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Fertility of Postpartum Iraqi Cows Following Timed Artificial Insemination within Ovsynch or Presynch Protocols

Ahmed Ibrahim Alsuwaidawi* and Hani Muneeb Alrawi

Department of Surgery & Obstetrics, College of Veterinary Medicine, University of Fallujah, Iraq.

*Corresponding Author: ahmedalsuwaidawi@gmail.com

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Abstract

This investigation was carried out on privately owned dairy farms situated in Al-Anbar Province, Iraq, extending from June 2022 to March 2023. The objective of our study was to compare the calving interval (CI), days open (DO), and conception rate at first service (CRFS) between timed artificial insemination (TAI) within ovulation synchronisation protocols and AI at estrus detection. Total cows (n = 80) 50–60 days postpartum were randomly divided. (1) AIED group (Control, n = 40) untreated cows were received AI at the first observed estrus according to the a.m. /p.m. rule. (2) OVS group (n = 20) was treated with an classical Ovsynch protocol. (3) The PG+G group (n = 20) underwent presynchronization with PGF2 and GnRH at the same time 7 days later, then underwent an Ovsynch protocol. Pregnancy was confirmed using trans-rectal ultrasonography on day's 30-35 post-insemination. The results of this study show thatmean days of CI and DO for the control group were significantly longer than those in both the OVS group and the PG+G group ($P < 0.01$). Among all groups, the CRFS showed a significant difference (37.5%, $P < 0.05$). Separately, the CRFS for the control group was similar to the OVS group (respectively, 27.5% vs. 30%, $P > 0.05$). Whereas, the PG+G group had a 65% CRFS, which was significantly higher than the control ($P < 0.01$) and OVS ($P < 0.05$). In summary, ovulation synchronization protocols enable TAI earlier in the postpartum period, eliminating the need for estrus detection and resulting in shorter CI and DO. Additionally, the PG+G protocol increased the CRFS compared to both the OVS and the control groups.

Keywards: Conception Rate, Dairy Fertility, Ovsynch. Timed AI.

الخلاصة

أجريت هذه الدراسة في محافظة الأنبار، العراق وفي حقول ابقار خاصة، للفترة من حزيران 2022 لغاية اذار 2023. وهدفت الى مقارنة الفترة بين الولادتين (CI)، والأيام المفتوحة (DO)، ومعدلات الحمل بعد التلقيحة الأولى (CRFS) في الأبقار المحلية بعد التلقيح الاصطناعي الموقوت (TAI) ضمن بروتوكولات مزامنة الإباضة. شملت الدراسة 80 بقرة كانت ما بين 50-60 يومًا بعد الولادة، قسمت عشوائيا إلى ثلاث مجموعات. المجموعة الاولى (مجموعة السيطرة) شملت 40 بقرة غير معالجة لقحت اصطناعيًا عند أول شبق تمت ملاحظته بعد الولادة وفقا للقاعدة التقليدية "صباحا/ مساء". المجموعة الثانية شملت 20 بقرة عولجت هذه الأبقار ببروتوكول Ovsynch التقليدي وكالآتي [GnRH؛ 7 أيام؛ PGF2α؛ 48 ساعة؛ GnRH؛ 16-24 ساعة؛ TAI]. المجموعة الثالثة شملت 20 بقرة عولجت ببروتوكول PG+G و يتضمن علاج ما قبل المزامنة باستعمال $PGF_{2\alpha}$ و GnRH في آنٍ واحد, وبفاصل زمني 7 أيام قبل ان يبدا بروتوكول Ovsynch. بعد 30-35 يوما من التلقيح ولغرض تشخيص الحمل، فحصت الابقار عبر المستقيم بواسطة جهاز الموجات فوق الصوتية. اظهرت نتائج هذه الدراسة ان متوسط الفترة بين الولادتين والايام المفتوحة لمجموعة السيطرة بلغ 421 و 141 يومًا على التوالي، وكان الفرق معنويا ($P=0.001$) مقارنة بكل من المجموعة الثانية (343 و 63 يومًا على التوالي) والمجموعة الثالثة (348 و68 يومًا على التوالي). وبينت النتائج ايضا وجود فرق معنوي عال ($P=0.013$, 37.5%) بما يخص معدلات الحمل بعد التلقيحة الاولى للمجاميع الثلاثة. واظهرت النتائج ايضا عدم وجود فرق معنوي بين مجموعة السيطرة و المجموعة الثانية ($P=0.83$) (27.5٪ مقابل 30٪، على التوالي) ؛ وان معدلات الحمل بعد التلقيحة الاولى للمجموعة الثالثة اعلى بكثير مقارنة بكل من مجموعة السيطرة ($P=0.005$) (65٪ مقابل 27.5٪، على التوالي) ومجموعة الأبقار الثانية ($P = 0.027$) (65٪ مقابل 30٪, على التوالي). وخلصت هذه الدراسة، بان بروتوكولات مزامنة الإباضة تعمل على تمكين التلقيح الصناعي الموقوت TAI في وقت مبكر بعد الولادة، مما يلغي الحاجة إلى ضرورة اكتشاف الشبق, ويقلل من الفترة بين الولادتين والأيام المفتوحة. بالإضافة على ذلك, فان بروتوكول PG + G زاد من معدلات الخصوبة بشكل كبير مقارنة بكل من بروتوكول Ovsynch التقليدي ومجموعة السيطرة.

Introduction

In a dairy herd, estrus detection (ED) is essential for excellent reproductive performance. Because inaccurate ED can result in delayed insemination, which can subsequently lead to decreased conception rate (CR) and pregnancy rate (PR), as well as prolonged intervals between calving (1, 2). Particularly in dairy farms that employ AI techniques that depend on visual observation as a method for ED, which is a traditional and time-consuming method for detecting cows in heat (3, 4). Therefore, over the last 40 years, hormonal programmes has been developed that control the oestrus cycle and allow for TAI. These programmes have been added to reproductive management to reduce the number of oestrus cows that could be inseminated and become pregnant without being noticed. This makes oestrus detection unnecessary and saves staff time (5). One such strategy is the Ovsynch protocol, which uses a combination of gonadotropin-releasing hormones (GnRH) and prostaglandin F2 alpha (PGF2α) to induce ovulation and synchronise follicular growth and luteolysis, followed by TAI without oestrus signs (6). However, previous studies have shown that starting Ovsynch at a random point in the oestrus cycle leads to a poor ovulatory response to the first GnRH treatment. Treated cows could ovulate soon after, which would mean that the corpus luteum (CL) did not destruct enough because a new, young CL formed that did not respond to a single dose of PGF2α (day 7) of Ovsynch (7, 8, 9). As a result, there was poor synchronisation of ovulation on the day of TAI and, consequently, decreased fertility outcomes (10, 11).

Studies suggested using the presynchronization treatment before the Ovsynch protocol and starting the protocol in the early diestrus, when the ovary responds better to the first GnRH injection (12, 13). Other authors reported that a second injection of PGF2α 24 hours apart during the Ovsynch protocol is able to increase luteolysis and fertility outcomes compared with one injection of PGF2α (14, 15, 16). According to Yousuf et al. (17), who reported the simplest protocols based on presynchronization treatment with PGF2α and GnRH (PG+G) both injected at the same time a week before the first GnRH treatment (d0) of the Ovsynch protocol, Furthermore, to improve luteolysis, a second PGF2α on d8 was recommended through these protocols (18).

The aim of this study was to compare the calving interval, days open and conception rate at first service between Ovsynch, Presynch protocols and AI at estrus detection.

Materials and Methods

Experimental animals and design

This research was carried out on private dairy farms in Al-Anbar Province, Iraq, from June 2022 to March 2023. Cows were housed in free-stall barns and were 50–60 days postpartum when the study began, with a calving rate of about 1-6 per cow and a weight range of 300–400 kg. A total of 80 local dairy cows were divided into three groups (Figure 1). AIED group (n = 40) of control cows had no treatment and received AI at the first ED according to the traditional breeding rule (a.m./p.m.). OVS group (n = 20): Cows were subjected to a conventional Ovsynch protocol [1st GnRH: 7 days; PGF2α: 48 hours; 2nd GnRH: TAI: 16–24 hours later]. PG+G group (n = 20): Cows will get a presynchronization treatment with PGF2α and GnRH (PG+G) simultaneously, 7 days before starting an Ovsynch protocol with double PGF2α treatment 24 hours apart [PG+G; 7 days; 1st GnRH; 7 days; 1st PGF2α; 24 hours; 2nd PGF2α; 24 hours; 2nd GnRH; TAI 16–24 hours later]. In both protocols, hormonal treatments consist of GnRH (10.5 µg of Busereline acetate, 2 mL, Over, Argentina) and

PGF2α (0.150 mg of d-cloprostenol, 2 mL, Invesa, Spain).

Reproductive Parameters

Reproductive Parameters were calculated by collecting data on previous calving dates, treatment initiation dates, insemination dates, pregnancy diagnosis dates, and calving dates during the study, including: Conception Rate At First Service (CRFS): all pregnant cows after the first AI / all inseminated cows x 100 (19). Days open (DO): the interval between the calving and the first service date resulting in a confirmed pregnancy. Calving interval (CI): the number of days between the calving and the next calving. In this study, we calculated the CI as follows: CI day = DO (mean) + gestation period (280 ± 3 days), as reported by Gangwer et al. (20).

Pregnancy Diagnosis

At 30–35 days after AI, the pregnancy confirmed using trans-rectal ultrasound with a 7.5-MHz probe (China, CHISON Eco2). The presence of anechoic uterine fluid with mature CL or an embryo with a heartbeat could confirm pregnancy (Figure 2). Cows that return to oestrus just before the time of pregnancy diagnosis were considered negative cases.

Statistical Analysis

The Statistical Analysis System (SAS, 2018) program was used to detect the effect of different factors on study parameters. The chi-square test was used to evaluate differences in CR at 5% and 1% levels of significance (14, 21, and 22). The least significant difference (LSD) test (Analysis of Variation, ANOVA) was used to significantly compare between means (23).

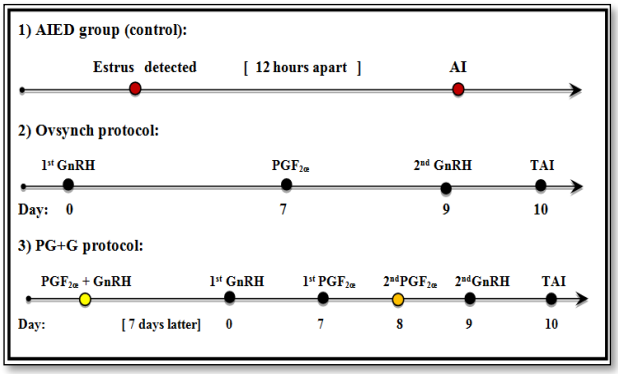

Figure 1: The control group received AI 12 hours after ED. The OVS group was treated with the Ovsynch protocol. The PG+G group received the Presynch protocol. Cows in both treatment groups received TAI 16–24 hours after the last GnRH injection.

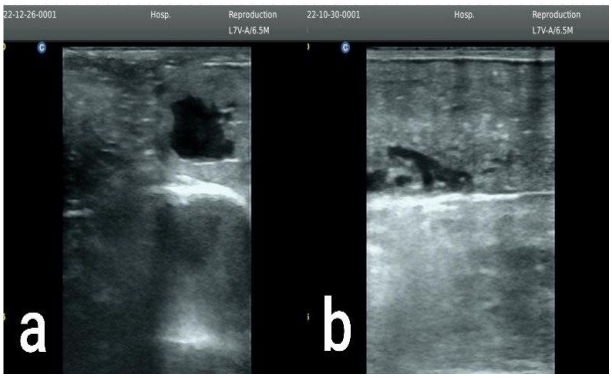

Figure 2: Ultrasonogram of the uterus indicates presence of pregnancy. a) Fluid (arrow) in the uterine lumen at day 30 post-insemination. b) Embryo (arrow) of 33 day-old as confirmed with B-mode.

Results and Discussion

Calving interval and Days open

In our study, the mean days of CI and DO were 421 ± 12 and 141 ± 12 , respectively, for control cows that received AI at their first ED postpartum (Table 1). This observation was comparable to another study finding that looked at data records from 150 Holstein herds in Tunisia and found that the average CI and DO were 444.2 and 150.9 days, respectively, (24). However, our finding was greater than the

standard values of CI and DO (> 400 and > 85 days, respectively) as reported by Rhodes et al. (25) and Stevenson and Britt (26). Although our observation was less than 470 and 228 mean days of CI and DO, respectively, reported for pure Holstein dairy cows in Ethiopia (27). The low rates of oestrus detection in dairy herds may have contributed to these results. The detection of oestrus is an essential factor that significantly affects the reproductive efficacy of dairy cattle, particularly in farming environments that employ AI techniques (4). Inaccurate ED can result in delayed insemination, which can subsequently lead to decreased CR and PR as well as prolonged intervals between calving to conception and calving to calving (2). Failure to identify oestrus is a significant problem contributing to expensive delays in the breeding of cattle in dairy herds, where cows exhibit shorter durations and fewer indications of oestrus and are frequently below 50% (28).This could be due to a number of physiological disorders like true anoestrus, anovulation, and metabolic disorders, as well as high milk production, which is linked to feed shortages and a negative energy balance, which suppresses pulsatile LH secretion and reduces ovarian responsiveness to LH stimulation, causing a delay in ovulation (29, 30, 31, 32, 33).

In the current study, the mean days of CI and DO for cows received TAI during OVS and PG+G protocols were (343 ± 2.8 and 63 ± 2.8) and (348 ± 1.9 and 68 ± 2), respectively (Table 1). The differences between TAI groups were not significant (P > 0.05). According to Kutlu and Dinç (34) was reported that the same findings were shown with G6G protocols, which did not differ significantly (P > 0.05) from PG-3-G presynchronization protocols in terms of mean days CI (334.1 vs. 333.7) and DO (54.1 vs. 53.7). On the other hand, these groups had significantly (P < 0.01) shorter mean days of CI and DO as compared with the control group. This result is in agreement with several previous studies (35, 36, 37) that demonstrated the ability of the Ovsynch protocol and its modifications to allow TAI at any time post-calving with better insemination rates may reached 100%, render heat detection unnecessary, and reduce days to first AI, DO, and CI as a result. Further, the Ovsynch protocol can also be used to treat cows suffering from postpartum reproductive disorders like anoestrus, anovulation, ovarian cysts, and heat stress, in addition to routine cycle synchronisation. (38, 12, and 34).

Table 1: CI and DO Following Timed AI within Ovsynch or Presynch Protocols in Iraqi cows

Groups / Item	Control (N=40)	OVS (N=20)	PG+G (N=20)
CI (mean± SE)	421 ± 12 a	343 ± 2.8 b	348 ± 1.9 b
DO (mean± SE)	141 ± 12 a	63 ± 2.8 b	68 ± 2 b

The values marked by different letters (a, b) show a statistically significant difference (P ≤ 0.01).

Conception Rate At First Service (CRFS)

In current study, control cows were had a 27.5% (11/40) CRFS on days 30–35 post-AI at the first ED postpartum (Figure 3). This finding were similar to other studies (16, 39) who observed CRFS at ED as found to be 32% and 30%, respectively. In contrast, our result were lower than 83% CRFS observed at PGF2α-induced oestrus (40). Also, a study by Cordoba and Fricke (36) found a higher CR of 45.6%, used AI based on Heat Watch as an advanced tool for the detection of oestrus. However, previous

study by Keister et al. (41) have been reported 15.9% pregnancy rate as described to be lower than our observation.

In our study, the cows in OVS group had a 30% (6/20) CRFS. The previous CRs findings was around our result (15, 42), which observed CRs a 30.6% and 30%, respectively, However, our observation following OVS protocol were lower than (43, 44) was reported of 46.8% and 48.6%, respectively, at 31 days post-AI. On the other hand, cows were treated with PG+G presynchronization protocol had a 65% (13/20) CRFS. This result was consistent with previous studies (17, 45), who reported percentages of 50% and 58%, respectively. But our observations were a little bit higher than those of previous studies (18, 22), which reported rates of 43% and 45.2%, respectively.

The CRFS for control cows that received an AI at first ED was similar to OVS cows that received TAI within the Ovsynch protocol (27.5% vs. 30%, $P \geq 0.05$) respectively. Our finding aligns with previous studies (43, 46), in which it was reported that CRFS did not differ significantly ($P \geq 0.05$) between those who had AI at ED and those who received TAI during the Ovsynch protocol. In contrast, Cordoba and Fricke (36) reported significantly better fertility ($P < 0.01$) for cows receiving AI after ED using Tail Paint, achieving higher CR compared to the Ovsynch protocol (47.3% vs. 27.3%), respectively.

On the other hand, TAI following the PG+G presynchronization protocol resulted in higher CRFS than both the control group (respectively, 65% vs. 27.5%, $P \leq 0.01$) and the OVS group (respectively, 65% vs. 30%, $P \leq 0.05$). Similar findings were reported by a recent study (47), who found that cows treated with PG+G had a higher CRFS ($P < 0.01$) than those detected in heat during presynchronization treatment just before the 1st GnRH (d0) of the Ovsynch protocol (44.6% vs. 32.8%). Additionally, Saini et al. (22) found that cows treated with PG+G as a presynchronization regimen had a higher CRFS ($P < 0.05$) than cows receiving only the Ovsynch protocol at random times of the oestrus cycle (45.2% vs. 29.6%).

These results suggest that low rates of detecting oestrus in the control group are a major cause of lower fertility in dairy herds, especially when AI is based solely on visual observation (48). This means that cows are less likely to submit to the first AI, which leads to a lower CR and PR per AI (49). The OVS group, On the other hand, where cows were received the Ovsynch/TAI, the CRFS did not show improvement, which may be attributed to certain physiological limitations associated with initiating the treatment at random stages of the oestrus cycle that can negatively impact the synchronized emergence of a new follicular wave, which is important to prevent the development of persistent follicles. Prolonged dominance of these follicles can lead to decreased oocyte quality and early embryonic development cessation, which can negatively impact fertility in lactating dairy cows (50).

The ovulatory response to the first GnRH (d0) of the Ovsynch protocol is associated with the concentration of circulating progesterone (P4) at this time. Previous reports showed that in ideal conditions for starting an Ovsynch protocol, cows would have CL with intermediate P4 (> 0.5 to < 7 ng/ml) and intermediate follicle size (12–19 mm) that positively impacts ovulation to the 1st GnRH and subsequently improves pregnancy outcomes following TAI protocols in multiple studies (48, 13). Additionally, incomplete luteolysis following single PGF2α treatment at (d7) of the Ovsynch protocol due to the formation of young CL, which is responsive to single PGF2α, leads to the presence of even small amounts of circulating P4 near TAI, which often have negative effects on ovulation

at the end of a TAI protocol and dramatically reduce fertility (51, 52).

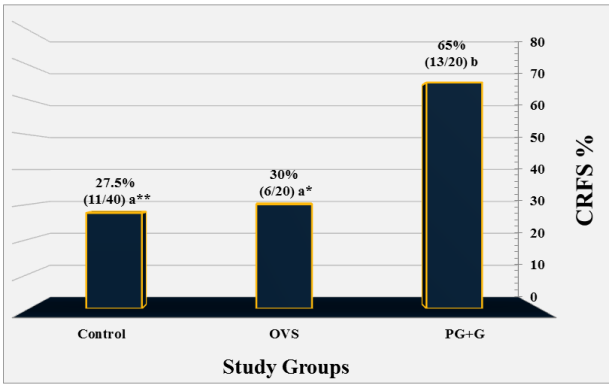

Figure 3: Percentage of conception rates at first service (CRFS) following Timed AI within Ovsynch or Presynch Protocols in Iraqi cows. The values marked by different letters (a, b) show a statistically significant difference **($P \leq 0.01$) and *($P \leq 0.05$).

Our results for cows that received PG+G/TAI could be related to the ability of Pre- PGF2α and GnRH treatments to cause luteolysis in cyclic cows and stimulate rebound ovaries in postpartum cows, as well as cause ovulation in anovular cows or those in the follicular phase of their cycle, since most of these cows would have started an oestrus cycle in the 7 days before starting the Ovsynch protocol (17, 18). According to previous studies, 5-7 days of the oestrus cycle provide the ideal conditions for starting an Ovsynch protocol with good synchronization rates, as mentioned above. Moreover, the PG+G protocol had a second PGF2α treatment on (d8) of Ovsynch, which could increase the proportion of cows with complete luteolysis. According to other studies (53, 46, 47), 90–97% of treated cows had complete luteolysis after double PGF2α doses 24 h apart (on days 7 and 8 of Ovsynch), which was confirmed by a decrease in P4 concentration (P4 > 0.5 ng/ml) at day final GnRH (d 9) of Ovsynch. Thus, the previous reports could provide logical explanations for the high fertility outcomes observed in the PG+G protocol compared to classical Ovsynch protocol.

Conclusion

This study concluded that both Ovsynch and PG+G protocols resulted in shorter intervals from the first day of breeding to conception in comparison to the control group. Although Ovsynch did not improve CRFS compared to the control group, this synchronisation protocol effectively manages AI in lactating dairy cows without requiring oestrus detection. In addition, PG+G presynchronization protocol improved CRFS over Ovsynch protocol ($P = 0.027$) and control group ($P = 0.005$).

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Palmer MA, Olmos G, Boyle LA, Mee JF. Oestrus detection and oestrus characteristics in housed and pastured Holstein–Friesian cows. Theriogenology [Internet]. 2010 Jul 1;74(2):255–64. Available from: https://doi.org/10.1016/j.theriogenology.2010.02.009

2. Mebratu B. Embryo transfer in Cattle Production and its principle and applications. International Journal of Pharmacy & Biomedical Research [Internet]. 2020 Feb 28;7(1):40–54. Available from:

http://dx.doi.org/10.18782/2394-3726.1083

3. Firk R, Stamer E, Junge W, Krieter J. Automation of ooestrus detection in dairy cows: a review. Livestock Production Science [Internet]. 2002 Jul 1;75(3):219–32. Available from: https://doi.org/10.1016/S0301-6226(01)00323-2

4. Reith S, Hoy S. Review: Behavioral signs of oestrus and the potential of fully automated systems for detection of oestrus in dairy cattle. Animal [Internet]. 2018 Jan 1;12(2):398–407. Available from: https://doi.org/10.1017/s1751731117001975

5. Moskáľová L, Pošivák J. Synchronization of ovulation and timed insemination in lactating dairy cattle. Folia Veterinaria [Internet]. 2023 Mar 1;67(1):91–7. Available from: https://doi.org/10.2478/fv-2023-0010

6. Pursley J, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology [Internet]. 1995 Nov 1;44(7):915–23. Available from: https://doi.org/10.1016/0093-691x(95)00279-h

7. Brusveen DJ, Souza AG, Wiltbank MC. Effects of additional prostaglandin F2α and estradiol-17β during Ovsynch in lactating dairy cows. Journal of Dairy Science [Internet]. 2009 Apr 1;92(4):1412–22. Available from: https://doi.org/10.3168/jds.2008-1289

8. Dewey ST, Mendonça LGD, Lopes G, Rivera F, Guagnini F, Chebel RC, et al. Resynchronization strategies to improve fertility in lactating dairy cows utilizing a presynchronization injection of GnRH or supplemental progesterone: I. Pregnancy rates and ovarian responses. Journal of Dairy Science [Internet]. 2010 Sep 1;93(9):4086–95. Available from: https://doi.org/10.3168/jds.2010-3233

9. Stevenson JTM. Physiological predictors of ovulation and pregnancy risk in a fixed-time artificial insemination program. Journal of Dairy Science [Internet]. 2016 Dec 1;99(12):10077–92. Available from: https://doi.org/10.3168/jds.2016-11247

10. Pursley JR, Martins JP. Impact of circulating concentrations of progesterone and antral age of the ovulatory follicle on fertility of high-producing lactating dairy cows. Reproduction, Fertility and Development. 2012;24(1):267. DOI: 10.1071/RD11917

11. Carvalho P, Wiltbank MC, Fricke PM. Manipulation of progesterone to increase ovulatory response to the first GnRH treatment of an Ovsynch protocol in lactating dairy cows receiving first timed artificial insemination. Journal of Dairy Science [Internet]. 2015 Dec 1;98(12):8800–13. Available from: https://doi.org/10.3168/jds.2015-9968

12. A N, Baranski W, Baryczka A, Janowski T. OvSynch protocol and its modifications in the reproduction management of dairy cattle herds – an update. Journal of Veterinary Research [Internet]. 2017 Sep 19;61(3):329–36. Available from: https://doi.org/10.1515/jvetres-2017-0043

13. Carvalho P, Santos VJC, Giordano JO, Wiltbank MC, Fricke PM. Development of fertility programs to achieve high 21-day pregnancy rates in high-producing dairy cows. Theriogenology [Internet]. 2018 Jul 1;114:165–72. Available from: https://doi.org/10.1016/j.theriogenology.2018.03.037

14. Wiltbank MC, Baez GM, Cochrane F, Barletta RV, Trayford CR, Joseph RM. Effect of a second treatment with prostaglandin F2α during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. Journal of Dairy Science [Internet]. 2015 Dec 1;98(12):8644–54. Available from:

https://doi.org/10.3168/jds.2015-9353

15. Barletta RV, Carvalho P, Santos VJC, Melo LC, Consentini CEC, Netto AS, et al. Effect of dose and timing of prostaglandin F2α treatments during a Resynch protocol on luteal regression and fertility to timed artificial insemination in lactating Holstein cows. Journal of Dairy Science [Internet]. 2018 Feb 1;101(2):1730–6. Available from: https://doi.org/10.3168/jds.2017-13628

16. Neisari N, Kaveh A, Pooladzadeh P, Hajibemani A, Mirzaeei F. Effect of Treatment with Additional Doses of Prostaglandin F2 Alpha during the Middle Stage of the Ooestrus Cycle on Luteolysis Process and Reproduction Performance in Holstein Dairy Cows. Iranian Journal of Applied Animal Science [Internet]. 2022 Sep 1; 12(3) : 471-478. Available from: https://dorl.net/dor/20.1001.1.2251628.2022.12.3.6.9

17. Yousuf MA, Martins J, Ahmad N, Nobis K, Pursley J. Presynchronization of lactating dairy cows with PGF2α and GnRH simultaneously, 7 days before Ovsynch have similar outcomes compared to G6G. Theriogenology [Internet]. 2016 Oct 1;86(6):1607–14. Available from: https://doi.org/10.1016/j.theriogenology.2016.05.021

18. Martins J, Acevedo MJT, Cunha TM, Piterini C, Pursley J. The effect of presynchronization with prostaglandin F2α and gonadotropin-releasing hormone simultaneously, 7 d before Ovsynch, compared with Presynch-10/Ovsynch on luteal function and first-service pregnancies per artificial insemination. Journal of Dairy Science [Internet]. 2017 Jun 1;100(6):5107–16. Available from: https://doi.org/10.3168/jds.2016-11628

19. Souames S, Berrama Z. Factors affecting conception rate after the first artificial insemination in a private dairy cattle farm in North Algeria. Veterinary World [Internet]. 2020 Jan 1; Available from: https://doi.org/10.14202/vetworld.2020.2608-2611

20. Gangwer M I. 10 important measures : understanding dairy herd performance measurements from the Agri-Tech Analytics DHIA herd total report [Internet]. ScholarsArchive@OSU. 1993. Available from: http://hdl.handle.net/1957/14775

21. Kuru M, Kaçar C, Oral H, Kaya S, Çetin N, Kaya D, et al. Effect of two prostaglandin F2α injections administered 24 hours apart on the pregnancy rate of Simmental cows subjected to the Ovsynch or Ovsynch + Controlled internal drug release (CIDR) protocols. Medycyna Weterynaryjna [Internet]. 2020 Jan 1;76(10):6451–2020. Available from: https://doi.org/10.21521/mw.6451

22. Saini G, Kumar S, Pandey A, Singh H, Virmani M. Presynchronization with simultaneous administration of GnRH and PGF2α 7 days prior to Ovsynch improves reproductive profile in Hariana zebu cow. Research Square (Research Square) [Internet]. 2022 May 31; Available from: https://doi.org/10.21203/rs.3.rs-1612052/v1

23. Behr A, Effa K, Mummed Y, Tadesse M. Reproductive and Productive Performance of Holstein Friesian and Crossbreed Dairy Cattle at Large, Medium and Small Scale Dairy Farms in Ethiopia. Int J Adv Res Biol Sci. [Internet]. 2019 Jan 1;6:15-29. https://www.researchgate.net/publication/335096195

24. M'hamdi N, Aloulou R, Brar SK, Bouallegue M, Hamouda B I. Phenotypic and genetic parameters of reproductive traits in Tunisian Holstein cows. Biotechnology in Animal Husbandry [Internet]. 2010 Jan 1;26(5–6):297–307. Available from: https://doi.org/10.2298/bah1006297m

25. Rhodes FM, McDougall S, Burke CJ, Verkerk GA, Macmillan KL. Invited Review: Treatment of Cows with an Extended Postpartum Anestrous Interval. Journal of Dairy Science [Internet]. 2003 Jun 1;86(6):1876–94. Available from: https://doi.org/10.3168/jds.s0022-0302(03)73775-8

26. Stevenson JT, Britt JH. A 100-Year Review: Practical female reproductive management. Journal of Dairy Science [Internet]. 2017 Dec 1;100(12):10292–313. Available from: https://doi.org/10.3168/jds.2017-12959

27. Mengistu DW, Wondimagegn KA. Evaluation of the reproductive performance of Holstein Friesian dairy cows in Alage ATVET college, Ethiopia. International Journal of Livestock Production [Internet]. 2018 Jun 30;9(6):131–9. Available from: https://doi.org/10.5897/ijlp2018.0469

28. Gordon IR. Reproductive technologies in farm animals [Internet]. CABI Publishing eBooks. 2017. Available from: https://doi.org/10.1079/9781780646022.0000

29. Cardoso F, LeBlanc SJ, Murphy MR, Drackley JK. Prepartum nutritional strategy affects reproductive performance in dairy cows. Journal of Dairy Science [Internet]. 2013 Sep 1;96(9):5859–71. Available from: https://doi.org/10.3168/jds.2013-6759

30. Mimoune N, Messai C R, Khelef D, Salhi O, Azzouz M Y and Kaidi R. Reproductive parameters and metabolic profile of repeat breeder cows. Livestock Research for Rural Development [Internet]. 2017 Aug; 29(8): 5115–26. Available from: http://www.lrrd.org/lrrd29/8/nora29146.html

31. Mengistu DW, Wondimagegn KA. Evaluation of the reproductive performance of Holstein Friesian dairy cows in Alage ATVET college, Ethiopia. International Journal of Livestock Production [Internet]. 2018 Jun 30;9(6):131–9. Available from: https://doi.org/10.5897/ijlp2018.0469

32. Szenci O. Importance of monitoring the peripartal period to increase reproductive performance in dairy cattle [Internet]. 2018. Available from: https://hrcak.srce.hr/223178

33. Garcia-Ispierto I, De Rensis F, Pérez-Salas JA, Nunes JM, Pradés B, Serrano-Pérez B, et al. The GnRH analogue dephereline given in a fixed-time AI protocol improves ovulation and embryo survival in dairy cows. Research in Veterinary Science [Internet]. 2019 Feb 1;122:170–4. Available from: https://doi.org/10.1016/j.rvsc.2018.11.020

34. Kutlu M, Dinç DA. Comparison of the effects of two pre-synchronization protocols (G6G and PG-3-G) on some reproductive performance parameters in Holstein cows. Eurasian Journal of Veterinary Sciences [Internet]. 2020 Dec 4;36(4):248–54. Available from: https://doi.org/10.15312/eurasianjvetsci.2020.305

35. Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized oestrus. Journal of Dairy Science. 1997;80(2):295–300. doi:10.3168/jds.s0022-0302(97)75937-x

36. Cordoba MC, Fricke PM. Initiation of the breeding season in a Grazing-Based dairy by synchronization of ovulation. Journal of Dairy Science [Internet]. 2002 Jul 1;85(7):1752–63. Available from: https://doi.org/10.3168/jds.s0022-0302(02)74249-5

37. Akoz M, Ibrahim A, Ali DD. Efficacy of the presynch-ovsynch program on some

reproductive parameters in postpartum dairy cows. Acta Veterinaria-beograd [Internet]. 2008 Jan 1; Available from: https://doi.org/10.2298/avb0806477a

38. Gundling N, Drews SJ, Hoedemaker M. Comparison of two different programmes of ovulation synchronization in the treatment of ovarian cysts in dairy cows. Reproduction in Domestic Animals [Internet]. 2009 Mar 11;50(6):893–900. Available from: https://doi.org/10.1111/j.1439-0531.2009.01342.x

39. Eidan SM, Al-Jashami AS. Effect of ovulation synchronization with Ovsynch program on reproductive performance and blood parameters in Holstein cows. Al-Anbar J Vet Sci. 2016;9(2):31-44. https://www.iasj.net/iasj/article/120940

40. Majeed, A. F., & Alhiti, A. S. A. Use of prostaglandin PGF2α for treatment of persistent corpus luteum in dairy cattle. Al-Anbar J Vet Sci. 2016;9(1):31-44

41. Keister ZO, Denise SK, Armstrong DS, Ax RL, Brown MA. Pregnancy outcomes in two commercial dairy herds following hormonal scheduling programs. Theriogenology [Internet]. 1999 Jun 1; Available from: https://doi.org/10.1016/s0093-691x(99)00101-6

42. Hussien A, Sharawy H, Lenis YY, James D, Turna O, Risha E, et al. The impact of different oestrus synchronization programs on postpartum Holstein dairy cow reproductive performance. Journal of Veterinary Medical Researches (Print) [Internet]. 2021 Sep 1;22(3):124–30. Available from: https://doi.org/10.21608/mvmj.2021.93141.1078

43. Gümen A, Keskin A, Yilmazbas-Mecitoglu G, Karakaya E, Alkan A, Okut H, et al. Effect of presynchronization strategy before Ovsynch on fertility at first service in lactating dairy cows. Theriogenology [Internet]. 2012 Nov 1;78(8):1830–8. Available from: https://doi.org/10.1016/j.theriogenology.2012.07.021

44. Yilmazbas-Mecitoglu G, Karakaya E, Keskin A, Gümen A, Koc V, Okut H. Comparison of synchronisation and fertility after different modifications of the ovsynch protocol in cyclic dairy cows. Acta Veterinaria Hungarica [Internet]. 2014 Mar 1; Available from: https://doi.org/10.1556/avet.2013.045

45. CENARIU M, PALL E, BORZAN M, CHIOREAN R, GROZA IS. Fertility improvement in lactating dairy cows using a presynchronization protocol before OVSYNCH [Internet]. Scientific Works. Series C. Veterinary Medicine; 2020 [cited 2023 Jul 16]. Available from: https://veterinarymedicinejournal.usamv.ro/index.php/scientific-papers/19-vol-2019-issue-1/1689-fertility-improvement-in-lactating-dairy-cows-using-a-presynchronization-protocol-before-ovsynch

46. McDougall S. Effects of treatment of anestrous dairy cows with gonadotropin-releasing hormone, prostaglandin, and progesterone. Journal of Dairy Science [Internet]. 2010 May 1;93(5):1944–59. Available from: https://doi.org/10.3168/jds.2009-2305

47. Martins JPN, Cunha TO, Martinez W, Schmitt JS. Presynchronization with prostaglandin F2α and gonadotropin-releasing hormone simultaneously improved first-service pregnancy per artificial insemination in lactating Holstein cows compared with Presynch-14 when combined with detection of oestrus. Journal of Dairy Science [Internet]. 2023 Jul 1;106(7):5115–26. Available from: https://doi.org/10.3168/jds.2022-22651

48. Bisinotto R, Ribeiro ES, Santos JEP.

Synchronisation of ovulation for management of reproduction in dairy cows. Animal [Internet]. 2014 Jan 1;8:151–9. Available from: https://doi.org/10.1017/s1751731114000858

49. Consentini CEC, Wiltbank MC, Sartori R. Factors That Optimize Reproductive Efficiency in Dairy Herds with an Emphasis on Timed Artificial Insemination Programs. Animals [Internet]. 2021 Jan 25;11(2):301. Available from: https://doi.org/10.3390/ani11020301

50. Monteiro PP, Borsato M, Silva F, Prata AB, Wiltbank MC, Sartori R. Increasing estradiol benzoate, pretreatment with gonadotropin-releasing hormone, and impediments for successful estradiol-based fixed-time artificial insemination protocols in dairy cattle. Journal of Dairy Science [Internet]. 2015 Jun 1;98(6):3826–39. Available from: https://doi.org/10.3168/jds.2014-9040

51. Heidari F, Dirandeh E, Pirsaraei ZA, Colazo MG. Modifications of the G6G timed-AI protocol improved pregnancy per AI and reduced pregnancy loss in lactating dairy cows. Animal [Internet]. 2017 Jan 1;11(11):2002–9. Available from: https://doi.org/10.1017/s1751731117000520

52. Wiltbank MC, Baez GM, Cochrane F, Barletta RV, Trayford CR, Joseph RM. Effect of a second treatment with prostaglandin F2α during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. Journal of Dairy Science [Internet]. 2015 Dec 1;98(12):8644–54. Available from: https://doi.org/10.3168/jds.2015-9353

53. Hubner AM, Peixoto PMG, Hillesheim JC, Canisso IF, Lima FB. Effect of GNRH 7 days before presynchronization with simultaneous PGF2α and GNRH on reproductive outcomes in Holstein dairy cows. Frontiers in Veterinary Science [Internet]. 2020 Oct 22;7. Available from: https://doi.org/10.3389/fvets.2020.574516