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Impact of presynchronization and Ovsynch on early postpartum ovarian activity and fertility of dairy cows in Libya

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Abstract

Background: Reproductive efficiency affects dairy cow profitability. Ovarian function in postpartum (P.P.) has been better understood using ultrasound and hormonal assays. Optimizing ovulation synchronization and carefully timing artificial insemination (TAI) can greatly enhance reproductive rates in dairy cows.

Aim: This experiment was designed to investigate the reproductive performance and ovarian activity in early postpartum lactating dairy cows using the Presynch-PGF2α, Ovsynch protocol, and TAI.

Methods: Randomly the cows were assigned to a control group and a treatment group, based on the chronological order of their calving date. On day 14 P.P., both groups received two cloprostenol treatments, 14 days apart. Ultrasonographic inspections were conducted on day 14 to check ovarian activity and uterine contents. On day 11, after presynchronization, cows in the treatment group were given 100 µg IM. of cystorelin, followed by a luteolytic dose of 500 µg IM., cloprostenol on day 7, and a second dose of cystorelin on day 8 (36 hours later). After the second cystorelin injection by 16–20 hours, cows were inseminated, while the control group had all cows displaying spontaneous estrus between day 0 and day 28 were artificially inseminated.

Results: Ovarian activity began to improve at 82.61% on day 19 P.P., with complete recovery between days 24 and 27 P.P. The second cloprostenol injection approached, causing follicular size to reach 8.41 ± 1.04 mm. After the second injection, ovarian activity switched from follicular to luteal, with corpus luteum rates of 23.91% and 26.1%. The presynchronized PGF2α regimen significantly enhanced ovarian activity from days 19–35 P.P. Ovulation and pregnancy rates in the Ovsynch group were 54.2% and 41.7% at the first timed artificial insemination (TAI), compared to 54.5% and 31.8% in the control group. There was no significant impact between them; it was just high in the presynchronized Ovsynch group. However, the P.P. period was minimized to 47–49 days till the first AI reached a 41.7% pregnancy rate and 20.8% at the second AI, for an overall 62.5%.

Conclusion: The current study concludes that presynchronization during preservice in clinically normal P.P. dairy cows reduces P.P. duration, increases ovarian activity performance, and reduces ovarian dysfunctions from day 19 to day 35 P.P., as well as improves the pregnancy rate.

Keywords: Postpartum cow, Ovarian activity, Presynchronization, Ovsynch, TAI.

Introduction

The sustainability and profitability of a dairy farm over the last 20 years in Libya have been real issues. Lactating dairy cows' reproductive effectiveness has continuously deteriorated due to insufficient estrus detection and AI-recorded pregnancy. Given that most managed dairy cow efforts fail, the subject matter is crucial to their management and long-term survival in the area. Many authors were concerned about identifying estrus, treating postpartum (P.P.) anoestrus, and decreasing the time between births, the most stressful aspects of production may be reduced or avoided altogether (Rhodes *et al*., 2003; Wiltbank *et al*., 2006; Crowe *et al*., 2014; Santos *et al*., 2016; Sakaguchi *et al*., 2023).

Reproduction must be efficient to maintain breeding populations. Healthy cow reproductive cycles include ovulation, mating, pregnancy, calving, and restoring oestrous and uterine functions. Body condition, energy balance (milk and dry matter intake), parity, season, and illness also matter (Vanholder *et al*., 2006; Crowe *et al*., 2014; Rodríguez *et al*., 2022; Fricke *et al*., 2023). It is clear that a good detection of estrus is critically important (Roelofs *et al*., 2010). In big dairy farms, estrus detection and animal identification are so difficult that "barn" workers make breeding management choices

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for individual cows after limited periods of unrestricted contact (Macmillan, 2010). For years, reproductive experts have wanted a hormonally synchronized world where all animals ovulate and become pregnant. Proper reproductive hormone synchronization with corpus luteum (CL) and follicle development is needed (Meglić *et al*., 2023). Synchronization programs, particularly those that give every cow in a herd a planned set of preliminary injections or treatments, are popular under these circumstances. These programs incorporate a brief, intense period of insemination connected with an identified estrus or a set time without estrus signs and timing artificial insemination (TAI) (Wiltbank and Pursley, 2014; Fricke *et al*., 2023).
Using GnRH and PGF2α, a regimen has been established that synchronizes the timing of ovulation within an 8-hours period (24 to 32 hours after the second injection of GnRH) with pregnancy per artificial insemination (P/AI) that is analogous to a.m.–p.m. breeding (Pursley *et al*., 1998; Wiltbank and Pursley, 2014; Hölper *et al*., 2023; Martins *et al*., 2023). Therefore, this experiment was designed to investigate the reproductive performance and ovarian activity using the Presynch-PGF2α, Ovsynch protocol and TAI in early P.P. lactating dairy cows.

Materials and Methods

Experimental animals

Four commercial dairy farms in Alzawia (60 km west of Tripoli, 30.54 N0 latitude) from August 2022 to December 2022 were used. Out of the total 155 cows, only 46 cows were participated in this experiment. Daily veterinary monitoring was performed for all cows. The study excluded cows with assisted birth, twining, retained placenta, primary metritis, or ketonuria in the first or second weeks of pregnancy. Cows having mastitis, lameness, digestive problems, aberrant vaginal discharges, and reproductive system pathological abnormalities palpable per rectum were also eliminated from the trial. All animals were kept in open stalls half-sheltered and provided a complete mixed feed for milking cows (VL8/HP Althadee Alsamed for animals feed company), water, and minerals ad libitum to suit lactation needs and body score.
The four farms had weekly reproductive health programs, so all animals were healthy and fit when estrus and insemination occurred. Pre- and post-partum body condition scores were taken on a five-point scale (1 = thin, 5 = fat). Age ranged from 2 to 6 years. On average, cows milked 30 l three times daily.

Experimental design

Transrectal ultrasonography (a portable machine B-mode scanner. RKU10 Handheld, equipped with a 5.0 to 7.5 MHz transducer Vet. Palm. Ultrasound KEEBOMEM, CHINE), and rectal palpation were used to check uterine involution, ovarian activity, and aberrant uterine discharge in cows following parturition for 14 days.
Starting in the second week P.P. (day 0: day 14 P.P.), all 46 cows were presynchronized by calving date. Two intramuscular doses of 500 µg cloprostenol (Synchromate, Bremer Pharma, GMBH, Cloprostenol 250 µg/ml IM) were administered 14 days apart. Cows were randomly selected for treatment and control groups.On day 11, following the second cloprostenol injection (presynchronization protocol), all 24 cows in the treatment group (Ovsynch protocol) were given 100 µg of cystorelin (IM) (Gonadoreline, GnRH., diacetate tetrahydrate 50 mcg/ml IM., IV., Ceva Sante animale, 10, va. De La Ballastiere, 33,500 libourne, France) in the early morning (7 am), followed by a luteolytic dose of cloprostenol (500 µg) via IM injection on day 7, and a second dose of cystorelin 36 hours later (at 7 pm) on day 8. 16–20 hours following the second cystorelin injection, these cows were inseminated (Fig. 1). In the control group (n = 22), all the cows displaying spontaneous estrus between day 0 and day 28 were artificially inseminated.

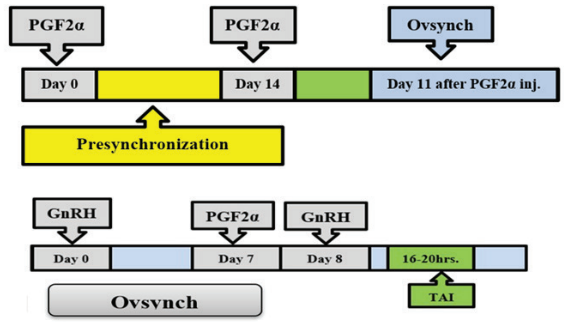

Fig. 1. Experimental design.

The ovulation rate was evaluated by ultrasound on day 11 following the first AI, detecting a CL in the ovaries. If a cow returned to estrus between days 8 and 30 (after the end of ovsynch protocol), examining per rectum verified estrus (ultrasonography and estrus signs) and re-insemination was done without medication. All cows were tested for pregnancy on day 45 following insemination by ultrasound and reported as positive or negative (0 = negative, 1 = positive). On day 70, rectal palpation and ultrasonography confirmed pregnancy. The presence of the CL and the level of progesterone during the presynchronization period were investigated according to the P.P. day and the day of receiving treatment to investigate the luteal activity.
Progesterone was determined using the Eagle Biosciences Progesterone ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the direct quantitative determination of progesterone to investigate ovarian activity during P.P. The sensitivity of the test is 0.1 ng/ml, dynamic range of 0.3–60 ng/ml. All the blood samples were collected from the jugular vein in 10 ml serum vacutainers with disposable needles kept at room temperature for 30 minutes, sera were separated by centrifuging at 3000 rpm for 15 minutes, and all the collected serum was made and numbered according to each cow, P.P. day and the day of protocol.

Statistical analyses

The statistical analysis was carried out using SPSS.2007, version 16. The data were analyzed using descriptive statistics for estrus response, ovulation rate, return rate, pregnancy rate (1st and 2nd AI), and ovarian activity and were analyzed by *t*-test and chi-squared test, and the level of significance was set at $p \leq 0.05$.

Ethical approval

The study was approved by the ethical committee of Zagazig University in accordance with the guidelines of the National Institutes of Health for the Care and Use of Animals.

Results

The ovarian activity was monitored in all animals throughout the presynchronization phase, starting with the first PGF2α injection on day 14 P.P., either via follicular or luteal activity.

The ovarian dynamic activity started to restore on day 19 P.P after the first PGF2α injection, which was 82.61% (38/46) to reach 100% during the next 24, 27, 35, 38, and 41 P.P., while it was 13.04% (6/46) at the beginning (day 14 P.P.) (Fig. 2). In contrast, the luteal activity (presence of CL) contributes 8.69, 6.52, and 23.91% at the first PGFα injection (days 14, 19, and 24 P.P., respectively) and reaches 26.1% at the second PGF2α injection (day 27 P.P.) (Table 1).

The shifting of the ovarian activity toward the luteal activity gradually gives an advantage to the treatment work and brings most of the cows into the middle of the cycle (between 6 and 10 of the estrus cycle). The significant impact of the CL presence was high ($p = 0.036$) between the first and second days of the PGF2α treatment (days 14 and 27 P.P.). The mean

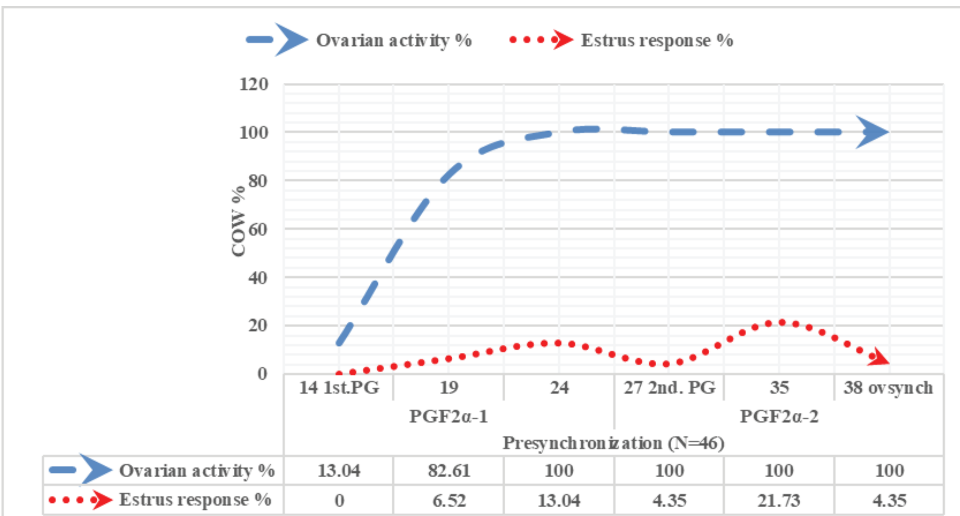

Fig. 2. The ovarian activity during P.P. under the effect of presynchronization.

Table 1. Effect of presynchronization treatment on ovarian dynamic according to P.P. days.

Items	**Presynchronization (N = 46)**					
	PGF2α-1			**PGF2α-2**		
Day of P.P.	14 1st.PG	19	24	27 2nd. PG	35	38 ovsynch
Ovarian activity %	13.04^a (6)	82.61^b (38)	100^b (46)	100^b (46)	100^b (46)	100^b (46)
Number of follicle (Means ± SD)	1.83 ± 0.75	2.23 ± 0.70	1.40 ± 0.63	2.23 ± 0.43	2.00 ± 0.81	1.85 ± 0.72
Size of follicle (mm., Means ± SD)	3.82^e ± 1.39	7.57^f ± 2.68	6.30^f ± 1.39	8.41^f ± 1.04	5.42^g ± 0.64	5.10^g ± .90
Presence of CL %	8.69^c (4)	6.52^c (3)	23.91^d (11)	26.1^d (12)	13.04^c (6)	28.26^d (13)
Estrus response %	0 (0)	6.52^h (3)	13.04^i (6)	4.35^h (2)	21.73^i (10)	4.35^h (2)

The values in the same row with different subscribe are significantly different $p \leq 0.05$.

of the follicular wave (number of follicles) does not affect either treatment or P.P. day and ranges from 1 to 3 waves. In return, the follicular size significantly (p = 0.038) changed from the first injection of the treatment to the second injection. The estrus response was high and significant (p = 0.04) on day 24 P.P. after the first injection of PGF2α (13.04%), and it was highly significant ($p \leq 0.001$) on day 35 P.P. (21.73%) after the second injection of PGF2α (Table 1).

The progesterone level was increased significantly ($p \leq 0.05$) toward the time of second injection of PGF2α, which was 0.56 ± 0.05, 0.64 ± 0.01, 1.45 ± 0.19, 2.50 ± 0.20, 4.80 ± 0.27, 5.11 ± 0.29, and 5.30 ± 0.30 ng/ml; (14,19, 24, 27, 35, 38, and day 41 P.P, respectively). On days 24 and 27 P.P., the correlation between the two variables was strong and positive at the 0.05 level. On days 35, 38, and 41 P.P., the significance level rose to 0.01 (Fig. 3).

The results in Table 2 show the comparative reproductive performance of Ovsynch and control groups after presynchronized by PGF2α 14 days apart. The pregnancy rates at first AI were 41.7% and 31.8%, respectively, and showed no statistically significant difference, it was just higher in Ovsynch group with overall 62.5% and 50% in the control group, either in ovulation rate was found almost equal (54.2% *vs* 54.5%). The estrus response was significantly higher in the control group (54.5% *vs* 29.17%). The estrus return was significantly higher in the Ovsynch group compared with the control group (33.3% *vs* 18.2%).

Discussion

Maximum reproduction is crucial for adequate output in both modern dairy farming and traditional farming methods. Over the course of the previous several decades, advances in reproduction control techniques have allowed for their widespread use on dairy farms. Dairy cows' health and milk production are dependent on the time period after calving before their ovaries return to a regular cycle. Changes in the cow's physiology and metabolism make them more susceptible to illness. To ensure the cow's continued health and milk production, this phase must be carefully managed.

Submission of cows for first P.P. TAI treatment and starting procedures on day 14 P.P., was a major obstacle in the current study. The cows were scheduled so that the first two injections of cloprostenol occurred 14 days apart from the presynchronization. The ovarian activity was recorded according to the presence of follicular or luteal activity on both ovaries of the cows using an ultrasonography machine. Ovarian function was shown to begin recovering on day 19 P.P. at an 82.61% rate, with full recovery occurring between days 24 and 27 P.P. At day 27 P.P., just around the

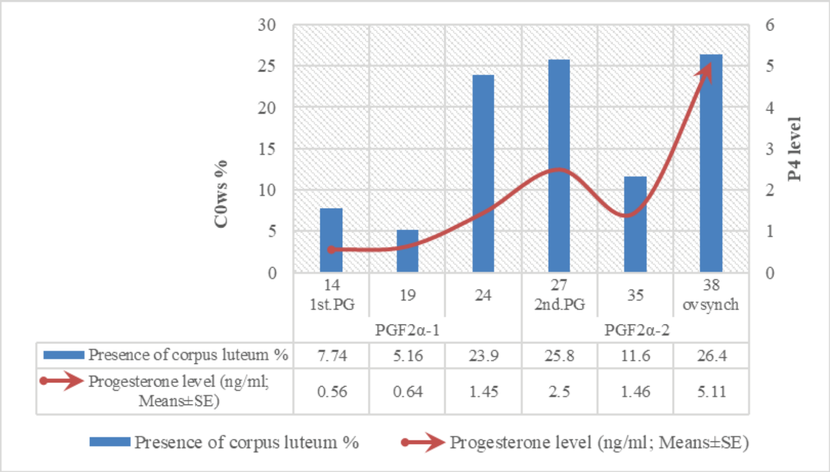

Fig. 3. The luteal activity during P.P. under the effect of presynchronization.

Table 2. Comparative of reproductive performance of the presynchronized Ovsynch and control groups.

Groups	No. cows	Estrus response % (n)	Ovulation % (n)	Pregnancy to 1st TAI % (n)	Return to estrus % (n)	Pregnancy to 2nd AI % (n)	Pregnancy overall % (n)
Ovsynch	24	29.17 (7)	54.2 (13)	41.7 (10)	33.3 (8)	20.8 (5)	62.5 (15)
Control	22	54.5 (12)	54.5 (12)	31.8 (7)	18.2 (4)	18.2 (4)	50 (11)

time of the second cloprostenol injection, the follicular size had increased to 8.41 ± 1.04 mm. On days 24 and 27 P.P., after the second injection of cloprostenol, the ovarian activity shifted from follicular to luteal, with respective existence of CL rates of 23.91% and 26.1%.

Presynchronized cows showed much higher luteal activity on P.P. day 38. This suggests that presynchronized cows may respond better to the estrus synchronization next protocol. After the second cloprostenol injection, the estrus response was satisfactory (21.73%) on day 35 P.P., compared to 6.16% and 13.04% after the first injections at days 19 and 24 P.P. The presynchronized PGF2α protocol significantly improved ovarian activity from days 19 to 35 P.P.

Authors analyzed ovarian cyclicity in P.P. dairy and beef cows, revealing that LH pulse frequency is crucial for recovery, with dominant follicle fate determined by LH pulsatility within the first wave (Crowe *et al*., 2014). LH pulses during the dominant phase of the follicular wave, the size of the dominant follicle, and the bioavailability of IGF-I all influence the capacity for oestradiol secretion, crucial for the survival of the dominant follicle (Austin *et al*., 2001; Canty *et al*., 2006).

Most cows begin follicular development between 7 and 10 days P.P., while FSH levels increase between 3 and 5 days. Between the same timeframe (Crowe, 2008; Crowe *et al*., 2014). Ovulation of the first P.P. dominant follicle occurs in 30%–80% of dairy cows, whereas 15%–60% atresia or develop cystic, and 1%–5% do not ovulate (Sartori *et al*., 2004). This is what brings our findings in line with what has been described in the above discussion.

This brief lag time improvement in this research helps bring more cyclic cows into the estrus synchronization next step and enhance fertility. These findings may be related to efforts to minimize the impact of puerperal clinical circumstances on ovarian activity. Thus, our results mostly reflect prostaglandins' direct effects on ovarian function, with only subclinical abnormalities as complicating variables. Long luteal periods before service, which usually resume in the early P.P. period, are common ovarian disturbances. The study's strategy and effort were similar to previous studies and could reduce P.P. problems on cow reproductive efficiency (Opsomer *et al*., 1998; Smith and Wallace, 1998; Opsomer *et al*., 2000; López-Gatius *et al*., 2003; Martins *et al*., 2023; Moskáľová and Pošivák, 2023).

Delay in luteolysis of the CL in the first days of P.P. during the study may result from a pre-clinical uterine infection that may delay CL luteolysis in the first days P.P. Abnormal uterine function may disrupt the luteolytic cascade, which generates prostaglandin F2α and activates oxytocin receptors via estradiol (Roche *et al*., 2000). A second prostaglandin dose at the same presynchronization method might be given on day 27. Cleaning the uterus and encouraging luteolysis of a remnant CL coincide now. These findings matched López-Gatius *et al*. (2003). These findings matched those of López-Gatius *et al*. (2003), who used two dosages of prostaglandins after P.P. in dairy cows. These dosages may be used to synchronize them for scheduled artificial insemination or to help maintain their reproductive health and improve ovarian activity.

Starting the timed AI regimen with GnRH around days 5–8 of the estrous cycle is best since cows are most fertile then (Stevenson and Jeffrey, 2016). These limitations correspond with the majority of research employing PGF2α as a presynchronization approach. Timing the first AI service after calving when cows are most estrous may increase pregnancy rates. Cows that started the timed AI program between days 5 and 12 of the estrous cycle had increased fertility and GnRH responses (Stevenson and Jeffrey, 2016). Also, Ambarcioglu *et al*. (2023) found that Presynch+Ovsynch increases first-insemination pregnancy rates significantly compared to other synchronization methods.

In the current study, the early estrus response at first GnRH, 8 from 24 cows enrolled in the presynchronized Ovsynch protocol (3 cows at day 40 P.P and 5 cows at day 42 P.P.). Several early findings suggested that cows who did not react to presynchronization might be impacted by the initial the first injection of gonadotropin releasing hormone (GnRH1) during Ovsynch protocol with early estrus response (Galvão *et al*., 2007; Fricke *et al*., 2014; Borchardt *et al*., 2016; Kim *et al*., 2022; Hölper *et al*., 2023).

The therapy causes 29.17% of the cows to exhibit estrus at TAI, proving its efficacy. The CL is essential for the emergence of mature follicles, leading to improved ovulation and conception rates after PGF2α injection. Authors suggest that a dominant follicle increases circulating estrogen (E2), causing lactating dairy cows to show estrus 48 hours after PGF2α treatment. A final GnRH treatment at 48 hours after PGF2α treatment induces a surge of LH and synchronizes ovulation, allowing for proper AI timing before ovulation (McDougall, 2010; Wiltbank and Pursley, 2014; Carvalho *et al*., 2018; Borchardt *et al*., 2020). This study shows agreement with these research's ideas that it is possible to increase the percentage of ovulation and pregnancy rate.

Lopez-Gatius (2021) suggested boosting the standard PGF2α dose to increase the estrus rate. More CL decreased estrus response, but increasing the dosage increased it. In conjunction with this research, estrus response was modest (29.17%) at AI. Following GnRH1 injection, the accessory CL rose by 25, 37.5, and 25% on days 40, 42, and 44 P.P. This is definitely a result of the first injection of GnRH1 of the protocol. However, the study found that the ovulation and pregnancy rates up to 54.2% and 41.7% at first TAI compared with 54.5% and 31.8% in the control group. There was no significant impact found in between. It was just high in the presynchronized Ovsynch group, but the importance in this study was that the time of P.P.

minimized to 47–49 days until the first AI to achieve a 41.7% of pregnancy rate plus 20.8% pregnancy rate at the second round of AI. The improvement of P/R in the control group could be due to the effect of the cloprostenol injection in both groups, which increases ovarian activity and helps to improve uterine health. These results are in contrast to those recorded by Pursley *et al*. (1997) on average, P/AI following Ovsynch procedures and P/AI after identified estrus had equal pregnancy risks, despite some variation across studies. Also, Rabiee *et al*. (2005) mentioned that Ovsynch may not improve pregnancy rates compared to other programs (natural breeding, single, double, or triple prostaglandin injections, Select Synch, Heat Synch, and modified Ovsynch) and that labor and hormone costs should be considered when using it routinely. According to Bover *et al*. (2019), utilizing Ovsynch as a synchronization approach may decrease fertility since the CL does not regress after prostaglandin F2α injection. Repeated PGF2α treatment may accelerate complete luteolysis. Besides pre-synchronization, Ovsynch and its different forms may quickly resynchronize inseminated and sonographically diagnosed non-pregnant cows, enhancing farm efficiency. Progesterone implants boost Ovsynch procedures. Along with managing reproduction, Ovsynch and its adaptations have helped dairy cows with anovulatory disorders and cystic ovarian illness (Meglić *et al*., 2023). A review study by Elmetwally *et al*. (2021) mentioned that many studies in Egypt indicate that Ovsynch+P4 and Presynch-Ovsynch both effectively improve fertility in cows with a CL 15 mm, with no difference in pregnancy/artificial insemination. The review included several ovulation synchronization techniques and variables influencing management program selection.

However, various variables, including herd, management, clinical illnesses, and milk production, may significantly affect normal ovarian cyclicity during the second month of P.P. This may help explain why studies on the effects of prostaglandin medication in the immediate P.P. period have shown conflicting results. Most studies have used a single dosage of prostaglandin provided during the first 30 days P.P. (Burton and Lean, 1995). A second dosage administered after 14 days is likely to enhance outcomes. Here, we investigated ovarian function in otherwise healthy cows and discovered that the pregnancy rate in treated cows was comparable to that seen in control cows (Moreira *et al*., 2001; López-Gatius *et al*., 2003; Martins *et al*., 2023). In a review paper by Pérez-Marín and Quintela (2023), therapeutic procedures for fixed TAI (FTAI) that depend on ovulation synchronization (like Ovsynch) or pre-synchronization have gained popularity as a means of obtaining high fertility in the first cycle after delivery. Treatments like these may enhance and synchronize ovulatory processes such as follicular growth, ovulation, and CL production.

It would seem that presynchronization saves money. Whether used for presynchronization before a timed AI regimen or as a tool for P.P. reproductive control of nursing dairy cows, our findings are comparable with those of earlier studies. To reduce the incidence of P.P. anestrus and other P.P. complications at the start of the service period. In addition, further studies are needed to improve dairy cow management practices in Libya.

Acknowledgments

None.

Authors' contributions

AB and AH designed the experiments; NM performed the experiments and wrote the first draft of the manuscript; and HAS revised and edited the manuscript. AA analyzed the data.

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Data availability

All data are provided in the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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