Menoufia Veterinary Medical Journal (MVMJ)

Menoufia Veterinary Medical Journal
https://vmmj.journals.ekb.eg

MVMJ. Vol. 1 (1): … September 2025

Menoufia University

Double Ovisynch protocol enhanced the conception rate in Holstein Dairy Cattle

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Key words:
Synchronization, Dairy cows, Conception rate.

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Article History
Received: 17 Jully 2025.
Accepted: 9 Aug 2025

ABSTRACT

The study aimed to determine the optimal timed artificial insemination protocol for high-yield Holstein dairy cows, examining the correlation between parity number, season, and post-synchronization fertility. Four oestrous synchronization protocols were applied: Double Ovisynch, Cider synch, Resynch, and Ovisynch56. During the winter season, the conception rates for mixed parity cows were (50.66%, 39%, 36.33%, and 35%) for Double Ovisynch, Cider synch, Resynch, and Ovisynch56 protocols, respectively. In first lactation cows, the rates were (56%, 46%, 44%, and 37%), and in second lactation season cows, the rates were (46%, 36%, 35%, and 34%). With Double Ovisynch, there were significant differences (p 0.05). During the summer season, the conception rates in mixed parity cows were (36%, 30%, 28%, and 22%) for Double Ovisynch, Cidersynch, Resynch, and Ovisynch56 protocols, respectively. In first lactation cows, the rates were (40%, 37%, 35%, and 33%), and in second lactation cows, the rates were (36%, 26%, 33% and 33%). In conclusion, it was found that the Double Ovisynch protocol was associated with a higher conception rate compared to the other protocols, regardless of parity or season.

1. INTRODUCTION

Timed artificial insemination (TAI) programs and estrous cycle synchronization have become essential strategies in dairy management. By helping farmers attain maximum reproductive performance, these programs have greatly increased the rate of conception. Furthermore, TAI has been found to be an affordable way to control reproduction, particularly in dairy cows with high yields and fewer estrous symptoms [1]. The two primary pharmacological bases for timed artificial insemination (TAI) programs are estradiol (E2) plus progesterone (P4)-based programs and Ovisynch-type programs (based on prostaglandin F2α [PGF]and gonadotropin-releasing hormone [GnRH) [2].

When applied to the entire herd, the Ovisynch technique increases the conception rate; however, it is not effective for cows that have specific reproductive problems [3]. The Ovisynch protocol's first insemination rate (FIR) is approximately 35%, indicating that it is a viable substitute for heat detection [4].

TAI increased the pregnancy rate to 45% 16 hours after the second GnRH injection [5]. The more accurate technique for pre-synchronizing dairy cows' oestrus cycles and causing cyclicity in anovular cows is the Double Ovisynch procedure [6]. Pregnancy rate increased at 33 and 63 days after initial insemination when lactating Holstein cows were subjected to a Double Ovisynch regimen with TAI, which produced 64% and 58% more pregnant cows, respectively [7].

There may be advantages to using a Controlled Internal Drug Release (CIDR) device in GnRH-based treatments. The main advantage of using the CIDR in GnRH-based treatments is that it guarantees progesterone exposure for females from day 1 to day 8 [8]. The CIDR also aids in avoiding the early heats (days 6 to 9) that are frequently linked to these systems. Between days 1 and 9, the CIDR's progesterone efficiently suppresses ovulation and estrus [8].

Non-pregnant cows conceived faster due to the resynchronization program. For herds that use a combination of a Resynch technique and the detection of estrus following the initial timed artificial insemination (TAI), this is a good choice [9]. The Resynch technique, based on ovarian structures, can be used in herds that do not include the detection of estrus following the initial TAI, as explained by [10].

Due to the pituitary gland's inability to release gonadotropin, fertility declines with age. In advanced age, a hormonal imbalance or deficit may result in a decline in the quality of eggs ovulated during subsequent fertilization, which could lead to uterine failure or the loss of embryos or fetuses [11].

Egypt already has summer weather that weather conditions that can put livestock at risk for heat stress [12]. This heat stress negatively affects dairy farms' reproductive performance, leading to increased age at first service, postpartum insemination time, and lowered conception and pregnancy rates. It also increases the occurrence of reproductive disorders like premature abortions, dystocia, retained fetal membranes, and weaker calves [13].

The present study aimed to improve fertility in dairy farms using oestrous synchronization protocols and to study the correlation between parity number, season, and post-synchronization fertility.

2. MATERIALS AND METHODS

2.1 Animals

Holstein dairy cows (lactation seasons 1-5) used in the study belonged to a private dairy farm in Ismailia, Egypt. The study was carried out between July 2022 and April 2023. Every animal was regularly immunized in accordance with a planned vaccination schedule and was found to be free of any infectious or contagious diseases. They received a total mixed ration (TMR) per NRC recommendations. All cows in this experiment were checked rectally, by ultrasonography, and visual examination of their mucous to determine if any cow had abnormalities or endometritis. The voluntary waiting period for postpartum animals was 60 days.

2.2. Experimental design

Four estrous synchronization protocols were utilized in this study: Double Ovisynch, Cidersynch, Resynch, and Ovisynch56. The varying number of groups is due to the farm's reproductive management practices. The Double Ovisynch protocol was applied to a larger group of cows compared to the other protocols, and this choice was based on several methodological and practical considerations. The Double Ovisynch protocol is widely regarded as one of the most effective and reliable fixed-time artificial insemination (FTAI) programs for improving fertility in lactating dairy cows. Numerous studies have shown that it yields higher pregnancy rates compared to other protocols [14]. As a result, it served as the reference or control group in this study, necessitating a larger sample size to ensure accurate statistical comparisons. By allocating more animals to the reference group, we enhance the statistical power of the study and reduce variability in estimating the effect of the Double Ovisynch protocol. This approach is common in experimental designs when one treatment serves as the control or standard for comparison [15].

2.2.1. Double Ovisynch Protocol:

During the winter season, there were 650 cows with mixed parity, 216 cows in their first lactation, and 200 cows in their second lactation. During the summer season, there were 364 cows with mixed parity, 162 cows in their first lactation season, and 117 cows in their second lactation season over the summer. The synchronization protocol started with an injection of 2 mL of GnRH (Gonavet Veyx, 50 µg/mL, Gonadorelin [6-D-Phe], Veyx-Pharma GmbH). Seven days later, a 2 mL injection of PGF2α (PGF Veyx, cloprostenol 0.0875 mg/mL, Veyx-Pharma GmbH) was administered. Seventy-two hours after the PGF2α injection, a second dose of GnRH was given. Seven days later, the Ovsynch 56 protocol was initiated, and all cows were timed for insemination [16].

2.2.2. Cider synch Protocol

During the winter season, there were 86 cows with mixed parity,18 cows in their first lactation season, and 25 cows in their second lactation season. During the summer season, there were 63 cows with mixed parity, 30 cows in their first lactation season, and 13 cows in their second lactation season. All cows received the EAZI-BREED CIDR (which contains 1.38 grams of progesterone in molded silicone over a flexible nylon spine, provided by Zoetis Company) on day zero. A 2 mL injection of GnRH was administered simultaneously. The CIDR was removed after 7 days, followed by an injection of 2 mL of PGF2α. A second dose of GnRH was given 56 hours later, and the cows were inseminated 16 hours later [17].

2.2.3. Resynch Protocol

During the winter season, there were 168 cows with mixed parity, 50 cows in their first lactation season, and 54 cows in their second lactation season. During the summer season, there were 187 cows with mixed parity, 58 cows in their first lactation season, and 77 cows in their second lactation season. Resynchronization began on day 28 following artificial insemination (AI) with a 2 ml injection of GnRH administered to all cows that had been inseminated. On day 35, non-pregnant cows received a dose of 2 ml of PGF2α. A second injection of GnRH was given 56 hours after the PGF2α dose. Timed artificial insemination (TAI) was then performed 16 hours after the final GnRH administration for the non-pregnant cows [18].

2.2.4.Ovisynch 56 protocol:

During the winter, there were 116 cows with mixed parity, 26 cows in their first lactation season, and 48 cows in their second lactation season. During the summer season, there were 91 cows with mixed parity, 34 cows in their first lactation season, and 34 cows in their second lactation season. The cows are given an initial injection of 2 ml of GnRH, followed by another 2 ml of PGF2α after 7 days. A second GnRH injection is administered 56 hours later, with timed insemination taking place 16 hours after this injection [5].

2.3. Mating and pregnancy diagnosis:

Cows were inseminated with frozen-thawed semen from approved sires from the "Worldwide Company, USA," and tested by CASA. According to [19], pregnancy was diagnosed by ultrasonography at about 30- 32 days after AI to establish the conception rate.

2.4 Statistical Analysis:

Version 27 of the SPSS statistical program was used to analyze the data statistically (SPSS, 2020). Differences in conception rates between treatment protocols were also investigated using the chi-square. The chi-square test is used to examine whether there is a statistically significant association between the synchronization protocols. Overall, a P-value of less than 0.05 was considered significant.

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3. RESULTS

The results during the winter season revealed that the conception rates for the protocols in mixed parity Holstein dairy cows were (50.66, 39.00, 36.33, and 35.00) for the Double Ovisynch, Cider synch, Resynch, and Ovisynch56, respectively, as shown in Table 1. After studying the effect of parity on fertility, the conception rates of the synchronization protocols in first lactation season cows were (56%, 46%, 44%, and 37%) for the

Double Ovisynch, Cider synch, Resynch, and Ovisynch56, respectively, as shown in Table 2. In comparison, the conception rates of the protocols in second lactation season cows were (46%, 36%, 35%, and 34%) for the Double Ovisynch, Cider synch, Resynch, and Ovisynch56, respectively, as shown in Table 3. According to the summer results, the protocols' conception rates in mixed-parity Holstein dairy cows were (36%, 30%, 28%, and 22%), respectively, for the Double Ovisynch, Cider synch, Resynch, and Ovisynch56. Studying the effect of parity on fertility and the results of synchronization protocols revealed that the conception rates of the synchronization protocols in first lactation season cows were (40%,37%,35%, and 33%) for the Double Ovisynch, Cider synch, Resynch, and Ovisynch56, respectively, as shown in Table 5. The conception rates of the protocols in second lactation season cows were (36%,26%,33%, and 33%) for the Double Ovisynch, Cider synch, Resynch, and Ovisynch56, respectively, as shown in Table 6.

Table 1: The effect of different synchronizing protocols on the conception rate of Holstein dairy cows with mixed parity during the winter season.

Synchronization protocol	Inseminated Cows	Concepted Cows	Non-Concepted Cows	Conception Rate%	X2	p-value
Double Ovi synch	650	329	321	50.66	3.1405	0.778
Cider synch	86	33	53	39.00		
Resynch	168	61	107	36.33		
Ovisynch56	116	41	75	35.00		

The result is non-significant at $p < 0.05$

Table 2: The effect of different synchronizing protocols on conception rate of Holstein dairy cows in the first lactation season during the winter season.

Synchronization Protocol	Inseminated Cows	Concepted Cows	Non-Concepted Cows	Conception Rate%	X2	P-value
Double Ovisynch	216	121	95	56%	2.78	0.425
Cider synch	18	8	10	46%		
Resynch	50	19	31	37%		
Ovisynch 56	26	11	165	44%		

The result is non-significant at $p < 0.05$

Table 3: The effect of different synchronizing protocols on conception rate of Holstein dairy Cows in the second lactation season during the winter season.

Synchronization Protocol	Inseminated Cows	Concepted Cows	Non-Concepted Cows	Conception Rate%	X2	p-value

Double Ovisynch	**200**	**92**	**108**	**46%**	**3.567**	**0.312**
Cider synch	**25**	**9**	**16**	**36%**		
Resynch	**54**	**18**	**36**	**34%**		
Ovisynch56	**48**	**17**	**31**	**35%**		

The result is non-significant at *p* < 0.05

Table 4: Effect of different synchronizing protocols on conception rate of Holstein dairy cows with mixed parity during the summer season.

Synchronization Protocol	**Inseminated Cows**	**Concepted Cows**	**Non-Concepted Cows**	**Conception Rate%**	**X2**	**P-value**
Double Ovisynch	**364**	**131**	**233**	**36%**	**15.24**	**.001**
Cider synch	**63**	**19**	**44**	**30%**		
Resynch	**187**	**41**	**146**	**22%**		
Ovisych 56	**91**	**25**	**66**	**28%**		

The result is significant at *p* < 0.05

Table 5: Effect of different synchronizing protocols on conception rate of Holstein dairy cows in the first lactation season during the summer season.

Synchronization Protocol	**Inseminated Cows**	**Concepted Cows**	**Non-Concepted Cows**	**Conception Rate%**	**X2**	**P-value**
Double Ovisynch	**162**	**65**	**97**	**40%**	**6.156**	**0.104**
Cider synch	**30**	**11**	**19**	**37%**		
Resynch	**58**	**19**	**42**	**33%**		
Ovisynch 56	**34**	**12**	**22**	**35%**		

The result is non-significant at *p* < 0.05

Table 6: Effect of different synchronizing protocols on conception rate of Holstein dairy cows in the second lactation season during the summer season.

Synchronizatio n	**Inseminated cows**	**Number of Concepted Cows**	**Non-Concepted Cows**	**Conception Rate%**	**X2**	**P-val**

Protocol						**ue**
Double Ovisynch	**117**	**42**	**75**	**36%**	**3.670**	**0.299**
Cider synch	**13**	**3**	**10**	**26%**		
Resynch	**77**	**25**	**52**	**33%**		
Ovisynch56	**34**	**11**	**23**	**33%**		

The result is nonsignifict at $p < 0.05$

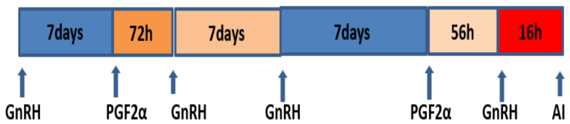

Figure 1: Double Ovisynch in dairy cattle.

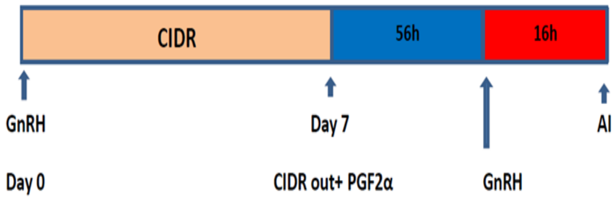

Figure 2: Cider synch protocol in dairy cattle

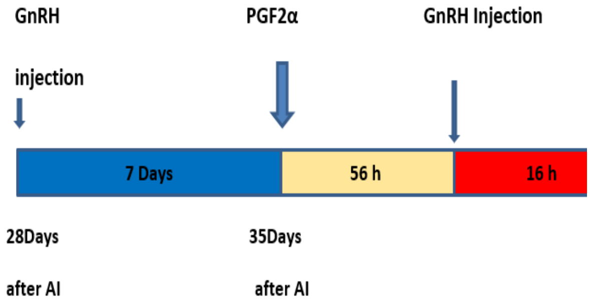

Figure 3: Resynch protocol.

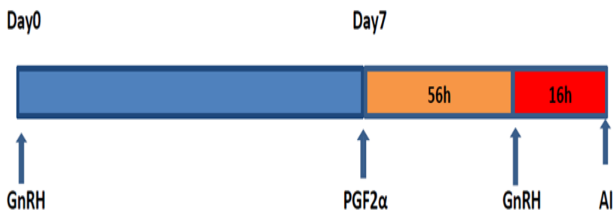

Figure 4: Ovisynch 56

Figure 5: Ultrasonographic image of a 31-day pregnancy in a Holstein dairy cow**.**

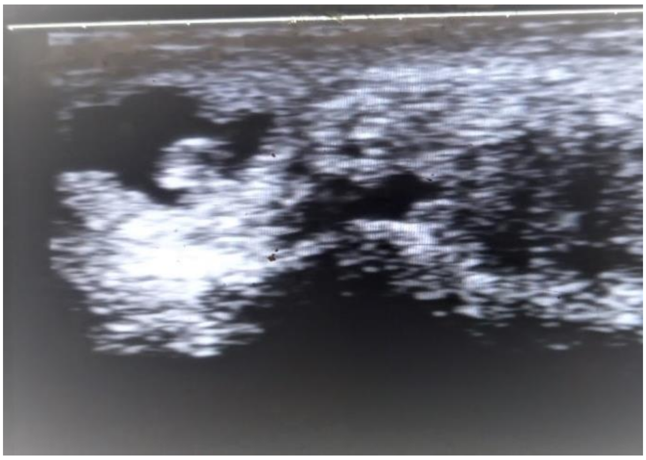

Figure 6: Ultrasonographic image of a 32-day pregnancy in a Holstein dairy cow.

4. DISCUSSION

Estrus synchronization in dairy cows includes reduced time devoted to estrus detection and reduced variability in days from parturition to first service, leading to reduced variability and length of calving intervals within a herd [20].

In the current study, the conception rates during the winter season for various synchronization protocols in mixed parity cows were as follows: 50.66% for Double Ovisynch, 39% for Cider synch, 36.33% for Resynch, and 35% for Ovisynch56. The Double Ovisynch protocol gives higher conception rates compared to other protocols. This finding aligns with Souza *et al*. [21], who demonstrated that the Double Ovisynch protocol enhances synchronization accuracy, follicular wave control, and corpus luteum development, resulting in higher conception rates. The positive effect of the Double Ovisynch protocol may be attributed to its beneficial impact on anovular non-cyclic cows, which is linked to hormonal changes during follicular development. Elevated levels of circulating progesterone (P4) during this phase could decrease LH pulsatility, potentially improving the competency of the dominant follicle and/or the quality of the ovulated oocyte, as noted by [22]. The Ovisynch 56 protocol has demonstrated a lower conception rate compared to other protocols. This aligns with findings from Pursley *et al*. [23], who reported that the first insemination rate (FIR) in the Ovisynch protocol is around 35%. This indicates that it is a viable alternative to relying on heat detection. The lower conception rate may be attributed to issues such as failure to ovulate or inadequate luteolysis after the first ovulation. Conversely, Pursley *et al***.** [24] reported a higher conception rate for the Ovisynch 56 protocol, estimating it at 44%. However, Stevenson *et al*. [25] documented a further decline in conception rates for the Ovisynch protocol, down to 27%. The variations in conception rates observed by different studies regarding the Ovisynch 56 protocol may be linked to differences in the animals' responses to the initial GnRH injection, as well as their cyclicity status at the start of treatment. This discrepancy in conception rates may also be due to the possibility that the first GnRH injection triggered the development of a new [26]. In a comparison between the Cider synch and Ovisynch56 protocols, Cider synch yields a higher conception rate. which agrees with Patel *et al*. [27] who said that in cows treated by Cider synch, it improves and increases fertility rate in the flock, which is due to gonadotropin-releasing hormone, which leads to a simultaneous new follicular wave during removal of CIDR. While Bhoraniya *et al*. [28] reported higher conception rates (66.66% and 33.33%) for Cider synch and Ovisynch56, respectively. The disparity in conception rates between the two authors may be attributed to the environmental conditions and the source of PGF2α and GnRH [29]. There was no significant difference in conception rates between the Ovisynch56 and Resynch protocols. This finding is consistent with the study by Alkar *et al*. [30], which reported that resynchronization with GnRH seven days prior to Ovisynch56 did not improve conception rates. One possible explanation is that cows that do not ovulate in response to the GnRH injection may experience a prolonged dominance of the ovulatory follicle, potentially leading to the production of lower-quality embryos. As a result, the risk of iatrogenic abortion was somewhat reduced with earlier resynchronization, which numerically decreased the median days to pregnancy. However, it is important to note that neither transrectal ultrasonography nor palpation is 100% accurate [31]. In contrast, Mendonca *et al*. [32] found that resynchronization with GnRH seven days before pregnancy diagnosis resulted in better pregnancy outcomes in herds with efficient heat detection. This could be attributed to a higher ovulatory response to the first GnRH injection in this group, as they were in the early stages of the estrous cycle when a dominant follicle capable of ovulation is present [33].

In this study, we compared the conception rates of four oestrous synchronization protocols in first-lactation dairy cows. The conception rates were as follows: 56% for Double Ovisynch, 46% for Cider synch, 37% for Resynch, and 44% for Ovisynch56. For second-lactation cows, the conception rates were 46% for Double Ovisynch, 36% for Cider synch, 34% for Resynch, and 35% for Ovisynch56. Our findings indicate that the Double Ovisynch protocol yields a higher conception rate than all other protocols across all parities. Additionally, first-lactation cows exhibit higher conception rates than second-lactation cows. This aligns with the findings of Pryce *et al*. [34] who reported a lower conception rate among animals with higher parity. They suggested that this may be due to a decrease in ovulation rate resulting from insufficient gonadotropin release from the pituitary gland. Moreover, the quality of eggs ovulated may deteriorate with age, leading to fertilization issues that can result in embryonic or fetal loss or uterine failure due to hormonal imbalances or deficiencies. Conversely, Shorten *et al*. [35] reported higher conception rates in multiparous cows compared to primiparous cows. This discrepancy might be attributed to the increased incidence of dystocia and metritis in primiparous animals, which could contribute to their lower conception rates [36].

The current study showed that conception rates were lower during the summer compared to winter across all groups. In mixed parity cows, the conception rates for the different synchronization protocols were as follows: 36% for Double Ovisynch, 30% for Cider synch, 28% for Ovisynch56, and 22% for Resynch. In Egypt, the climatic conditions significantly stress Holstein cows, leading to decreased fertility during the summer [37]. The decline in reproductive parameters during heat stress may be linked to reduced blood levels of essential metabolic hormones and growth factors necessary for optimal follicular development, which drop to suboptimal levels [38]. When comparing the conception rates of synchronization protocols in first-lactation dairy cows, the rates were 40% for Double Ovisynch, 37% for Cider synch, 35% for Ovisynch56, and 33% for Resynch. In second-lactation cows, the conception rates were 36% for Double Ovisynch, 26% for Cider synch, 33% for Ovisynch56, and 33% for Resynch. These findings align with Lopez-Gatius, who reported lower conception rates for services performed in hot months or during periods with higher temperature-humidity index (THI) values [39]. However, these results differ from those of Nabenishi *et al*. [40], who found that cows inseminated during the winter had lower pregnancy rates following the second and later artificial inseminations compared to those inseminated during the summer or autumn. This discrepancy could be explained by the exposure to cooler temperatures and inclement weather during the winter months. Additionally, the negative energy balance that affects the quality of developing oocytes might impair fertility, as the energy requirements for maintenance increase during the cold season prior to artificial insemination [41].

5. CONCLUSIONS

The Double Ovisynch protocol appears to be the most effective synchronization method under the studied conditions. Regardless, Seasonal heat stress and parity. Although this method incurs higher hormonal costs, the resulting increase in conception rates makes it a worthwhile investment, ultimately enhancing reproductive efficiency and economic profitability.

6. ACKNOWLEDGEMENTS

We would like to express our gratitude to all of the Ismailia Dairy Farms' owners and employees for their gracious assistance during the practical portion of our project.

Authors’ declarations

Publication consent

Each author has demonstrated their consent for the publication of the current manuscript.

Data and material availability:

All data of this study is provided.

Conflict of interests.

All authors have stated the absence of any conflicts of interest.

Funding.

This research did not receive funding from any specific grant.

REFERENCES

[1]. **Nishi S A (2024)** "Uprising the effective estrus synchronization protocols with hormones in anestrus dairy and beef cows guided by rectal palpation." Meat Research, 4(2): 1-5.

[2]. **Consentini CE, Wiltbank MC, Sartori R (2021)** Factors that optimize reproductive efficiency in dairy herds with an emphasis on timed artificial insemination programs. *Animals*, *11*(2), 301.

[3]. **Pursely JR, Kosorok MW, Wiltbank MC (1997)** Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci.,80: 301-306.

[4]. **Vasconcelos JLM, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC (2001)** Reduction in the size of the ovulatory

follicle reduces subsequent luteal size and pregnancy rate. Theriogenology, 56: 307–314.

[5]. Pursely JR, Silcox RW, Wiltbank MC (1998). Effect of time of insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J. Dairy Sci.,81: 2139-2144.

[6]. Ayres H, Ferreira RM, Cunha AP, Araújo RR, Wiltbank MC (2013). Double Ovsynch in high-producing dairy cows: effects on progesterone concentrations and ovulation to GnRH treatments. Theriogenology, 79(1): 159–164.

[7]. Santos V G P D, Carvalho C, Maia B, Carneiro A, Valenza P M, Fricke. (2017) Fertility of lactating Holstein cows submitted to a Double-Ovsynch protocol and timed artificial insemination versus artificial insemination after synchronization of estrus at a similar day in milk range. J. Dairy Sci., 100:8507-85

[8]. Fonseca FA, Britt B T, McDaniel J C, Wilk A H, Rakes J H (2004) Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci., 66: 1128-1147.

[9]. Wijma RM M, Perez M, Masello M L, Stangaferro J O, Giordano (2018) A resynchronization of the ovulation program based on ovarian structures present at nonpregnancy diagnosis reduced time to pregnancy in lactating dairy cows. J. Dairy Sci.,101:1697-1707.

[10]. Carvalho P DV G, Santos J O, Giordano M C, Wiltbank P M, Fricke (2018). Development of fertility programs to achieve high 21-day pregnancy rates in high-producing dairy.Theriogenology,114:165-172.

[11]. Al-Amin MAN, Arman SM, Afrose M, Rahman MM, Bhattacharjee J, Bhuiyan MMU (2018) Pregnancy rate and associated factors in dairy cows of Bangladesh. The Bangladesh Veterinarian, 35(1-2): 25-31.

[12]. Gad AE, Emara SS, Eid SY, El-Zaher HM (2021) Studying Resistance of Some Dairy Cattle Breeds to Heat Stress in Relation to Milk Yield. Adv. Appl. Physiol., 6 (1):14-22.

[13]. Rodríguez-Godina IJ, García JE, Morales JL, Contreras V, Véliz FG, Macías-Cruz U, Avendaño-Reyes L, Mellado M (2024) Effect of heat stress during the dry period on milk yield and reproductive performance of Holstein cows. Int. J. Biometeorol., 68: 883–890.

[14]. Herlihy MM, Giordano J O, Souza A H, Ayres H, Ferreira R M, Keskin A, Wiltbank M C (2012) Presynchronization with Double Ovsynch improves fertility at first postpartum timed AI in lactating dairy cows. J. Dairy Sci., 95(12):7003–7014.

[15]. Dohoo I R, Nielsen C R, Emanuelson U (2016) Multiple imputation in veterinary epidemiological studies: a case study and simulation. *Preventive veterinary medicine*, *129*: 35-47.

[16]. Borchardt S, Haimerl P, Pohl A, Heuwieser W (2017) Evaluation of prostaglandin F-2 alpha, versus prostaglandin F-2 alpha, plus gonadotropin-releasing hormone as Presynch methods preceding an Ovsynch in lactating dairy cows: A meta-analysis. J. Dairy Sci.,100:4065-4077.

[17]. Silva LACL, Simoes LMS, Bottino MP, Santos APC, Santos G, Martinez IYH, Souza JC, Baruselli PS, Sales JNS (2018). Presynchronization by induction of the largest follicle using a progesterone device in a GnRH-based ovulation synchronization protocol in crossbred dairy cows. Theriogenology, 119:233-237.

[18]. Bartolome JA, Silvestre FT, Kamimura S, Arteche ACM, Melendez P, Kelbert D, McHale J, Swift K, Archbal LF, Thatcher WW (2005) Resynchronization of ovulation and timed insemination in lactating dairy cows I: use of the Ovsynch and Heat synch protocols after non-pregnancy diagnosis by ultrasonography. Theriogenology, 63: 1617–1627.

[19]. Kasimanickam RR, Hall JB, Estill CT, Kastelic J P, Joseph C, Abdel Aziz RL, Nak D (2018) Flunixin meglumine improves pregnancy rate in embryo recipient beef cows with an excitable temperament. Theriogenology, 107: 70-77.

[20]. Zeuh V L, Mopate D, Nadjilem, D. Djonret., 2014. Evaluation of two methods of estrus synchronization of cattle in Chad. Open Journal of Animal Sciences, 4(1): 13-14.

[21]. Souza AH, Ayres H, Ferreira RM, Wiltbank MC (2008) A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology,70(2):208–215.

[22]. Giordanon JO, Wiltbank MC, Guenther JN, Pawlisch R, Bas S, Cunha AP, Fricke PM (2012) Increased fertility in lactating dairy cows resynchronized with

Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. J. Dairy Sci., 95(2):639–653.

[23]. Pursley JR, Kosorok MR, Wiltbank MC (1997) Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci., 80: 301–306.

[24]. Pursley JR, Mee MO, Wiltbank MC (1995) Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology,44: 915–923.

[25]. Stevenson JS, Pursley JR, Garverick HA, Fricke PM, Kesler DJ, Ottobre JS, Wiltbank MC (2006) Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. J. Dairy Sci., 89: 2567-h 2578.

[26]. Kaim M, Bloch A, Wolfenson D, Braw-Tal R O S E M B E R G, Rosenberg M, Voet H, Folman Y (2003) Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses, and conception. J. Dairy Sci., 86(6): 2012-202.

[27]. Patel KR, Dhami AJ, Hadiya KK, Savalia KK, Sarvaiya NP (2013) Effect of CIDR and Ovsynch protocols on estrus response, fertility and plasma progesterone and biochemical profile in true anoestrus crossbred cows. Indian J. Anim. Prod. Mange., 29(3-4): 50-58.

[28]. Bhoraniya HL, Dhami AJ, Naikoo M, Parmar BC, Sarvaiya NP (2012) Effect of oestrus synchronization protocols on plasma progesterone profile and fertility in postpartum anoestrus Kankrej cows. Trop. Anim. Health Prod., 44: 1191-1197.

[29]. Akbarabadi MA, Shabankareh HK, Abdolmohammadi A, Shahsavari M H (2014) Effect of PGF2α and GnRH on the reproductive performance of postpartum dairy cows subjected to synchronization of ovulation and timed artificial insemination during the warm or cold periods of the year. Theriogenology, 82(3):509-516.

[30]. Alkar A, Tibary A, Wenz JR, Nebel RL, Kasimanickam R (2011) Presynchronization with GnRH 7 days prior to resynchronization with CO-Synch did not improve pregnancy rate in lactating dairy cows. Theriogenology,76:1036-1041.

[31]. Silva E R A, Sterry D, Kolb N, Mathialagan M F Mc,Grath J M, Ballam P M Fricke (2007) Accuracy of a pregnancy associated glycoprotein ELISA to determine pregnancy status of lactating dairy cows twenty-seven days after timed artificial in semination. J. Dairy Sci., 90:4612–4622.

[32]. Mendonca LGD, Rocha LS, Voelz BE, Lima GT, Scanavez ALA, Stevenson JS (2019) Presynchronization strategy using prostaglandin F-2 alpha, gonadotropin-releasing hormone, and detection of estrus to improve fertility in a resynchronization program for dairy cows. Theriogenology, 124:39-47.

[33]. Vasconcelos J L M, Silcox R W, Rosa G J M, Pursley J R, Wiltbank M C (1999) Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology*, *52*(6):1067-1078.

[34]. Pryce J E, Royal M D, Garnsworthy P C, Mao I L (2004) Fertility in high-producing dairy cow. Livest. Prod. Sci.,86: 125-135.

[35]. Shorten P R, Morris C A, Cullen N G (2015) The effects of age, weight, and sire on pregnancy rate in cattle. J. Animal Sci., 93(4):1535-1545.

[36]. Mee J F (2008) Prevalence and risk factors for dystocia in dairy cattle: A review. *The Veterinary Journal*, *176*(1):93-101.

[37]. Kilany AA, El-Darawany AHA, EL-Tarabany AA, Al-Marakby KM (2023) Effect of environmental thermal stress on reproductive performance of Holstein cows in Egypt. Biol. Rhythm Research, 54(12):770-781.

[38]. De Rensis F, Lopez-Gatius F, García-Ispierto I, Morini G, Scaramuzzi R (2017) Causes of declining fertility in dairy cows during the warm season. Theriogenology, 91:145-53.

[39]. López-Gatius F (2003) Is fertility declining in dairy cattle? a retrospective study in northeastern Spain. Theriogenology, 60(1):89-99.

[40]. Nabenishi H, Yamazaki A (2017) Impaired reproduction in Japanese Black cattle under cold environmental conditions. Reprod. Domst. Anim., 52:371-775.

[41]. Leroy J L, De Bie J, Jordaens L, Desmet K, Smits, A, Marei W F,Van Hoeck, V (2018) Negative energy balance and metabolic stress in relation to oocyte and embryo quality: An update on possible pathways reducing fertility in dairy cows. Animal Reproduction, 14(3): 497-506.