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Comparison of pregnancy outcomes using either an Ovsynch or a Cosynch protocol for the first timed AI with liquid or frozen semen in lactating dairy cows

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ABSTRACT

The objective of this study was to evaluate fertility to the first timed AI (**TAI**) using either liquid semen or frozen semen after an Ovsynch or a Cosynch protocol in lactating dairy cows. The hypothesis was that there is an increase in fertility to the first TAI when cows are inseminated with liquid semen compared to that when frozen semen is used in a Cosynch protocol. Lactating dairy cows (n = 1724; 540 primiparous, 1184 multiparous) from 9 commercial dairy farms were enrolled on a weekly basis to facilitate first timed AI. Two experiments were conducted. In experiment 1, all cows received GnRH, 7 d later $PGF_{2\alpha}$, and then received one of the following treatments: 1) GnRH + TAI with liquid semen 56 h after $PGF_{2\alpha}$; 2) GnRH + TAI with frozen semen 56 h after $PGF_{2\alpha}$; 3) GnRH 56 h after $PGF_{2\alpha}$ + TAI with liquid semen 12–16 h after the second GnRH; 4) GnRH 56 h after $PGF_{2\alpha}$ + TAI with frozen semen 12–16 h after the second GnRH. In experiment 2, all cows received GnRH, 7 d later $PGF_{2\alpha}$, and then received treatments 3 or 4 as described for experiment 1. Number of sperm per straw was 20×10^6 sperm/straw and 10×10^6 sperm/straw for frozen and liquid semen, respectively. Pregnancy diagnosis was performed by ultrasound scanning at 39 d after TAI. In experiment 1 (n = 1263), there was an interaction of semen preservation method by TAI protocol. Cows inseminated with liquid semen concurrently with the second GnRH (Cosynch-56) achieved greater pregnancy per AI (**P/AI**) than cows inseminated with frozen semen using the same synchronization protocol (20.0% vs. 27.5%; $P = 0.032$). There was no effect of semen preservation method (liquid semen 32.3% vs. frozen semen 28.6%; $P = 0.330$) when cows were inseminated approximately 16 h after the second GnRH injection (Ovsynch-56). Parity affected P/AI with primiparous having a greater P/AI than multiparous cows (34.8% vs. 20.2%; $P = 0.001$). In experiment 2 (n = 377), there was no effect of semen preservation method (liquid semen 26.5% vs. frozen semen 25.5%; $P = 0.846$) when cows were inseminated approximately 16 h after the second GnRH injection (Ovsynch-56). Parity affected P/AI with primiparous having a greater P/AI than multiparous cows (37.0% vs. 17.3%; $P = 0.001$). The results of this study provide evidence that liquid semen achieved greater P/AI in a TAI protocol with a long time interval between insemination and ovulation (Cosynch-56) compared with frozen semen indicating that liquid semen might have a longer viability in the reproductive tract.

1. Introduction

Long-term storage of semen in liquid nitrogen using plastic straws has been one of the key factors for the widespread adoption of artificial insemination (**AI**) in the dairy industry [1]. In 2007, about 72% of the dairy operations in the U.S. were using frozen semen for AI [2]. The process of freezing and thawing, however, irreversibly affects a significant proportion of sperm with typical estimates of 50% survival postthawing [3,4]. This issue might be resolved by using liquid semen for AI. Liquid semen has compelling advantages such as reduced insemination doses [5,6], less damage to sperm [7], more widespread use of genetically superior sires, limiting the negative effect of sexing technology [6], lower cost of storage, and simple handling [8]. In contrast, the major disadvantage for liquid semen is its limited fertile life span of approximately 3 days [8]. Liquid semen only accounts for 5% of worldwide AI [9],

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predominantly in seasonal calving dairy systems such as New Zealand, Australia, and Ireland. The use and production of liquid semen can only be justified by the increase in fertility when compared with frozen semen.

Synchronization protocols for timed artificial insemination (**TAI**) are widely adopted in the dairy industry [10]. The most common synchronization protocol is a 7 d Ovsynch [11]. The interval from induction of ovulation to insemination influences fertility of dairy cows. Pursley et al. [12] demonstrated maximum pregnancy and calving rates when cows received TAI approximately 16 h after the second GnRH injection (**GnRH2**). Prolonged time from insemination to ovulation (>24 h) appears to reduce pregnancy per AI (**P/AI**) for cows inseminated at the onset of estrus [13,14] or at the time of the GnRH2 mediated LH surge [12,15]. Decreased fertilization rates have been reported when cows were inseminated at the onset of estrus compared with breeding 12 or 24 h later [14]. A loss of sperm viability is likely responsible for the declines in fertilization rate and P/AI that have been observed in several [12,14,16] but not all [17] studies that examined extended intervals between AI and ovulation. Despite these results, some dairy farms utilize protocols in which TAI is done concurrently with GnRH2 of the 7 d Ovsynch protocol, i.e., Cosynch protocol [18]. This protocol requires one less animal intervention thus decreasing the number of potentially stressful events and lock-up times as well as labor costs. For liquid semen a higher viability in the female reproductive tract compared to cryopreserved semen has been postulated [19]. Bucher et al. [20] obtained similar P/AI in beef cows using liquid semen (n = 736; 51.5%) at a concentration of 3×10^6 sperm/straw compared with frozen semen (n = 719; 50.4%) at a concentration of 20×10^6 sperm/straw. In another study using beef cows, liquid semen achieved greater P/AI (n = 430; 59.9%) compared with frozen semen (n = 408; 49.4%) using the same concentration of 25×10^6 sperm/straw in a TAI protocol [7]. In lactating dairy cows using liquid semen might be more convenient for TAI regarding the duration of the AI process because multiple inseminations can be performed more efficiently as there is no need for thawing. Controlled randomized trials (**RCT**) comparing liquid and frozen semen in TAI protocols for lactating dairy cows, however, are missing.

Therefore, the objective of this study was to compare liquid and frozen semen using either an Ovsynch or a Cosynch protocol for the first TAI in a 2 × 2 factorial design. The main hypothesis of the present study was that there is an increase in P/AI for the first TAI when cows are inseminated with liquid semen compared with frozen semen in a Cosynch protocol. Additionally, we hypothesized that using an Ovsynch protocol, liquid semen achieves comparable P/AI compared with frozen semen.

2. Material and methods

The experimental procedures reported herein were conducted with the approval of the Institutional Animal Care and Use Committee of the Freie Universität Berlin.

2.1. Farms and animals

This experiment was performed on 9 commercial dairy farms in Mecklenburg-Vorpommern, Germany from April 2016 to October 2016. Lactating dairy cows (n = 1724; 540 primiparous, 1184 multiparous) were housed in free-stall facilities and had ad libitum access to fresh feed and water. Diets were typical for Mecklenburg-Vorpommern, using corn silage as the major forage and balanced by a professional nutritional consultant for protein, vitamins, and minerals. Cows were fed a total mixed ration twice daily that consisted of corn silage and grass silage as forage with a corn, soybean meal, and canola meal-based concentrate. All total mixed rations were balanced to meet or exceed minimum nutritional requirements for dairy cows [21]. Cows were milked either 2 (3 farms) or 3 (6 farms) times daily. Lists for scheduled injections (completed by AI personnel) and pregnancy examination (completed by the herd veterinarian) for individual cows were generated weekly using a commercial on-farm software program (5 farms used HerdeW, version 5.9, dsp-Agrosoft Ltd., Ketzin, Germany; 1 farm used DairyPlan C21, GEA, Düsseldorf, Germany; 1 farm used Dairy Comp 305; Valley Agricultural Software, Tulare, USA; 2 farms used FULLEXPERT, Lemmer-Fullwood Ltd., Lohmar, Germany). These programs were also used to track and record reproductive outcomes and individual cow events for each cow enrolled in the experiment.

2.2. Experimental design

This study was designed as a controlled randomized study using a 2 × 2 factorial design (experiment 1). The first main effect was the type of semen preservation method used for TAI (i.e., conventional frozen semen or liquid semen). The type of semen used for TAI changed every other week on each farm (i.e., week 1: frozen semen; week 2: liquid semen; week 3: frozen semen). The second main effect was the time of insemination relative to the second GnRH injection in a 7 d TAI protocol. Cows were inseminated either 12–16 h after the second GnRH injection (**Ovsynch-56**) or concurrently with the second GnRH injection (**Cosynch-56**). Cohorts of cows were randomly assigned to 1 of 2 synchronization protocol on a weekly basis to facilitate first postpartum TAI (i.e., Ovsynch-56 or Cosynch-56). Animals were assigned to synchronization protocols based on the last digit of their unique 10-digit animal identification number. Odd-numbered cows were assigned to Ovsynch-56 and even-numbered cows were assigned to Cosynch-56.

In September 2016, we compared P/AI between the Cosynch-56 and the Ovsynch-56 using pregnancy information of approximately 800 TAI. There was a marked reduction in P/AI for cows inseminated with frozen semen concurrently with GnRH2. In order to prevent pregnancy losses due to the synchronization protocol enrollment of cows into the Cosynch-56 was terminated and the farms continued with Ovsynch-56 only (experiment 2). The procedures for using the 2 semen preservation methods continued as described with type of semen used for TAI changing every other week.

2.3. Reproductive management of the farms

None of the farms used a presynchronization protocol before the first TAI. Reproductive management of the first postpartum AI (i.e., VWP, DIM at enrollment) is summarized in Table 1. Days in milk when cows received the first GnRH injection represented study day 0. As illustrated in Fig. 1, animals assigned to Ovsynch-56 (n = 1054) received 2 injections of GnRH (100 μg of Gonadorelin, Gonavet Veyx, Veyx Pharma Ltd., Germany) on day 0 and 9 (56 h after $PGF_{2\alpha}$). Prostaglandin $F_{2\alpha}$ (500 μg Cloprostenol, PGF Veyx forte, Veyx Pharma Ltd., Germany) was administered on day 7. TAI was performed 12–16 h after the second GnRH injection on a Friday morning. Animals assigned to Cosynch-56 (n = 670) received the same injection schedule as described for Ovsynch but were inseminated concurrently with the second GnRH injection Thursday evening (Fig. 1). On each farm, a single professional AI technician performed all inseminations.

Pregnancy diagnosis was performed by transrectal ultrasonography. Pregnancy status was evaluated by the herd veterinarian 39 d after TAI. A positive pregnancy diagnosis was based on visualization of an embryo with a heartbeat.