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Is Doublesynch protocol a new alternative for timed artificial insemination in anestrous dairy cows

Ö.A. Öztürk, Ü. Cirit *, A. Baran, K. Ak

Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Istanbul University, Avcilar, Istanbul, Turkey

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Abstract

This is the very first report that suggests high pregnancy rates can be obtained with use of the Doublesynch protocol in anestrous dairy cows. Recently, a new synchronization method has been developed (Doublesynch) that resulted in synchronized ovulations both after the first and second gonadotropin-releasing hormone (GnRH) treatments. It was suggested that this protocol has the potential to increase the pregnancy rates in primiparous dairy cows. The aim of the current study was to confirm the success of the Doublesynch protocol and further to investigate the effect of this method on pregnancy rates in anestrous cows. Lactating primiparous Holstein (Bos taurus) cows (n = 165) between 60 and 172 d postpartum were monitored twice with 10-d intervals (on Days –10 and 0) by ultrasonography, and blood samples were collected. Cows were classified as anestrous if both blood samples had progesterone (P4) concentration <1 ng/mL and as cyclic if at least one of the two samples had P4 concentration ≥1 ng/mL. Cyclic cows were classified again as cyclic-high P4 (having an active corpus luteum) if the second blood samples had P4 concentrations ≥1 ng/mL and as cyclic-low P4 if P4 concentrations were <1 ng/mL on Day 0. Then, the cows classified as anestrous (n = 51), cyclic-high P4 (n = 63), or cyclic-low P4 (n = 51) were put into two treatment groups (Ovsynch or Doublesynch) randomly to establish six groups. Cows in the Ovsynch group were administered a GnRH (lecirelin 50 μg, im) on Day 0, PGF (Prostaglandin F2 alpha, D-cloprostenol 0.150 mg, im) on Day 7, and a second dose of GnRH 48 h later. Cows in the Doublesynch group were administered a PGF on Day 0, GnRH on Day 2, a second PGF on Day 9, and a second GnRH on Day 11. Timed artificial insemination (TAI) was performed 16 to 20 h after the second GnRH in both treatment groups. Pregnancy diagnosis was conducted (by ultrasonography) 45 ± 5 d after TAI. In anestrous cows and those with high and low progesterone concentration at treatment onset, Doublesynch treatment led to markedly increased pregnancy rates with respect to Ovsynch treatment ($P < 0.05$). On the overall analysis of data, it was revealed that the Doublesynch method increased pregnancy rates by 43 percentage units (29.8% vs. 72.8%, $P < 0.0001$) in relation to Ovsynch. Pregnancy rates of cows having small, medium, or large follicles at the day of second GnRH administration were similar in the Doublesynch group (70.4%, 85.2%, and 63.0%, respectively; $P > 0.05$), whereas pregnancy rates reduced dramatically as follicle size increased in the Ovsynch group, particularly in cows with follicles greater than 16 mm (45.5%, 28.1%, and 5.3%, respectively; $P < 0.05$). Our results confirm and support observations that the Doublesynch protocol increases the pregnancy rates in postpartum primiparous cows as reported previously. Our data also demonstrate that the Doublesynch method increases the pregnancy rates in anestrous cows. Thus, these data suggest that the Doublesynch protocol can be used to obtain satisfactory pregnancy rates after TAI in both anestrous and cycling primiparous dairy cows regardless of stage of estrous cycle.

Keywords: Anestrous cows; Doublesynch; Primiparous; Synchronization; Timed artificial insemination

* Corresponding author. Tel.: +90 212 473 70 70 (17264); fax: +90 212 473 72 41.
E-mail address: ucirit@istanbul.edu.tr (Ü. Cirit).

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1. Introduction

The ability to control the time of ovulation precisely after a period during which follicular development and corpus luteum (CL) regression have been programmed sequentially permits a timed insemination. Such programs are essential in high-producing dairy cows that experience a reduction in estrus intensity that contributes to undetected heats, reoccurring luteal phases without estrus expression, or reoccurring waves of follicles that fail to ovulate [1]. In 1995, a protocol termed Ovsynch was developed allowing timed artificial insemination (TAI) without the need for detection of estrus in lactating dairy cows [2]. Later studies have demonstrated that there are several stages of the estrous cycle when initiation of the Ovsynch program causes reduced pregnancy rates [3–5]. For example, initiation of the program between Days 13 and 17 of the cycle is a time during which spontaneous regression of the CL occurs prior to the time that PGF (Prostaglandin F2 alpha) is administered at 7 d after the administration of gonadotropin-releasing hormone (GnRH). Such cows ovulate prematurely relative to insemination and are not likely to conceive [6]. A second problematic stage occurs early in the estrous cycle (e.g., Days 2 to 4). At this stage, spontaneous ovulation has already occurred, and the potentially new dominant follicle is too small to ovulate in response to GnRH administration [4]. As a consequence, the dominant follicle at the second administration of GnRH is considered aged [1], and cows that are included in the Ovsynch program in the early estrous cycle are less fertile [3,4]. Strategies have been developed to minimize the proportion of cows in these problematic stages of the cycle for initiation of the Ovsynch program. Researchers who try presynchronization treatment with PGF administration 7 d before Ovsynch have usually observed that it did not have any effect on increasing pregnancy rates [7,8]. Another approach is to treat cows twice with PGF at an interval of 14 d and to make the first GnRH administration of the Ovsynch program 12 d after the second administration of PGF. This Presynch-Ovsynch program increased pregnancy rates 12 percentage units (37% vs. 49%) [9] and 18 percentage units (25% vs. 43%) [10] in lactating cyclic cows. However, in this presynchronization method, the duration between the beginning of treatment and AI is approximately 36 d.

Success of the Ovsynch protocol is dependent on whether lactating dairy cows are anestrous or cycling [1,11,12]. It has also been demonstrated that ovulation of the follicle in response to the first GnRH administration is a prerequisite for the success of the Ovsynch program [1,8,13]. Pregnancy rates were lower in cows that were not cycling at the time the Ovsynch program was initiated. If anestrous cows ovulate at the first and second GnRH treatments of the Ovsynch program, then pregnancy rates appear to be normal (e.g., 39%) [1].

Primiparous cows have a delayed onset of cyclicity and a greater frequency of anestrus (35.6%) compared with multiparous cows (16.9%), mainly due to the fact that first lactation cows not only have to ensure milk production for their offspring but also their own growth [6,14,15]. Generally, primiparous cows account for approximately 30% to 50% of the lactating cattle on dairy farms. In addition, in developing countries, the newly established farms often consist of pregnant heifers, and thus they initially have to deal with the some of the problems associated with primiparous cows and newborn calves. These management problems generally include parturition of so many cows during a very short time, the care of newborn calves, training of primiparous cows to milking machines, postpartum health problems, and training of inexperienced personnel. As they try to overcome these above-mentioned problems, they generally have limited time for estrus detection and reproductive management of the herd. These problems collectively cause a decrease in profitability of the farm. If new estrus synchronization protocols can be developed to provide satisfactory pregnancy rates after TAI, this would facilitate the reproductive management of dairy farms, especially the new ones.

Recently, Cirit et al. [8] have developed a new synchronization method (-2PGPG) by administering an additional PGF 2 d before the Ovsynch protocol (GPG). This new program increased pregnancy rates by 22.2 percentage units (50.0% vs. 72.2%); however, statistical significance was not obtained ($P > 0.05$) because of the low number of cows used. Researchers have attributed this higher pregnancy rate to the significant increase in ovulation rates after the first GnRH administration by -2PGPG treatment (88.9% vs. 38.9%). This method has now been called Doublesynch (abbreviation of double synchronization) as it resulted in synchronized ovulations both after the first GnRH (88.9%) and second GnRH (94.5%) treatments [8].

To our knowledge, there is no published report on anestrous cows using the Doublesynch method. The aim of this study was to confirm the success of the Doublesynch protocol and to investigate the effect of this method on pregnancy rates in anestrous cows.