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A COMPARATIVE STUDY OF PRE-SYNCHRONIZATION PROTOCOLS G7G AND eCG PROTOCOL IN DAIRY COWS IN ANBAR GOVERNORATE, IRAQ

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ABSTRACT

This study was conducted on privately-owned dairy farms in Al-Anbar Province, Iraq, from July 2023 to January 2024. The aim of the study was to evaluate the impact of timed artificial insemination (TAI) on the calving interval (CI), days open (DO), and conception rate at first service (CRFS) compared to artificial insemination (AI) based on oestrus detection. A total of 40 cows, selected between 60 and 80 days postpartum, were divided into three groups. The control group (n= 20) untreated cows that received AI at the first detected oestrus according to routine a.m./p.m. breeding rule. The treated cows (n = 20) were equally distributed between the G7G protocol [$PGF_{2\alpha}$ -4d-GnRH-7d-GnRH-7d-$PGF_{2\alpha}$-1d-$PGF_{2\alpha}$-1d-GnRH, and TAI after 16-24h] and the eCG Protocol [eCG-7d-GnRH-7d-$PGF_{2\alpha}$-1d-$PGF_{2\alpha}$-1d-GnRH, and TAI after 16-24h]. Transrectal ultrasound confirmed the pregnancy on days 30-35 post-insemination. The average days of CI and DO for the control [430 and 150], respectively, were significantly longer compared to both treated groups [G7G (372 and 92) and eCG Protocol (386.2 and 88.2), $P<0.01$], respectively. The overall CRFS showed a significant difference across all groups [45%, $P\leq0.05$]. Separately, the CRFS for control cows differed significantly compared to both treated protocols [25% vs. G7G (70%) and eCG (60%), $P\leq0.05$], respectively. However, CRFS between the treated groups did not differ significantly. In summary, the TAI eliminates the need for oestrus detection and enhances reproductive fertility earlier in the postpartum period, resulting in shorter CI and DO.

Keywords: *Presynch, ovsynch,* and *oestrus synchronization*.

INTRODUCTION

Optimizing reproductive efficiency in a dairy herd is essential for determining the global profitability of dairy cattle. Key factors influencing cows' reproductive success include number of services per conception, calving interval (CI), days open (DO), as well as management practices such as accurate heat detection, proper insemination techniques, and conception rate (Gross *et al.,* 2011; Rahawy, 2021).

Failure to detect oestrus at the optimal insemination time (Bihon and Assefa, 2021) is a major cause of lower conception rates (CR) and overall reproductive success, which can lead to financial losses (Madureira *et al.,*

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2021; Wicaksono *et al.,* 2024). Many factors impact oestrus detection, including environmental and physiological variables like flooring type and milk production (Rivera *et al.,* 2010; Tippenhauer *et al.,* 2021). Furthermore, cows experience silent oestrus or become anovulatory before the voluntary waiting period ends (Sauls *et al.,* 2017, Tippenhauer *et al.,* 2023).

The Ovsynch protocol, created by Pursley *et al.* (1997), enables timed artificial insemination (TAI) without requiring detection of oestrus (Jeong *et al.,* 2023). Previous research has shown that giving an extra dose of prostaglandin F2 alpha ($PGF_{2\alpha}$) on day eight will help the corpus luteum (CL) regression (Carvalho *et al.,* 2015). This is because 90–100% of cows that got the second dose of $PGF_{2\alpha}$ 24h had complete luteolysis (Karakaya-Bilen *et al.,* 2019; Kuru *et al.,* 2020; Alsuwaidawi and Alrawi, 2024).

Research conducted by specific authors demonstrates that the Ovsynch protocol achieves optimal success when implemented in the early stages of dioestrus. Presynchronizing is one way to begin ovsynch in cows during the dioestrus phase (Pursley *et al.,* 1997; Moreira *et al.,* 2001, Cardoso Consentini *et al.,* 2021). The Presynch-Ovsynch technique entails the presynchronization of cows through the administration of $PGF_{2\alpha}$ with two doses, spaced 14 days apart (Vasconcelos *et al.,* 1999). This method enhanced pregnancy per artificial insemination following Ovsynch from 29.4% to 42.8% (Carvalho *et al.,* 2015). The Double-Ovsynch protocol demonstrated a greater increase in pregnancy per artificial insemination relative to Presynch-Ovsynch (Souza *et al.,* 2008; Li *et al.,* 2024).

The G6G or G7G protocols are a reproductive approach that begins with $PGF_{2\alpha}$ treatment for cows, followed by GnRH injection either 2 or 4 days later, and subsequently six to seven days prior to the commencement of the classic ovsynch-protocol (Khalil, 2019). These strategies enhance the proportion of cows in the optimal phase of the oestrous cycle (Dirandeh *et al.,* 2015). The incorporation of GnRH in presynch-ovsynch may offer benefits for anovular cows. (Heidari *et al.,* 2017). Cows subjected to the G6G protocol exhibited significantly higher ovulation rates at the initial GnRH treatment 85% vs. 54%, enhanced responsiveness to $PGF_{2\alpha}$ 96% vs. 69%, improved synchronization rates 92% vs. 69%, and increased P/AI rates 50% vs. 27% compared to cows administered Ovsynch on random days of the oestrous cycle (Bello *et al.,* 2006).

Administering eCG at the end of oestrus synchronization protocols may improve fertility in dairy cows (Souza *et al.,* 2009). The effects of eCG are linked to three primary outcomes: a) an increase in the diameter of the small follicle (Pulley *et al.,* 2013) b) enhancement of ovulation rate and pregnancy (Sá Filho *et al.,* 2010); and c) increase in plasma P4 level throughout the new luteal phase (Ferreira *et al.,* 2013). Furthermore, the administration of eCG to dairy cows found to reduce the number of atretic follicles, which are ovarian follicles that began to mature but did not develop into the dominant follicle and have involuted, while simultaneously enhancing the follicle growth rate (Lakher *et al.,* 2019).

Administering eCG seven days prior to the initial Ovsynch protocol injection promotes the development of larger follicles (Păcală *et al.,* 2010). Research indicates that the inclusion of eCG in a GnRH protocol enhances reproductive outcomes (Sales *et al.,* 2016). The administration of eCG may enhance cyclicity and provide advantages for older cows or those with inadequate nutrition, which could otherwise hinder successful breeding (Small *et al.,* 2009; Yenİlmez, and Özdemİr, 2020)

This study aimed to improve reproductive performance by implementing TAI without oestrus detection, utilizing two presynchronization protocols, and comparing these findings to AI at oestrus detection in dairy cows.

MATERIALS AND METHODS

Animals* and *study area

This study conducted on 40 Holstein dairy cows from July 2023 to January 2024 at various private diary farms in Al Anbar Province, Iraq. The cows enrolled in the study were in the 60–80 day postpartum period, weighing around 350 kg. and average age was 4 years, as determined using the dentation approach (Torell *et al.,* 2003). The tape method was employed to ascertain the weight of cows utilizing Schaeffer's formula: [body weight (W lbs) = length of the animal from the shoulder to the pin bone (L inches) × {chest girth of the animal * 2} (G2 inches) / 300]. Subsequently, the ensuing formula was applied to convert weight from pounds (lbs) to kilograms (kg): Weight (kg) = Weight (lbs) * 0.4536 (Riaz *et al.,* 2023, Wangchuk *et al.,* 2018).

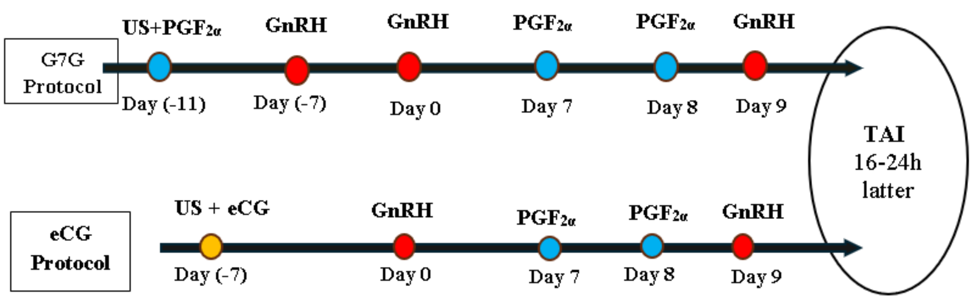
Experimental design

Forty dairy cows are divided into three groups: one control group and two treatment groups. Control cows (n = 20) received no treatment and were inseminated based on the AM/PM rule following visual detection of oestrus signs. The remaining cows (n = 20) were equally divided into two treatment groups, which were treated with GnRH (10.5 µg of Busereline acetate, 2.5 mL, I/M, Over, Argentina), $PGF_{2\alpha}$ (0.150 mg of d-cloprostenol, 2 mL, I/M, Invesa, Spain), and eCG (1000 IU PMSG 10 mL, IM, Oviser, Spain) depending on their ovarian status.

The cyclic cows were treated with the G7G protocol:

[$PGF_{2\alpha}$ -4d - GnRH-7d-GnRH-7d-$PGF_{2\alpha}$-1d-$PGF_{2\alpha}$ -1d-GnRH, and TAI after16-24h].

Noncyclic cows were treated with eCG Protocol (n = 10):
[eCG-7d-GnRH-7d-$PGF_{2\alpha}$-1d-$PGF_{2\alpha}$-1d-GnRH, and TAI after 16-24h] (Figure 1). The semen used for AI is frozen (-196°C), stored, and distributed in 0.25 ml plastic straws and was obtained from the artificial insemination center in Abu-Grab at Baghdad.



Ovarian ultrasonography (US) was used to classify the cows into two groups based on their ovarian state. The G7G Protocol cows have undergone CL protocol. The eCG Protocol consists of cows with follicles <8 mm.

Ultrasonography of the ovaries* and *pregnancy diagnosis

ovarian conditions were assessed on the first day of presynchronization treatment using trans-rectal ultrasonography with a 6.5-7.5 MHz linear array probe and a portable ultrasound device (CHISON, Eco 2). The presence of a corpus luteal (CL) (Figures 2 and 3) was considered indicative of an active ovary (cyclic). In contrast, if the ovaries were inactive, no CL present and follicles <8 mm they were classified as inactive ovaries (non-cyclic) (Widodo *et al.,* 2022). The diameter of each follicle and CL area was measured with built-in calipers (Martins *et al.,* 2023).

The pregnancy was confirmed at 30-35 days after TAI using a trans-rectal ultrasound with a 6.5-7.5 MHz linear probe (CHISON Eco2, China). Pregnancy was confirmed by detecting an embryo's heartbeat or the identification of anechoic uterine fluid, along with the presence of a mature CL (Aziz and Al-Watar, 2022).

Statistical analysis

SAS (version 9.6) was used for statistical analysis. The significance threshold for CRFS was set at a probability of 0.05, and percentages were compared using the Chi-square test. The least significant difference (LSD) test, a component of analysis of variance (ANOVA-1), was used to compare means (Al-Ali and Rahawy *et al.,* 2022).

RESULTS

The mean days of CI in the control, G7G, and eCG protocol groups were 430±10, 372±2.7, and 368.2±3.3, respectively. The mean DO for the control, G7G, and eCG Protocol was 150±10, 92±2.7, and 88.2±3.3, respectively (Table 1). Statistical analysis revealed no significant differences between the treatment groups in the CI and DO ($P \geq 0.05$). However, these groups exhibited a significantly shorter mean duration of CI and DO, compared to the control group ($P \leq 0.05$).

In our study, pregnancy was confirmed using trans-rectal ultrasonography performed 30–35 days after the first AI (Figures 2 and 3). A notable significant difference in CR was observed between groups (Table1) (45%; $P \leq 0.05$). The CR for the control, G7G, and eCG protocol were 25%, 70%, and 60%, respectively. The statistical analysis showed no significant differences between the G7G and eCG protocol groups ($P \geq 0.05$; Table 1), however both the G7G and eCG protocol groups exhibited significant differences when compared to the control group ($P<0.05$).

Table 1: Mean days for calving interval (CI), days open (DO), and Conception Rate at First Service (CRFS).

Groups	No.	CI ± SE	DO ± SE	(N) CRFS%
Control	20	430 ± 10^{a}	150 ± 10^{a}	(5) $25\%^{a}$
G7G	10	372 ± 2.7^{b}	92 ± 2.7^{b}	(7) $70\%^{b}$
eCG protocol	10	368.2 ± 3.3^{b}	88.2 ± 3.3^{b}	(6) $60\%^{b}$

Values within the same column denoted by different superscript letters (a, b) indicate a statistically significant difference y ($P < 0.05$).

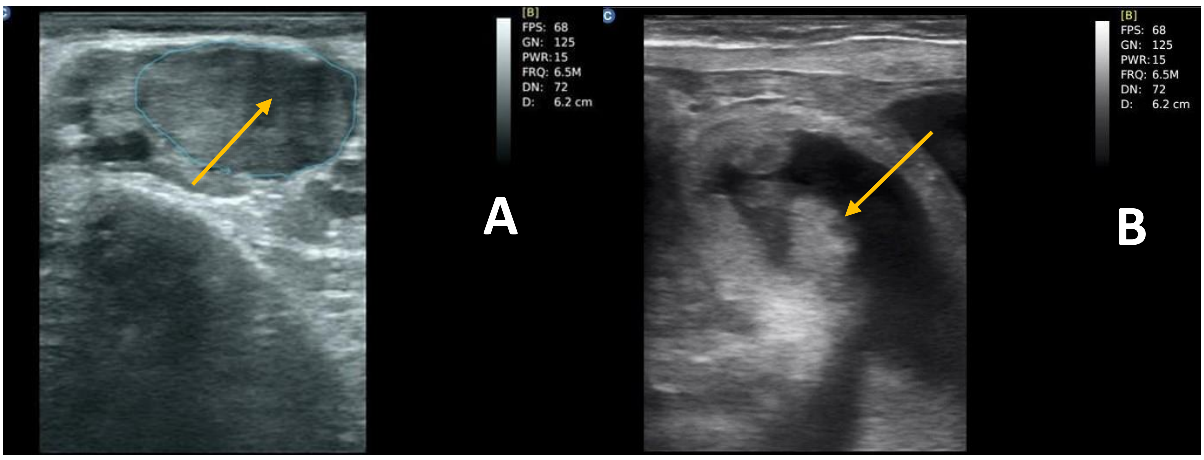


Figure 2: Ultrasound examination of cow in G7G group showing:
(A) an ovary with mature corpus luteum (before treatment),
(B) uterine horn showing a 35-day-old embryo (after treatment).

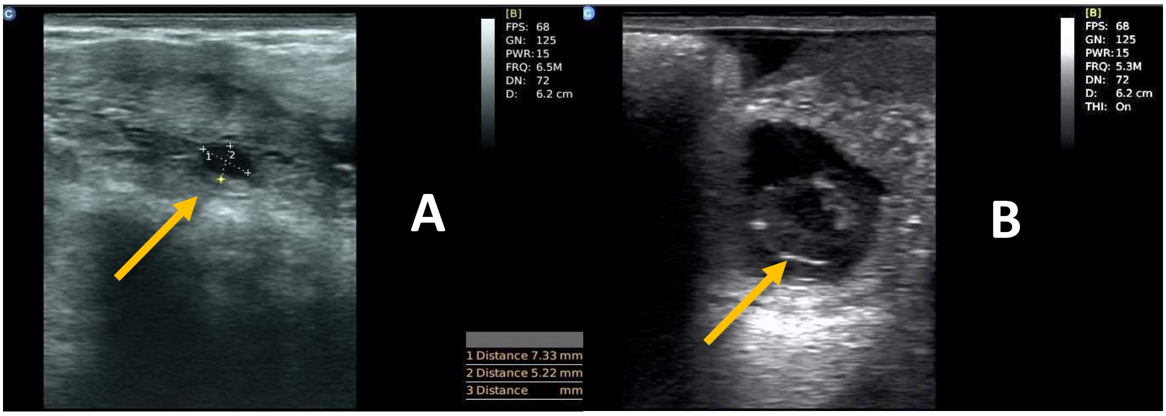


Figure 3: Ultrasound examination of cow in eCG protocol group showing:
(A) an ovary with small follicle with 6.3 mm diameter (before treatment),
(B) uterine horn showing 31-day-old embryo (after treatment).

DISCUSSION

Detection of oestrus is a critical factor that significantly affects the reproductive efficacy of dairy cattle, particularly in farming environments that use AI techniques (Reith and Hoy, 2018). Several challenges complicate oestrus detection, especially in large herds (Ranasinghe et al 2010, Uhm *et al.,* 2020; Jeong *et al.,* 2023).

In this study, control cows that received AI on the first postpartum oestrus detection had greater days of calving interval (CI) and days open (DO) than standard values (≤400 and ≤100 days, respectively) (Rhodes *et al.,* 2003; Stevenson and Britt, 2017). This observation aligns with the findings Kutlu and Dinç (2020), which reported an average CI and DO of 421 and 141 days, respectively. However, in this study, the treated cows underwent TAI, became pregnant, and had CI and DO within the optimal ranges (Alsuwaidawi and Alrawi, 2024). Furthermore, treatment groups showed significantly reduced CI and DO, compared to the control group ($P \leq 0.05$). In a study by Alsuwaidawi and Alrawi (2023), TAI protocols demonstrated significant improvements in reproductive performance compared to untreated cows and those managed through estrus detection, which aligns with the findings of this study. The study evaluated key reproductive metrics, such as CI, DO, and conception rate, with first service (CRFS) across different TAI protocols.

CI and DO and CRFS results can vary between research studies due to factors, such as synchronization techniques (e.g.: G6G, Ovsynch, and their modified forms), environmental condition, cow health, and management practices (Galvão *et al.,* 2007; Dirandeh *et al.,* 2015). Ovulatory responses and luteal regressions can also affect the period from calving to successful pregnancy (Tibary *et al.,* 2019).

Heat stress, especially during summer, reduces fertility and lengthens CI by affecting follicular development (Heidari *et al.,* 2017). Cows with delayed oestrous cycles require more time to conceive, thus extending DO (Sahithi *et al.,* 2019). Health, metabolic condition, and reproductive outcomes in multiparous and primiparous cows also contribute to these disparities. Healthy cows have fewer DO and recover to fertility faster (Siddiqui *et al.,* 2013).

In this study, G7G protocol exhibited a higher CRFS, aligning with previous research, where the reported CRFS was 71.43% (Ahmed and Doley 2017) and 64.7% (Kutlu and Dinç 2020). However, certain studies have reported a lower CR value of 38%, 50%, 37.7%, and 45% (Dirandeh *et al.,* 2015; Heidari *et al.,* 2017; Dirandeh *et al.,* 2018, Bakhtoo *et al.,* 2021).

The eCG protocol improved CRFS aligns with previously reported data, including the 55.6% complete response (Small *et al.* 2009). However, other studies have reported varying CRFS of 40% (Kavousi Nodar *et al.,* 2018), 50% (Lakher *et al.,* 2019), 45% (Mohammadsadegh, 2019), and 30.77% (Malik *et al.,* 2021), respectively.

Although, the G7G and eCG protocol led to higher CRFS; the difference between the two treatment groups was not statistically significant (P>0.05). This suggests that both methods provide comparable effectiveness in achieving successful pregnancies.

The G7G protocol is similar to the G6G protocol, but with a different timing and sequence of hormone administration. It uses GnRH and $PGF_{2\alpha}$ for presynchronization to enhance follicular wave synchronization before starting Ovsynch. This protocol has proven particularly effective in large herds or under heat stress conditions, where estrous expression is reduced, and labor-intensive estrus detection is impractical. G7G achieved a 32.7% conception rate at 32 days, outperforming standard Ovsynch (19.7%) and significantly reducing pregnancy loss, 24.1% compared to 50% in estrus detection (Kumar *et al.,* 2016). Furthermore, by synchronizing ovulation more precisely, the G7G protocol reduces days open and shortens the calving interval, improving the overall herd reproductive efficiency (Heidari *et al.,* 2017; Yousuf *et al.,* 2016).

The eCG protocol uses eCG to enhance follicular growth and ovulation in cows with suboptimal ovarian function or metabolic stress. This procedure increases CRFS and reduces treatment intervals, making it effective in controlling anestrous cows or those with poor ovulation synchronization, it also improves luteal function post-insemination and pregnancy retention (Yenİlmez and Özdemİr, 2020). A study by Kavousi Nodar et al. (2018), who compared the eCG-Ovsynch and 2PGF2α-Ovsynch protocols in dairy cows, found no notable differences in CRFS, CI, or DO (45% vs. 40%, respectively). In the eCG-Ovsynch group, a greater percentage of cows demonstrated elevated progesterone (P4) levels at the time of $PGF_{2\alpha}$ injection, with 85.4% compared to 69% in the 2PG-Ovsynch group. This suggests that eCG may increase P4 levels and improve efficiency in TAI. As mentioned earlier, initiating the Ovsynch protocol within the first 5–7 days of the oestrous cycle is crucial for achieving high CRFS and optimizing reproductive outcomes

Initiating the Ovsynch protocol within the early follicular phase, specifically during the first 5-7 days of the estrous cycle, is critical for achieving high CRFS and optimizing reproductive outcomes. (Kuru *et al.,* 2020; Alsuwaidawi and Alrawi, 2024). During this phase, cows typically have a dominant follicle primed for ovulation. Hormonal interventions like GnRH and eCG can effectively induce ovulation at this stage, leading to better synchronization of the follicular wave. (Păcală *et al.,* 2010; Kumar *et al.,* 2016; Mohammadsadegh, 2019).

The idea is based on the finding that 10 to 20% of lactating dairy cows subjected to an Ovsynch treatment do not attain complete luteal regression, leading to diminished fertility during TAI. The hypothesis proposed that administering a second $PGF_{2\alpha}$ treatment 24 hours after the initial dose intended to raise the number of cows achieving complete luteal regression, thereby enhancing the pregnancy per artificial insemination to improve overall fertility outcomes (Brusveen *et al.,* 2009).

Both the G7G and eCG protocols include a second of $PGF_{2\alpha}$ dose on day 8 of Ovsynch, intending to increase the number of cows achieving full luteolysis. Additional research indicated that 90–97% of cows administered two doses of $PGF_{2\alpha}$ 24 hours apart (on days 7 and 8 of Ovsynch) experienced complete regression of the corpus luteum (Riaz *et al.,* 2023, Yousuf *et* *al.,* 2016, Malik *et al.,* 2021; McDougall, 2010, De Rensis *et al.,* 2024).

Effective presynchronization techniques for reducing reliance on oestrus identification are essential for enhancing reproductive success and fertility in dairy cattle. These methods allow for a precise TAI, reducing the need for natural oestrus detection (Dirandeh *et al.,* 2018). G7G protocol significantly decreases pregnancy losses by enhancing ovulation synchronization, thereby optimizing TAI. Research indicates that reproductive performance under G7G protocol is boosted, resulting in increased CRFS, and decreased early pregnancy loss, particularly in cows subjected to heat stress, where oestrus behaviour is less apparent, ultimately enhancing herd reproductive performance (Dirandeh *et al.,* 2018; Heidari *et al.,* 2017).

CONCLUSION

The G7G and eCG protocols are highly effective tools for controlling dairy herd reproduction, providing reliable techniques for synchronizing ovulation, increasing conception rates, reducing days open, and shortening the calving interval. Both methods are for use on dairy farms that rely on artificial insemination without the need for estrus detection.

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CONFLICT OF INTEREST

There is no conflict of interest.

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دراسة مقارنة لبروتوكولات ما قبل التزامن G7G و eCG Protocol في الأبقار الحلوب في محافظة الأنبار، العراق

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أجريت هذه الدراسة على أبقار في حقول أهلية في محافظة الانبار للفترة من تموز2023 لغاية كانون الثاني 2024. هدفت هذه الدراسة إلى مقارنة فترة الولادة (CI) والأيام المفتوحة (DO) ومعدل الحمل بعد اجراء اول تلقيح لها (CRFS), وذلك باستخدام بروتوكولين لمزامنة الاباضة المعتمدة على طريقة التلقيح الصناعي الموقوت (TAI) , لمقارنتهما مع طريقة التلقيح الصناعي التقليدية المعتمدة على كشف الشبق. اجريت هذه الدراسة على 40 بقرة بين 80-60 يوما بعد الولادة. لقحت الابقار التابعة الى المجموعة الضابطة والبالغ عددها 20 بقرة بواسطة التلقيح الصناعي المعتمد على قاعدة الصباح/المساء بعد ظهور علامات الشبق عليها وقسمت الابقار المتبقية والبالغ عددها 20بقرة بالتساوي الى مجموعتين حيث خضعت الى برنامجين من التلقيح الصناعي الموقت هما G7G and eCG protocol . تم تشخيص الحمل من خلال الفحص بواسطة جهاز الموجات فوق الصوتية بعد 35-30 يوم من التلقيح. اظهرت النتائج وجود فرق معنوي (P≤0.01) حيث بلغ معدل الحمل بعد اجراء اول تلقيح (CRFS) 25% في المجموعة الضابطة مقابل 65% في المجموعات التي خضعت لبرامج التلقيح الصناعي الموقوت .بشكل منفصل. ، كانت نتائج CRFS مختلفة بشكل ملحوظ (P≤0.05) بين المجموعة الضابطة 25% ومجاميع العلاج 70% و 60% لل G7G و eCG Protocol على التوالي. مع ذلك، لم يختلف بروتوكول G7G بشكل كبير عن eCG Protocol P>0.05) وكان متوسط الايام بين ولادة واخرى وعدد الايام المفتوحة للمجموعة الضابطة (١٥٠ و ٤٣٠) أطول بشكل ملحوظ (P ≤0.01) من تلك الموجودة في كل من G7G (٩٢ و ٣٧٢) و eCG Protocol (٨٨,٢ و ٣٨٦,٢) . باختصار، فإن تقنية التلقيح الصناعي الموقوت (TAI) تغني عن الحاجة إلى كشف لشبق وتعزز الكفاءة التناسلية بوقت مبكر بعد الولادة، مما يؤدي إلى تقليل عدد الايام بين ولادة واخرى وتقليل عدد الايام المفتوحة.