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Meta-Analysis: The effect of using fertility hormones with single ovulation synchronization and double ovulation synchronization methods on reproductive traits in dairy cows

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ABSTRACT

The aim of this study was to evaluate the reproductive parameters of dairy cows treated with reproductive hormones using the single ovulation synchronization (ovsynch) and double ovulation synchronization (double ovsynch) methods. This study used 31 article journals with parameters estrus percentage, ovulation percentage, corpus luteum formation percentage, pregnancy rate, conception rate and progesterone level. The article journals used of latest 35 years from 1990 to 2025. The methods used is meta-analysis. R Studio 4.4.2. were used for tools of statistical analysis. The results showed that the double ovsynch method is significantly effective ($P<0.05$) compared with ovsynch for estrus percentage, ovulation percentage and conception rate. Meanwhile, regarding the corpus luteum formation percentage, pregnancy rate and progesterone level showed that the double ovsynch method have no significant differences ($P>0.05$) compared with ovsynch. The conclusion of this research is the double ovsynch method was optimum than ovsynch method.

Introduction

The production of dairy cows is crucial to ensure sufficiency of protein from animal sources, especially milk and supporting the economy of livestock farmers. Reproductive performance is the key factor in the productivity and production of dairy cows. Nevertheless, estrus cycle disorders, low fertility rates, and affected to the low cattle reproductive performance (Setyorini *et al.*, 2020). Optimal reproductive performance not only supports consistent milk production but also ensures proper calving intervals and herd replacement.

The reproductive performance of dairy cows can be evaluated through several parameters that serve as indicators of reproductive efficiency. These include the timing of the first mating post-calving, service per conception (S/C), days open (DO), mating period, and calving interval, all of which may be influenced by hormonal imbalances (Imlak *et al.*, 2023). Hormonal imbalance in cows refers to alterations in the endocrine system that can result in reduced estradiol levels, decreased Luteinizing Hormone (LH) concentrations, and diminished progesterone secretion, ultimately disrupting the estrus cycle in dairy cows (Tao and Dahl, 2013). Hormonal protocols for estrus and ovulation synchronization (ovsynch) are the solution to overcome these issues.

Synchronization of estrus can be performed with the injection of gonadotropin releasing hormones (GnRH), estrogen, progesterone, and prostaglandin (PGF2α) or by administrating a combination of all four. The administration of GnRH during the estrus cycle can cause regression and ovulation of the dominant follicle and initiate a new follicular (Akbarabadi *et al.*, 2014). However, the success rate of ovsynch can still be low, especially in cows with poor reproductive status, such as those in negative energy balance or early postpartum. The double ovulation synchronization (double ocsynch) protocol was introduced to enhance the effectiveness of synchronization (Berean *et al.*, 2025). This method involves an initial Ovsynch sequence (pre-synchronization) followed by the standard ovsynch protocol (Calvano *et al.*, 2015). The purpose is to better prepare the ovarian follicles and uterine environment before insemination, which may lead to improved ovulation response and conception rates. Meanwhile, the administration of PGF2α can help lower progesterone levels to the lowest point, thereby triggering the secretion of estrogen from the dominant follicle cells, which can lead to estrus. Previous research has shown differences in results between cattle given fertility hormones and those not given fertility hormones regarding reproductive performance.

Several studies have reported that double ovsynch yields higher pregnancy rates compared to the standard ovsynch protocol, particularly in high-producing dairy cows or cows with delayed resumption of ovarian activity postpartum. However, double ovsynch requires additional time, labor and hormonal costs, raising the question of whether the increased reproductive outcomes justify the additional resources. According to the research conducted by Giordano *et al.* (2012), cows administered reproductive hormones through the ovsynch and double ovsynch techniques exhibited estrus of 55.75±1.78% and 50.50±1.63%, respectively. In the study conducted by Chebel *et al.* (2025), it was noted that cows administered reproductive hormones through the ovsynch and double ovsynch methods exhibited estrus of 31.60±1.48% and 37.30±1.70%, respectively. In the research of Berean *et al.* (2025), it was reported that ovsynch and double ovsynch resulted in 42.6 % and 64.8 %, respectively. This concluded that administering reproductive hormones through the ovsynch or double ovsynch methods in cows resulted in varying results regarding estrus percentage. Consequently, a meta-analysis is essential to deliver more precise results concerning the impact of hormone administration on fertility through ovsynch and double ovsynch methods on reproductive parameters.

Materials and methods

Materials

The materials used in the meta-analysis research consisted of internationally accredited article journals accessed through Google Scholar, Scopus, Science Direct, and other relevant academic research. The literature research resulted in 33 article journals that could be used in the meta-analysis.

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Methods

The method used in this study was quantitative meta-analysis. Data selection based on inclusion and exclusion criteria. The data search using keywords such as dairy and beef cattle, reproductive hormones, CR reproductive appearance, estrus, ovulation, pregnancy rate, and progesterone hormones resulted in 242 articles. Articles were selected based on inclusion and exclusion criteria. The inclusion criteria were met if the articles were from international journals published between 1990 and 2025, contained mean data and within standard deviation (SD), included moderator data such as dosage, resulting in a final total of 31 articles journal to be used in the meta-analysis study. The data collected was processed using Microsoft Excel. This step involved adding data about the authors, year of publication, mean data, standard deviation, sample size (n), and moderator data. Article selection was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, utilizing Review Manager 5.4.1, as illustrated in Fig. 1.

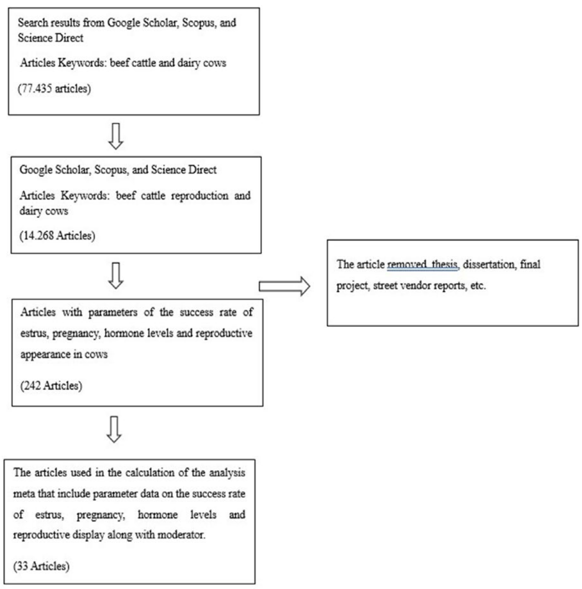

Figure 1. PRISMA diagram

Statistical analysis

Data that were included and excluded were then subjected to meta-analysis using R Studio 4.4.2. Heterogeneity tests can be categorized into a random effects model if the p-value is significantly different ($p<0.05$), while they can be categorized into a fixed-effects model if the p-value is not significantly different ($p>0.05$). Study heterogeneity was assessed using the I^2 value, which ranged from 0 to 100% and indicated the level of data variation in the studies. The effect size measurement was carried out by comparing the variance analysis results between the control and experimental groups. The effect size was calculated using the standardized mean difference to determine the summary effect. In measuring the summary effect, there are three categories: strong if the value is ≥ 0.5, moderate if the value is 0.5 – 0.3, and weak if the value is 0.3 – 0.1 (Cohen, 1988). Moderator analysis was performed using meta-regression to evaluate the RUP dosage administration in dairy cows. Publication bias evaluation in the meta-analysis uses funnel plots and Egger's regression test to assess publication bias in the meta-analysis.

Results

Estrus percentage

The number of studies regarding estrus percentage in Table 1 is 20. The ovsynch data shows the percentage of cows experiencing estrus is 50.03%, while the data using the double ovsynch method shows 51.19%. The results of the meta-analysis indicate a significant difference, as the p-value is < 0.05 (p = 0.001), with heterogeneity among studies (I2 = 99.86%), indicating that the obtained data is heterogeneous. Additionally, the meta-regression findings in Table 2 show that the moderator variable in the dosage given does not have a significant effect (p>0.05) on estrus presentation, with a slope value of 136.96, which means there is a tendency that increasing hormone dosage can increase the proportion of cows experiencing estrus, although it is not statistically significant.

Ovulation percentage

The number of studies regarding ovulation percentage parameters in Table 1 is 10. The ovsynch data shows the percentage of cows that experienced ovulation is 58.59%, while data using the double ovsynch method shows 63.32%. The results of the meta-analysis indicate a significant difference, as the p-value = 0.05 (p = 0.05), with heterogeneity among the studies (I2 = 99.76%), indicating that the obtained data is heterogeneous. Additionally, the meta-regression findings in Table 2 show that the moderator variable in the dosage given does not have a significant effect (p>0.05) on the ovulation percentage, with a slope value of 71.352 which indicates a tendency that increasing hormone doses may increase the proportion of cows that experience ovulation, although it is not statistically significant.

Table 1. Meta-Analysis calculation results for production parameters.

Parameters	Number of studies	Ovsycnh	Double Ovsynch	Model Estimates	
				ES	p-value
Estrus (%)	10	50.03	51.19	-3.53	<.0001
Ovulation (%)	10	58.59	63.32	-3.09	0.05
Corpus Luteum (%)	10	48.02	66.8	-3.15	0.08
Pregnancy Rate (%)	10	38.49	42.23	0.96	0.39
CR (%)	10	46.41	52.13	-1.08	<.001
Progesterone (ng/ml)	10	5.44	5.71	-1.62	0.59

Corpus luteum formation percentage

The number of studies regarding the corpus luteum (CL) formation percentage in Table 1 is 10. The ovsynch data shows the presentation of cows experiencing CL is 48.02%, while the data using the double ovsynch method shows 66.80%. The results of the meta-analysis indicate that there is no significant difference, as the p-value is > 0.05 (p = 0.5923), with heterogeneity among the studies (I2 = 99.48%), indicating that the data obtained is homogeneous. Furthermore, the meta-regression findings in Table 2 show that the moderator variable in the dosage given does not significantly affect CL (p > 0.08) with a slope value of 131.882, indicating a trend that higher doses of hormones administered will lead to greater formation of CL and can increase progesterone levels in dairy cows.

Table 2. Meta-Regression Calculation Results for Production Parameters.

Parameters	Number of studies	Model Estimates		
		Slope	SE Slope	p-value
Estrus (%)	10	136.96	32.45	2.43
Ovulation (%)	10	71.35	82.27	0.39
Corpus Luteum (%)	10	87.75	131.88	0.51
Pregnancy Rate (%)	10	-120.80	201.45	0.55
CR (%)	10	10.55	36.37	0.77
Progesterone (ng/ml)	10	42.57	79.48	0.59

Pregnancy rate

The number of studies regarding the pregnancy rate in Table 1 is 10. The ovsynch data shows a presentation of cattle that experienced a pregnancy rate of 38.49%, while the data using the double ovsynch method shows 42.23%. The meta-analysis results indicate no significant difference, as the p-value is > 0.05 (p = 0.386), with heterogeneity among the studies (I2 = 99.84%), indicating that the obtained data is homogeneous. Furthermore, the meta-regression findings in Table 2 show that the moderator variable in the dosage of administration does not have a significant impact (p>0.08) on the presentation of the pregnancy rate, with a slope value of -120.801, which means there is a tendency for a decrease in the pregnancy rate as the hormone dosage increases, although this relationship is not statistically significant.

Conception rate

The number of studies regarding the conception rate (CR) in Table 1 is 10. The ovsynch data shows a presentation of cows that experienced a pregnancy rate of 46.61%, while the data with the double ovsynch method shows 52.13%. The results of the meta-analysis indicate a significant difference, as the p-value < 0.05 (p = 0.001), with heterogeneity among studies (I2 = 91.70%), indicating that the obtained data is heterogeneous. Additionally, the meta-regression findings in Table 2 show that the moderator variable in the dosage administered does not have a significant effect (p>0.08) on the presentation of the pregnancy rate with a slope value of 10.552, meaning that the increase in hormone dosage administered does not yet have a sufficiently strong relationship with the response level.

Progesterone level

The number of studies regarding the progesterone level in Table 1 is 10. The ovsynch data shows the presentation of cows that experienced ovulation at 5.44 ng/ml, while the data with the double ovsynch method

Table 3. Summarizing the characteristics of the included studies.

Author	Year	Parameter	Treatment
Laplacette *et al*.	2025	Estrus, Ovulation, Progesterone, CL, CR, Pregnancy	Reproduction Hormone
Giordano *et al*.	2012	Estrus, Ovulation, Progesterone	Reproduction Hormone
Hölper *et al*.	2023	Progesterone, CL, Pregnancy	Reproduction Hormone
Masello *et al*.	2019	Estrus, Ovulation, Progesterone	Reproduction Hormone
Santos *et al*.	2017	Estrus, Progesterone, Pregnancy, CR, S/C	Reproduction Hormone
Consentini *et al.*	2024	Ovulation, Pregnancy	Reproduction Hormone
Souza *et al.*	2008	Ovulation, Progesterone, CL	Reproduction Hormone
Ayres *et al.*	2013	Ovulation, CL	Reproduction Hormone
Consentini *et al.*	2025	Ovulation, Pregnancy	Reproduction Hormone
Barletta *et al.*	2018	Progesterone, CL, Pregnancy, Ovulation	Reproduction Hormone
Mee *et al.*	2009	CL, Pregnancy	Reproduction Hormone
Wiltbank *et al.*	2015	CL, Pregnancy	Reproduction Hormone
Chebel *et al.*	2025	Estrus	Reproduction Hormone
Gümen *et al.*	2003	Estrus	Reproduction Hormone
Ahmadi *et al.*	2007	Estrus	Reproduction Hormone
Wijma *et al*.	2018	Estrus	Reproduction Hormone
Stevenson *et al.*	2018	Estrus, CL, Progesterone, pregnancy rate	Reproduction Hormone
Lauber *et al*.	2021	Estrus	Reproduction Hormone
Dirandeh *et al*.	2015	Ovulation	Reproduction Hormone
Dewey *et al*.	2010	Ovulation	Reproduction Hormone
Bello *et al*.	2006	Progesterone, CL	Reproduction Hormone
Sitko *et al.*,	2023	Progesterone, CL	Reproduction Hormone
Herlihy *et al.*	2012	Pregnancy	Reproduction Hormone
Xu and Burton	2000	CR	Reproduction Hormone
Gowtham *et al.*	2024	CR	Reproduction Hormone
Binversie *et al.*,	2012	CR	Reproduction Hormone
Ahmed *et al.*	2016	CR	Reproduction Hormone
Sartori *et al.*	2004	CR	Reproduction Hormone
Rheinberger *et al.*	2020	CR	Reproduction Hormone
Guo *et al.*	2020	CR	Reproduction Hormone
Lean *et al.*	2003	CR	Reproduction Hormone

shows 5.71 ng/ml. The results of the meta-analysis indicate no significant difference, as the p-value is > 0.05 (p = 0.5923), with heterogeneity among studies (I2 = 99.85%), indicating that the obtained data is homogeneous. Furthermore, the meta-regression findings in Table 2 show that the moderator variable in the dose administration does not have a significant effect (p > 0.05) on the progesterone hormone levels, with a slope value of 42.566, meaning that increasing the hormone dosage will indirectly affect the increase in progesterone levels in dairy cows.

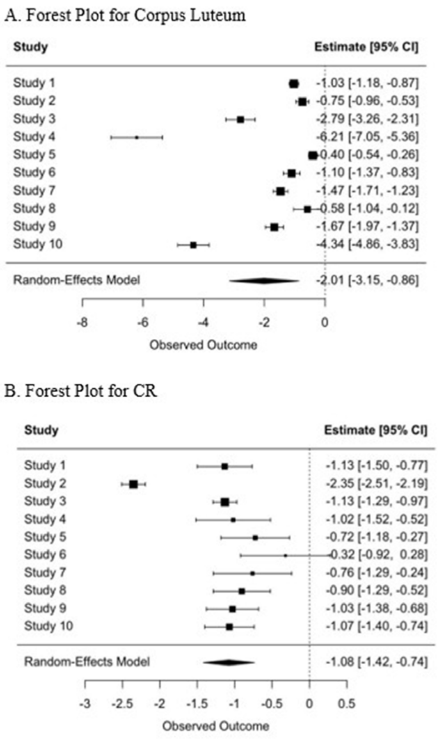

Fig. 2. Forest Plot Corpus Luteum and Conception Rate.Ovulation, Estrus, Progesterone, and Pregnancy.

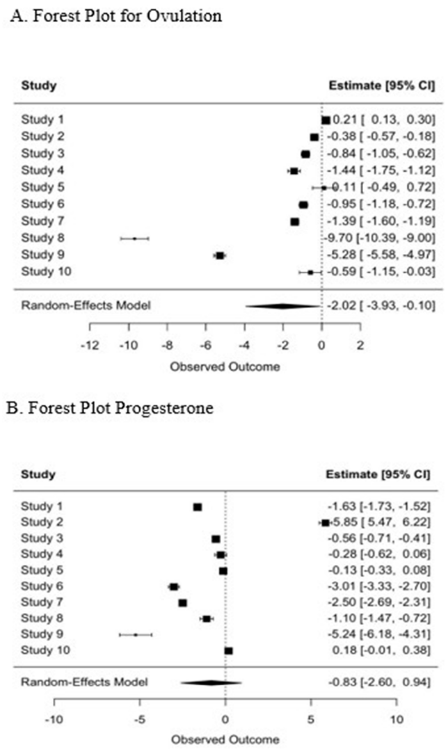

Fig. 3. Forest Plot ovulation and progesterone.

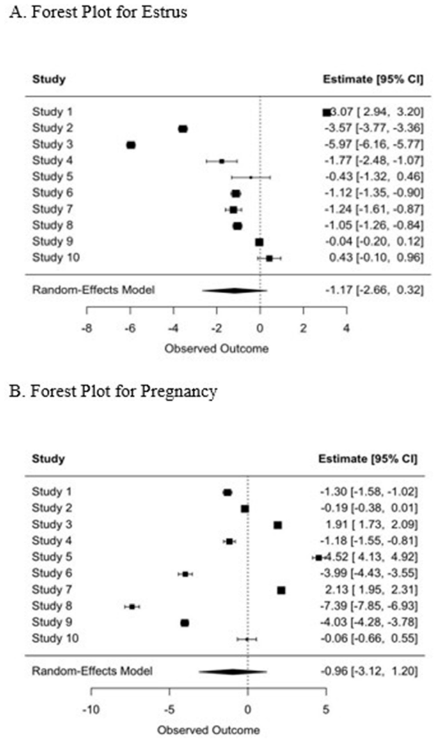

Fig. 4. Forest Plot for Estrus and Pregnancy.

Discussion

The estrus cycle is defined as the cyclical pattern of ovarian activity in female animals that transitions between reproductive receptivity and non-receptivity, allowing for the potential establishment of pregnancy following mating. It consists of two phases: the luteal phase and the follicular phase, with a typical duration of 18–24 days in cattle. estrus detection is very important for the profitability of dairy cow. The ovsynch protocol is a hormone therapy that synchronizes estrus and ovulation in cows and heifers by strategically administering Gonadotropin-Releasing Hormone (GnRH) and Prostaglandin (PGF2α) (Mariol *et al*., 2024). Based on the results of a meta-analysis of 10 studies, the estrus rate in dairy cows subjected to ovsynch treatment was recorded at 50.03%. This indicates that half of the population given hormones showed significant signs of estrus. The effectiveness of this method is greatly influenced by the physiological condition of the cows, particularly the status of the reproductive cycle. Cows that are in anestrus (not cycling) or in the luteal phase when given the first hormone tend to show a low estrus response because the dominant follicle is not ready or the CL is still active. According to Sahu *et al*. (2014), in the ovsynch method, the condition of the cows after being given reproductive hormones is still in the luteal phase or anestrus when the treatment begins, thus the response to the hormones is not as optimal as in double ovsynch. Dairy cows have an estrus cycle that lasts an average of 21 days. The estrus cycle consists of four main phases: prestrus, estrus, metestrus, and diestrus. The estrus phase typically lasts for 12–18 hours, with variations influenced by nutrition, stress, and the physiological status of the cow (Reith and Hoy, 2018). The double ovsynch method provides additional time and more optimal early hormonal treatment, allowing cows to reach a more physiologically prepared condition for experiencing estrus and ovulation synchronously. According to Li *et al*. (2023), early hormonal treatment in double ovsynch helps induce early ovulation and improves corpus luteum function as well as progesterone levels, so that when the main ovsynch program begins, the cows are already in an active estrus cycle and responsive to hormonal stimulation.

Ovulation is the process of releasing a mature egg (oocyte) from the dominant follicle in the ovary into the oviduct, where fertilization occurs. The ovulation process occurs due to a surge of luteinizing hormone (LH) triggered by an increase in estradiol levels from the dominant follicle. This indicates that half of the population given hormones effectively responded to the administration of GnRH and $PGF_2\alpha$ in the ovsynch protocol, with the release of oocytes from the dominant follicle. Ovulation typically occurs 24–32 hours after the peak LH surge and about 28–30 hours after the onset of estrus (Rensis *et al*., 2024). Failure in the release of LH or disturbances in follicle maturation can lead to non-ovulation, which results in infertility. Meanwhile, with the double ovsynch treatment, it was recorded at 63.32%. This indicates that dairy cows with the double ovsynch protocol show that this method is more effective in improving ovulation percentage compared to the ovsynch. This is very beneficial in improving reproductive efficiency and success in artificial insemination in dairy cow, especially those in low reproductive performance such as early postpartum or anestrus. Several studies have shown that double ovsynch can significantly increase the ovulation rate and CL formation, especially in cows with anestrus or early postpartum status (Herlihy *et al*., 2012). Factors that may influence ovulation in dairy cows include environmental conditions, the feed provided, age, and metabolic status.

Progesterone is the primary steroid hormone produced by the corpus luteum (CL) after a cow ovulates, and it is crucial for regulating the reproductive cycle and maintaining pregnancy by preparing the uterus for an embryo and sustaining its development (Sakumoto, 2016). Table 1 is 10. The ovsynch data shows the progesterone level of cows that experienced ovulation at 5.44 ng/ml, while the data with the double ovsynch method shows 5.71 ng/ml. This hormone is also produced by the placenta during pregnancy. Progesterone works by preparing the uterus for embryo implantation, suppressing estrus and subsequent ovulation, and maintaining pregnancy by creating an intrauterine environment that supports embryo development. The Double Ovsynch protocol is capable of stimulating the formation of a more optimal corpus luteum (CL) and produces slightly higher levels of progesterone compared to the standard Ovsynch, thus increasing the chances of reproductive success. The progesterone hormone produced by the corpus luteum after ovulation plays a role in preparing the uterus to support implantation. According to Bulletti *et al*. (2022), the hormone progesterone is essential for embryo implantation by preparing the endometrium and for maintaining pregnancy until birth, demonstrating its critical role in reproduction.

The CL is a transient endocrine ovary gland that regulates the length of the estrus cycle and produces progesterone to create a suitable uterine environment for pregnancy. The CL originates from the transformation of granulosa and theca follicular cells into large and small luteal cells triggered by the preovulatory LH surge. The corpus luteum has a predetermined life span of approximately 14 days. The CL rapidly develops in the first 4–5 days of the cycle, reaching maximum size around day 7, and begins to produce progesterone in high quantities (Pugliesi *et al*., 2023). The double ovsynch protocol includes a presynchronization stage before the main treatment, which helps prepare cows in the appropriate cycle phase to respond to hormonal treatment. In dairy cows, the formation and normal function of the CL are closely related to reproductive success, as an imbalance in progesterone production can lead to implantation failure or early embryo death. If fertilization does not occur, the CL will undergo luteolysis by the action of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) secreted by the uterus, resulting in a decrease in progesterone and the resumption of the estrous cycle. Hormonal protocols like Ovsynch and Double Ovsynch aim to increase the likelihood of CL formation by synchronizing ovulation in a controlled manner. GnRH in these protocols can stimulate the ovulation of existing follicles, while $PGF_2\alpha$ is used to eliminate old CL, thereby allowing for new ovulation to occur, resulting in functional CL (Bisinotto *et al*., 2010). The hormone treatment using Ovsynch and Double Ovsynch methods is designed to induce ovulation in the synchronous formation of CL. Double Ovsynch has an advantage because it provides a presynchronization phase that helps cows enter the cycle before the Ovsynch treatment, thus increasing the chances of forming functional CL.

The pregnancy rate is the result of multiplying the insemination rate by the conception rate, which means that it is necessary to increase one of the two factors, or both depending on the farm current situation, in order to obtain an increase in the pregnancy rate. This parameter reflects the success of reproductive management, including the success of estrus detection, timing of insemination, semen quality, and the physiological condition of the cows (Fricke *et al*., 2014). In the Ovsynch protocol, the success rate of pregnancy heavily depends on the cows' response to the first GnRH (i.e., undergoing ovulation and forming a corpus luteum). If there is no mature dominant follicle during the treatment, the chances of successful pregnancy become lower. This indicates that the Double Ovsynch Protocol is significantly more effective than the standard Ovsynch protocol in increasing the pregnancy rate in dairy cows. This is because the double ovsynch protocol adds one cycle of Ovsynch before the main Ovsynch to ensure that the cows are in optimal reproductive condition before insemination. Conversely, in the Double Ovsynch protocol, an additional Ovsynch cycle is added before the main Ovsynch to ensure that the cows enter an optimal reproductive condition before insemination.

The conception rate (CR) value is closely related to the condition of the recipient livestock. However, optimizing other reproductive management factors is still necessary to achieve higher results. This factor is not only seen from the performance of the recipients but also there are several factors that should be considered, such as the level of stress in livestock, reproductive status, and genetic quality. The increase in CR with the Double Ovsynch method is achieved by the presence of a presynchronization phase that helps more cows to be in an optimal physiological condition during hormonal treatment. According to Bisinotto *et al*. (2010), to increase the proportion of cows that experience timely ovulation, appropriate hormonal actions should be taken so that the timing of insemination is more aligned with the time of ovulation, which is a key factor in the success of conception. An important factor in the success of CR is the environmental factors; during times of heat stress, it is also influenced by nutrition, health, the knowledge of the breeder in detecting estrus, proper breeding management, as well as genetics in the livestock. In addition, the experience and skills of the inseminator, and the quality of the frozen semen being inseminated can affect the high/low value of CR (Marques *et al*., 2014).

Conclusion

The conclusion of this study is that the double ovsynch method is significantly more effective in increasing estrus percentage, ovulation percentage and conception rate compared to ovsynch. This condition especially in cows with less than ideal reproductive performance such as anestrus or early postpartum phase.

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Conflict of interest

The authors have no conflict of interest to declare.

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