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Evaluation of Ovsynch and CIDR + Ovsynch Protocols to Improve Reproductive Efficiency in Lactating Dairy Cows

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AHS, AS, NA and DN conceived and designed the study. AHS and YN performed experimental work. AS and IA analyzed the data and wrote the article.

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Lactating dairy cows, OVP0, P4 profile, Ovulatory follicle diameter, Reproductive efficiency.

ABSTRACT

Appropriate postpartum reproductive management plays a vital role in dairy farm economics. Primary objective of the present study was to compare the efficiency of standard Ovsych protocol (OVP0) and its modified forms (OVP5 and OVP7) as postpartum reproductive management tools in cyclic dairy cows. In total, 167 Holstein cows were randomly divided into three treatment groups. The OVP0 group was comprised of 58 cows. Other two groups, OVP5 (n=55) and OVP7 (n=54), were similar to OVP0 except the intravaginal insertion of controlled internal drug release (CIDR) inserts for 5 or 7 days, respectively. Pregnancy was diagnosed using ultrasonography on d30, d60 and d90 post AI. Ovulatory follicle diameter was measured at timed AI and progesterone profile (ng/mL) on d30 and d60 post AI. Pregnancy rate was analyzed by Chi-square procedure while ovulatory follicle diameter and Progesterone profile by one way ANOVA (α=0.05). Ovulatory follicle diameter (Mean±SEM) was 15.19±0.17 (OVP0), 15.30±0.21 (OVP5) and 15.24±0.19 (OVP7), respectively. The P4 concentration has significant (P<0.05) difference among OVP0 (6.52±0.32), OVP5 (7.75±0.38) and OVP7 (7.58±0.26) on d30 post AI. This difference was non-significant (P > 0.05) on d60 post AI in OVP0 (6.37±0.49), OVP5 (6.75±0.36) and OVP7 (6.80±0.41), respectively. On d30 post AI, pregnancy rate was 39.70, 42.60 and 45.50% in OVP0, OVP5 and OVP7 groups, respectively (P=0.48). Corresponding pregnancy rate on d60 (P=0.39) and d90 (P=0.61) was 36.20, 32.80% in OVP0, 43.80, 41.80% in OVP5 and 37% in OVP7 group. Overall pregnancy loss was 17 (OVP0), 08 (OVP5) and 13% (OVP7), respectively (P=0.62). In conclusion, although pregnancy rate has non-significant difference among all three breeding protocols but numerically, improved pregnancy rate and reduced pregnancy loss was observed in OVP5 group.

INTRODUCTION

Major responsible factors for compromised reproductive efficiency in modern dairy operations are prolonged postpartum anovulatory anestrus, silent estrus and poor estrus detection practices (Crowe *et al*., 2015). During postpartum period, reproductive management around day 50-postpartum is comprised of estrus detection and insemination at a proper time along with the occasional use of $PGF_{2\alpha}$ or P4 injection in cows that have not been observed in estrus at day 60-postpartum (Pursley *et al*., 1997). Improved reproductive management (IRM) is a vital responsible component for optimal returns for dairy operations. This IRM is necessary for optimal dairy business profitability. Similarly, estrus synchronization is an important tool for IRM. Successful estrus synchronization involves the control of both the follicular and luteal phases of the estrous cycle.

The possibility of modifying the estrous cycle through hormonal treatments has been resulted in a variety of synchronization protocols to reduce inter-calving interval and the first service conception rate. Estrus synchronization protocols can be divided into four main categories: i) prostaglandins ($PGF_{2\alpha}$), ii) gonadotropin-releasing hormone (GnRH), iii) progesterone based, and iv) combination of different hormones. All these protocols have their own merits and limitations. Responsive luteal tissue is prerequisite for prostaglandins' based protocols. The GnRH-$PGF_{2\alpha}$-GnRH procedure was used with the aim of ovulation synchronization of cows to get rid of laborious estrus detection methods. This procedure has two unique methodologies: Ovsynch and Cosynch. Ovsynch (GnRH-$PGF_{2\alpha}$-GnRH) is the primary synchronization protocol in dairy cattle since its inception. The timeline of this

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protocol involves $PGF_{2\alpha}$ administration 7d after GnRH injection while 2nd GnRH is given 2d after the $PG_{2\alpha}$ and FTAI 16 hrs after last GnRH. Cosynch involves same time line as Ovsynch with the only difference of FTAI along with second GnRH. Enrolled animals are required to be in cyclicity for the introduction of Ovsynch protocol.

Although the Ovsynch protocol is preferred for cyclic postpartum animals in terms of economic benefits, many modifications of this protocol are in practice nowadays. High progesterone (P4) profile preceding FTAI has resulted in improved ovulation synchrony, better quality embryos and subsequent pregnancy rate (Rivera *et al*., 2011; Chebel *et al*., 2010; Galvão *et al*., 2004). In case of dairy cows, it is well documented that inadequate P4 profile during developmental stages of the ovulatory follicle is the main hindrance leading to reduced fertility in high producing cows submitted to FTAI (Bisinotto *et al*., 2014; Wiltbank *et al*., 2014). Furthermore, negative uterine function is likely to occur in animals with low P4 prior to AI causing increased estrogen receptor-α in the uterus resulting in elevated $PGF_{2\alpha}$ synthesis in the subsequent diestrous leading to premature corpus luteum (CL) regression (Cerri *et al*., 2011; Mobashar *et al*., 2018). This suboptimal P4 profile also disturbs normal expression of several endometrial genes (Forde *et al*., 2012).

Similarly, lactating dairy animals have reduced P4 profile during diestrus than non-lactating (Sangsritavong *et al*., 2002; Wiltbank *et al*., 2014) resulting in enhanced growth rate of graffian follicle (Cerri *et al*., 2011) and blight embryos (Rivera *et al*., 2011) leading to reduced pregnancy rate (PR). To maintain a higher level of P4 before AI, standard Ovsynch plus P4 insert in lactating dairy cows has resulted in improved PR (Stevenson *et al*., 2006). El-Zarkouny *et al*. (2004) also used CIDR inserts in the Ovsynch protocol for 7d prior to GnRH till the $PGF_{2\alpha}$ and obtained increased PR at d29 post AI in comparison with Ovsynch protocol alone in lactating dairy cattle. Keeping in view the benefits of elevated P4 profile during follicular development, it was hypothesized that the introduction of P4 (CIDR) in Ovsynch protocol would result in tight follicular wave synchrony and ultimately enhanced PR as compared to standard Ovsynch protocol. Therefore, the objective of this study was to evaluate OVP0, OVP5 and OVP7 protocols in postpartum dairy cattle through the measurement of PR and plasma P4 concentration as a postpartum reproductive management tool.

MATERIALS AND METHODS

Location

This study was carried out on a commercial dairy farm in Yenişehir, Bursa Province, Turkey (40°15′52″N 29°39′11″E) during February-June. Holstein cows (N=167) with 45-100 days in milk were enrolled. Total mixed ration was presented twice daily with *ad libitum* access to water. Balanced feed, in accordance with NRC, was made available as per requirements for dairy cattle, Milking was done thrice daily. Rolling herd average was 8500 kg; average daily yield of milk was 28.0 kg with 3.79% fat and 3.01% protein. Before study, all cows were subjected to body condition score (BCS) and cows with BCS ranging from 2.50 to 3.25 were enrolled. Cows that have palpable and evident CL (either d0 or d11) were considered to be cyclic. Animals without CL at both examinations were declared acyclic and deleted from the study.

Group I (OVP0; n=58)

Animals in this group were given 2 mL I/M injection of GnRH (Dalmarelin, Lecirelin 25ug/mL, Fatro®Italy) on d0 and d9 (day of 1st GnRH injection was marked as d0). Seven days after 1st GnRH injection, all cattle were administered 2 mL I/M injection of $PGF_{2\alpha}$ analogue (Dalmazine, d-cloprostenol 0.075 mg/mL; Fatro®Italy) and were bred through FTAI approximately 16 h after the 2nd GnRH injection (Fig. 1). Ovulatory follicle diameter (OFD) was measured before FTAI through ultrasonography.

Group II (OVP5; n=55)

Cows in OVP5 group were treated same as in group I but CIDR was inserted at d2 and removed on d7 of the experiment (Fig. 1). The OFD was measured before FTAI through ultrasonography likewise OVP0.

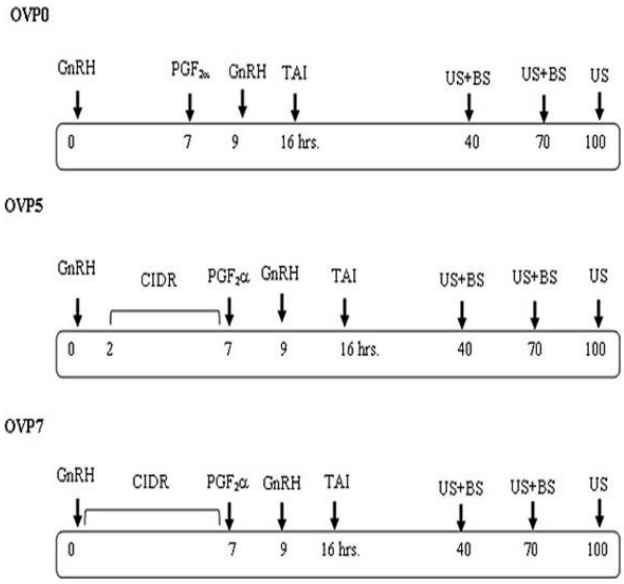

Fig. 1. Timeline (in days) of experimental groups of postpartum Holstein-Friesian cows.

Group III (OVP7; n=54)

Animals in OVP7 group were treated same as in group I except CIDR insertion on d0 (day of start of the experiment) and removal on d7 of the experiment (Fig. 1). The OFD was also measured before FTAI through ultrasonography likewise OVP0.

Pregnancy diagnosis

Pregnancy was diagnosed through ultrasonography on d30, d60 and d90 post FTAI. It was confirmed by the presence of amniotic vesicle (AV), the heartbeat of the embryo and intraluminal uterine fluid as pregnancy markers. Fetal loss was recorded by difference between second and third pregnancy check. In this way, PR (%), and fetal loss (%) were recorded.

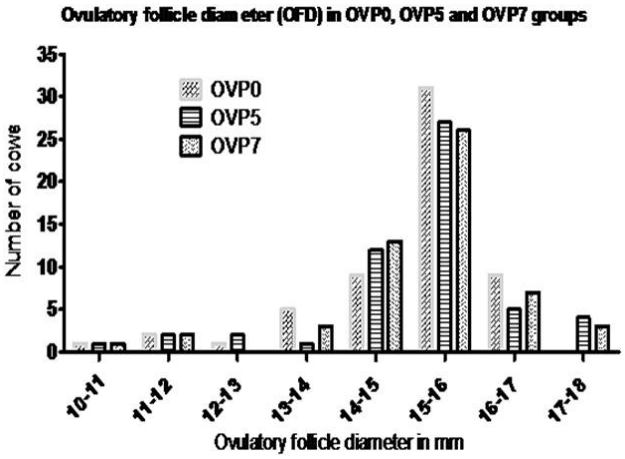

Fig. 2. Distribution of ovulatory follicle diameter (OFD) in mm at the time of AI in lactating dairy cows in different synchronization protocols.

Blood sampling

Median caudal vein was punctured for collection of blood samples (10 mL) with vacutainers (BD, Franklin Lakes, NJ, USA) at d30 and d60 post FTAI from all cows. Soon after collection, the blood sample was centrifuged @ 2800X g for 20 min and harvested plasma was labeled according to individual cow identification and stored at -20°C till P4 analysis with a solid-phase, radioimmunoassay kit (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA).

Statistical analysis

Data were analyzed using SPSS Statistics 21.0 for Windows (SPSS Inc. Illinois, USA). Data are expressed as Mean±SEM (Steel *et al*., 1997). Effect of treatments on PR (in percentage) was calculated by using Chi-square procedure. Differences were considered to be statistically significant at α=0.05. Both OFD and Progesterone concentrations were subjected to one way ANOVA. To see variations among treatments, DMRT was applied. $P<0.05$ was considered to be statistically significant.

RESULTS

The major aim of the present study was to evaluate the effect of progesterone supplementation during standard Ovsynch protocol for different durations on PR in cyclic lactating cows. Progesterone concentration was also determined at d30 and d60 post AI in all three groups. Pregnancy losses were diagnosed by transrectal ultrasonography on d60 and d90 post FTAI as shown in Table I.

On d30 post FTAI pregnancy rates in OVP0, OVP5 and OVP7 groups were 39.7, 45.5 and 42.6%, respectively. Similarly, on d60 post FTAI, the PR in corresponding groups was 36.2, 43.6 and 37.0%, respectively, while these corresponding values on d90 post FTAI were 32.8, 41.8 and 37.0%, respectively (Table I). Pregnancy losses upto d60 and d90 post FTAI in these groups were 8.7 *vs*. 9.5, 4.0 *vs*. 4.2 and 13.0 *vs*.0.0%, respectively, while overall pregnancy loss upto d90 post FTAI in these groups were 17.0, 8.0 and 13.0%, respectively (Table I). Size of preovulatory follicles of cows in all the treatment groups was also measured through ultrasonography and it was found to be 15.19±0.17, 15.30±0.21 and 15.24±0.19 mm (Mean±SEM), respectively (Table II). Distribution of OFD has been shown in Figure 2. Although the values of

Table I.- Effect of different synchronization protocols on pregnancy rate (%) in lactating dairy cows.

Variable	Pregnancy rate (%)			P-Value
	OVP0 (n = 58)	OVP5 (n = 55)	OVP7 (n = 54)	
Pregnancy rate at day 30 post FTAI	39.7 (23/58)	45.5 (25/55)	42.6 (23/54)	0.48
Pregnancy rate at day 60 post FTAI	36.2 (21/58)	43.6 (24/55)	37.0 (20/54)	0.39
Pregnancy rate at day 90 post FTAI	32.8 (19/58)	41.8 (23/55)	37.0 (20/54)	0.61
Pregnancy loss upto 60 post FTAI	8.7 (2/23)	4.0 (1/25)	13.0 (3/23)	0.67
Pregnancy loss upto 90 post FTAI	9.5 (2/21)	4.2 (1/24)	0.0 (0/20)	0.34
Overall Pregnancy loss upto day 90 post FTAI	17.0 (4/23)	8.0 (2/25)	13.0 (3/23)	0.62

all these variables in OVP0, OVP5 and OVP7 groups differed numerically, statistically there was no significant (P>0.05) difference among all three treatment groups (Tables I, II).

Table II.- Effect of different synchronization protocols on ovulatory follicle diameter (OFD; Mean ± SE) in Lactating dairy cows.

Treatment	OFD (mm)	P- value
OVP0	15.19± 0.17	0.711
OVP5	15.30± 0.21	
OVP7	15.24± 0.19	

Pregnancy diagnosis was done with ultrasonography. On the basis of pregnancy outcome, animals were divided into either pregnant or non-pregnant group. Progesterone (P4) concentration in all treatment groups was measured on d30 and d60 post AI. The values of P4 concentrations were significantly (P<0.05) lower in OVP0 (6.52±0.32 ng/mL) as compared to those of OVP5 (7.75±0.38 ng/mL) and OVP7 (7.58±0.26 ng/mL) groups, respectively on d30 post FTAI but values between the latter two groups differed non-significantly (P > 0.05).

On the other hand, progesterone concentration differed non-significantly (P > 0.05) among all treatment groups at d60 post FTAI. These values in OVP0, OVP5 and OVP7 groups were 6.37±0.49, 6.75±0.36 and 6.80±0.41 ng/mL, respectively (Table III).

Table III.- Plasma P4 concentrations (ng/mL; Mean ± SEM) in pregnant cows of different synchronization treatment groups on day 30 and 60 post FTAI.

Parameter	OVP0	OVP5	OVP7
Day 30 Post FTAI	6.52± 0.32^{b}	7.75± 0.38^{a}	7.58± 0.26^{a}
Day 60 Post FTAI	6.37± 0.49^{a}	6.75± 0.36^{a}	6.80± 0.41^{a}

^{a,b}, different superscripts in same row indicate significant differences in the same column (P < 0.05).

DISCUSSION

Ovsynch is one of the most acceptable FTAI estrus synchronization protocol for lactating dairy cows with erratic results (Pursley *et al*., 1997). The core objective of the current project was to appraise P4 supplementation in addition to standard Ovsynch protocol for 5 or 7 days in comparison with OVP0 in lactating cyclic Holstein-Frisian cows. It is well established fact that supplemented progesterone has a reflective effect on follicular development, LH pulsatility and finally, ovulatory response in dairy cattle. A positive relationship has been documented between P4 profile during follicular development prior to AI and the succeeding PR, suggesting that P4 profile is critical for fertility (Folman *et al*., 1990). Studies with P4 supplementation have resulted in tight ovulation synchrony in prepubertal heifers and anestrus dairy cows as well. On the other hand, progesterone releasing intravaginal device (PRID) insertion in luteal phase has been found to enhance PR in cattle with low plasma P4 profile as compared to those having elevated P4 profile during estrus (Folman *et al*., 1990).

In present study, all the cattle were cyclic at the beginning of the experiment. The reason for selection criteria of cyclic animals in present study was based on previous findings of Bisinotto *et al*. (2013) indicating that 90-95% of cows in diestrus phase of cycle at the introduction of FTAI protocol are anticipated to have CL at the time of $PGF_{2\alpha}$ inj. Contrary to those without CL have 75% chances for the presence of CL at $PGF_{2\alpha}$. In present study, the pregnancy rates at d30, d60 and d90 post FTAI in OVP5 group of cows were non-significantly (P>0.05) but numerically higher as compared to those of OVP0 and OVP7 groups. Although, Melendez *et al*. (2006) has observed that CIDR inclusion to FTAI programs has resulted in an increased PR but the cows under treatment were presynchronized with two shots of $PGF_{2\alpha}$. Circulatory P4 profile has profound impact on pregnancy establishment and its maintenance as well. Its subnormal profile could be a major risk factor which necessitates exogenous P4 supplementation in lactating dairy cattle. Stevenson *et al*. (2006) evaluated 634 dairy cows for Ovynch+P4 supplementation at 6 different geographical locations in the USA and observed that overall PR was non-significantly (P>0.05) improved in CIDR supplemented group as compared to Ovsynch at day 28 (50 *vs*. 40%) and day 56 (38 % *vs*. 33 %), respectively.

Similarly, Kawate *et al*. (2007) also reported improved PR (58 *vs*. 50%) in Ovsynch+CIDR treated beef cows as compared to those of single $PGF_{2\alpha}$ treated group. In a similar study, Peeler *et al*. (2004) recorded higher PR in CIDR inserted cows as compared to FTAI protocol using Estradiol Cypionate, $PGF_{2\alpha}$ and GnRH. Without active CL, supplementation with P4 has resulted in reduced fertility due to persistent follicle development leading to aged oocyte resulting in poor PR after estrus synchronization. They also reported that cattle with elevated P4 profile at the start of standard Ovsynch protocol had an optimal PR (43%) in comparison with other group having low circulatory P4 profile (31%) and anovular group (30%). In another experiment, the cows were subjected to presynchronization with two shots of $PGF_{2\alpha}$ at 14-days interval and breeding protocol was started on either 3rd

or 10th day of $PGF_{2\alpha}$. This controlled breeding protocol was resulted in ovulatory response either from 1st or 2nd follicular wave at FTAI. Higher PR (42%) and P4 profile was observed in cows ovulated during 2nd follicular wave as compared to those with low P4 profile (30%) ovulated during 1st follicular wave (Bisinotto *et al*., 2013). In another study Cerri *et al*. (2011) documented that cattle with low P4 profile were found to have higher LH concentration and resulted in not only altered follicular pattern but also uterine origin $PGF_{2\alpha}$ production. This changed hormonal profile has a responsible role in reduced PR in cattle with low P4 profile. The exact phenomenon responsible for conclusion is still lacking conclusive clarification.

The P4 profile during follicular development was 3.0 ng/mL higher in cows having CL as compared to anovular or cyclic animals with a stage other than diestrus (Bisinotto *et al*., 2014; Rivera *et al*., 2011). Elevated P4 profile during the development of ovulatory follicle decreases follicular growth rate (Cerri *et al*., 2011), improves IGF-1 concentration in follicular fluid as well as embryo quality (Rivera *et al*., 2011) and subsequent PR (Bisinotto *et al*., 2013). In lactating dairy cow, this increase in P4 profile was 0.8 ng/mL after single P4 device insertion (Lima *et al*., 2009). This was considered as low and inadequate compared with the cows having CL (Cerri *et al*., 2011) to alter the OFD in cows submitted for FTAI (Colazo *et al*., 2013; El-Zarkouny *et al*., 2004) or AI at detected estrus (Bisinotto *et al*., 2015; Lima *et al*., 2009).

In present study, overall pregnancy loss on d90 post FTAI was lower in OVP5 and OVP7 groups (8 and 13 %) as compared to OVP0 group (17%). In a similar study, Wiltbank *et al*. (2011) has observed significantly ($P<0.05$) reduced pregnancy loss (6.8%) in cows having elevated P4 profile in comparison with elevated loss (14.3%) in group with reduced P4 concentration. Stevenson *et al*. (2006) found no beneficial effect of P4 supplementation on pregnancy loss, but concluded that this loss was minimal in cyclic cows (16%) as compared to non-cyclic cows (31%). In another study, Chebel *et al*. (2010) compared pregnancy loss in 7 lactating dairy (n=3248) herds. They observed pregnancy loss of 0-17% in Ovsynch protocol as compared to CIDR supplemented cows with 4-12%. Recently, it has been documented that 19.70% pregnancy loss ($p>0.05$) was observed in dairy cows (n=2207) synchronized with Ovsynch protocol in comparison with Ovsynch+CIDR insert (from 0-7 days of Ovsynch protocol) where pregnancy loss was reported to be 18.7% (El-Tarabany, 2016).

In present study, OFD was non-significantly different (P=0.0711) in all three synchronization groups (OVP0, OVP5 and OVP7). The PR was, although, 7.4% higher in OVP5 group than OVP0 group. Percentage PR was comparable in OVP7 and OVP0 groups. The reason behind this similar PR is might be due to the same physiological status, cyclic, of enrolled animals in all treatment groups. In a previous study, Souza *et al*. (2007) observed that higher PR (52.6%) was achieved on d60 post AI in animals' group with medium sized follicles, 15-19 mm, in comparison with smaller, ≤13 mm, and larger sized, ≥20 mm, follicle groups having a PR of 38.2 and 34.4%, respectively. In this study, they used E2 (estradiol-17β) 8h prior to second GnRH.

In present study, progesterone concentration was investigated on d30 and d60 post FTAI. On d30 and d60 post AI, lower P4 values were measured in the cows of OVP0 group as compared to OVP5 and OVP7 groups, but these differences at d30 post FTAI were statistically significant ($P<0.05$) while on d60 post FTAI, these were found to be non-significant ($P>0.05$). In a similar research Chebel *et al*. (2010) found that inclusion of P4 in FTAI protocol has been resulted in enhanced ovulation synchrony. On the other hand, no beneficial impact of CIDR supplementation was observed in comparison with control group (Lima *et al*., 2009). In the same study, they found that when Ovsynch protocol was supplemented with CIDR, it was encountered by less chances of premature luteal tissue regression. As reduced P4 profile prior to ovulation and insemination has resulted in premature upregulation of endometrial receptors for E2 and oxytocin which are key factors for $PGF_{2\alpha}$ synthesis and subsequent CL regression (Inskeep, 2004).

Reduced circulatory P4 profile during the estrous cycle preceding insemination has been shown to result in decreased fertility in dairy cows. In similar fashion, Townson *et al*. (2002) has shown that dairy cows ovulated in third follicular wave have been resulted in higher PR as compared to two waves (81 *vs*. 63%, respectively; P=0.058). There was a clear positive linear correlation between circulatory P4 profile on the day of luteolysis and successive embryonic viability (Diskin *et al*., 2002). Shaham-Abalancy *et al*. (2001) has shown that the outcome of reduced P4 profile was impediment in stimulatory effect on uterine sensitivity to oxytocin during the late luteal phase of the subsequent cycle. As a consequence increased $PGF_{2\alpha}$ may hinder CL maintenance during early embryonic stages. Optimal P4 profile is compulsory for pregnancy establishment and its maintenance but there is lack of information about the minimum threshold level of P4 and embryonic losses. For normal pregnancy maintenance, no cut-off value of P4 has been established hitherto. Many researchers have clearly demonstrated that reduced circulatory or delayed rise of P4 after ovulation is a key factor responsible for embryonic survivability in cattle. Transport of P4 to the uterus is supposed to occur via a

local, countercurrent mechanism, arrangement. This was supported by the fact of elevated P4 profile in ipsilateral horn (Weems *et al*., 1988). Similarly, Stronge *et al*. (2005) observed that milk P4 profile from d4-d7 post-ovulation has a positive association with embryonic survival in both dairy cattle and heifers. The present study is different from the above study as progesterone concentration was evaluated after d30 and d60 post FTAI.

CONCLUSION

Proper reproductive management has a vital role for optimal PR in postpartum dairy cattle worldwide. In order to verify P4 level during follicular development, as developing follicle has a profound impact on future reproduction, CIDR inserts were used for a variable period of time with OVP0 protocol. In conclusion, OVP5 protocol can be used as a postpartum reproductive management tool to increase PR. Results of current study have shown an advantage of P4 supplementation as a part of the Ovsynch protocol in cyclic animals either for 5 or 7 days. In both treatment groups, P4 profile was significantly higher than the control group on d30 post FTAI. To minimize the cost, synchronization protocol must be economical and practical as well. The average price of OVP0 protocol is about 1800 PKR and OVP5 or OVP7 is 3000 PKR. As residual conc. of P4 is 0.74 mg after single use for a 7 day period, reuse of autoclaved or disinfected CIDR supplementation (Muth-Spurlock *et al*., 2016) can reduce the price at 2400 PKR.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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