

Impact of Timed Artificial Insemination within Presynchronization Protocol on Reproductive Performance in Dairy Cows

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Abstract

The aims of this study were to compare of some reproductive performance after timed artificial insemination (TAI) or using artificial insemination (AI) at oestrus detected. Fifty anoestrus cows, selected between 75 and 100 days postpartum, were separated into two equal groups. The control group (n = 25) consisted of cows that received no treatment and underwent artificial insemination when the first postpartum oestrus was identified. The treated group (n = 25) of cows underwent the presynchronization protocol [Day 0 (GnRH); Day 7 (GnRH); Day 14 (PGF_{2α}); Day 15 (PGF_{2α}); Day 16 (GnRH); TAI 16-24 hours latter]. Trans-rectal ultrasonography confirmed the pregnancy on days 35-40 after insemination. The results of this study show pregnancy rate (PR) for the treated group was significantly higher than (P=0.04) to that of the control group (56% vs. 28%, respectively). Furthermore, mean days of days open (DO) and calving interval (CI) for the treated group (104 ±2.8 and 384 ±2.8, respectively) were significantly (P=0.001) shorter than those in the control group 156 ±17.3 and 436 ±17.3, respectively). In conclusion, using TAI within presynchronization protocol eliminate the requirement for oestrus detection and improve reproductive performance at first service in postpartum dairy cows.

Key words: Calving interval, Days Open, Pregnancy rate, Presynch, Timed Artificial Insemination

I. Introduction

The identification of oestrus is increasingly recognized as a significant challenge in dairy herds (Holman *et al.*, 2011). Cows show fewer oestrus behavioral signs, which are diminishing and pose challenges for farmers to identify cow in heat (Dobson *et al.*, 2007; Somers *et al.*, 2015). This decline in oestrus detection efficiency has led to lower pregnancy rates (PR), prolonged days open (DO), and calving interval (CI), consequently, economic losses; specifically, in dairy farms using artificial insemination (AI) techniques rely on visual observation method for oestrus detection for identifying cows in heat (Reith and Hoy, 2018).

Ovulation synchronization protocols (Ovsynch), which do not require oestrus monitoring, can be applied in farms with low oestrus detection rates. The aim of Ovulation synchronization is to initiate treatment early after calving, rather than waiting for cows to exhibit oestrus after the voluntary waiting period. These protocols enhance reproductive efficiency by synchronizing ovulation with prostaglandin F_{2α} (PGF_{2α}) and gonadotropin-releasing hormone (GnRH), using Timed Artificial Insemination (TAI), cows are inseminated at fixed time without the need to observe their oestrus (Pursley, Mee and Wiltbank, 1995; Pursley *et al.*, 1997).

However, previous studies (Pursley and Martins, 2011; Carvalho, Wiltbank and Fricke, 2015) have shown that starting Ovsynch at a random point in the oestrus cycle leads to poor synchronisation of ovulation

on the day of TAI; consequently, decreased fertility outcomes. Other studies indicate that the Ovsynch protocol is most successful when performed between days 5 and 7 of the oestrous cycle in lactating dairy cows, one approach is to presynchronize the oestrous cycle (Stevenson, 2016; Mariol *et al.*, 2024).

Therefore, authors was developed many presynchronization methods that increase the effectiveness of TAI protocols. The main idea of presynchronization protocols that were developed based on presynchronization with PGF_{2α} such as Presynch-14 protocol (Moreira *et al.*, 2001) or presynchronization based on GnRH such as Double Ovsynch protocol (Souza *et al.*, 2008), was to synchronize cows' oestrous cycles to the early distrust phase before the Ovsynch protocol began (Cardoso Consentini, Wiltbank and Sartori, 2021).

This study was aimed to evaluate the efficiency of TAI within presynchronization program on some reproductive performance in postpartum local dairy cows at first service postpartum compared to AI at oestrus detection.

II. Materials and Methods

Study animals, housing and design

The study was conducted from Jan 2024 to Mar 2025. on private rural filed in Khalidiya and Ramadi cities, Anbar Province, Iraq. Postpartum anoestrus cows (n=50) were housed in free stalls barns and provided with clean, fresh water were milked twice daily. The cows were selected between 75–100 DIM at a ranges of parity (3-6), and weight (200-400 kg).

The control group (n = 25) untreated cows were monitored for oestrus signs (only cows being mounted) two times/day by farm owners (Figure 1). Cows observed in estrus in the AM received AI in the PM and vice versa. The Presynch group (n = 25) treated cows were subjected to ovarian ultrasound examination at first day treatment, to ensure the ovarian activity. After that, cows received treatments of GnRH [Busereline acetate (Gestar®), 10.5 µg, 2.5 mL, Over, Argentina] and PGF_{2α} [Cloprostenol (Alfaglandin® C), 500 mcg, 2 mL, Alfasan, Holland] according to presynchronization scheduled (Figure 1).

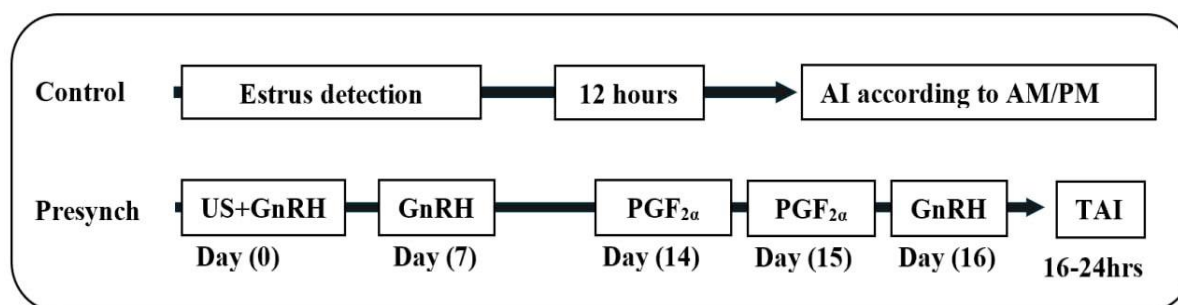


Figure 1: US (ultrasonography to confirm the presence of the follicles size ≥ 8 mm in diameter. Presynch protocol [Day 0 (GnRH); Day 7 (GnRH); Day 14 (PGF_{2α}); Day 15 (PGF_{2α}); Day 16 (GnRH)]. 16-24 hours latter TAI (timed artificial insemination)].

Reproductive parameters

The pregnancy rate (PR%) is defined as the number of pregnant cows following the first service / the total number of cows inseminated ($\times 100$). In this study, the calving date obtained by asking the breeders. The days open was the time from calving date to the date AI that result in confirmed pregnancy. The calving interval was obtained by adding the pregnancy period (280 days) to days open.

Ultrasound examination

The present study evaluated the ovarian status of treated cows on the initial day of treatment through trans rectal ultrasonography employing a 6.5-MHz linear probe to detect large follicles (≥ 8 mm) in diameter. The follicle diameter was measured using an electronic calliper based on the equation (follicle height + follicle width) / 2, as described by (Widodo *et al.*, 2022).

Pregnancy diagnosis was conducted between days 35 and 40 post-insemination for all cows using trans-rectal ultrasound with a 6.5-MHz linear probe. Pregnancy can be confirmed if an embryo with a heartbeat is seen or if there is clear fluid in the uterus along with a developed corpus luteum (CL) (Dixit and Haloi, 2022).

Statistical analysis

Study parameters' influence of several variables was found using the statistical analysis system (SAS, 2018) software. The chi-square test was used to evaluate changes in CR at 5% and 1% significant levels. Means were compared substantially using the least significant difference (LSD) approach.

Results and Discussion

Pregnancy rate (PR%)

In the present study, the untreated cows in control group which receive AI at first estrus detected using traditional observation method, achieved a first service PR of 28% (7/25) (Table 2, Figure 2). This result is consistent with previous findings that reported PR of 27.5%, 31% and 32% (Fricke *et al.*, 2014; Alsuwaidawi and Alrawi, 2023; Strickland *et al.*, 2024). Conversely, Keister (Keister *et al.*, 1999) and Lakher (Lakher *et al.*, 2019) was observed a lower PR of 15.9% and 16.6%, respectively.

In contrast, previous studies was achieved a higher findings of PR after using advanced tools for oestrus detection compared to the traditional method that used in this study. For instance, (Lauber and Fricke, 2024) reported a 43.5% PR for tail paint method. Another study by Jobst (SM, 2000) who was found a 45.6% PR for heat watch method. Variability in PR among studies may be attributed to differences in nutritional status, body condition score, reproductive status (e.g., cyclicity or anestrus), and estrus detection methods.

In the current study, cows treated with a presynchronization protocol followed by TAI exhibited a significantly higher PR of 56% (14/25) (Table 1, Figure 2). This outcome aligns with previous research reporting PR of 57% and 51.4% following TAI within the G6G and PG-3-G presynchronization protocols, respectively (Yousuf *et al.*, 2016; Kutlu and Dinç, 2020). These results surpass other studies where first-service PR of 33%, 40%, and 45% were achieved using different TAI strategies (Hubner *et al.*, 2020; Consentini *et al.*, 2022, 2025).

However, the current PR was lower than that reported by (Alsuwaidawi and Alrawi, 2024) who found a 65% PR at first service post-calving in cows treated with the PG+G protocol. The PG+G protocol involves the concurrent get of GnRH and PGF_{2α} seven days prior to initiating the Ovsynch regimen, potentially enhancing its efficacy in cyclic and non-cyclic cows (Martins *et al.*, 2017). Similarly, Oosthuizen (Oosthuizen *et al.*, 2025) demonstrated a PR of 60.9% in cows treated with a control internal drug release (CIDR) device combined with PGF_{2α} 7 days before beginning the CO-Synch protocol.

The observed differences in PR between studies can be attributed to the variation of synchronization protocol and hormone combinations, sample size, herd management practices, and the expertise of inseminators as well as cow-related factors (age, breed, parity).

Table 1: Comparison of first-service pregnancy rate between study groups.

Item	Groups		P-value
	Control (n=25)	Presynch (n=25)	
Pregnancy rate% (n/n)	28% (7/25)	56% (14/25)	P=0.04

There is a significant difference ($P \leq 0.05$) between groups in terms of pregnancy rate.

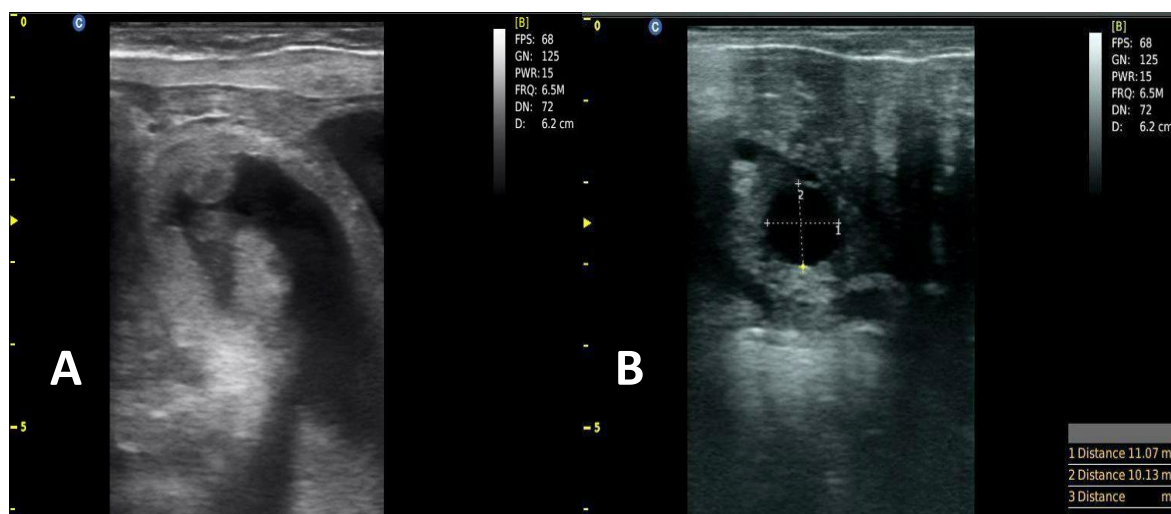


Figure 2: Ultrasound images show (A) 35-day-old embryo is contained in the uterine horn. (B) an ovary with a large follicle measuring 10.56 mm in diameter.

As shown in Table 1, the statistical analysis indicated a significant difference ($P = 0.04$) in first-service PR between the control group and the Presynch group, with rates of 28% and 56%, respectively. This observation aligns with previous research reporting significantly improved PR in presynchronized cows compared to untreated controls (Lauber and Fricke, 2024; Strickland *et al.*, 2024).

In contrast, previous studies have found no significant difference ($P \geq 0.05$) in pregnancy outcomes at 35 days post-insemination between TAI and estrus-based AI strategies (DeJarnette and Marshall, 2003; Rabiee, Lean and Stevenson, 2005). Furthermore, other authors have reported fertility outcomes for cows that receive AI at detected oestrus was higher ($P \leq 0.05$) PR compared to the TAI within classical Ovsynch regime (Cordoba and Fricke, 2002).

The results of this study suggested that low oestrus detection rates may be considered a primary factor contributing to lower fertility in the control group. It has been reported that the rate of oestrus detection with the traditional observation method did not reach 50% in the first and second lactation periods due to the low intensities and short durations of oestrus behaviour caused by increased milk production, which is associated with feed shortages and a negative energy balance (Bisinotto *et al.*, 2015).

The rise in fertility seen in the cows that received treatment might be due to TAI-based protocols eliminating the need for detecting oestrus. By synchronising ovulation and scheduling insemination at a fixed time, these protocols ensure a 100% service rate for all treated cows, thereby standardising breeding efficiency and reducing the labour and time investments associated with traditional oestrus observation (Fricke and Wiltbank, 2022).

Furthermore, all cows in the treated group were selected to be in the follicular phase of their oestrous cycle, exhibiting active ovaries (follicle size ≥ 8 mm) on the initial day of treatment (Figure 2). According to Bello (NM, 2006), GnRH-based presynchronization protocols are reported to be more beneficial for cows in the follicular phase or those that are anovulatory at the time of treatment. Administering GnRH seven days before the Ovsynch procedure stimulates the release of LH and FSH, promoting ovulation and the initiation of a new follicular wave—essential aspects for achieving synchronisation success.

Moreover, presynchronization with GnRH generally establishes the ideal physiological conditions for starting the Ovsynch protocol, characterised by a functional CL with moderate serum progesterone levels (0.5–7 ng/mL) and a dominant follicle measuring 12–19 mm (Wiltbank and Pursley, 2014).

Calving interval (CI) and Days open (DO)

Calving interval (CI) and days open (DO) are critical reproductive performance metrics in dairy cattle management. CI, defined as the duration between two consecutive calvings in the same cow, comprises two essential components: the gestation period (approximately 280 days) and the open period (Temesgen *et al.*, 2022;

Cakircali *et al.*, 2023). The open period is the time from parturition until successful conception and is highly influenced by postpartum reproductive physiology, including the anoestrus phase, ovarian cyclicity, and breeding management practices (Sari, Hartono and Suharyati, 2017).

According to industry benchmarks, ideal reproductive targets include a DO period ranging between 85–115 days and a CI of 365–395 days. These values are considered optimal for sustaining one calf per cow annually, a key goal for maximizing herd productivity and economic returns (Stevenson and Britt, 2017; Damiran *et al.*, 2018; Senbeta, Abebe and Gibe, 2024). Optimal reproductive performance is generally achieved when the first estrus is observed within 40 days postpartum, and the first service occurs after 60 days in healthy cows (Dinkissa and Guye, 2022).

In the current study, untreated control cows were inseminated at their first detected spontaneous estrus showed average CI and DO values of 436 ± 17.3 and 156 ± 17.3 days, respectively (Table 2). These values notably exceed the ideal ranges, indicating suboptimal reproductive performance. Similar elevated CI and DO values have been reported in various studies, respectively, 444.2 and 151 days (M'hamdi *et al.*, 2010), 665.1 and 199.8 days (Dinkissa and Guye, 2022), and 421 and 121 days (Alsuwaidawi and Alrawi, 2023).

Conversely, more favorable reproductive metrics have been documented. For instance, Sari (SARI, AYAŞ and SELVİ, 2025) reported a mean CI of 374.73 days and DO of 95.61 days in a broad survey involving 2,234 cows across 49 farms. Likewise, (Yılmaz and Sariözkan, 2020) observed CI and DO values of 369.9 and 82.9 days, respectively, figures aligning well with the recommended targets. Teseuggesting the persistence of reproductive inefficiencies in multiple herds under diverse management conditions.

In this study, cows in the treatment groups exhibited average days of CI and DO was 348 and 104, respectively (Table 2). These values align closely with those reported by (Kutlu and Dinç, 2020) who observed 333 and 53 days in cows subjected to a presynchronization protocol. Moreover, the present results corroborate findings from local study evaluating different presynchronization protocols documented CI and DO values of 343 and 63 days, respectively (Alsuwaidawi and Alrawi, 2024).

The data analysis showed a significant difference ($P = 0.001$) in CI and DO between the control and treatment groups (Table 2). These findings confirm that the application of TAI within an ovulation synchronization protocol effectively improves reproductive performance, resulting in CI and DO values that fall within the desired industry benchmarks.

Table 2: Effects of TAI protocol on calving interval and days open in dairy cows.

Item	Groups		P-value
	Control	Presynch	
Days open	156 ± 17.3	104 ± 2.8	$P=0.001$
Calving interval	436 ± 17.3	384 ± 2.8	$P=0.001$

Values: mean days $\pm$ standard error. There is highly significant difference ($P \leq 0.01$) between groups in terms of days open and calving interval.

The suboptimal reproductive performance observed in this study's control group may be attributed to various factors, including management factors such as inaccurate oestrus detection, substandard housing, hygiene conditions and inadequate nutritional support. Additional risk factors influencing CI and DO include parity, calf sex, body condition score (BCS), age at first conception, milk production levels, and the presence of postpartum disorders such as dystocia, metritis, and retained placenta, alongside seasonal heat stress (Hafilah and Solihati, 2024; Senbeta, Abebe and Gibe, 2024).

Moreover, the prolonged postpartum anoestrus period can delay breeding (which represents the interval from calving to the first observed oestrus), leading to an extended DO and negatively impacting CI and overall reproductive efficiency. This period is affected by several interrelated factors, including the duration required for complete uterine involution, the timely resumption of normal ovarian cyclicity, the occurrence of silent ovulations, and challenges in oestrus detection and insemination timing (SARI, AYAŞ and SELVİ, 2025).

Presynchronization protocols based on the strategic use of exogenous hormones (GnRH and PGF_{2α}) can address many reproductive issues by promoting the resumption of ovarian activity, resolving postpartum disorders (e.g., anoestrus, anovulation, ovarian cysts), and mitigating the effects of environmental and physiological stressors, such as heat stress (Wiltbank and Pursley, 2014; Temesgen *et al.*, 2022).

Conclusion

Using TAI within presynchronization protocol significantly improved reproductive performance in postpartum dairy cows compared to estrus-based AI. These findings support the integration of TAI protocols into reproductive management strategies, particularly in herds with poor estrus detection efficiency.

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Conflict of interest

The authors affirm that they have no conflicts of interest.

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