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A Comparative Analysis of Double-Ovsynch and Ovsynch-Heat-Synch Protocols on Pregnancy Rates in Primiparous Dairy Cows

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Abstract Today, ovulation synchronization methods are widely used to improve fertility in dairy cows. In this regard, this research was conducted in one of the dairy herds in East Azerbaijan province, Iran. The cows were randomly divided into two groups. Ovsynch-Heat-Synch Group: This group included 30 primiparous cows. One month after calving, provided the uterus was clean, the synchronization program commenced. On the first and ninth days, they received GnRH, and on the seventh day, PGF2α intramuscularly. One week later, they received GnRH again; seven days after that, they received PGF2α, and 24 hours later, 1 mg of estradiol benzoate intramuscularly. Cows that showed estrus after the estradiol injection were inseminated using the AM/PM method, while those that did not show estrus 48 hours after the estradiol injection were artificially inseminated (Timed Artificial Insemination, TAI). Double-Ovsynch Group: This group also included 30 primiparous cows. One month after calving, provided the uterus was clean, the synchronization program began. On the first and ninth days, they received GnRH analogue; on day seven, PGF2α was administered intramuscularly. One week later, they received GnRH analogue again; seven days later, PGF2α was given, and 48 hours later, they received GnRH analogue intramuscularly. Forced insemination was performed 16 to 24 hours after the last GnRH injection. Pregnancy diagnosis was conducted using ultrasound between days 30 to 35 and by rectal palpation between days 40 to 45 after artificial insemination. The results showed that the fertility rate was 43.3% (13 cows) in the Ovsynch-Heat-Synch group and 60% (18 cows) in the Double-Ovsynch group. In the statistical analysis, this difference was not significant ($p>0.05$). This study indicated that the new Ovsynch-Heat-Synch method does not have a significant superiority over the Double-Ovsynch method.

Introduction

Domestic cattle, buffalo, and wild species of the Bovidae family exhibit distinct reproductive characteristics. Sexual maturity in female cattle occurs when they are capable of releasing gametes and displaying complete sexual behaviors. Key factors influencing sexual maturity include body size, nutrition, and seasonality. Dairy and beef cattle, due to domestication, have evolved into non-seasonal breeders, capable of reproductive activity year-round [1].

During the estrous cycle, the ovaries undergo hormonal and structural changes. Follicles grow in a wave-like pattern, with only one follicle ovulating per cycle. Follicular growth is regulated by hormones such as FSH and LH. Nutritional

and hormonal factors, including IGF-1, play significant roles in follicular development [2].
Hormones like progesterone and estrogen influence follicular growth and regression, while ovulation is closely associated with inflammatory-like mediators such as interleukin-1. Additionally, the hormone oxytocin, by stimulating prostaglandin production, plays a crucial role in regulating ovulation [2].

Low fertility in dairy cows leads to a decrease in reproductive efficiency and the profitability of herds [3]. One common issue is the detection of estrus in cows, which can be addressed using ovulation synchronization programs such as Ovsynch and Heat-Synch [2]. The Ovsynch program induces synchronized ovulation in cows through GnRH and PGF2α injections, whereas Heat-Synch uses estrogen to induce ovulation. Research has shown that these methods can generally improve fertility in cows; however, they may not yield favorable results in first-lactation cows [1]. This study aims to compare the effects of two new ovulation synchronization methods, namely Double-Ovsynch and Ovsynch Heat-Synch, on the fertility of first-lactation dairy cows

Materials and methods

This study was conducted on a dairy herd in East Azerbaijan province, Iran. A total of 60 Holstein-Friesian dairy cows, ranging from 30 to 200 days in milk (DIM) and producing an average milk yield of 25 kg per day, were included in the study. The cows' diets were formulated based on their milk production and provided as Total Mixed Ration (TMR). The cows were randomly assigned to two groups: Ovsynch Heat-Synch group: This group consisted of 30 first-lactation cows. The synchronization protocol started one month after calving, provided the uterus was clean. The cows received 10 micrograms of GnRH analogue (Vetarolin, Abureyhan Pharmaceutical Company, Iran) on days 0 and 9, and 500 micrograms of PGF2α (Vetaglandin, Abureyhan Pharmaceutical Company, Iran) intramuscularly on day 7. On day 16, another 10 micrograms of GnRH analogue was administered, followed by 500 micrograms of PGF2α seven days later, and 1 milligram of estradiol benzoate (Vetaestrol, Abureyhan Pharmaceutical Company, Iran) intramuscularly 24 hours after PGF2α [4]. Cows that showed estrus after the estradiol injection were inseminated using the AM/PM method, while those that did not exhibit estrus were inseminated 48 hours after the estradiol injection (Timed Artificial Insemination, TAI). Double-Ovsynch group: This group also consisted of 30 first-lactation cows. The synchronization protocol began one month after calving, provided the uterus was clean. The cows received 10 micrograms of GnRH analogue (Vetarolin; Aburihan Pharmaceutical Company, Iran) on days 0 and 9, and 500 micrograms of PGF2α (Vetaglandin; Aburihan Pharmaceutical Company, Iran) intramuscularly on day 7. On day 16, another 10 micrograms of GnRH analogue was administered, followed by 500 micrograms of PGF2α seven days later. A second dose of 10 micrograms of GnRH analogue was administered 48 hours after the second PGF2α injection. Forced insemination was performed 16 to 24 hours after the last GnRH injection [4]. Pregnancy diagnosis was performed using ultrasonography between days 30 to 35 and by rectal palpation between days 40 to 45 after artificial insemination.

Statistical analysis

To statistically analyze the data, fertility rates observed in both groups were compared using the Chi-square test implemented in SPSS version 20.

Results

Pregnancy rates were 43.3% in the Ovsynch Heat-Synch group and 60.0% in the Double-Ovsynch group (Table 1). Statistical analysis using the Chi-square test indicated no significant difference between the two groups (χ^2 = value, p = 0.196). Since the p-value exceeded the conventional threshold of 0.05, the difference in pregnancy rates was not considered statistically significant. Additionally, the Phi correlation coefficient was 0.167, which also did not reach statistical significance ($p>0.05$), indicating a

Table 1. Number and frequency of pregnant and non-pregnant cows in each research group

Group	Pregnant (No)	Non-Pregnant (%)	Non-Pregnant (No)	Pregnant (%)	Total (No)	Total (%)
Ovsynch Heat-Synch	13	43.3	17	56.7	30	100
Double-Ovsynch	18	60	12	40	30	100
Total	31	51.7	29	48.3	60	100

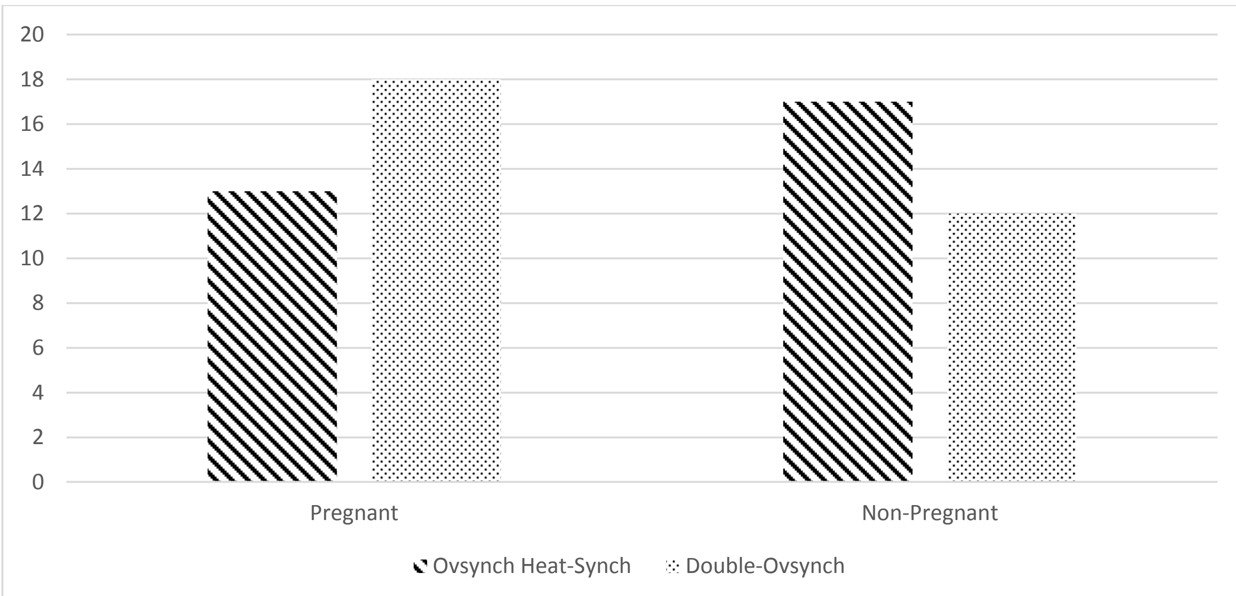

Fig 1. Number of pregnant and non-pregnant cows in each research group

weak and non-significant association between the treatment protocol and pregnancy outcome (Figure 1).

Discussion

Pursley and colleagues (1995) demonstrated that the pregnancy rate in the Ovsynch program averages between 30% and 40% [4]. In a study by Pancari and colleagues (2002), the pregnancy rates of two treatment groups, Ovsynch and Heat-Synch, were evaluated. Both groups underwent two injections of PGF2α, administered 14 days apart and 14 days before the start of the program. The pregnancy rate for the Ovsynch group was reported as 37.1 ± 5.8%, compared to 29 ± 3.5% in the Heat-Synch group. However, the overall pregnancy rates between the two groups did not differ significantly [5]. Results of previous studies showed that the Ovsynch protocol produced varying pregnancy rates in dairy cows, ranging from 37.8% to 42.3%, depending on factors such as days postpartum, geographic region, and climate [3]. Stevenson observed that a limited number of cows exhibited estrus in the Ovsynch program. Between 8% and 16% of cows showed estrus near the PGF2α injection, and over 30% exhibited estrus at or after the second GnRH injection [6]. Given the positive feedback of estradiol on LH surge during the proestrus phase and the cost-effectiveness of estradiol-17 beta estrus, efforts were made to replace the second GnRH injection in Ovsynch with estradiol compounds [7, 8]. In the Heat-Synch protocol, one of the estradiol-17 beta compounds (EB) is injected 24 hours after PGF2α, followed by mandatory artificial insemination (AI) 48 hours later. This protocol has two major differences compared to Ovsynch because firstly, estradiol is administered instead of the final GnRH injection, and secondly estradiol is given 24 hours post-PGF2α injection. To improve fertility, cows exhibiting estrus after estradiol administration are inseminated according to the AM/PM rule, while non-estrus cows are subjected to mandatory AI 48 hour's post-estradiol injection. The Heat-Synch protocol induces estrus in more cows, and its pregnancy rate is comparable to that of the Ovsynch protocol. Stevenson et al. compared Ovsynch and Heat-Synch, reporting that Heat-Synch-synchronized cows showed more estrus compared to Ovsynch cows, despite slightly lower ovulation rates (91% vs. 100%; P=0.09)(6). However, the pregnancy rates in both groups were similar, showing no significant difference [6]. In a study conducted by Herlihy et al. (2011), three synchronization programs, Ovsynch, CIDR_Observation, and CIDR_TAI, were evaluated in grazing dairy herds with seasonal calving systems [9]. They observed that all three programs reduced the interval from calving to first AI compared to the control group. Additionally, CIDR_Observation and CIDR_TAI improved first AI pregnancy rates compared to Ovsynch [6]. The latter also demonstrated lower first AI pregnancy rates compared to the control group. In another study, Herlihy et al. (2012) evaluated Presynch-Ovsynch and Double-Ovsynch protocols for improving fertility during the first postpartum AI in lactating dairy cows [10]. They found that the Double-Ovsynch program decreased the proportion of cows with low progesterone levels at the time of the first GnRH injection in the second Ovsynch cycle, subsequently improving first AI pregnancy rates. The key limitation of the Ovsynch protocol is variability in synchronization responses, with 10% to 30% of cows failing to synchronize after the final GnRH injection [11]. The success of the Ovsynch protocol depends on the stage of the estrous cycle at the first GnRH injection. Initiating Ovsynch during the early luteal phase (days 5–12 of the cycle) is optimal [12]. To address limitations, pre-synchronization strategies like Double-Ovsynch ensure that more cows are in early diestrus phase at the start of the main synchronization protocol, enhancing pregnancy rates [12]. Further research with larger sample sizes and studies under heat stress conditions is recommended for validating and optimizing these protocols.

Conclusion

The Ovsynch protocol and its modifications, such as Heat-Synch and Double-Ovsynch, have become widely used tools for synchronizing ovulation and improving reproductive management in dairy cows. While Ovsynch typically achieves pregnancy rates between 30% and 40%, studies show that protocols like Heat-Synch can induce estrus in a greater proportion of cows, though overall pregnancy rates remain statistically similar between the two methods. The effectiveness of Ovsynch is influenced by factors such as the stage of the estrous cycle at protocol initiation, body condition, lactation number, and postpartum management, leading to variability in synchronization and conception rates. Pre-synchronization strategies, such as Presynch or Double-Ovsynch, have demonstrated improved pregnancy outcomes by ensuring more cows are optimally staged for synchronization. Despite these advances, a notable proportion of cows still fail to synchronize or conceive, highlighting the need for further research and protocol optimization, particularly under varying management and environmental conditions

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Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

All aspects of this research were conducted in strict accordance with ethical guidelines.

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