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Fertility Response of Cyclic and Acyclic High Lactating Holstein Dairy Cows to PRID-Modified Ovsync Protocol

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Abstract

Objective: This study aimed to evaluate the fertility response and concurrent follicular dynamics and hormonal profiles of cyclic and acyclic high lactating Holstein cows to PRID Modified (PRID-synch) protocol in comparison with Basic-Ovsynch protocol (control). **Methods:** Forty cyclic and forty acyclic cows were subjected to modified PRID-sync and Basic-Ovysynch (control) protocols. The cows subjected to the modified PRID-synch included 20 out of cyclic cows (cPRID-synch) and 20 out of acyclic cows (acPRID-synch). Similarly, the cows subjected to Basic-ovsync included 20 out of the cyclic cows (cBasic-ovsynch) and 20 out of the acyclic cows (acBasich-ovsynch). The fertility response and concurrent follicular dynamics, circulating levels of progesterone (on Days 0, 3, and 5), E2, and insulin (on Days 7, 8, 9, and 10) were evaluated. **Results:** In cyclic cows, the conception rate was numerically higher in cPRID-synch than cBasic-ovsynch (50% versus 30%). The conception rate did not differ between acPRID-synch and acBasic-ovsynch (30% versus 20%) in acyclic cows. The largest follicle diameter (LFD) was greater (P<0.05) in cBasic-Ovsynch than cPRID-synch on Days 7, 8, and 9 but did not differ between the two groups on Day10. The LFD did not differ (P>0.05) between acPRID-synch and acBasic-Ovsynch on Days 9 and 10 but, on Days7 and 8, it was greater (P<0.05) in acBasic-Ovsynch than acPRID-synch. **Conclusion:** It could be concluded that supplementation of P4 via PRID insertion, on Days 0-7 in the ovsynch protocol in cyclic or acyclic cows improves fertility response with higher response in cyclic cows than acyclic cows.

Keywords: Cow, Fertility, cPRID-synch, acPRID-synch

1. Introduction

Reproductive efficiency plays a key role in the economic success of dairy herds as it influences milk production per day of calving interval and culling policies (Vries, 2006 and Santo et al, 2016). Since cows have their highest milk production during early lactation, the efficiency of milk production can be increased by maximizing the number of cows that become pregnant early in lactation (Rhodes et al, 2003 and Consentini et al, 2020). Also, the economic benefits may come from reducing reproductive costs such as semen costs, reproductive hormones, and veterinary costs (Cabrera et al, 2014 and Ricci et al, 2020) and culling rates or shift in culling to cows with lower milk production (Middleton et al, 2019). Higher reproductive efficiency will cause a greater percentage of cows to enter a high fertility cycle leading to many benefits in terms of health, production, and reproduction (Middleton et al, 2019). Fixed-time artificial insemination following ovulation synchronization (FTAI-Ovsynch protocols) became an integral part of reproductive management in many dairy herds (Colazo et al, 2014) as tools to improve reproductive efficiency by reducing calving interval and increasing the first service pregnancy rate (PR) in cows (Shahzad et al, 2019). FTAI-Ovsynch protocols can be used in cyclic and acyclic cows but the fertility response was significantly worse in acyclic cows (Cartmill et al, 2001).

Anovulatory anestrous was diagnosed in 11-38% of cows at the start of the service period in year-round calving dairy herds (Ribeiro et al, 2013). Decreased fertility in anovular cows subjected to FTAI program is largely mediated by insufficient P4 concentration during the growth of the preovulatory follicle (Santos et al, 2016). A pregnancy rate of 21% was recorded at 74 days following TAI in anestrous cows treated with Basic-Ovsync protocol (Moreira et al, 2000). Supplementation of P4 via insertion of intravaginal implants could improve reproductive efficiency in dairy cows submitted to FTAI-Ovsync protocols (Bisinotto et al, 2015 and Melo et al, 2018). In FTAI-Ovsync protocols, it is possible to manipulate circulating P4 by controlling CL-lifespan through administration of GnRH or PGF (Prusley et al, 1995 and Colazo et al, 2017) or by exogenous supplementation of P4 through the insertion of intravaginal implants, providing a gradual and controlled release of P4 (Silva et al, 2020).

Intravaginal insertion of PRID insert increased serum P4 concentration at the time of PGF2α treatment and increased PR at 32 days after FTAI by 11.2 percentage points. Nonetheless, insertion of progesterone releasing device (PRID, 1.55g) increased the circulating

P4 (Van Werven et al, 2013). The major role of insertion of P4 on Days 0 to 7 in an Ovsync protocol is to control follicular growth, avoid early ovulation, and provide dominant follicle (DF) with adequate size, estradiol production, and ovulatory capacity at the time of TAI (Shahzad et al, 2019). We hypothesized that insertion of PRID concurrent with the injection of GnRH1on day 0 and its removal concurrent with PGF2α injection on Day 7 of the Basic-Ovsync protocol would enhance fertility response in cyclic and acyclic cows by optimizing both follicular growths to adequate size and enhancing its oestrogenic activity. This study aimed to assess the efficacy of modifying Basic-ovsync protocol by incorporating PRID for 7 days, from Day 0 (Day of GnRH1 treatment) to Day 7 (Day of PGF2α treatment) on the follicular dynamics, hormonal profiles, and subsequent fertility response of cyclic and acyclic cows in comparison to cyclic and acyclic cows treated with Basic-Ovsync protocol as a control.

2. Materials and methods

The current study was performed on the farm of Alexandria-Copenhagen Company for Milk and Beef production at Kilo 76 Cairo-Alexandria desert road during the period extending from 2016-2017. All procedures applied to the animals used in this research were approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

2.1. Animals and Management

A total of 80 high lactating Holstein Fresian cows aged 4-6 years and had an average parity of 3.6± 1.1 and a BCS ranging from 2.5-3.5 on a scale of 1-5 (1= Emaciated and 5= Obese cows) were enrolled in the current study. Cows were housed in open barns with fans, bedded with sand and 50% of their area was sheltered. Cows were fed a TMR-formulated diet for cows producing 44kg of 3.5% fat corrected milk according to NRC (2001) recommendations. The main ingredients of feed were corn silage, Egyptian clover (fresh and dried), ground corn, wheat bran, soya bean meal 44%, cottonseed mail, bypass dry fat, and minerals mixture. Cows had free access to water. Only cows, free from detectable reproductive disorders and clinical diseases, were enrolled in the current study.

2.2. Study design

The cows enrolled in the current study included 40 cyclic (>60 days postpartum) and 40 acyclic cows (>60 - ≤180 days postpartum). The acyclicity anestrous was predicated by the absence of estrous signs for at least 21 days and confirmed by the absence of CL in two transrectal ultrasonographic examinations done at 10-days intervals. Cows in the cyclic or acyclic groups were subjected to modified PRID-synch and Basic-Ovysynch (control) protocols. The cows subjected to the modified PRID-synch included 20 out of cyclic cows (cPRID-synch) and 20 out of acyclic cows (acPRID-synch). Similarly, the cows subjected to Basic-ovsync included 20 out of the cyclic cows (cBasic-ovsynch as a control group for cPRID-sync) and 20 out of the acyclic cows (acBasic-ovsynch as a control group for acPRID-synch). The Basic-ovsynch protocol consisted of two GnRH agonist treatments (20 µg Buserelin, 5ml Receptal, MSD Company) on Days 0 (GnRHa1) and 9 (GnRHa2) with a single injection of PGF2α analog (500µg cloprostenol sodium, 2 ml Estrumate MSD Company) in-between on Day 7. The modified PRID-synch included the same treatment regime utilized in the Basic-ovsync but was modified by intravaginal insertion and removal of PRID (progesterone releasing intravaginal device, 1.55 gm of P4, Ceva Company, France) on Days 0 and 7 respectively. The cows were inseminated at 16 h after the GnRH2 treatment.

2.3. Ultrasonography examinations

Transrectal Ultrasonography (TUS) of ovaries was performed by an ultrasound device (SonoscapeA5, China) equipped with a 7.5 MHz transducer on Days -10 and 0 (Day 0 is the day of GnRH1 treatment) to assess cyclicity status. The absence of CL in the two TUS examinations done at 10-days intervals confirmed acyclicity in acyclic cows while the presence of a CL in one of the two TUS confirmed cyclicity in the cyclic cows. Ovaries of both cyclic and acyclic cows were examined by TUS on Days 0, 3, 5, 7, 8, 9, and 10 in both Modified PRID-synch and Basic-ovsynch protocols to measure the diameter of the largest follicle (LFD). Two perpendicular diameters of the largest follicle were measured in frozen sonograms and their average was recorded. Also, the uterus was examined by TUS on Day 32 post-TAI for pregnancy diagnosis. The presence of anechoic amniotic vesicle containing embryo proper experiencing heartbeats is a positive sign of pregnancy. The Pregnancy rate (PR) was calculated by dividing the number of pregnant cows on the number of cows subjected for FTAI in both cyclic (cPRID-synch and cBasic-ovsynch) and acyclic (acPRID-synch and acBasic-ovsynch) cows.

2.4. Blood sampling

Blood samples were collected by jugular venipuncture in plain Vacutainer tubes on Days 0, 3, and 5 for progesterone assay and Days 7, 8, 9, and 10 for estradiol (E2) and insulin assays. The sampling tubes were placed in a refrigerator for 3 hours and centrifuged at 4°c for 20 minutes at 1500xg. The serum was preserved at -20°c until the requested hormonal assays were performed.

2.5. Serum progesterone assay

Serum progesterone concentrations were estimated by progesterone EIA using steroid EIA- progesterone Kits (ALKOr Bio. Inc. Company, 192148, P. O. Box 243, St. Petersburg, Russia). The sensitivity of the test was 5.5 nmol / L. The Intra- and Inter-assay Coefficient of variations were 8.8% and 11.5% respectively.

2.6. Serum estradiol assay

Serum estradiol concentrations were measured by enzyme Immunoassay using Estradiol (E2) enzyme Immunoassay KIT (Catalog number, BC 111, Biocheck, Inc 323 Vintage Park Dr. Foster City, (A.94404). The cross-reactivities are Estrone 2.10%, Estriol 1.50%, 17 an Estriol 0.30%, and 0.01 for each of Cortisol, Progesterone, Testosterone, DHEA-Sulphate, and 5a – Dihydrotestosterone. The Intra- and Inter-assay Coefficient of variations were 4.9% and 6.60% respectively.

2.7. Serum insulin assay

Serum insulin concentrations were measured by Enzyme Immunoassay test using the Insulin ELISA KIT (Immunospec Corporation, 14155 Farmington Rd, Livonia M1 48154 – 5422 USA. The assay sensitivity was 2.0 u1 u/ml.

2.8. Statistical Methods

Variables were presented as mean ± standard error of mean. All data were analyzed with SAS (version 9.1.3; SAS Institute Inc., Cary, NC). Analyses were performed separately on cyclic and acyclic cows as well as on pooled data. Repeated measures analysis of variance was used to analyze variables with multiple measurements per cow using the mixed procedure of SAS. Fisher's exact test was used to assess differences in count data (pregnancy rate). In all tests, statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Pregnancy rates

In the cyclic cows, the conception rates were numerically higher in cPRID-synch than cBasic-ovsynch (50% versus 30%). In the acyclic cows, the conception rates did not differ between acPRID-synch and acBasic-ovsynch (30% versus 20%). The fertility response to the PRID-synch was numerically greater in cyclic cows (cPRID-synch, 50%) than in the acyclic cows (acPRID-synch, 30%). However, the fertility response to Basic-ovsync protocol in acyclic cows (acPRID-synch, 30%) was similar to the response of cyclic cows to Basic–ovsync in cyclic cows but was slightly higher than its counterpart value in acBasic-ovsynch (20%) (Table1). Regardless of the type of the applied ovsync protocol, the pregnancy rate in cyclic cows was numerically greater than acyclic cows (40% versus 25%). Also, regardless of the cyclicity status of cows, the conception rates did not differ between PRID-synch-treated cows and Basic-ovsynch-treated cows (40% versus 25%) (Table1).

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Table1: Pregnancy rates in cyclic and acyclic cows in different synchronization protocols

Ovsync Protocol	Cyclic cows (n=40 cows)			Acyclic cows (n=40 cows)			Total (n=80)	
	Specific name of protocol	pregnant	%	Specific name of protocol	pregnant	%	Total NO. of pregnant	Total %
PRID-synch (n=40)	cPRID-sync (n=20)	10	50	acPRID-synch (n=20)	6	30	16	40
Basic-ovsynch (n=40)	cBasic-Ovsynch (n=20)	6	30	acBasic-ovsynch (n=20)	4	20	10	25
Total		16	40		10	25	26	32.5

Cyclic versus acyclic cows, Fisher's exact test, P > 0.05 in all protocols. PRID-treated cows versus Basic-ovsynch-treated cows, Fisher's exact test, P > 0.05. cPRID-synch= cyclic cows treated with PRID-synch, cBasic-ovsync= cyclic cows treated with basic ovsync protocol, acPRID-synch= acyclic cows treated with PRID-synch protocol, and acBasic-ovsynch= acyclic cows treated with Basic-ovsynch protocol. Basic-ovsynch protocol= The cows were treated with IM injection of GnRH on days 0 and 9 with an IM injection of PGF on Day 7 and FTAI at 16 hr after the 2nd injection of GnRH. The PRID-synch is the same as the Basic ovsynch but with insertion of PRID (Progesterone releasing intravaginal device) on day0 and its removal on Day 7.

3.2. Follicular dynamics

In cyclic cows, the LFD did not differ (P>0.05) between cPRID-synch and cBasic-Ovsynch on Days 0, 3, 5, and 10. On Days 7, 8, and 9, the LFD was greater (P<0.05) in cBasic-Ovsynch in comparison with cPRID-synch in cyclic cows. In acyclic cows, the LFD did not differ (P>0.05) between acPRID-synch and acBasic-Ovsynch on Days 0, 3, 9, and 10. However, on days 5, 7, and 8, the LFD in acBasic-Ovsynch was greater (P<0.05) than acPRID-synch in acyclic cows (Table 2). Regarding the effect of treatment day on the LFD in cyclic cows, it was observed that although the LFD did not differ (P>0.05) between Days 7 and 8, it was greater (P<0.05) on Day 9 in comparison with Day 8 in either cPRID-synch or cBasic-Ovsynch. Between Days 9 and 10, it was observed that while the LFD did not differ (P>0.05) between days 10 and 9 in cBasic-Ovsynch, it was larger (P<0.05) on Day 10 compared with day9 in cPRID-synch (Table2).In acyclic cows, it was observed that while the LFD did not differ (P>0.05) either between days 7 and 8 or between days 9 and 10, it was larger (P<0.05) on days 9 and 10 in comparison with days 7 and 8 acPRID-synch. However, in acBasic-Ovsynch, the LFD did not differ on Day 7 versus day 8 and Day 8 versus day 9, and Day 9 versus day 10 (Table 2).

Table 2: Largest follicle diameter in PRID-treated and Basic-ovsync-treated cows either cyclic (cPRID-synch and cBasic-Ovsynch) and acyclic cows (acPRID-synch and acBasic-Ovsynch).

Day of the Ovsync protocol	Cyclic cows		Acyclic cows	
	cPRID-synch	cBasic-Ovsynch	acPRID-synch	acBasic-Ovsynch
0	1.52 ± 0.04^{a}	1.39 ± 0.10^{a}	1.30 ± 0.13^{a}	1.34 ± 0.09^{a}
3	1.13 ± 0.17^{a}	1.11 ± 0.08^{a}	0.97 ± 0.09^{a}	1.21 ± 0.14^{a}
5	1.16 ± 0.17^{a}	0.93 ± 0.12^{ab}	0.85 ± 0.05^{b}	1.15 ± 0.17^{a}
7	$0.61 \pm 0.03^{b\ C}$	$1.01 \pm 0.11^{a\ B}$	$0.83 \pm 0.07^{b\ B}$	$1.18 \pm 0.14^{a\ C}$
8	$0.78 \pm 0.03^{b\ C}$	$1.14 \pm 0.15^{a\ B}$	$0.95 \pm 0.02^{b\ B}$	$1.29 \pm 0.11^{a\ BC}$
9	$1.11 \pm 0.06^{b\ B}$	$1.43 \pm 0.11^{a\ A}$	$1.31 \pm 0.08^{ab\ A}$	$1.48 \pm 0.10^{a\ AB}$
10	$1.33 \pm 0.07^{b\ A}$	$1.60 \pm 0.10^{ab\ A}$	$1.39 \pm 0.04^{a\ A}$	$1.64 \pm 0.09^{a\ A}$

Values are means ± standard errors. Within the same row, values within cyclic or acyclic cows and carrying different small letters differ significantly at $^{a\text{-}b \text{ or } b\text{-}c}$P<0.05, $^{a\text{-}c}$ P<0.01. Within the same column, values carrying different capital letters are significantly different at $^{A\text{-}B \text{ or } B\text{-}C}$ P<0.05, $^{A\text{-}C}$ P<0.01 cPRID-synch (cyclic cows treated with PRID-synch), cBasic-ovsync (cyclic cows treated with basic ovsync protocol), acPRID-synch (acyclic cows treated with PRID-synch protocol) and acBasic-ovsynch (acyclic cows treated with Basic-ovsynch protocol). Basic-ovsynch protocol= The cows were treated with IM injection of GnRH on days 0 and 9 with an IM injection of PGF on Day 7 and FTAI at 16 hr after the 2nd injection of GnRH. The PRID-synch is the same as the Basic ovsynch but with insertion of PRID on day0 and its removal on Day 7.

3.3. Hormonal profiles in PRID-treated cyclic cows and PRID-treated acyclic cows.

3.3.1. Serum progesterone concentration

The serum P4 concentration was greater in the cPRID-synch than cBasic-Ovsynch on Days 0 and 5 (P<0.05) and on Day3 (P<0.01) in the cyclic cows (Table2). In the acyclic cows, the serum P4 did not differ (P>0.05) between acPRID-synch and acBasic-Ovsynch on Day 0. On Days3 and 5, the serum P4 was greater (P<0.05) in acPRID-synch in comparison with acBasic-Ovsynch treated (Table 3).

3.3.2. Serum estradiol concentration

The serum E2 concentrations did not differ (P>.0.05) between cPRID-synch and cBasic-Ovsynch-treated cyclic cows (Table2) on Days 7-10. In the acyclic cows, the serum E2 concentration in acPRID-synch-treated cows was greater (P<0.05) than acBasic-Ovsynch-treated cows on Day7. In contrast, on Day 8, the serum E2 concentration in acBasic-Ovsynch-treated cows was greater (P<0.05) than acPRID-synch -treated cows. However, the serum E2 concentrations did not differ (P>.0.05) between acPRID-synch and acBasic-Ovsynch on Days 9 and 10 (Table 3).

3.3.3. Serum insulin concentration

The serum insulin concentrations did not differ (P>.0.05) between cPRID-synch and cBasic-Ovsynch-treated cyclic cows except on Day10 where, the serum insulin concentrations was greater in cPRID-synch –treated cows in comparison with cBasic-Ovsynch-treated cows (Table2). In acyclic cows, the serum insulin in acPRID-synch-treated cows was greater (P<0.05) than acBasic-Ovsynch-treated cows on days 7 and 9. However, there was non-significant variation in the serum insulin concentrations between acPRID-synch-treated cows and acBasic-Ovsynch-treated cows on Days 8 and 10 (Table 3)

Table 3: Hormonal profiles in PRID-treated and Basic-ovsync-treated cows either cyclic (cPRID-synch and cBasic-Ovsynch) and acyclic cows (acPRID-synch and acBasic-Ovsynch).

Type of hormone	Day	Cyclic cows		Acyclic cows	
		cPRID-sync	cBasic-Ovsynch	acPRID-synch	acBasic-Ovsynch
Serum progesterone (ng/ml)	0	$8.77 \pm 0.53^{a\ B}$	$5.37 \pm 1.10^{b\ B}$	$0.47 \pm 0.07^{a\ C}$	$0.43 \pm 0.07^{a\ B}$
	3	$13.4 \pm 1.33^{a\ A}$	$6.51 \pm 0.69^{c\ B}$	$4.81 \pm 0.76^{a\ B}$	$1.16 \pm 0.14^{b\ B}$
	5	$14.3 \pm 0.86^{a\ A}$	$11.0 \pm 0.87^{b\ A}$	$10.2 \pm 0.71^{a\ A}$	$6.50 \pm 0.82^{bc\ A}$
Serum Estradiol (pg/ml)	7	$8.48 \pm 0.88^{a\ B}$	$8.21 \pm 0.81^{a\ A}$	$11.1 \pm 0.23^{a\ A}$	$6.32 \pm 0.83^{b\ B}$
	8	$11.0 \pm 0.74^{a\ A}$	$9.38 \pm 0.94^{ab\ A}$	$8.92 \pm 0.62^{b\ B}$	$11.2 \pm 0.51^{a\ A}$
	9	$5.22 \pm 0.49^{a\ C}$	$4.38 \pm 0.68^{a\ B}$	$3.95 \pm 0.72^{a\ C}$	$4.41 \pm 0.80^{a\ C}$
	10	$3.06 \pm 0.17^{a\ D}$	$2.40 \pm 0.34^{a\ C}$	$2.26 \pm 0.37^{a\ D}$	$1.74 \pm 0.22^{a\ D}$
Serum insulin (ng/ml)	7	$1.16 \pm 0.10^{a\ BC}$	$1.07 \pm 0.11^{a\ AB}$	$1.13 \pm 0.11^{a\ AB}$	$0.80 \pm 0.08^{b\ AB}$
	8	$0.91 \pm 0.11^{a\ C}$	$0.70 \pm 0.09^{a\ C}$	$0.84 \pm 0.13^{ab\ B}$	$0.58 \pm 0.12^{b\ B}$
	9	$1.24 \pm 0.14^{a\ AB}$	$0.92 \pm 0.08^{a\ BC}$	$1.15 \pm 0.11^{a\ A}$	$0.63 \pm 0.09^{b\ B}$
	10	$1.49 \pm 0.22^{a\ A}$	$1.22 \pm 0.11^{b\ A}$	$1.29 \pm 0.12^{a\ A}$	$1.02 \pm 0.08^{a\ A}$

Values are means ± standard errors. Within the same row, values, within cyclic or acyclic cows, and carrying different small letters differ significantly at $^{a\text{-}b\ or\ b\text{-}c}$ P <0.05, $^{a\text{-}c}$ P<0.01). Within the same column, values carrying different capital letters are different at $^{A\text{-}B\ or\ B\text{-}C}$ P<0.05, $^{A\text{-}C}$ P<0.01. cPRID-synch= cyclic cows treated with PRID-synch, cBasic-ovsync= cyclic cows treated with basic ovsync protocol, acPRID-synch= acyclic cows treated with PRID-synch protocol and acBasic-ovsynch= acyclic cows treated with Basic-ovsynch protocol. Basic-ovsynch protocol= The cows were treated with IM injection of Gonadtropin releasing hormone (GnRH) on days 0 and 9 with an IM injection of PGF on Day 7 and fixed time insemination (FTAI) at 16 hr after the 2nd injection of GnRH. The PRID-synch is same as the Basic ovsynch but with insertion of PRID (Progesterone releasing intravaginal device) on day0 and its removal on Day 7.

3.4. Comparative hormonal profile between cyclic (cPRID-synch) and acyclic (acPRID-synch) modified PRID-synch treated cows

3.4.1. Serum P4 concentrations

The serum P4 concentration in the cPRID-synch was greater (P<0.01) than the acPRID-synch on Days 0, 3, and 5 (Fig. 1A).

3.4.2. Serum E2 concentrations

The serum E2 concentrations in the cPRID-synch-treated-cows were greater than in acPRID-synch-treated cows on Days 7 (P<0.01) and 8 (P<0.05). However, the serum E2 did not differ (P>0.05) between cPRID-synch-treated-cows and acPRID-synch-treated cows on Days 9 and 10 (Fig. 1B)

3.4.3. Serum insulin concentrations

There were non-significant variations in the serum insulin concentration between PRID-treated cyclic cows (cPRID-synch) and PRID-treated acyclic cows (acPRID synch) on Days 7, 8, 9, and 10 (Fig. 1C).

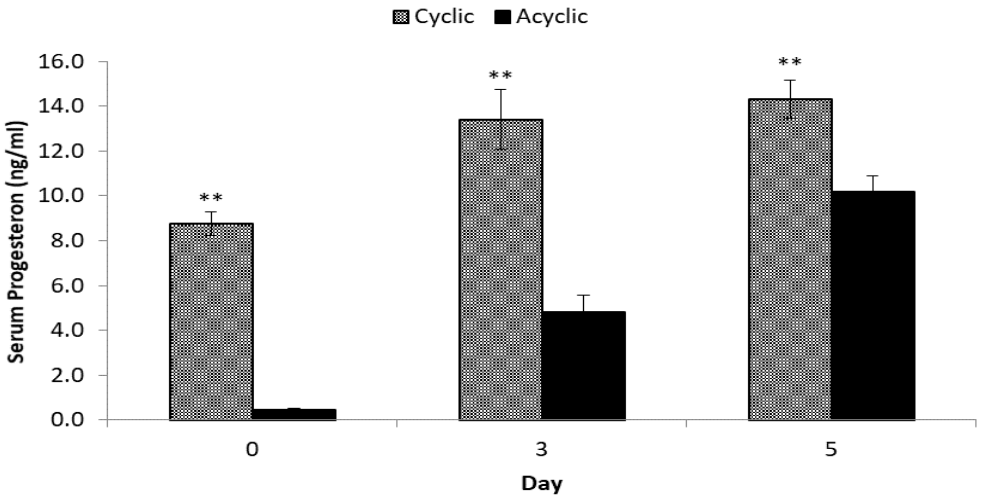

Figure1A. Serum progesterone concentrations in PRID-treated cyclic cows (cPRID-synch) versus PRID-treated acyclic cows (acPRID synch-treated cows).

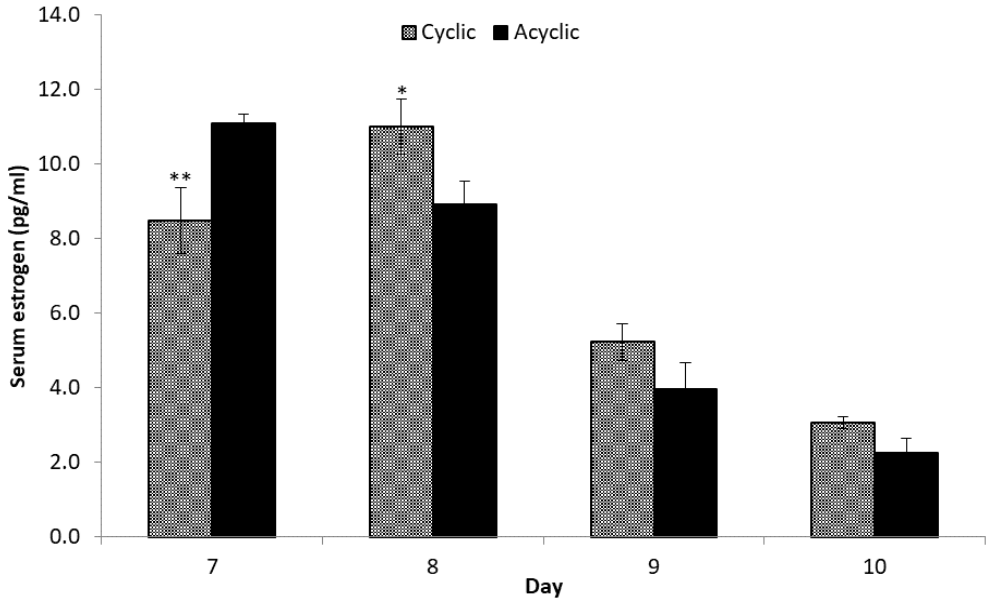

Figure1B. Serum estradiol concentrations in PRID-treated cyclic cows (cPRID-synch) *versus* PRID-treated acyclic cows (acPRID-synch-treated cows).

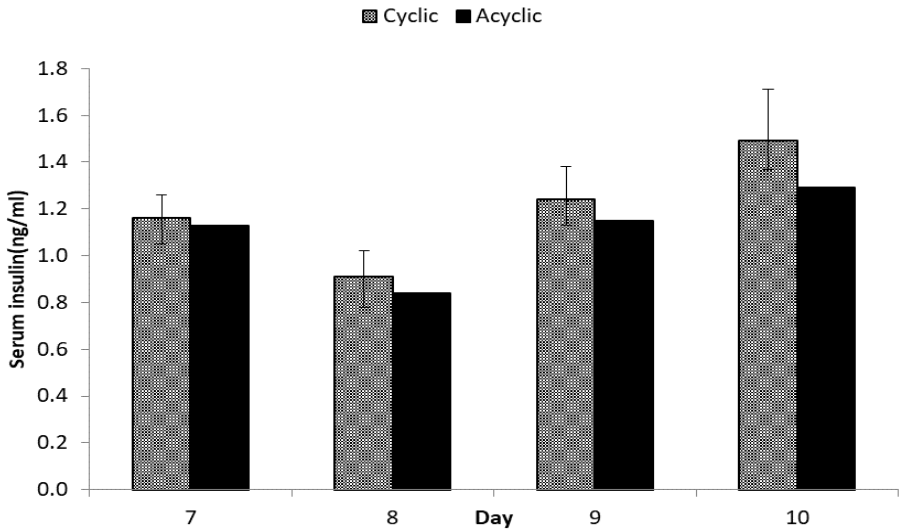

Figure1C. Serum insulin concentrations in PRID-treated cyclic cows (cPRID-synch) *versus* PRID-treated acyclic cows (acPRID synch-treated cows).

4. Discussion

This study evaluated the fertility response to modified PRID-synch protocol in cyclic (cPRID-synch) and acyclic (acPRID-synch) in comparison with their counterpart control groups: cBasic-ovsynch and acBasic-ovsynch respectively. Also, the concurrent follicular dynamics and hormonal profiles were studied.

In cyclic Cows, the greater ($p>0.05$) pregnancy rate in cPRID-treated cows in comparison with cBasic ovsynch treated cows (50% Versus 30%) confirmed the beneficial effect of supplemental P_4 on Days 0-7, period of DF development, in improving pregnancy rate (PR). This result came in accordance with Consentini et al (2020) who reported that supplementation of P_4 in the period extending between days 0 and 7 in the GnRH-based TAI protocol in the presence of CL increased PR by 15-24%. However, the fertility response of the acyclic cows to PRID-synch (acPRID-synch) in comparison with acBasic-ovsynch was similar to results of Colazo and Mapletoft (2014) who recorded PR of 31% in cows treated with ovsync plus P_4 implant versus 21 % in cows treated with ovsync protocol alone. In the same respect, Moreira et al (2001) recorded a PR of 21 % in acyclic cows treated with ovsync-TAI protocol only. The greater difference (20%) in PR between cPRID-synch treated cows (PR equals 50%) and cBasic-ovsynch treated cows (PR equals 30%) versus smaller differences (10%) in the PR between acPRID-synch (PR equals 30%) and acBasic–ovsynch (PR equals 20%) may be supported by the results of Bisinotto et al (2014) and Lima et al (2009) who concluded that supplemention of P_4 via insertion of P_4 Vaginal implants is more effective in improving PR in cyclic than acyclic cows. The similarity of PR between acPRID-synch and cBasic-ovsynch is an indicator of the efficacy of P_4 in improving the fertility response of acyclic cows to be similar to that of the cyclic cows to Basic-ovsynch protocol. Also, comparable PRs were recorded in cyclic (Colazo et al, 2013) and in acyclic cows (Colazo and Mapletoft, 2014) treated with P_4 ovsynch versus ovsynch alone protocol (41% versus 25% in cyclic cows and 33% versus 17% in acyclic cows respectively).

The gradual reduction in the diameter of LF (DF of the terminated follicular wave, tFW) from day 0 to day 7 and the gradual increase in the diameter of LF (DF of synchronized follicular wave, SFW) from day 8 to day 10 may be explained in the light of the fact that the follicles which did not ovulate in response to GnRH1 (~50% Lopez et al, 2013 and Melo et al, 2016) underwent atresia during the interval from day 0 to day 7 while the DF of the SFW would have been still in the early dominant phase. The DF which underwent atresia here in-between day 0 and day 7 might be present in cows that were in metestrous (1-4 days) at the time of the first GnRH treatment. This DF may not ovulate and begin to undergo atresia at the approximate time of PGF injection (Martinez et al, 2002 and Stevenson et al, 2008). Cows, that failed to ovulate at GnRH1, likely had either a follicle that was too young (small) to ovulate in response to the GnRH-induced LH surge (Sartori et al,2001) or had an atretic follicle (Atkins et al, 2010). However, the rate of regression of DF of tFW was faster in cPRID-synch or acPRID-synch compared with their counterparts cBasic-ovsynch or acBasic-ovsynch. This may be attributed to the higher levels of P_4 in cPRID-synch Versus cBasic-ovsynch and acPRID-synch vs acBasic-ovsynch in the current study. On the other hand, the gradual increase in the diameter of the LF on days 8-10 is due to recruitment of new SFW within 48 h after GnRH1 (Stevenson et al, 2004) and its diameter exceeded that of the regressing follicle of the tFW. Also, the rate of growth of the DF of SFW became faster after PGF injection on Day7 and subsequent luteolysis of CL (s) in cBasic-ovsynch and acBasic-ovsynch or simultaneous luteolysis of accessory CL and removal of PRID with a subsequent drop of P_4 to basal level. The low P_4 is followed by an increase in the LH pulse frequency and amplitude resulting in a higher growth rate of DF of SFW (Ginter et al,2001 and Colazo and Mapletoft, 2014).

The regression in the DF of the tFW simulates to a great extent that occurs to DF of the 1st FW in 2-waves cycle and 2nd and 3rd FWs in the 3-waves cycle which undergoes atresia in the presence of high serum P_4 characteristic of the diestrous (Corresponding to interval 0-7 day in the ovsynch). However, the growth of the DF of SFW simulates the growth of the preovulatory follicle (DF of 2nd wave in 2-waves cycle and DF of 3rd wave in 3-WaVes cycle during proestrus (corresponding to the interval from Day 7 to 10 in the present study (Gomez -leon et al, 2019). In partial agreement with our results, Ginther et al (2001) reported that administration of physiological doses of P_4 reduces LH level with a subsequent decrease in the diameter of the DF 31 hr after deviation. Moreover, Adam et al (1992) reported that suppression of LH as a consequence of P_4 secretion by the CL causes the DF to cease its metabolic activity after 2-3 days and begin to regress. The non-significant variation in the DF of SFW on Day l0 among all of the treatment groups in cyclic or acyclic either PRID-treated or Basic-ovsynch-treated may come in line with Bisinotto et al (2015) who reported that the diameter of the ovulatory follicle, in cows without CL and treated with TAI ovsynch protocol either with or without CIDR implant insertion, did not differ in comparison with cyclic cows treated with TAI ovsynch alone. Also, Perry et al (2005) reported that the diameter of the ovulatory follicle did not differ between cyclic and acyclic cows submitted to TAI-ovsynch, a finding which partially agreed with the results of our study with regard to the diameter of the ovulatory follicle on day 10 in cBasic-ovsynch versus acBasic-ovsynch.

The increased circulating serum P_4 levels from day 0 to day 3 and from day 3 to day 5 may be attributed to the gradual increase in the serum p_4 secreted from secondary CL in cyclic cows (cPRID-synch and cBasic-ovsynch) and CL formed after first ovulation in acyclic cows (acPRID-synch and acBasic-ovsynch). These CL(s) were formed due to ovulation (not detected in the current study) of DFs that were present on the Day of GnRH1 treatment. Bisinotto et al (2013) reported the presence of newly formed CLs (Secondary CLs) on Day 3 after GnRH1 treatment in 63.7% of cows without CL on Day 0 (control) and in 51.4% of 2 CIDR-treated cows without CL on Day 0 of ovsynch. The greater circulating P_4 in cPRID-synch compared with cBasic-synch on Days 0, 3, and 5 as well as in acPRID-synch compared with acBasic-ovsynch on Days 3 and 5 may result from the P_4 released from PRID implants present in the vaginae of the treated cows. This explanation was consistent with the findings of the previous studies (Bisinotto et al, 2015, Van Werven et al, 2013 and Silva et al, 2020). Also, Bisinotto et al (2013) recorded higher plasma P_4 concentration in 2CIDR-treated cows without CL at the time of GnRH1 in comparison with cows without CL nor CIDR at the time of GnRH1.

The higher circulating P_4 concentration in cPRID-synch compared wih acPRID-synch on Days 0, 3 and 5 might be due to the primary corpora lutea that were present at the time of initiation of PRID-synch in cyclic cows but not present at the time of initiation of PRID-Synch in acyclic cows (acPRID-synch). This explanation came in agreement with Denicol et al (2012) who observed reduced P_4 concentration during the growth of DF follicle in TAI-ovsynch protocol initiated in

the absence of CL at the time of the first GnRH treatment. Also, Bisinotto et al (2013) recorded lower P_4 from day 0 to day 3 in cows submitted to ovsynch without a CL at the time of first GnRH treatment compared with those having mature CL on the Day of initiation of the ovsynch protocol.

The serum E_2 concentration did not differ ($p>0.05$) neither between cPRID-synch and cBasic-ovsynch nor between acPRID-synch and acBasic-ovsynch on Days 7, 8, 9 and 10. However, perry et al (2007) reported that the size of the ovulatory follicle did not affect circulating concentration of E_2 in ovsynch-treated cows on Day 9 of Co-synch-TAI protocol between cyclic (6.8 ± 0.36 pg /mL) and acyclic (5.80 ± 0.32 Pg/mL) cows. Also, AtKins et al (2010) recorded comparable E_2 Concentrations (2.5 to 3,4 Pg/mL) in CIDR-synch-treated cows on Day of GnHR2 treatment to those recorded in the present study in PRID-synch on Day l0 (2.26 ± 0.37 in acPRID-synch and 3.06 ± 0.17 in cPRID-synch). Regardless of the cyclicity status of cows at the time of the initiation of the TAI-ovsynch protocols in the present study, it seems that the higher serum P_4 levels in cPRID-synch versus cBasic-synch and acPRID synch versus acBasic-ovsynch on Days 0-5 and possibly on Day 7 (not estimated in the current study) are believed to be the main cause of greater PR in cPRID-synch versus cBasic-ovsynch in cyclic cows and in acPRID-synch versus acBasic - ovsynch in acyclic cows. Bisinotto et al (2010) found that cows that began ovsynch with high P_4 had greater pregnancies per AI (43%) than cyclic cows that had Low P_4 (31.3%) or that were anovulatory (29.7%) at the time of initiation of ovsynch. Moreover, the same author added that cows ovulating dominant follicle of the 2nd follicular wave (developed in high P_4 milieu) had higher PR than cows ovulating DF of first follicular wave (Developed in low P_4 milieu) (41.7% Vs 30.4%).

The adequate P_4 concentration, especially in high lactating cow, during the development of preovulatory follicle is critical for gamete development and supplementation of P_4 at this developmental phase increase the pregnancy rate (Binelli et al, 2014 and Bisinotto et al, 2013). Moreover, decreased oocyte quality and consequently embryonic quality may result from premature maturation of the oocyte (Revah and Butler, 1996) in cows with increased pulse frequency of LH because of low circulating P4 during DF development (Adam et al, 1992). It could be concluded that supplementation of P4 via PRID insertion, on Days 0-7 in the ovsynch protocol in cyclic or acyclic cows improves fertility response with higher response in cyclic cows than acyclic cows.

Conflict of interest

The authors declare that they have no conflict of interest.

Research Ethics Committee Permission

This study was approved by the local Ethics and guides of the Faculty of Veterinary Medicine, Kafrelsheikh University University Egypt.

Authors' contribution

Adel A. Ramoun; Tarek S. Zarara; Ismail I. EL-Kon and Nabil I. Mansour designed and conducted the study. Adel A. Ramoun shared with Tarek S. Zarara in performing the practical part of the study. All authors collected and analyzed the data. Adel A. Ramoun revised and approved the final version of the manuscript.

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