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Impact of three different ovulation synchronization protocols on first-service conception rates in postpartum cows

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Abstract

The study was conducted on 60 Holstein dairy cows during 50 and 60 days postpartum in different rural areas in Al-Anbar Province, Iraq. This study evaluated the effects of two ovulation synchronization protocols on postpartum fertility performance compared to the conventional Ovsynch protocol. All cows were treated with GnRH (10.5 µg) and $PGF_{2\alpha}$ (0.150 mg) through three different synchronization protocols: (1) Ovsynch protocol (n=20) as the control treatment [GnRH-7d-$PGF_{2\alpha}$-48h-GnRH]; (2) GPPG protocol (n=20) as (1), but with an extra dose of $PGF_{2\alpha}$-24h after the first one. (3) PG+G protocol (n=20) as (2), but with presynchronization treatment using $PGF_{2\alpha}$ and GnRH simultaneously, seven days before the GPPG protocol. All cows were subjected to timed artificial insemination (TAI) 16-24 hours after the final GnRH injection. Ultrasound confirmed the pregnancy at 30-35 d post-TAI. There was no significant difference between the groups (P>0.05) regarding calving interval and open days. Overall, first-service conception rate (FSCR) tends to be significant (45%, P=0.07). Separately, FSCR for groups 1, 2, and 3 were 30, 40, and 65%, respectively. The difference between groups 1 and 3 was significant (P=0.02); however, group 2 did not differ from groups 1 (P=0.50) and group 3 (P=0.11). In conclusion, implementing TAI protocols during the 50-60-day postpartum period lowers the calving interval to standard limits. Furthermore, the PG+G presynchronization protocol significantly enhances FSCR compared to the traditional Ovsynch during the postpartum period.

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Introduction

The reproductive efficiency of a herd is an essential component of dairy cattle's global profitability. Factors such as age at first calving, calving interval, days open, and number of services per conception, as well as management factors (accuracy of heat detection, use of proper insemination techniques, and appropriate herd health policies), also influence a cow's reproductive success (1,2). Oestrus detection in dairy farms is considered one of the most important key components in fertility management programs. Effective and accurate oestrus identification is essential for excellent reproductive performance. The low or insufficient detection of oestrus in dairy cows hurts their reproductive efficiency, resulting in considerable economic losses (3,4). An alternative approach is the implementation of timed artificial insemination (TAI) in conjunction with ovulation synchronization techniques. This strategy aims to enhance cattle production to surpass established benchmarks for profitability and efficiency. These programs have become one of the most widely used reproductive technologies developed in the dairy industry. Moreover, implementing these strategies can potentially promote economic revitalization (5,6). The Ovsynch protocol (7), which is the most common TAI protocol, begins at a random stage of the oestrus cycle using a sequence of GnRH and $PGF_{2\alpha}$ treatments [day 0: 1st GnRH; day 7: $PGF_{2\alpha}$; day 9: 2nd GnRH; TAI: 24 h later]. Using $PGF_{2\alpha}$- GnRH protocol can regulate ovulation, follicular development, and the corpus luteum (CL) regression, all of which are critical processes in the Ovsynch protocol (8). Therefore, TAI can happen without oestrus detection (9,10). Unfortunately, there are at least two disadvantages observed with the Ovsynch protocol that led to decreased fertility outcomes: (i) failure of the first GnRH on (d 0) to induce ovulation of the dominant follicle to initiate a new ovarian follicular wave; (ii) incomplete CL regression due to the presence of a young CL, which is unresponsive to a single dose of PGF$_{2\alpha}$ on (d 7) of the Ovsynch protocol. Incomplete CL regression, defined as plasma P4 concentrations > 0.5 ng/ml at TAI, reduces pregnancy rates. Therefore, Ovsynch has been modified numerous times to improve its reproduction outcomes (11,12). Several studies have shown that starting the Ovsynch protocol between days 5 and 12 of the oestrus cycle when the best P4 environment and ovulatory responses were present, led to more P/AI than starting the protocol at the random stage of the cycle (13). Since then, many presynchronization strategies have been developed, either using PGF$_{2\alpha}$ (e.g., Presynch-11) (14) or combining GnRH and PGF2α (e.g., PG-3-G) (15) to equalize or introduce the oestrus cycle into the early diestrus stage. Moreover, (16) has reported that a significant modification to Ovsynch protocols is the recommendation to include a second PGF$_{2\alpha}$ treatment 24 h after the first one (on days 7 and 8) of the Ovsynch protocol, which is highly recommended for GnRH and PGF$_{2\alpha}$ type presynchronization protocols used for the first TAI. Thus, an additional PGF$_{2\alpha}$ dose could induce adequate CL regression and minimize P4 levels near the time of TAI (17).

This study aimed to determine whether adding a second PGF$_{2\alpha}$ injection on day 8 of the Ovsynch protocol with or without a presynchronization treatment by using PGF$_{2\alpha}$ and GnRH simultaneously seven days before the initiation of treatment, affects the first-service conception rate in postpartum Iraqi cows compared to the classical Ovsynch protocol.

Materials and Methods

Ethical approve

The University of Fallujah College of Veterinary Medicine evaluates and approves the experiment's protocols No. 18, dated 2/10/2023.

Animals, housing and location

This study was conducted from June 2022 to March 2023 in different rural areas in Al-Anbar Province, Iraq. 60 Holstein dairy cows were housed in free-stall barns and provided with clean, fresh water. The cows were enrolled in the study during the (50-60 days) postpartum period at age (3-6 years), parity (2nd to ^{fifth}), and weight (300-400 kg).

Treatments protocols and timed AI

Total cows were divided equally into three groups for each one (n=20), as shown in Figure 1. All cows were treated with GnRH (10.5 µg of Busereline acetate, 2.5 mL, I/M, Over, Argentina) and PGF$_{2\alpha}$ (0.150 mg of d-cloprostenol, 2 mL, I/M, Invesa, Spain). The semen used for AI is frozen (-196°C), stored, and distributed in plastic straws (0.025 ml) and was obtained from the artificial insemination cantering in Abu-Grab at Baghdad. The first group received classical Ovsynch protocol as control: [day 0: first GnRH; day 7: PGF$_{2\alpha}$; day 9: second GnRH]. The second group, cows treated with GPPG protocol, is an Ovsynch protocol just with adding a second PGF$_{2\alpha}$ dose 24 h after the first one: [day 0: first GnRH; day 7: first PGF$_{2\alpha}$; day 8: second PGF$_{2\alpha}$; day 9: second GnRH]. The third group: cows treated with PG+G presynchronization protocol, using PGF$_{2\alpha}$ concurrently with GnRH (seconds apart), seven days before a GPPG protocol was started: [PGF$_{2\alpha}$ and GnRH, seven days apart, GPPG protocol]. Timed AI was performed 16-24 hours after the final GnRH injection.

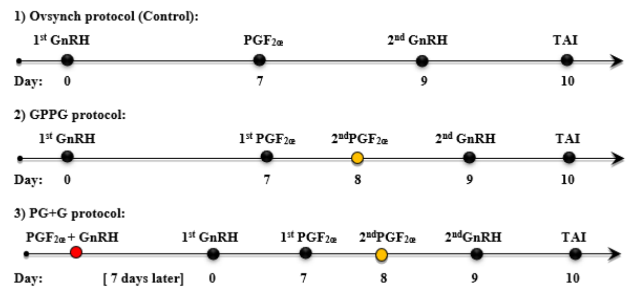

Figure 1: Schematic illustration of synchronization protocols. (1) Ovsynch Protocol: The control group was treated with GnRH, followed by seven days with PGF$_{2\alpha}$, and then 48 hours with GnRH. (2) GPPG Protocol: administration of additional PGF$_{2\alpha}$ injections (24 h) after the first PGF$_{2\alpha}$ injection. (3) PG+G Protocol: administration of pre-synchronization treatment using PGF$_{2\alpha}$ and GnRH simultaneously seven days before the GPPG protocol. All cows received TAI (16-20 h) after the final GnRH injection.

Pregnancy diagnosis

At day 30-35 post-TAI, the pregnancy was confirmed using trans-rectal ultrasound with a 6.5-7.5 MHz linear probe (China, *CHISON Eco2*). Confirmation of pregnancy can be achieved through the identification of anechoic uterine fluid in conjunction with the presence of either a mature corpus luteum or an embryo exhibiting a detectable heartbeat (18). Cows that return to oestrus just before the time of pregnancy diagnosis were considered negative cases.

Statistical analysis

Statistical analysis was performed with SAS (version 9.6). The Chi-square test (test of independence) was used to compare percentages of FSCR (a significant level was set at

0.05 probability) in this study. The least significant difference (LSD) test (analysis of variance, ANOVA-1) was used to compare between means significantly (19,20).

Results

Calving interval and day open

The mean days of calving interval in the control, GPPG, and PG+G were 343, 346, and 348, respectively. The mean days from calving to the first conception interval (days open) for the control, GPPG, and PG+G were 63, 66, and 68, respectively. The statistical analysis revealed no statistically significant difference between the groups in the calving interval and days open (P≥0.05; Table 1). In our present investigation, pregnancy was established through the utilization of transrectal ultrasonography performed 30-35 days after timed artificial insemination (TAI), as depicted in figure 2. There is a notable disparity in FSCR between the various groups (45%; P=0.07). The FSCR for the control, GPPG, and PG+G were 30%, 40%, and 65%, respectively. The statistical analysis revealed substantial differences between the control and PG+G groups (P=0.02). In contrast, the GPPG group exhibited no significant differences compared to the control group (P=0.50) and the PG+G group (P=0.10), as indicated in table 2.

Table 1: Mean days for calving interval and days open

Group	Calving interval	Days open	± St er.
Control	343	63	2.8
GPPG	346	66	1.3
PG+G	348	68	1.9
P-value	(P≥0.05)	(P≥0.05)	0.0

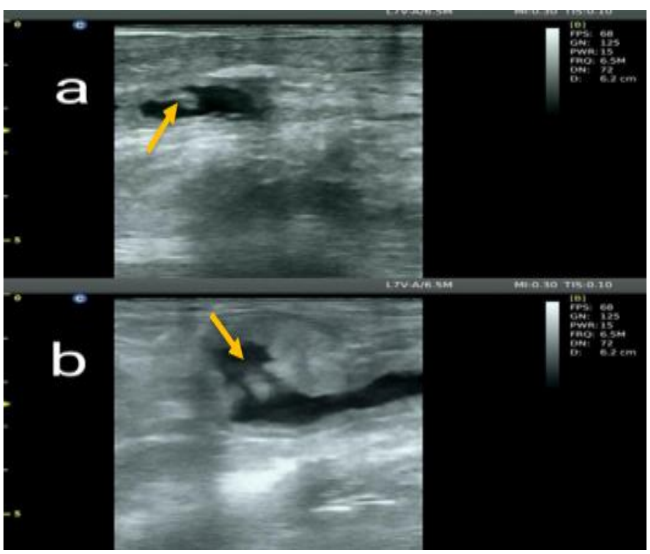

Figure 2: Ultrasonogram at 30-35 days post-TAI showing (a) 32-day-old embryo (arrow). (b) 35-day-old embryo (arrow).

Table 2: The first-service conception rates (FSCRs) at 30-35 days post-TAI of study groups

Groups	NO.	FSCR	
		Frequency	Percentage
Ovsynch	20	6	30% [b]
GPPG	20	8	40% [ab]
PG+G	20	13	65% [a]
Total	60	27	45%*

Values in the same column with superscripts, [ab] differed from Ovsynch (Control): Chi-square ([a b]=P < 0.05). * FSCR tends to differ significantly (45%, P=0.07).

Discussion

It is common knowledge that the calving interval is an essential determinant of reproductive performance in dairy farms. To attain a typical calving interval of 365-400 days, it is necessary to maintain a range of 85 to 115 open days (21,22). In the present study, all TAI protocols resulted in typical calving intervals and days open; additionally, there were no significant differences between the groups. This study showed that cows in any groups subjected to TAI and became pregnant had calving intervals and days open within the optimal values. Additionally, the differences between study groups were statistically insignificant.

The current study was parallel with a recent study by Kutlu and Dínç (23), who reported that cows treated with two different presynchronization protocols G6G vs. PG-3-G had similar values of calving interval 334.1 and 333.7, respectively and days open 54.1 vs. 53.7, respectively. However, another study Hussien (24) reported longer days open for cows subjected to the Ovsynch protocol than the Presynch protocol 167.3 vs. 117.43, respectively. As seen by various authors, these values could be related to differences in the time of treatment beginning at the postpartum period and the number of services per conception.

Multiple studies have reported that Ovsynch hormones GnRH and $PGF_{2\alpha}$ can treat cows suffering from postpartum reproductive disorders, such as cows with inactive ovaries or persistent CL (25,26). Also, the ability effects of the Ovsynch protocol to allow TAI at any time post-calving with high insemination rates may reach 100%. As a result, reduce days to first service and decrease days open and calving interval (9). We hypothesize that the management strategy that included Ovsynch and its modification protocols would shorten the pregnancy and improve the postpartum reproductive performance in local dairy cows.

Several studies have reported that providing an optimum P4 environment during the preovulatory follicle growth is essential for fertility (27). A study using a dataset with 6144 cows evaluated P4 concentration at 1st GnRH. The results showed that cows having intermediate P4 levels (> 0.5 to < 7 ng/ml) at 1st GnRH (d0) had the greatest pregnancy per AI (28). Therefore, the ideal conditions for starting an Ovsynch protocol are cows having a functional CL with P4 (≥ 3 ng/ml) or intermediate P4 concentration with follicle size 12-19 mm that positively impacts ovulation to the 1st GnRH (6,29). The inducing ovulation of the dominant follicle results in the emergence of a new follicular wave, and a new CL will be formed on (d7) that is associated with an increasing P4 level during the growth of the preovulatory follicle, which has a positive effect on oocyte quality and, subsequently, fertility outcomes (13).

Control cows that received Ovsynch at random stages of the oestrus cycle had the lowest FSCR, as observed in this study. Similar findings have been reported by other studies following the Ovsynch protocol (30,31), which observed that FSCR was 35%, 30.6%, and 30%, respectively. These could be related to the initiation of the treatment randomly at the oestrus cycle, increasing the chance of the failure of the first GnRH (d0) to induce ovulation, as well as decreased luteolysis rate (d7), leading to poor synchronization of ovulation near TAI (32). Furthermore, the single dose of $PGF_{2\alpha}$ (d7) from the Ovsynch protocol cannot cause complete luteal regression in cows with young CL that do not respond, resulting in a small amount of P4 close to TAI (12).

In contrast, studies have observed a higher FSCR of 40% and 44%, respectively, as described to be in constituent with our observation (33,34). However, a recent study has reported that the pregnancy rate was 21.7% following an Ovsynch protocol, which is lower than our observation (5). The differences in FSCR seen by various authors with the Ovsynch protocol could be related to differences in responsiveness to the first GnRH injection and the animal's cyclicity status at the beginning of treatment (34). This discrepancy in Ovsynch conception rates could be attributed to the fact that the first GnRH may have triggered the growth of a new wave of follicles. Moreover, incomplete CL regression, defined as plasma P4 concentrations > 0.5 ng/ml at TAI, results in reduced pregnancy rate due to the negative effects of residual P4 on ovulation at the end of a TAI protocol and on gamete transport, hampering fertilization efficiency leads to decreases in fertility (28).

In the present study, incorporating a second $PGF_{2\alpha}$ 24-h interval as part of the GPPG protocol had a 10% improvement in FSCR at 30-35 days post-TAI compared to the Ovsynch protocol. These results attributed to extended luteolytic effect over an extra 24 hours improved luteolysis rates, reduced P4 secretion, eliminated the negative effects of P4, and ensured a higher synchronization rate at the end of the protocol. Similarly, another study also showed that two doses of $PGF_{2\alpha}$ treatment-24h apart increased the FSCR at 30 days following TAI compared to a single $PGF_{2\alpha}$ treatment during Ovsynch protocol, but these increases were (P>0.05) insignificantly (35,36). Previous studies have supported that the idea of the additional $PGF_{2\alpha}$ dose at (d8) will induce more an adequate CL regression if a young CL is present on the (d7) protocol, as shown 90-100% of cows receiving the second dose of $PGF_{2\alpha}$-24h had complete luteolysis (37,38).

Notably, the highest FSCR in the current study was obtained after applying the PG+G presynchronization protocol. This may be because presynchronization strategies change the cow's oestrus cycle to early diestrus (especially days 5-7), which is considered the best time to use the Ovsynch protocol (39). Our results align with those of other research that has employed the PG+G presynchronization technique and showed 50% and 57.5%, respectively (40,41). Moreover, the results of the present study showed that the FSCR significantly differed between groups. The group PG+G reported the highest rate of FSCR compared to the control. However, the difference was not significant when compared to the GPPG group.

This result agrees with a recent local study Alsuwaidawi (42) that compared PG+G as a presynchronization regimen to the Ovsynch protocol at random stage of the oestrus cycle. This result also supported by an Indian study in which the FSCR following PG+G protocol was higher than Ovsynch protocol (45.2 vs. 29.6%, respectively) as mentioned by Saini *et al.* (43). Furthermore, another study Cenariu (41) who reported that presynchronization treatments with PG+G protocol allowed an improved pregnancy rate (by 15%) at 35 days post-TAI compared to the GPPG protocol (57.5 vs. 42.5%), respectively.

This improvement in PG+G FSCR could be attributed to the fact that the first GnRH may have triggered the growth of a new wave of follicles because GnRH induces the release of gonadotrophin hormones, which are essential for growth, maturation, and ovulation (44). In addition, studies have reported that ovulatory response following the first GnRH (d0) in the PG+G group was 73.8% to 90.6% (45,46). Their results indicate that the PG+G treatment successfully synchronized the oestrus cycle into days 5-7 because, according to other previous studies, the ovulated response to the first GnRH during days 5, 6, and 7 of the oestrus cycle was 67%, 94%, and 82%, respectively (32). Moreover, another study has evaluated the synchronization rate (cows with complete luteolysis) and size of the dominant follicle diameter at the time of final GnRH, which were significantly higher for cows that received the PG+G presynchronization protocol in comparison to those that received the Ovsynch protocol alone (43).

Conclusion

This study concluded that TAI protocols resulted in a calving interval and days open within the optimal values. Although GPPG did not improve FSCR compared to the control group, the PG+G presynchronization protocol improved FSCR over the control group.

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Conflict of interest

The authors assert that they possess no conflicts of interest.

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تأثير ثلاث بروتوكولات مختلفة لمزامنة الإباضة على معدلات الإخصاب بعد التلقيح الأول في الأبقار بعد الولادة

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الخلاصة

أجريت هذه الدراسة في محافظة الأنبار، العراق في حقول خاصة على ٦٠ بقرة حلوب بين اليوم ٥٠ و٦٠ بعد الولادة. هدفت هذه الدراسة تقييم تأثير نظامي مزامنة التبويض على أداء الخصوبة بعد الولادة مقارنةً بنظام مزامنة الإباضة التقليدي. بلغت الجرعات لجميع الأبقار (١٠,٥ ميكروغرام) من هرمون مطلق لموجهة الغدد التناسلية و(٠,١٥٠ ملي غرام) من هرمون البروستاغلاندين عن طريق ثلاث برامج علاجية: (١) نظام مزامنة الإباضة التقليدي (٢٠ بقرة) استخدمت كمجموعة سيطرة هرمون مطلق لموجهة الغدد التناسلية -٧ أيام- هرمون البروستاغلاندين - ٤٨ ساعة- هرمون مطلق لموجهة الغدد التناسلية. (٢) نظام البروستاغلاندين المضاعف (٢٠ بقرة) كما في النظام الأول مع إضافة جرعة ثانية من هرمون البروستاغلاندين بعد ٢٤ ساعة من الجرعة الأولى. (٣) نظام ما قبل مزامنة الإباضة (٢٠ بقرة) يشبه النظام الثاني، ولكن عولجت الأبقار باستخدام هورموني (البروستاغلاندين والمطلق لموجهة الغدد التناسلية) في وقت واحد قبل سبعة أيام من البدا ببروتوكول (٢). جميع الأبقار خضعت للتلقيح الاصطناعي الموقوت بعد ١٦-٢٤ ساعة من حقن أخر جرعة في النظام العلاجي. تم تأكيد الحمل عن طريق الفحص بالموجات فوق الصوتية خلال الفترة ٣٠-٣٥ يومًا بعد التلقيح الاصطناعي. لم يختلف معدل الأيام بين الولادتين والأيام المفتوحة بين المجموعات. من حيث معدل الأخصاب بعد التلقيح الأول للمجموعات ١ و٢ و٣,على التوالي ,٣٠٪ و٤٠٪ و٦٥٪. ,45%) ,P=0.07) وبشكل منفصل، كان الفرق بين المجموعتين ١ و ٣ كبيرا (P=0.02) ؛ ومع ذلك، لم تختلف المجموعة ٢ عن كل من المجموعة ١ (P=0.50) والمجموعة ٣ (P=0.11). في الختام، فإن تنفيذ أنظمة TAI خلال فترة ما بعد الولادة يقلل من فترة بين الولادتين إلى الحدود القياسية. علاوة على ذلك، فإن برنامج ما قبل مزامنة الإباضة يزيد من نسبة الحمل للتلقيح الأول بشكل معنوي مقارنة ببرنامج المزامنة التقليدي.