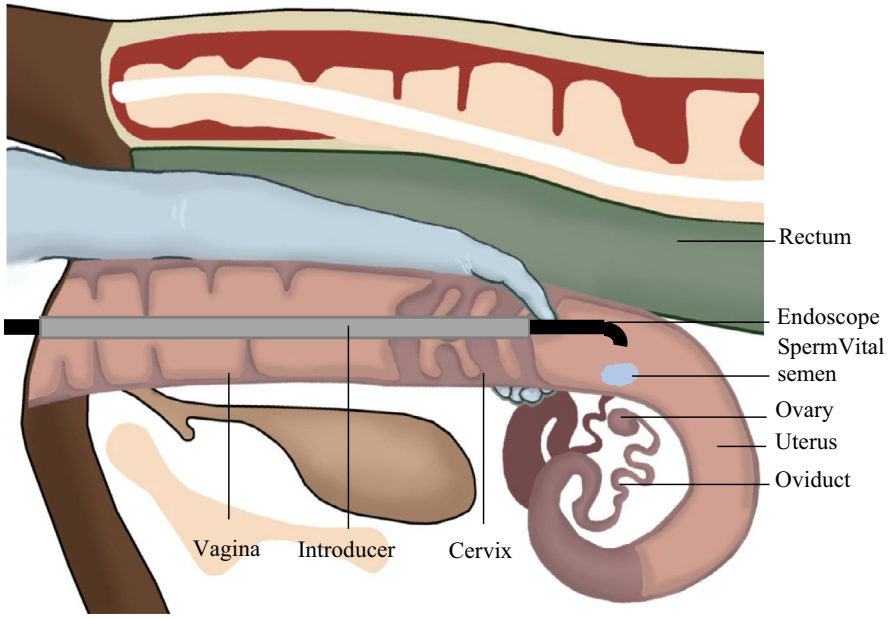

FIGURE 2 Genital tract endoscopy: introducer enabling passage of the endoscope through the cervix; SpermVital semen deposited by standard AI before endoscopy for in vivo monitoring

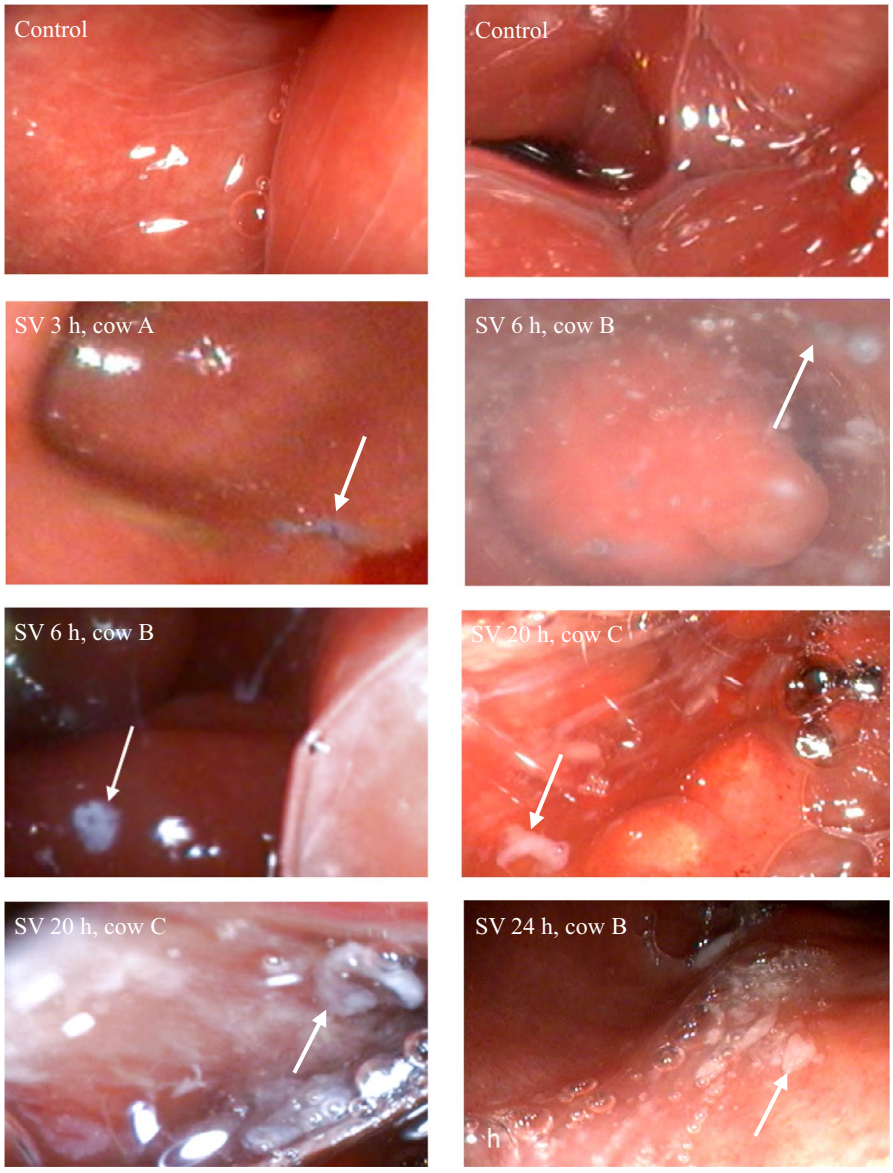

FIGURE 3 In vivo luminal view of the uterus; oestrous mucosa without stained SV fragments (transparent mucus with low viscosity, Control); SpermVital semen (arrows) stained with Blue Dextran 3 hr (SV 3 hr, cow A), 6 hr (SV 6 hr, cow B), 20 hr (SV 20 hr, cow C) and 24 hr (SV 24 hr, cow B) following AI

one location in one of the uterine horns in each cow. A dilator was then inserted through the cervix until reaching the posterior parts of the uterine body. To facilitate penetration of the flexible endoscope (Karl Storz Silver Scope) through the tortuous cervix, an introducer was thread onto the cervical dilator until reaching the anterior parts of the cervix (Figure 1).

By removing the dilator, passage of the endoscope through the cervix and access to the uterus was enabled (Figure 2). Dissolution of the SV gel was monitored by GTE 3, 6, 20 and 24 hr following AI. Images of the oestrous uterine mucosa without SV gel fragments were taken for control purposes.

2.6 | Spermatozoa quality in utero ex vivo

Bovine reproductive organs in pro-oestrus (n = 3) were inseminated with 2 SV doses in each uterine horn for incubation at room temperature. A section was made in the uterine horns 20 hr post-insemination followed by sampling of the inseminated SV gel into a pre-warmed SV dilution buffer. The remaining gel from one AI dose was diluted in SV dilution buffer (500 µl, ~37°C) and incubated for 15 min at ~37°C with gentle tilting. Semen (5 µl) was placed on a pre-warmed microscope slide, and subjective motility was examined by phase-contrast microscopy at 37°C and 100× magnification. An aliquot of the SV semen sample was incubated for 10 min in room temperature with 37.5 µM PI for examination of viability by fluorescence microscopy as described (Alm-Kristiansen et al., 2018).

3 | RESULTS

Fragments of SV gel were found in vivo in the uterine horns at 3 (cow A), 6 (cow B), 20 (cow C) and 24 hr (cow B) after AI. In vivo, these fragments were discerned from the uterine tissue and the transparent oestrous mucus discharge by the Blue Dextran staining and their indented shape (Figure 3; video provided in Video S1). Since

fragments of gel were difficult to extract in vivo, sperm motility and viability was only examined after incubation in utero ex vivo. Initial measurements of post-thaw motility and viability means (*SD*) were 52% (3) and 53% (5), while for sperm from gel incubated overnight in utero ex vivo the corresponding values were 38.3% (12) and 52% (2), respectively. The SV gel was partly dissolved following overnight incubation.

4 | DISCUSSION

In this study, non-invasive GTE was used in vivo to monitor SV semen following AI. A gradual dissolution of the alginate gel was visualized in 4 recordings 3–24 hr after AI. The gradual dissolution of the alginate gel was also observed ex vivo after uterine incubation. This indicates a slow release of spermatozoa from the solid alginate gel as shown previously (Alm-Kristiansen et al., 2018), and as in the present study demonstrating viable and motile spermatozoa following overnight incubation. A study applying cryopreserved canine semen to compare processing with alginate gel microencapsulation to standard processing shows similar results, with elevated motility and viability in the former after incubation at body temperature (Shah et al., 2011).

Limitations of the GTE technique were encountered reaching the uterine lumen in animals with a highly tortuous cervix. Careful operating of the GTE equipment was required to avoid abrasions in the genital tract mucosa. The effective discrimination of SV semen from uterine tissue and exudate by GTE depended on the blue staining of the alginate gel. Decolourising of the gel was observed, particularly >6 hr' post-insemination and most prevalent in vivo, likely due to the extensive perfusion of the uterus in oestrus.

This case study showed that SV gel can be retrieved in vivo 24 hr after insemination. Uterine incubation of the semen ex vivo demonstrated that high motility and viability was maintained overnight. The results indicate a prolonged release of immobilized spermatozoa expressing high reproductive potential, possibly resulting in an extended window of time for successful AI in cattle.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

HFB prepared trial design, performed animal clinical work, in utero ex vivo examinations, preparation of manuscript; HCLS performed clinical work, ex vivo examinations, semen in vitro analysis, preparation of manuscript; BH preparation of manuscript; ER preparation of manuscript; AHAK performed semen in vitro analysis, preparation of manuscript; EK prepared trial design, preparation of manuscript.

DATA AVAILABILITY

Additional data, for example endoscopy video material can be acquired on request by contacting the first author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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