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Presynchronization with CIDR improved the efficiency of ovsynch in the cyclic and acyclic Postpartum Pluriparous Cows

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Abstract

The postpartum period is considered an important period in the reproductive life of dairy cows. This study was aimed to evaluate the effect of progesterone (P_4)-CIDR supplementation in the course of Ovsynch regimens in postpartum Holstein-Friesian dairy cows. Eighty Holstein Friesian dairy cows were used. All animals were gynaecologically examined for uterine involution and ovarian resumption by rectal ultrasound scan. Those with history of endometritis, caesarean section, dystocia, retained placenta, acute mastitis, lameness or other unhealthy were excluded. Fifty-two cows were cyclic and twenty eights were non-cyclic. Animals were divided into three synch-regimens. The 1st group (N=27), used as a control, is the ovsynch regimen only in which the animals were treated with GnRH-$PGF_{2\alpha}$-GnRH (GPG), the 2nd group (N=26) in which the GPG was presynchronized with controlled internal drug release (CIDR) device before (CIDR-GPG), and the 3rd group (N=27) in which CIDR device was incorporated into GPG, after the first GnRH injection (G-CIDR-PG). Blood samples were collected 5 times (day -7, 0, 7, 14 and 50) for measuring P_4 using enzyme-immunoassay (EIA) technique. Ultrasound examinations were performed on days -7, 0, 7 and 9 for following up the ovarian changes, and repeated at 35, 45 and 50 days post-insemination for pregnancy diagnosis. Conception rate was variable among groups. It was increased by 69.23, 51.85 and 40.74% in CIDR-GPG, G-CIDR-PG regimens treated groups and GPG-control group, respectively. The conception rate was higher in the cyclic versus acyclic GPG group (44.4 vs. 33.3 %). On the other hand, acyclic cows were highly responsive than cyclic cows in CIDR-GPG group (70.0 vs. 68.8%) and G-CIDR-PG group (55.6 vs. 50.0%). In conclusion, we recommend that the CIDR-GPG regimen is improving fertility and conception rate in postpartum dairy cows.

Keywords: CIDR, Cow, Conception, Ovsynch

Introduction

The animal's reproductive performance greatly affects its productivity and subsequently its profitability [1]. The calving interval should average one year [2]. Therefore, the interval between parturition and first service should be less than 90 days [3]. It could be achieved by the early resumption of ovarian activity and higher conception rates. However, most of dairy farms depend on artificial insemination (AI) in reproduction, conception rates were dramatically decreased from 66% to about 35% since 1973 till 2000 parallel to the use of AI and higher dry matter intake [4,5]. Resumption of the ovarian activity is controlled by hormonal and environmental factors. Cows respond to the pulsatile release of luteinizing hormone (LH) generally exhibited a corpus luteum (CL) and subsequently raise the circulating progesterone (P_4) within the first month after parturition [4]. Many different treatment regimens are applied to synchronize estrus. Mostly, a luteolytic agent prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) is included either alone or in combination with a regulatory agent of the follicular development. Depending on the fact that progesterone (P_4) is necessary to fertility and its level before insemination was found to be positively correlated to the subsequent conception rate [6,7].

The alternative regimens such as, P_4, $PGF_{2\alpha}$ and follicle wave regulating agent were widely applied. Our hypothesis was that P_4

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enhances a rebound phenomenon of the hypothalamic-pituitary-gonadal (HPG) axis. Using P_4 before the ovsynch protocol, could improve the follicular development, oocyte maturation and conception rate. The controlled-internal drug release (CIDR; a pessary progestin-containing device) was incorporated with the ovsynch protocol and found to improve the conception rates of first-lactation cows [8,9]. The aim of the present study was to evaluate the effect of presynchronization with P_4 before ovsynch or incorporation P_4 with the ovsynch on the conception rate in cyclic and acyclic cows.

Material and methods

Animals and management

This study was conducted on governmental dairy farm in South Egypt (Qena province) containing pure Holstein Friesian dairy cows during the period from May to November (ambient temperature, 34-40 ^{o}C and humidity, 60-70%). Animals were fed a total mixed ration according to the National Research Council [10] with continuously available water. After calving, the newborn calves were allowed to suckle their dams for five days. Cows were milked twice daily at 12 h interval. The external signs of estrous were checked twice daily by herdsmen. All animals were bred by natural mating at the first observed estrus (usually 46 days post-partum). Pregnancy was diagnosed by rectal palpation 50-60 days after mating. Eighty healthy pluriparous cows with history of normal calving (age, 4.2 ± 0.1 years old; milk yield average, 6405 ± 0.36 kg/year/cow) were used. The body condition score (BCS) of cows averaged 3.0 ± 0.04 (BCS, 1= emaciated and 5 = obese) [11], the values were presented as mean ± SEM. The experiment started at the 30th days post-partum (P.P) after detecting the cyclicity state of the animals.

Hormonal drugs

Gonadotropin-releasing hormone (GnRH) (Receptal, Intervet Egypt Co. for animal health, 1 mL contains 0.0042 mg buserelin acetate equivalent to 0.004 mg buserelin) [12]. Prostaglandins-$F_{2\alpha}$ ($PGF_{2\alpha}$) (Lutalyse, Zoetis Animal Health, 1 mL contains 5 mg dinoprost) [13]. The controlled internal drug-release (CIDR) (Pfizer Animal Health, silicon-coated devices containing 1.38 g of progesterone/device inserted intravaginally) [14].

Experimental design

Cows were classified based on the gynecological examination (rectal palpation for uterine involution), ovarian-ultrasound scan and vaginoscopy (for secretions) into cyclic (N=52) and acyclic (N=28) based on the presence of the corpus luteum (CL) or follicles [15] and the reports of the regular monthly examination for the state of the animals and confirmed by the ultrasonography and hormonal analysis that matching the previous report specific to each animal in the farm. The first ovarian examination by ultrasonography was started on the 20th day P.P and repeated 10 days later for detecting the CL and follicular activity. The start of the study was defined as day -7 and the cows were allocated to one of the following groups as shown in Fig. 1. The first group (GPG) contained twenty-seven animals (cyclic 18 cows and non-cyclic 9 cows) that incorporated into ovsynch protocol (GPG), while the second group (CIDR-GPG) contained twenty six animals (cyclic 16 cows and non-cyclic 10 cows) that incorporated into G-CIDR-PG protocol and the third group **(**G-CIDR-PG) contained twenty seven animals (cyclic 18 cows and non-cyclic 9 cows) that incorporated into G-CIDR-PG protocol. Blood samples were collected 5 times from jugular vein in heparinized tubes (Day -7, 0, 7, 14 and 50). The harvested plasma samples were stored at -20 ^{o}C until the estimation of P_4 concentrations. Plasma P_4 was measured by enzyme-linked immune sorbent assay (ELISA) [16] using progesterone Kits (Immunospec corporation, U.S.A).

Ultrasonographic examinations were carried out on days -7 (start of treatment), 0, 7, 9 and 50 for all animals in order to record the ovarian cyclicity, response to GnRH (Intervet Egypt Co. for animal health, 1ml contains 0.0042 mg buserelin acetate equivalent to 0.004 mg buserelin), luteolytic effect of $PGF_{2\alpha}$ (Zoetis Animal Health, 1 mL contains 5 mg dinoprost), and the pregnancy diagnosis, respectively. Pregnancy diagnosis was recorded by 34 days after mating or 50 days from the start of the protocol. Cows with detectable luteal tissue on Day -7 were considered cyclic. For recording the conception rate, pregnancy was determined at 35-45 days after mating by using the real time B-mode ultrasound scanner (DRAMINSKI ANIMAL profile scanner equipped with a 7.5 MHz linear transducer; DRAMINSKI Co., Poland).

Statistical analysis

Chi-square was used for analyzing the data of conception rates. All data of plasma P_4 concentrations were tested for significant difference by one-way ANOVA and confirmed by Newman-Keuls as a post-hoc test. All data of milk yield between cyclic and acyclic animals in the different groups of the study were tested for significant difference by two-way ANOVA and confirmed by Bonferroni as a post-hoc test. All data were presented as mean ± S.E.M. The differences were considered significant at $P<0.05$ using Graph Pad Prism software program (V.5.01, San Diego USA, 2007).

Table 1: Descriptive statistics of body condition score (BCS), parity and milk production (kg/day) in postpartum dairy cows at the start of treatments; ovsynch protocol (GPG), presynchronization of ovsynch with CIDR (CIDR-GPG) and incorporation on CIDR into the ovsynch (G-CIDR-PG). All values were presented as Mean ± S.E.M

Treatment	Number	B.C.S.	Parity
GPG group			
Non-cyclic	9	2.27 ± 0.12	1.44 ± 0.17
Cyclic	18	2.75 ± 0.08	1.22 ± 0.10
Total	27	2.59 ± 0.08	1.30 ± 0.09
CIDR-GPG group			
Non-cyclic	10	3.25 ± 0.83	1.70 ± 0.15
Cyclic	16	3.34 ± 0.08	1.94 ± 0.06
Total	26	3.31 ± 0.06	1.85 ± 0.07
G-CIDR-PG group			
Non-cyclic	9	3.22 ± 0.87	2.0 ± 0.00
Cyclic	18	3.25 ± 0.06	2.0 ± 0.08
Total	**27**	**3.24 ± 0.05**	**2.0 ± 0.05**

Results

The descriptive statistics of BCS, parity and milk production (kg/day/cow) of animals in the three groups; GnRH-$PGF_{2\alpha}$-GnRH (GPG), controlled-internal-drug-release (CIDR)-GPG and G-CIDR-PG groups in cyclic and acyclic cows were shown in table 1. No significant difference was recorded for milk production between the cyclic and acyclic cows within each protocol of study. Conception rates after natural mating following GPG, CIDR-GPG and G-CIDR-PG in cyclic and acyclic postpartum dairy cows were shown in table 2. The conception rate tended to be greater in CIDR-based protocols than in GPG-ovsynch protocol. Furthermore, conception rate varied in response to different groups. In GPG group, the acyclic animals showed lower conception rates than those cyclic (33.3 vs. 44.4 %) but the response was reversed in the CIDR-treated ovsynch groups where the acyclic animals showed higher conception rates compared to cyclic in the CIDR-GPG (70.0 vs. 68.8 %) and G-CIDR-PG (55.6 vs. 50.0 %) (Table 2). Regarding the milk yield, there was no significant difference between cyclic and acyclic cows among the different groups of the study.

Figure 2 shows the ultrasound images of ovarian structures, including; the follicle (Fig. 2A) and corpus luteum (Fig. 2B) on days – 7, 0, 7, 9 and 14, in addition to the amniotic vesicle (Fig. 2C) as a sign of pregnancy diagnosis was recorded on day 34 after mating that equals the day 50 from the start of the protocol. Plasma P_4 concentrations in cyclic and acyclic cows after three protocols of estrus synchronization were shown in Fig. 3. The data related to pregnant cyclic and acyclic animals were presented in Fig. 3A, and 3C, respectively. The plasma P_4 levels on day 0 were significantly higher ($P<0.05$) in CIDR-GPG protocol compared to other treatment groups. However, its levels on day 7 were significantly higher ($P<0.05$) in G-CIDR-PG protocol compared to other treatment groups. For the non-pregnant cyclic and acyclic animals (Fig. 3B, and 3D, respectively), the plasma P_4 profile from day 0 up to day 7 revealed the same results as mentioned for those cyclic. The cyclic animals, both pregnant and non-pregnant, the plasma P_4 levels on day 14 maintained significantly higher in CIDR protocols rather than non-CIDR ovsynch regimen ($P<0.05$) (Fig. 3A and 3B). However, the acyclic animals showed non-significant differences in response to all ovsynch regimens on the same day (Fig. 3C and 3D).

Table 2: Conception rates after natural mating following GPG, CIDR-GPG and G-CIDR-PG in cyclic and acyclic postpartum dairy cows

Experimental groups	Cyclic				Acyclic				Conception rate
	N	Pregnant	N	Non-pregnant	N	Pregnant	N	Non-pregnant	
GPG	8	44.4%	10	55.6 %	3	33.3 %	6	66.7 %	40.74 %
CIDR-GPG	11	68.8%	5	31.25%	7	70.0 %	3	30.0 %	69.23 %
G-CIDR-PG	9	50.0%	9	50.0%	5	55.6 %	10	44.4 %	51.85 %

N: Number; Chi square=8.33, **df** = 1, **P** = 0.0039**

Discussion

Several modifications of ovsynch were applied depending on hormonal supplementation of progestin, $PGF_{2\alpha}$ and gonadotropins [17,18]. One of the causes of poor fertility in high producing dairy cows is inadequate progesterone and 60-85% of dairy cows showed a suboptimal circulating P_4 for pregnancy [19]. On dependence on evaluating the effect of P_4 supplementation throughout the course of Ovsynch protocol in cyclic and acyclic P.P Holstein Friesian dairy cows, we recorded an increase in the conception rate when P_4 was used with the Ovsynch protocol. This result was in accordance with Kawate *et al.* [20] who reported that the addition of CIDR to the ovsynch protocol (G-CIDR-PG) improved the conception rates in postpartum Japanese Black beef cows.

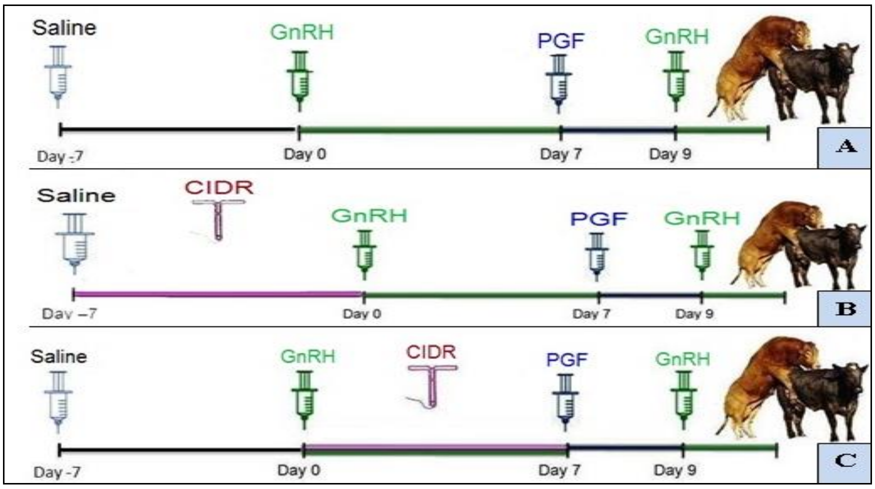

Figure 1: Experimental design for the studied groups: (A) GPG group: Day 0, GnRH (Receptal, 5 mL intramuscular injection); Day 7, $PGF_{2\alpha}$ (Lutalyse, 5 mL IM); Day 9, GnRH was repeated followed by natural mating after estrous detection that monitored 16-18 h after the last GnRH. (B) CIDR-GPG: Day -7, cows were initially treated with CIDR intravaginally for 7 days; Day 0, GnRH (Receptal, 5 mL IM) and removal of CIDR's devices; Day 7, $PGF_{2\alpha}$ (Lutalyse, 5 mL IM); Day 9, GnRH was repeated followed by natural mating after estrous detection that monitored 16-18 h after the last GnRH. (C) G-CIDR-PG: Day 0, GnRH (Receptal, 5 mL IM) and insertion of CIDR's devices for 7 days; Day 7, $PGF_{2\alpha}$ (Lutalyse, 5 mL IM) and removal of CIDR's devices; Day 9, GnRH was repeated followed by natural mating after estrous detection that monitored 16-18 h after the last GnRH.

The current investigation presented presynchronization with CIDR before GPG as a new regimen as an attempt to enhance the conception rate in comparison to ovsynch or G-CIDR-PG. Conception rate was variable among groups. It is likely that the addition of P_4 to the Ovsynch protocol may be affected by a number of variables such as age, post-partum interval and ovarian follicular growth. Pregnancy rates were positively associated with higher P_4 concentration in the luteal phase of the cycle preceding AI [21]. This proved by the findings that reported an improvement in the endometrial morphology following elevated P_4 concentrations of the preceding cycle. The high pregnancy rates were attributed to the quadratic effect of P_4 at the $PGF_{2\alpha}$ injection in ovsynch protocol. Cows with $P_4 \geq 1$ ng/mL at the $PGF_{2\alpha}$ injection had greater pregnancy rate (41%) than cows with $P_4 \leq 1$ ng/mL (12%) [22]. Variations in serum P_4 at the $PGF_{2\alpha}$ injection dramatically affected fertility in dairy cows. Higher P_4 concentrations prior to insemination reduced uterine secretion of $PGF_{2\alpha}$ (in response to oxytocin) during late luteal phase [23].

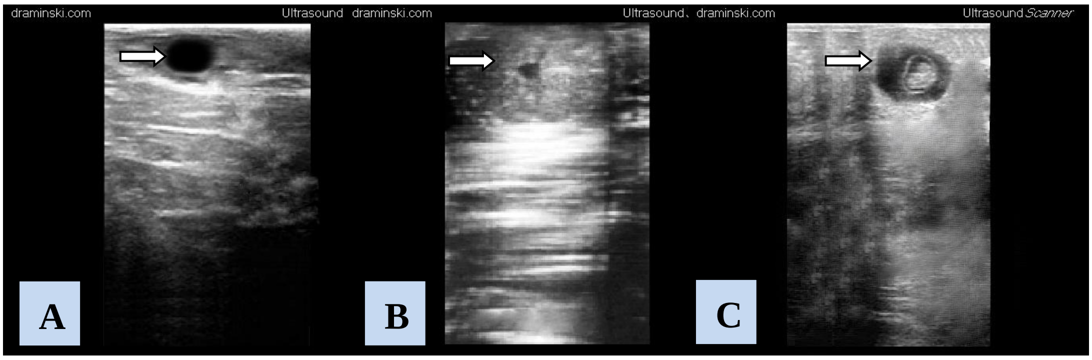

Figure 2: Representative ultrasound image of ovarian activity and follicular dynamics in synchronized cows of all groups at start of treatment (day -7) showing growing follicle (A), corpus luteum (B). Ultrasound image of uterine horn in a studied cow shows the embryonic vesicle at around day 40 post mating (C).

In addition, high P_4 levels at the time of $PGF_{2\alpha}$ injection and luteal regression shortly after $PGF_{2\alpha}$ treatment induced a greater response to GnRH and better fertility in lactating dairy cows [24]. Lamb *et al.* [25] reported that P_4-CIDR supplementation between GnRH and $PGF_{2\alpha}$ treatment could improve the pregnancy rate in cows with low P_4 concentrations at $PGF_{2\alpha}$ injection. Progesterone was considered a 'proxy variable' among other factors associated with conception rate. The fact that ovulation induced by GnRH administration followed by a high proportion of pre-mature luteal regression can be avoided by priming with exogenous P_4 [26]. Our results were incorporated with other studies that reported CIDR insertion improved the conception rates in non-cyclic cows and the exogenous supplement of P_4 served as a primer to facilitate ovulation of a follicle in response to the second GnRH injection [25-27]. The conception rates were higher in cyclic cows than in acyclic cows, which agree with Geary *et al*. [28] but contradict the report of Sakase *et al*. [29] who stated no effect of cyclicity on the conception rates in response to ovsynch.

In the present study, concentrations of P_4 in cows with or without an active CL were increased by the CIDR insertion and P_4 increased in both CIDR-GPG and G-CIDR-PG on day 0 and 7, respectively. These elevations are in agreement with those achieved by different authors [20,30,31] who indicated that P_4 concentrations were rapidly increased and reached its peak within 1h after CIDR insertion and then decreased rapidly up to 0 ng/mL 12 to 24 h once the CIDR was removed. Other studies concluded that CIDR insertion provided a relatively small increase (0.5 to 1 ng/mL) in serum P_4 concentrations [27]. The CIDR might maintain P_4 above 1 ng/mL for at least 7 days in cows without CL [28]. The increase in P_4 from day 0 to 7 was small in the ovsynch group. In cycling cows, plasma P_4 concentration tended to be higher on days 0 and 7 in the CIDR-GPG and G-CIDR-PG protocols than those in the ovsynch protocol. Moreover, in GPG protocol, the first GnRH treatment was given on approximately day 15 of the estrus cycle and the CL was regressed before the $PGF_{2\alpha}$ treatment and estrus occurred, resulting in ovulation before timed-AI [32].

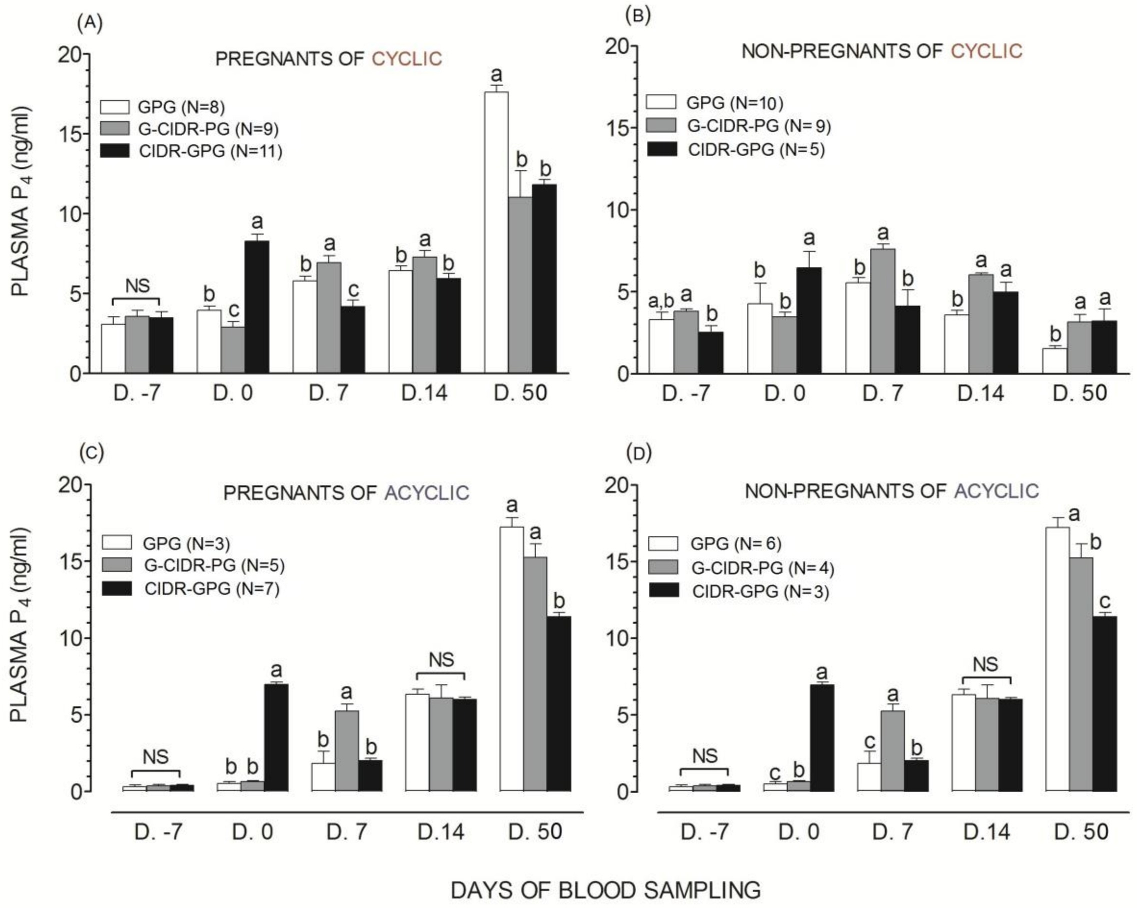

Figure 3: Plasma P_4 concentrations of pregnant cyclic (A), non-pregnant cyclic (B), pregnant acyclic (C), and non-pregnant acyclic (D) cows in response to treatment protocols of GPG, CIDR-GPG and G-CIDR-PG groups throughout the periods of study. The letters on bars (a, b and c) denote the significant difference at *P<0.05. NS means non-significant difference.

In acycling cows, plasma P_4 concentrations were clearly higher on day 0 in CIDR-GPG group than in the ovsynch group that could be due to the release of P_4 from CIDR. On day 7, plasma P_4 concentrations were clearly higher in G-CIDR-PG group than ovsynch and CIDR-GPG groups that may be related to the release of P_4 from CIDR, in addition to a possible ovulation or luteinization following the first GnRH treatment. The plasma P_4 concentration was (8.81 ± 0.71 ng/mL) at day 50 in all conceived cyclic cows, this result came in agreement with that obtained by Han *et al*. [33] who found that P_4 at 15 to 32 days after AI was consistently higher in pregnant (> 4 ng/mL) than non-pregnant cows.

In non-pregnant cows, P_4 concentrations in CIDR-GPG and G-CIDR-PG groups increased on days 0 and 7 than in the ovsynch protocol, which attributed to the release of P_4 from CIDR. The P_4 concentrations on day 14 were high (3 up to 6 ng/mL), which referred to the efficiency of the ovsynch protocols (with or without CIDR) in stimulating the resumption of ovarian activity and regardless the incidence of conception in both cyclic and acyclic groups. The variations of P_4 concentration among groups is related to the follicular size at ovulation. Induction of ovulation in cows with smaller dominant follicle at the second GnRH treatment resulted in smaller CL [34] and therefore, reduced the fertility and P_4 concentration [35].

In the pregnant cows, the P_4 concentration after insemination was lower in cows of non-CIDR ovsynch protocol than those subjected to CIDR and that conception rate varies among groups recording the lowest values in the non-CIDR's animals. Sartori *et al*. [36] and Wolfenson *et al*. [37] suggested that prolonged exposure to P_4 increase the rhythm of the follicular growth leading to larger follicles and consequently larger corpora lutea that produce more P_4 [38]. In addition, P_4 level is the most important factor affecting embryonic life in dairy cows. Previous studies showed that embryonic loss was high in cows with low levels of P_4 compared to those of high levels during the first week after insemination [39].

The results of the current study revealed that the overall conception rate in GPG and G-CIDR-PG groups were 40.74% and 51.85%, respectively that approximately similar to the results reported by Stevenson *et al*. [40] who stated that conception rates at the day 28th post insemination was 50% following ovsynch plus CIDR. On the other hand, presynchronization with CIDR before ovsynch protocol was improved the conception rate to 69.23 %. Prolonged effect of P_4 (CIDR and induced CL) lead to delay in LH release, which allow smaller follicles to fully mature and increased the ova number [41]. From the genetic view, prolonged maintenance of oocyte in the follicular environment could be beneficial to the accumulation of more maternal mRNA as a good factor for the embryonic growth [41, 42].

Conclusion

The current study presented that the CIDR-GPG protocol as a new ovsynch modification could be used for estrus synchronization in the postpartum dairy cows. It achieved a higher conception rate especially in acyclic cows, while the conception rates in all treatments was affected by the cyclic status of animals.

Conflict of interest

The authors declare no conflict of interest.

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الملخص العربي

تحسين معدل حدوث الحمل والخصوبة بإستخدام السيدار قبل نظام تزامن التبويض فى فترة ما بعد الولادة بالأبقار الحلابة

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تعتبر فترة ما بعد الولادة مهمة جدا للحياة التناسلية للأبقار الحلابة. تهدف الدراسة الحالية الي استخدام البروجيسترون (P4) والسيدار المهبلي في برنامج تزامن دورة التبويض والشبق في أبقار الهولشتاين فريزيان الحلابة. تناولت الدراسة عدد ٨٠ بقرة حلابة. جميع الحيوانات تم فحصها تناسليا من حيث عودة الجهاز التناسلى مثل الرحم واستعادة نشاط المبيض وذلك من خلال الجس والفحص بواسطة السونار من خلال فتحة المستقيم. وبعد اجراء الفحوصات الاكلينيكية والاطلاع على السجلات الصحية بالمزرعة يتم استبعاد الحيوانات التى تعانى من التهابات رحمية أو مهبلية أو سبق أن تعرضت لعسر ولادة وعمليات قيصرية واحتباس المشيمة والعرج والتهاب الضرع. اثنان وخمسون حيوانا منتظما فى دورة الشبق بينما ثمانية وعشرون حيوانا غير منتظم في دورة الشبق. تم تقسيم الحيوانات الى ثلاث مجموعات حسب تلقيهم المعاملات الدراسية الى الاتى:- المجموعة الأولى وعددها ٢٧ حيوان تلقت فقط النظام العادى أو جى-بى-جى بينما المجموعة الثانية وعددها ٢٦ حيوان تم معاملتها بالسيدار المهبلى قبل نظام الجى-بى-جى, أما المجموعة الثالثة وعددها ٢٧ حيوان تم معاملتها بالسيدار المهبلى بعد معاملة الحيوانات بالمحفز التناسلى GnRH لنظام الجى-بى-جى. تم أخذ عينات الدم خمس مرات فى اليوم السابع قبل المعاملات – اليوم صفر لبدء المعاملات – واليوم السابع والرابع عشر والخمسين لقياس البروجسترون.ثم تم الفحص بواسطة السونار فى اليوم السابع قبل المعاملات – اليوم صفر لبدء المعاملات – واليوم السابع والتاسع ثم إعادة الفحص بالأيام الخامس والثلاثين والخامس والأربعين لتأكيد الحمل. أظهرت النتائج تنوع معدل الحمل بين المجموعات حيث زاد بنسبة ٦٩,٢٣%، ٥١,٨٥% و ٤٠,٧٤% في كلا من المجموعه المعاملة الثانية والثالثة مقارنة بالمجموعه الضابطه الأولي على التوالي. وكان معدل الحمل في المجموعه النشيطة أعلي من المجموعة الخاملة في الحيوانات التي تلقت نظام جي-بي-جي (٤٤,٤% مقابل ٣٣,٣% على التوالي). بينما استجابت الحيوانات الخاملة أكثر من النشيطة فى المجموعه الثانية (٧٠ % مقابل ٦٨,٨ % على التوالى) وكذلك فى المجموعة الثالثة (٥٥,٦ % مقابل ٥٠ % على التوالى). بناءا على ما سبق فان نتائج البحث توصى باستخدام السيدار المهبلى قبل نظام الجى– بى– جى فى تحسين الخصوبة ومعدل الحمل فى الأبقار الحلابة فى فترة ما بعد الولادة.