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Original Research

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Efficacy of Co-synch Protocol on Normal Cyclic Dairy Cows with or Without Corpora Lutea

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Abstract

Improving reproductive efficiency, estrus synchronization and controlled breeding protocols are efficient management techniques in dairy cows. Therefore, the present study was designed to compare the efficacy of Co-synch protocol in the cows carrying corpora lutea (CL), without CL or with unexamined ovarian structure. Fifty-six cows were divided into 4 groups; control group1 (n=12) and Co-synch treated groups; group2: cows with unexamined ovarian structure group (n=9), group3: cows having only follicles without mature Cl (n=16) and group4: cows having mature Cl (n=19). Ultrasonography was performed before and after gonadotropin (GnRH) injection. Blood was collected for analysis of progesterone (P4) and estradiol 17-ß. Reproductive data was obtained regarding 1st service conception rate (1st SCR), number of services/conception (s/c) and days open. Ultrasonography revealed luteinization in cows without mature CL. While those having mature CL showed increase in the size of the CL or formation of accessory CL. Results revealed significant decrease in serum P4 before GnRH treatment in cows having no mature CL. Significant increase in serum P4 levels in all groups was recorded after GnRH treatment. Besides, the highest P4 level was assessed in cows having mature CL. Estradiol 17-ß was significantly decreased in cows having mature CL before and after treatment. Cows having mature CL revealed significant upgrade in the percentage of 1st SCR, decrease in s/c and shortening in days open as compared with other groups. In conclusion, applying Co-synch to the cows carrying CL is beneficial and promises protocol to improve reproductive performance in dairy cows.

KEYWORDS
Corpora lutea, Co-synch, Dairy cows, Gonadotropin, Timed insemination

INTRODUCTION

Effective breeding management is a crucial tool to enhance the reproductive and productive performance of dairy cattle (Cardoso Consentini *et al.*, 2021). Over the past few decades, milk yield per dairy cow has increased considerably due to continuous genetic selection and improvement of nutrition and herd management. Simultaneously with this selection for production characteristics, dairy cow fertility has declined significantly (Brito *et al.*, 2021). The implementation of timed artificial insemination (TAI) synchronizes follicular development and ovulation that subsequently proceed to acceptable pregnancy per TAI (Busch *et al.*, 2008).

One of the most common breeding protocols used in the dairy field is Co-synch (El-Zarkouny *et al.*, 2004). Initiation of synchronization protocol using gonadotropin-releasing hormone (GnRH) can induce ovulation by inducing luteinizing hormone (LH) release thus, initiate a synchronized new follicular wave 2 days later (Macmillan and Thatcher, 1991). Cyclic cows that respond to the first GnRH can generate accessory CL thus increases circulating progesterone (P4) during growth of the ovulatory follicular wave and consequently improves hormonal environment (Melo *et al.*, 2016).

This could be a principal advantage in GnRH-based protocol. However cows carrying existing CL will show increase in its size or formation of another accessory CL on the same or on the other ovary which will increase the level of circulating P4 followed by enhancement of the reproductive performance (Chen *et al.*, 2023).

Besides, in order to optimize reproductive performance and milk production in dairy farms, there is a great necessity to monitor the fertility indices (Mungube *et al.*, 2019). Moreover, the application of these controlled breeding programs reduced intervals from calving to the first postpartum services, reduced days open (DO) and consequently reduced the calving interval (Miller *et al.*, 2007).

The rationale for this protocol is that the first GnRH induce LH release and ovulation of a dominant follicle and emergences of a new follicular wave within 2 days. The administration of prostaglandin F2α (PGF 2α) 7 days later will induce luteolysis, and the second GnRH will induce LH release synchronizing ovulation of the new dominant follicle (Mapletoft *et al.*, 2018).

Studies have shown that the first GnRH resulted in ovulation in 44 to 54% of dairy cows (Bó and Mapletoft, 2014), 56% of beef heifers (Martinez *et al.*, 1999), and 60% of beef cows (Small *et al.*, 2010), and the emergence of a new follicular wave was synchronized only when treatment caused ovulation (Martinez *et al.*, 1999).

Hence, the current study elucidated the efficacy of Co-synch protocol in improving the reproductive indices of dairy cows with respect to serum P4, estradiol 17-ß, ovarian ultrasonographic images before and after treatment.

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MATERIALS AND METHODS

Animals

At the beginning, 64 dairy Holstein cows from ElBarka dairy farm were subjected to the experiment. The study was carried out from September 2020 to October 2021. Cows aged 2–3.5 years old (1 to 2 lactations). The average milk production per cow was about 9 tons /milking season. They were milked three times daily. The animals were free from brucellosis, leucosis and TB according to the certificate of origin and the regular veterinary health checks performed to them. Cows were kept in a free yard with sufficient shadow. All cows were fed total mixed ration according to NRC (2001). The water and feed ad libitum.

All the procedure of the experiment followed the guidelines of the ethical committee of the Faculty of Veterinary Medicine, Suez Canal University, Egypt (approval no. 2016104).

Clinical examination

The animals used in this investigation which were in the close up period 260 Days Carried Calf (DCC) were clinically examined regarding lameness and GIT disturbance (Smith *et al*., 2009).

Apparently normal cows were only used in this investigation. Experimented cows were selected with body condition score (BCS) 3.25 to 3.75 by visual observation and palpation. The body condition score was determined on a scale from 1 to 5 with a 0.25-unit precision where 1 is extremely thin and 5 is extremely fat (Edmonson *et al*., 1989).

Reproductive examination

At transition periods, cows that showed acute septic metritis or any puerperal or metabolic disorders were excluded from the study.

Starting from day 30 to 35 day after parturition, the animals were examined rectally and ultrasonography (B-mode, linear transrectal transducer, 5-8 MHz, Sonoscape M12, China). On the basis of ovarian structures and uterine involution (Aalseth, 2005; Upham, 1996), cows (*n*=56) were grouped in to 4 groups as follow: group 1; control group (n=12), group 2; cows with unexamined ovarian structure group (*n*=9), group 3 cows having only follicles without mature Cl (*n*=16) and group 4 cows having mature Cl (*n*=19).

All the treated groups were directed to the co-synch program in which 10µg GnRH (Receptal®, 1ml contains 4.0 µg buserelin acetate, MSD company, Germany) was injected at day 0. Then after 7 days, 500 µg PGF2α (Estrumate®, 1ml contains 250 µg cloprostenol, MSD company, Germany) was injected. Afterwards, ultrasonographic examinations were performed on both ovaries. Artificial insemination accompanied with GnRH injection were performed after 48-64 hours from PGF2α injection as described by Geary *et al*. (1998) and Rodriguez *et al*. (2023).

Sampling

Blood was obtained from tail vein of the studied cows. The blood was collected on plain tube for hormonal analysis. A total of 112 blood samples were collected from control at the same time of their herd mates as well as treated cows before and after the 7th day of GnRH injection. The samples were chilled on ice packs immediately after collection then transferred immediately to the lab to be centrifugated at 3500 round per minute for 10 minutes. Harvested sera were frozen at – 20ºC until analysis.

Estrus detection, insemination and pregnancy diagnosis

In the control group, estrus was detected by well-trained persons continuously throughout the day and night. Artificial insemination (AI) of those cows was performed by visual heat detection without any treatment by the am-pm rule i.e., cows came in heat in the morning were inseminated at the evening and others came in the evening inseminated in the next morning (Graves *et al*., 1997).

In the treated groups, AI was carried out by the same veterinarian at the due time of Co-synch program via Fixed time AI (FTAI). Moreover, animals returned to heat were inseminated by the same veterinarian again depending on heat detection (Michael and Thomas, 2005). Animals that came on heat during the different breeding protocols were not inseminated and left to be inseminated in the fixed time of each program. A frozen-thawed semen from approved sires was used for insemination of all the groups.

Other non-returned cows were checked for pregnancy by ultrasonography at the 32nd days after insemination, then confirmed rectally at the 55th days post insemination.

Hormonal analysis

Bovine ELISA kits were used for assessment of serum P4 and estradiol 17-ß (NEOGEN Co., USA). Analysis was performed according to manufacture instruction.

Reproductive indices

The reproductive data of the studied cows were collected regarding:

First service conception rate (1st SCR): number of pregnant cows after first AI / number of inseminated cows X 100 as mentioned by Falkenberg *et al*. (2008).

Number of services per conception (S/C): sum of all AI used for all cows / Number of cows confirmed pregnant as described by Upham (1991).

Days open: the period between parturition and the following conception of a dairy cow. (Harman *et al*., 1996).

Statistical analysis

All data was analyzed statistically using GraphPad Prism version 5.0 (GraphPad Software, San Diego, California USA). Statistical comparisons were made by one way ANOVA to compare different groups. The significant differences between groups were determined by using Tuke's test post HOC multiple comparison. The student's t-test was performed in each group before GnRH treatment and after GnRH treatment by 7 days. The obtained results expressed as mean ± SE. The results were considered significant when P ≤ 0.05.

RESULTS

Effect of Co-synch protocol on ultrasonographic ovarian images

Before GnRH treatment, ovarian ultrasonographic images of cows having CL showed the presence of growing follicle as anechoic structure less than 2cm on the left ovary. However, the right ovary revealed a CL as hypoechoic structure. However, at the 7th days post GnRH treatment the ultrasonographic examination revealed the presence of CL with central cavity in the left ovary. Moreover, a marked increase in the size of the present CL at the right ovary in cows diagnosed with CL or formation of another accessory CL on the same or on the other ovary as shown in Fig. 1.

On the other side, the ovarian ultrasonographic image in cows having no CL (Fig., 2) presented growing follicles on both ovaries before GnRH treatment. The Co-synch administration after 7 days forms a CL on the right ovary.

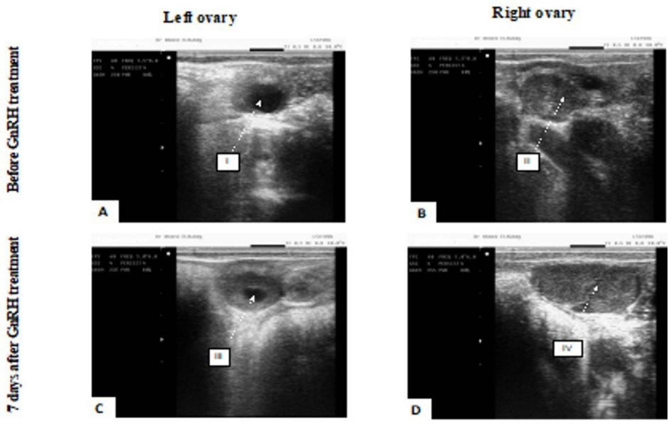

Fig. 1. Ultrasonographic images of ovaries with CL before and after GnRH treatment in cows treated with Co-synch protocol. Before GnRH treatment (A), (I) refers to growing follicle appears in the left ovary as an anechoic structure less than 2cm. In (B), (II) refers to corpus luteum (CL) on the right ovary as a hypo-echoic structure. After GnRH treatment by 7 days (C), the left ovary declares a CL with central cavity seems like a hypo-echoic structure with an anechoic antrum. In the right ovary (D), (IV) shows a pronounced increase in the size of CL that appears as a hypo-echoic structure.

Effect of Co-synch protocol on hormonal levels

Before GnRH treatment, serum P4 levels revealed highly significant (P < 0.001) variations between control and treatment groups. The highest P4 levels were recorded in Co-synch treated cows with unexamined ovarian structures and those having mature CL (1.49±0.1 and 1.46±0.41 ng/ml) as compared with other groups, respectively. However, the lowest serum P4 was in cows with no CL (0.26±0.1 ng/ml) as compared with other groups (Table 1).

The same trend noticed in P4 levels after 7 days from GnRH, control and Co-synch treated groups pronounced significant (P < 0.01) variations. Cows having CL exhibited significant (P < 0.01) elevation in serum P4 levels. On the other side, cows having only follicles without mature CL had significantly the lowest (P < 0.05) values of serum P4 levels.

Serum P4 showed significant (P < 0.05) variations within each group before and after 7 days from GnRH treatment. Dramatic upgrade in the serum P4 was assessed in cows having mature CL.

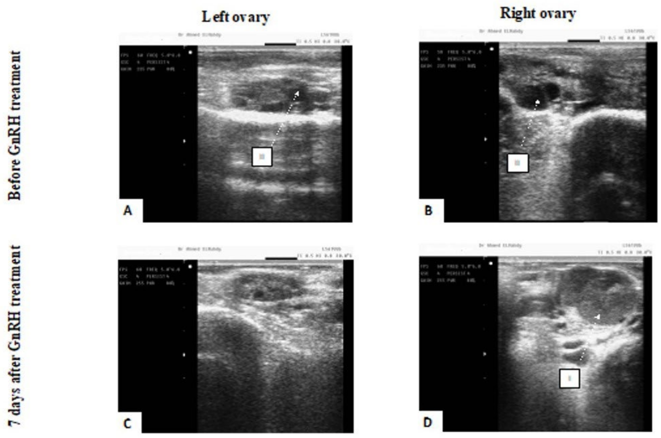

Fig. 2. Ultrasonographic images of ovaries without CL before and after GnRH treatment in cows treated with Co-synch protocol. Before GnRH treatment, the ovarian ultrasonographic image presented growing follicles on both ovaries (A and B, II). The Co-synch administration after 7 days forms a CL (D, I) on the right ovary.

Concerning serum estradiol 17-ß, significant variations (P < 0.01 and 0.05) were observed between all studied groups before and after GnRH treatment, respectively. Moreover, the lowest values (P < 0.01) were obtained in cows with CL as compared with control and other treated groups before and after GnRH treatment. Non-significant variation was noticed with each group before and after GnRH treatment.

Effect of Co-synch protocol on reproductive indices

As illustrated in Table 2, significant (P = 0.001) differences in the percent of the 1st SCR between different groups. The cows with mature CL recorded the highest (P < 0.05) 1st SCR as compared with other treated groups. Moreover, the control group revealed significant (P < 0.05) upgrade in the 1st SCR as compared with cows with unexamined ovarian structure and those having follicles without CL. However, the previous later treated groups did not show any significant (P >0.05) variations in the 1st SCR.

Concerning S/C, significant (P = 0.01) differences were observed between all groups. The lowest (P < 0.05) value of S/C was obtained in cows having mature CL as compared with control and treated groups. On the other hand, no significant variations were recorded in S/C between control and cows with unexamined ovarian structure as well as those having follicle without CL.

All the groups showed significant (P < 0.01) differences regarding the days open. Moreover, cows with mature CL revealed significant (P < 0.05) shortening in the interval between parturition and the following conception as compared with control and other treated groups. However, the control group exhibited a

Table 1. Serum progesterone and estradiol 17- ß levels in cows with unexamined ovarian structures and those with or without mature CL before and 7 days after GnRH treatment in Cosynch protocol.

		Control group (*n*=12)	Cosynch with unexamined ovarian structures (*n*=9)	Cosynch with known ovarian structures (*n*=35)		*P* value
				Cows having only follicles without mature CL (*n*=16)	Cows having mature CL (*n*=19)	
Progesterone (ng/ml)	Before GnRH treatment	1.5 ± 0.27 aB	1.49 ± 0.1aB	0.26±0.1bA	1.46 ± 0.41aA	0.0001
	7 days after GnRH treatment	2.2 ± 0.3 aA	2.8 ± 0.6 aA	1.26±0.41bB	3.65±0.25 aB	0.002
	P value	0.04	0.03	0.01	0.02	
Estradiol 17-ß (pg/ml)	Before GnRH treatment	36.83 ± 7.84b	31.10 ± 4.3b	33.8 ± 1.87b	26.8 ± 0.3a	0.003
	7 days after GnRH treatment	33.72 ± 6.45b	29.38 ± 5.26b	30.9 ± 1.3b	23.3 ± 1.2a	0.02
	P value	0.3 (NS)	0.3 (NS)	0.6 (NS)	0.1 (NS)	

Data are expressed as Mean± SE. Different small letters in the same rows are significant at P ≤ 0.05. Different capital letters in the same column are significant at P ≤ 0.05

Table 2. Reproductive data of cows with unexamined ovarian structures, cows having follicles without mature CL and those having mature CL treated by co-synch breeding program.

	Control Group (*n*=12)	Cosynch with unexamined ovarian structures (*n*=9)	Cosynch with known ovarian structures		P value
			Cows having follicles without mature CL (*n*=16)	Cows having mature CL (*n*=19)	
First service conception rate	(*n*=5) 41.6% [ab]	(*n*=3) 33.3% [b]	(*n*=5) 31.25% [b]	(*n*=10) 52.6% [a]	0.001
Service per conception	2.1 ± 0.4 [b]	2.2 ± 0.2 [b]	2.5 ± 0.22 [b]	1.64 ± 0.22 [a]	0.01
Day open (day)	170.0 ± 21.21 [c]	99.5 ± 0.50 [b]	85.8 ± 5.58 [b]	56 ± 2.56 [a]	0.003

Data are expressed as Mean± SE. Different small letters in the same rows are significant at $P \leq 0.05$.

significant ($P < 0.05$) prolongation in the days open as compared with other groups. Furthermore, both of cows with unexamined ovarian structures as well as those having follicle without CL did not prohibit any significant ($P > 0.05$) differences concerning days open.

DISCUSSION

Observing cows in estrus and inseminating them at the optimum time are necessary steps for effective reproductive management of a dairy herd (Rodriguez *et al*., 2023). However, large herd sizes can lead to reproductive inefficiency and decreased profits on dairy farms. Therefore, synchronization of estrus behavior through pharmacological control has been used to improve reproductive profitability (Mercadante and Lamb, 2023; Nebel and Jobst, 1998).

Reproductive failure is a major reason for economic losses in dairy farms. Thus, the main goal of any breeding program is to optimize the number of females that become pregnant (Perry, 2005). Hence, the current study elucidates the efficacy of Co-synch protocol in improving reproductive indices with respect to ovarian ultrasonographic images, serum P4 as well as estradiol 17-ß before and after GnRH treatment.

In the current study, ovarian ultrasonographic image revealed that administration of GnRH in Co-synch protocol to cows with and without CL resulted in luteinization as well as excessive formation of lutein tissue for the previously formed CL. These results came in harmony with those of Silva *et al*. (2023) and Stouffer (2006) and Mussard *et al*. (2007). Besides, Yoshimura *et al*. (2022) ensured a significant increase in the mean peak of LH after GnRH administration that proceed to l formation. On the other hand, Ambrose *et al*. (2004) stated that all cows developed a new follicle after CL formation in response to GnRH treatment additionally, a majority of these newly recruited follicles subsequently ovulated.

Before Co-synch-GnRH treatment, serum P4 levels significantly increased in cows with unexamined ovarian structures and those having mature CL. Moreover, administration of GnRH proceeded to significant elevation in serum P4 levels in all treated groups and within each group before and after treatment. On the other side, all the studied cows revealed significant variations in serum estradiol 17-ß levels before and after GnRH treatment. Furthermore, the lowest values were demonstrated in the cows having CL. These findings were in consistence with those of Motta *et al*. (2020) who related the upgrade of P4 and the downgrade of estradiol 17-ß following administration of GnRH. Moreover, Schoenemann *et al*. (1985) and Geary *et al*. (1998) reported the role of elevated P4 in inhibition of estradiol that prompted the excess in GnRH receptor expression from the pituitary gland.

The mechanism by which GnRH motivates the production of P4 from CL is that GnRH enhances the release of LH from anterior pituitary that bind to the specific G protein-coupled receptors on the plasma membrane of steroidogenic cells in the CL. That induce the adenylate cyclase system to produce cyclic adenosine monophosphate (cAMP). The later stimulates protein kinase A (PKA) that phosphorylates and prompts steroidogenic acute regulatory protein (StAR). It controls cholesterol transport from outer to inner mitochondrial membrane where cholesterol transformed to pregnenolone. Finally, pregnenolone is converted to P4 (Teeli *et al*., 2019). In addition, elevated P4 as a consequent to GnRH treatment intensely raise LH secretion as reported by Giordano *et al*. (2012). That consequently acts a vital role in formation, development as well as functioning of the CL.

In the present study, application of Co-synch protocol improved the fertility indices in cows having mature CL via significant increase in the 1st SCR, decrease S/C and shortening the days open as compared with control and other treated groups. The results of Giordano *et al*. (2015) assured that the administration of GnRH increased the proportion of inseminated cows rather than the non-treated group that in turn enhanced the reproductive performance. Moreover, application of TAI to the synchronized dairy cows improved reproductivity that declared in reduction of days to first AI hence, increased the number of pregnant cows after AI (Fricke *et al*., 2014).

The Co-synch-treated cows carrying CL showed significant enhancement in the reproductive pattern regarding the 1st SCR, S/C and days open as compared with those carrying growing follicles treated with the same program. It means that cows with mature CL resumed their ovarian cyclicity after parturition before GnRH treatment at least one time that was declared by elevation of serum P4 levels more than 1 ng/ml. These findings came in harmony with those of Darwash *et al*. (1997) and Smith and Wallace (1998) who clarified that resumption of ovarian activity plays an important role in the subsequent fertility. In addition, Stronge *et al*. (2005) reported that the inadequate P4 is one of the causes of poor fertility in high producing dairy cows and 60-85% of dairy cows showed a suboptimal circulating P4 for pregnancy. The explanation of improved reproductive performance in Co-synch treated cows having mature CL is that pregnancy rates were positively associated with higher P4 concentration in the luteal phase of the cycle preceding AI due to an improvement in the endometrial morphology following elevated P4 concentrations of the preceding cycle (Rosenberg *et al*., 1990). Additionally, Sterry *et al*. (2009) found that cows with P4 ≥1 ng/mL at the PGF2α injection in Co-synch protocol had greater pregnancy rate (41%) than cows with P4≤1 ng/mL (12%). That was confirmed in the current study for cows having CL and showed better reproductive pattern than others.

In contrast to our findings, McArt *et al*. (2010) reported no difference in days to conception and proportion of cows inseminated when compared between PGF2α injection to induce estrus and the Ovsynch protocol according to the presence or absence of CL.

CONCLUSION

Applying Co-synch protocol via administration of GnRH and PGF2α to dairy cows carrying CL is beneficial in enhancement of the reproductive performance and productive profitability that was manifested by shortening of days open postpartum.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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