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Progesterone-based hormonal treatments to induce and synchronize the onset of puberty in buffalo-heifers

Hassab-Allah A. Abou EL-Ghait

Mahallet Mousa Buffalo Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt

Correspondence:h.abouelghait@gmail.com

Abstract

Objective: The present work aimed to study the effect of progesterone (CIDR)-based GnRH treatments plus eCG (1000 or 500 IU) on inducing and synchronizing the onset of puberty in buffalo-heifers.

Methods: Buffalo-heifers (n=28) were randomly allocated into four treatment protocols (7 each): (G1, CIDR- eCG1000- GnRH), (G2, CIDR- eCG 500- GnRH), (G3, CIDR-GnRH) and (G4, CIDR- alone). The largest follicle diameter (LFD) and antral follicles counts (AFC) of small (≤ 4mm), medium (> 4mm- ≤8mm) and large follicles (>8mm) were estimated by transrectal ultrasonography on Days 0, 8, and 11. Also, the LFD was estimated on Day 13 as well.

Results: On Day 11, the AFC of both small and medium-sized follicles was higher in G1 compared with G2 ($P < 0.05$) and with both G3 and G4 ($P < 0.01$). The AFC of large follicles was the greatest ($P < 0.05$) in G1compared with all groups but did not differ among G2, G3, and G4 on Day 11. On Day 13, the LFD showed non-significant variation between G1 and G2 as well as between G3 and G4. It showed a significant increase in G1 ($P < 0.01$) and G2 ($P < 0.05$) compared with either G3 or G4. The conception rate was the highest ($P<0.05$) in G1 compared with all groups.

Conclusion: It could be concluded that the incorporation of eCG at a dose of 1000 IU in P4 (CIDR) based together with GnRH treatment protocol induces and synchronizes puberty in buffalo-heifers at the age of puberty.

Keywords: Puberty, buffalo- heifers, eCG, AFC, CIDR.

1. Introduction

Puberty in heifers is a process of acquiring reproductive competence (Hossein-Zadeh, 2011). The delayed and asynchronous onset of puberty, silent heat, and long postpartum ovarian quiescence are the main causes of poor reproductive performance in buffaloes (Verma et al., 2019). Great variations in the age at attaining puberty in buffalo heifers (Perera, 2008) lead to wide variation and delay in the age at first breeding (Ettema and Santos, 2004) and consequently in the age at first calving (Bhatti et al., 2007; Sarwar et al., 2009). Breeding buffalo-heifers at older ages affected the lifetime productivity in buffaloes (Batra et al., 2019).

Hormonal inadequacies and immaturity of the reproductive neuroendocrine axis is the main cause of delayed puberty in well-managed buffalo-heifers (Nanda et al., 2003). The initiation of high-frequency release of GnRH and consequently LH pulses is a prerequisite for the onset of puberty (Day et al., 1984). The GnRH plays an important role in the regulating secretion of LH, follicular development, and secretion of steroid hormones (Gupta and Carviel, 2016).

Exogenous hormonal treatments such as progesterone, GnRHa, and gonadotrophins either alone or in various combinations were used for induction of puberty (Chaudhari et al., 2012). Madgwick et al. (2005) reported that GnRH-treated heifers attained puberty earlier than control heifers with a high level of LH hormone and a greater number of LH pulses than the control. Administration of P4 can induce puberty by decreasing the estrogen receptors in the hypothalamus which in turn decreases the negative feedback effect of E2 on GnRH release with a subsequent increase in LH pulsatility (Day et al., 1984). Supplementation of P4 via CIDR insertion was more effective because of lower peripheral concentration of P4 which resulted in greater secretion and follicular growth during P4 treatment (Bergfeld et al., 1996b). Progesterone supplementation with or without eCG produced more favorable results in heifers (Khade et al., 2011; Pawshe et al., 2020). The eCG was used at doses of 300–800 IU for the induction of folliculogenesis, early onset of puberty, treatment of anoestrous, and induction of superovulation for embryo transfer (Murphy, 2018; Naseer et al., 2012). The current study hypothesized that the use of P4-based

eCG-GnRH treatment would induce and synchronize the onset of puberty in buffalo-heifers.

The present work aimed to study the effect of P4 (CIDR)-based GnRH treatments plus eCG (1000 or 500 IU) on inducing and synchronizing the onset of puberty in buffalo heifers aged 18-33 months.

2. Materials and methods

This study was conducted at Mahallet Mousa Buffalo Research Station, Kafr El-Sheikh, Province Egypt, during the period extending from November (2019) to March (2020), which coincides with a high breeding season for buffaloes in Egypt. The animals were handled in accordance with the guide of the Faculty of Veterinary Medicine, Kafrelsheikh University for care and use of agricultural animals for research purposes.

2.1. Animals and management

Twenty-eight buffalo-heifers, 18-33 months in age and 320-330kg in weight, were used for conducting the current study. They had body condition scores (BCSs) of 2.75 -3.25 (1-5, (Bhalaru et al., 1987). They were fed on a diet formulated from concentrates and Berceme (*Trifolium alexandrinum*) designed to meet growth requirements according to APRI (1997, unpublished data). They were kept indoors in open yards whereas 30% of the yard area was sheltered. They had open access to water. They were vaccinated against infectious diseases according to the vaccination program recommended by the General Authority of Veterinary Services. Before enrollment in the study, the heifers were confirmed to have no congenital affections of the reproductive system and still did not attain puberty by transrectal ultrasound examination. The absence of ovarian activity, indicative of attaining puberty, is confirmed by the absence of the corpus luteum in two transrectal ultrasound examinations of the ovaries conducted at 10 days intervals (Rodrigues et al., 2013). Also, the determination of basal serum progesterone levels, < 0.5 ng/ml, in two blood samples taken at a 10-days interval, confirmed that these heifers still did not attain puberty.

2.2. Experimental design

All heifers (n = 28) received intravaginal insertion of controlled internal drug release (CIDR) that contains 1.389 of P4 (Ezabrid Pfizer, Animal health) on Day 0 which remained in situ until removal on Day 10. Then the heifers were randomly assigned into four equal treatment groups (n=7 each). Each buffalo heifer in the first group (G1, CIDR- eCG1000- GnRH, Fig. 1A) received IM injection of 1000 IU eCG (2 vials Gonasir, each contains 500 IU eCG, MSD, Animal health company) on Day 10 and 20 µg buserelin acetate (equivalent to 5ml Receptal, MSD, Animal health company) on Day13. The second group (G2, CIDR- eCG 500- GnRH, Fig. 1B) received the same treatment regime applied in G1 but the dose of eCG was reduced to 500 IU. In the 3rd group (G3, CIDR-GnRH, Fig. 1C), the animals received an IM injection of 5ml saline instead eCG administrated in either G1 or G2 on Day 10. The animals in the 4th group (G4, CIDR- alone, Fig. 1D) received nothing more than the intravaginal insertion of the CIDR implant on Day 0.

2.3. Ovarian ultrasonography

For each buffalo-heifer, the two ovaries were monitored by transrectal scanning using a linear array transducer adjusted at a frequency of 7.5-MHz transducer (Sonoscape Co. LTD, Shenzen, China supplied with a multifrequency linear transducer 3.0-8.0 MHz). The antral follicles count (AFC) was determined on Days 0, 8, and 11 as has been previously described (Burns et al., 2005). In brief, each ovary was scanned from pole to pole, and images for different ovarian sections were captured and frozen. All antral follicles were mapped on both ovaries. Two perpendicular diameters were averaged for each follicle and the total number of follicles per pair of ovaries was categorized into small (≤4mm); medium (>4 -≤8 mm) and large (>8 mm). The number of follicles per ovarian pair was counted and recorded. The diameter of the largest follicles was recorded for each animal on Days 0, 8, 11, and 13 of the treatment. The ovaries were scanned for the presence of CL on days -10 and 0. Also, pregnancy diagnosis was performed by transrectal ultrasonic scanning of the uterus at day 35–40 post-insemination.

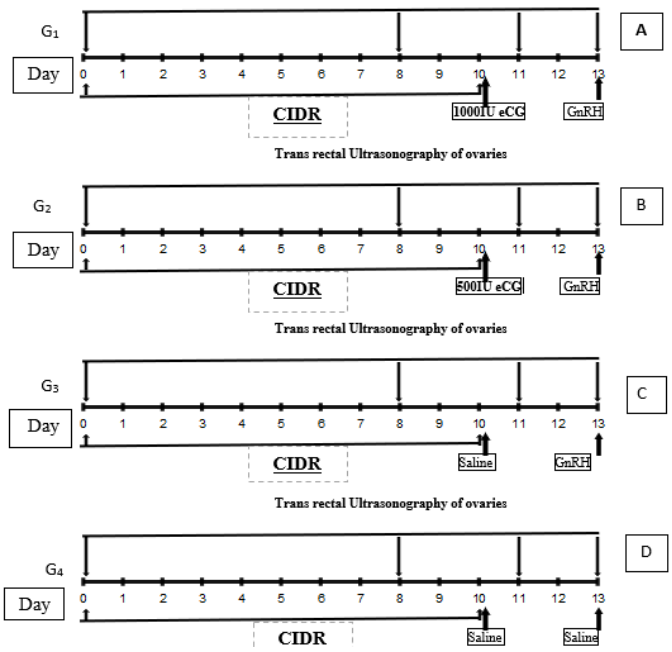

Figure 1. Experimental design. G1=CIDR- eCG1000- GnRH (A); G2= CIDR- eCG 500-GnRH (B); G3= CIDR-GnRH (C) and G4= CIDR- alone (D). D0= Day of CIDR insertion in all groups, D10= Day of CIDR removal in all groups, and injection of eCG at a dose of 1000 IU in G1 and 500 IU in G2, and D13= Day of injection of GnRH in G1, G2, and G3.

2.4. Blood sampling

Blood samples were collected via jugular vein puncture in vacutainer tubes on Days 0 (Day of CIDR insertion), 11 and 13 of treatment as well as on Day 12 post-breeding. The blood samples were centrifuged at 1000 rpm per minute for ten minutes. The serum samples were stored at -20C° until hormonal assays were conducted.

2.5. Hormonal assay

Serum progesterone concentration was estimated on Days 0 and 11 of treatment as well as on Day 12 post-breeding. Serum estradiol concentration was estimated in the blood sample taken on Day11. Serum P4 and E2 concentrations were assayed by Radioimmunoassay (RIA) using Beckman coulter RIA progesterone and Beckman coulter RIA estradiol kits (Immuno TECH, S.r.o Radiova 1-10277, Prague, Czech Republic) respectively, according to the procedures described in the catalog enclosed with the kits. The inter-and intra- assay coefficients of variations were 8.66% and 8.15% for progesterone and 14.5% and 14.4% for estradiol, respectively. The averages of sensitivity were 9.58 ng/ml for progesterone and 9.58 pg/ml for estradiol.

2.6. Reproductive management and fertility status

The Buffalo-heifers were observed twice daily, at 12-h intervals, by the herd man for at least 1 h for estrous signs (Rhodes et al., 2003). Buffalo-heifers were considered in estrous when stand and accept mounting by buffalo-bull teaser (Vale et al., 1990). Twelve hours after estrous detection, buffalo-heifers were inseminated by an experienced inseminator using fertile semen. Pregnancy diagnosis was conducted by transrectal ultrasonic examination at 35–40 days post-insemination. The conception rates were calculated by dividing the number of conceived heifers by the total number of inseminated heifers.

2.7. Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using SAS Software (2002). The AFC, largest follicle diameter (LFD), serum concentrations of P4 and E2 were analyzed by analysis of variance (one-way ANOVA). The mean differences between the groups within periods were compared by Duncan's multiple range tests (1955). Chi-square analysis was conducted to determine the effect of treatments on conception rates rate. Differences were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Antral follicles count (AFC)

The means ± SEM of small AFC (≤ 4mm) and medium AFC (> 4 mm - ≤8 mm) did not differ among treatment protocols on Days 0 and 8. On Day 11, the mean ± SEM of small AFC was higher in G1 at $P < 0.05$ compared with G2 and at $P < 0.01$ compared with either G3 or G4 (Fig. 2 A and B). Also, the mean ± SEM of small AFC was higher ($P < 0.05$) in G2 compared with either G3 or G4 (Fig. 2 A). The mean ± SEM of medium AFC was higher in G1 compared with G2 ($P < 0.05$) and G4 ($P < 0.01$). It was noted that while the mean ± SEM of medium AFC in G2 showed a significant ($P < 0.05$) increase compared with G4, it showed a non-significant ($P > 0.05$) increase compared with G3 (Fig. 2 B). The mean ± SEM of large AFC did not differ among treatment protocols but on Day 11, it was greater ($P < 0.05$) in G1compared with all groups. However, it did not differ among G2, G3, and G4 (Fig. 2 C).

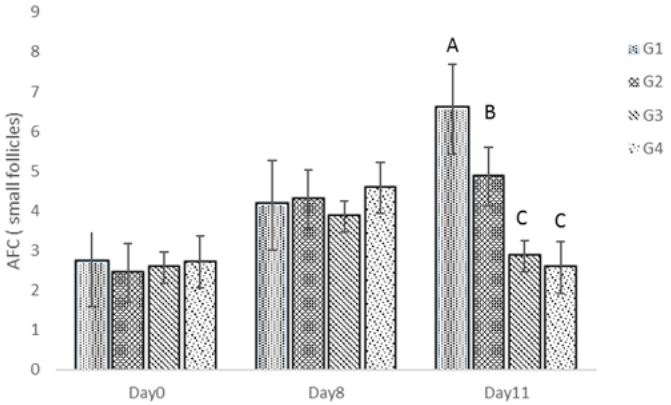

Figure 2A. The means ± SEM of small (< 4mm) AFC in G1 (CIDR- eCG 1000- GnRH, n=7); G2 (CIDR- eCG 500- GnRH, n=7); G3 (CIDR-GnRH, n=7) and G4 (CIDR alone, n=7) on Days: 0, 8 and 11 of treatment protocols A-B or B-C p<0.05; A-C p<0.01.

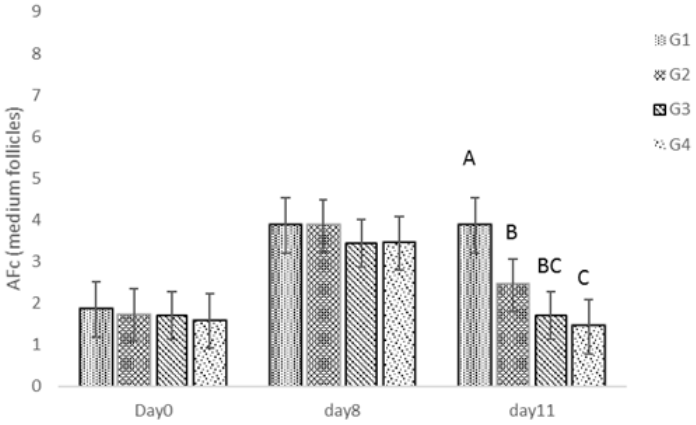

Figure 2 B. Means ± SEM of medium (> 4 - ≤ 8 mm) AFC in G1 (CIDR- eCG 1000- GnRH); G2 (CIDR- eCG 500- GnRH); G3 (CIDR-GnRH) and G4 (CIDR alone) on Days: 0, 8 and 11 of treatment protocols. A-B or B-C p<0.05; A-C p<0.01.

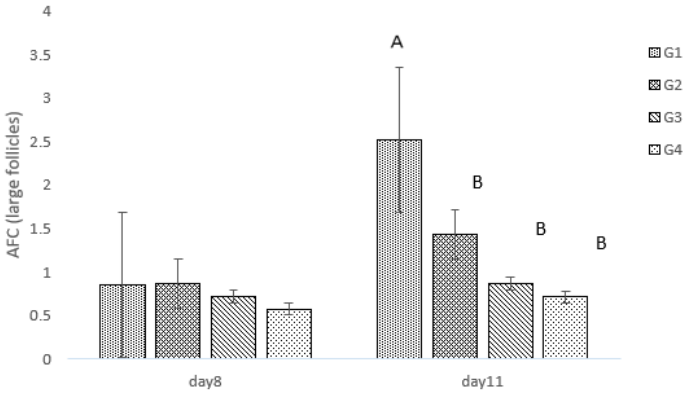

Figure 2C. Means ± SEM of large (> 8 mm) AFC in G1 (CIDR- eCG 1000- GnRH); G2 (CIDR- eCG 500- GnRH); G3 (CIDR-GnRH) and G4 (CIDR alone) on Days: 0, 8 and 11 of treatment protocols. A-B or B-C p<0.05; A-C p<0.01.

3.2. The largest follicle diameter

The Largest follicle Diameter (LFD) did not differ among four treatment protocols on either Day 0 or Day 8. On Day 11, there was a non-significant ($P > 0.05$) increase in the LFD between G1 and G2; G2 and G3 and G3 and G4 respectively. The LFD was larger in G1 compared with G3 ($P < 0.05$) and G4 ($P < 0.01$). In the same respect, the LFD was larger ($P < 0.05$) in G2 compared with G4 (Fig. 3). On Day 13, it was observed that the LFD showed a non-significant variation between G1 and G2 as well as between G3 and G4. The LFD showed a significant increase in G1 ($P < 0.01$) and G2 ($P < 0.05$) compared with either G3 or G4 (Fig. 3).

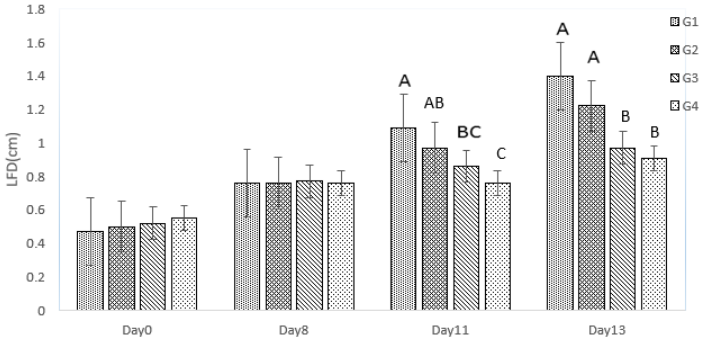

Figure 3. The largest follicle diameter (LFD) in G1 (CIDR- eCG 1000- GnRH); G2 (CIDR- eCG 500- GnRH); G3 (CIDR-GnRH) and G4 (CIDR alone) on Days: 0, 8, 11 and 13 of treatment protocols. A-B or B-C p<0.05; A-C p<0.01.

3.3. Serum P4 concentrations

The serum P4 concentrations did not differ among all treatment protocols on Day 0. On Day 13 of the treatment protocols, the serum P4 concentrations in either G1 or G2 showed a significant (P< 0.05) increase in comparison with both G3 and G4. On Day 12, post-breeding, although the serum P4 concentrations did not differ among G1, G2, and G3, it was significantly (P < 0.05) higher in G1 compared with G4 (Table 1).

3.4. Serum estradiol concentrations

It was observed that while the serum estradiol concentrations showed non-significant variations among G1, G2, and G3, it showed a significant (P< 0.05) increase in G1 compared with G4 (Table 2).

3.5. Fertility response

The conception rate was the highest (P<0.05) in G1 as compared to G2, G3, and G4 (Table 2).

Table 1. Means ± SEM of serum progesterone concentration (on the treatment Days 0 and13 and Day 12 post-breeding) and serum estradiol on Day 11 of the treatment

Hormone	Day	Treatment groups			
		G_1	G_2	G_3	G_4
P4(ng/mL)	Day_0	0.29 ± 0.03	0.35 ± 0.05	0.38 ± 0.04	0.35 ± 0.04
	Day_{13}	1.47 ± 0.11^{A}	1.48 ± 0.06^{A}	0.86 ± 0.06^{B}	0.87 ± 0.06^{B}
	$Day_{12\ PB}$	5.64 ± 0.52^{A}	4.36 ± 0.78^{AB}	3.88 ± 0.19^{AB}	3.50 ± 0.46^{B}
E2(pg/mL)	Day_{11}	55.63 ± 10.29^{A}	44.17 ± 6.38^{AB}	36.62 ± 3.06^{AB}	24.77 ± 1.05^{B}

G1= (CIDR- eCG 1000- GnRH); G2 (CIDR- eCG 500- GnRH); G3 (CIDR- GnRH) and G4 CIDR alone. PB= Post-breeding, CIDR= Controlled internal drug release, eCG= Equine chorionic gonadotropin and GnRH= Gonadotropin Releasing Hormone. A-B or B-C p<0.05; A-C p<0.01

Table 2. Conception rates after induction and synchronization of puberty in buffalo-heifers

Parameter	Treatment groups				
	G_I (n=7)	G_2 (n=7)	G_3 (n=7)	G_4 (n=7)	All group (n=28)
Conception rate	6/7	5/7	3/7	2/7	16/28
Percentage (%)	86	72	43	29	57
chi_ square	3.371*	1.289	0.143	1.086	8.857*
Sig	0.05	0.257	0.705	0.257	0.012

G1= (CIDR- eCG 1000- GnRH); G2 (CIDR- eCG 500- GnRH); G3 (CIDR- GnRH) and G4 CIDR alone. CIDR= Controlled internal drug release, eCG= Equine chorionic gonadotropin and GnRH= Gonadotropin Releasing Hormone. * P< 0.05.

4. Discussion

The present work aimed to study the effect of P4 (CIDR)-based GnRH treatments plus eCG (1000 or 500 IU) on inducing and synchronizing the onset of puberty in buffalo-heifers aged 18-33 months. Also, the effect of these hormonal treatments on antral follicles counts (AFC), largest follicle diameter, and concomitant steroid hormones levels were investigated. In the present study, the absence of any luteal tissue (Agarwal and Allamaneni, 2004) as being detected by the transrectal US of ovaries and low P4 (<0.5 mg/ml, Peter et al. 2009) on Day 0 confirmed ovarian acyclicity and that the heifers still did not attain puberty. Also, the absence of large follicles (>8 mm) on the ovaries of heifers in all groups on Day 0 ensured the acyclic status of ovaries since the growth of large follicles beyond the diameter of 8 mm depends mainly on adequate LH pulses frequency (Kumar et al., 2014). In support, Noakes et al. (2009) attributed the prepubertal anestrus to low LH pulses frequency with subsequent insufficient growth of dominant follicle and threshold for positive feedback effect of E2 on LH surge.

The non-significant variations in the LFD and AFC of all sizes (SF, MF, and LF) among all of the treatment groups on Day 8 while CIDR still in situ indicated that the low P4 level (Bergfeld et al., 1996a) controlled the ovarian follicular activity, in terms of LFD and AFC, at the same level in all groups. Concerning the size of the LFD, our results came in accordance with Edward et al. (2013) who found that a lower dose of P4 in the intravaginal progesterone releasing device (IPRD) did not affect the growth rate of the DF after wave emergence. On Day 11, the 2nd day after CIDR removal and the Day of eCG treatment in both G1 (1000IU eCG) and G2 (500IU eCG), the increase in the LFD in G1 compared with G3 and G4 and in G2 compared with G4 might be attributed to the stimulatory effect of eCG on the follicular growth (Bartolomeu et al., 2007) in both G1 and G2 in comparison with G3 and G4. This suggestion came in agreement with Bartolomeu et al., (2007) who reported that the growth rate of DF was greater throughout the interval extending from CIDR removal and onset of eCG treatment to ovulation in buffalo receiving CIDR based plus eCG treatment regime compared with those receiving CIDR only. Moreover,

Peter et al. (2009) found that eCG treatment improved the diameter of DF at the time of FTAI when administered to heifers treated with first-time use Intravaginal progesterone releasing device (IPRD). The non-significant increase in the diameter of the LF in G1 compared with G2 may be explained in the light of a higher dose of eCG used in G1 (tow fold) in comparison with G2. However, the lower eCG dose in G2 compared with G1 also could explain the significant and non-significant increase in LFD in G1 and G2 respectively in comparison with G3. The non-significant variation in the LFD between G3 and G4 on Day11 may be interpreted because heifers in both of the two groups received no hormonal treatment other than CDIR between day 10 and 13 when heifers in G3 received GnRH but heifers in G4 received no treatment at the end of the treatment period.

Doubtless, the increase in AFC of all follicles classes (SF, MF, and LF) in G1 in comparison with other treatment groups on Day 11 may be due to the higher dose of eCG used in G1 compared with G2 or non-inclusion of eCG in the treatment regimens applied in both G3 and G4. In the same respect, the significant increase in AFC of SF in G2 compared with G3 or G4 and MF in G2 compared with G3 could be also attributed to the inclusion of eCG even at a lower dose in the treatment regime applied in G2 but neither in G3 nor in G4.

The eCG-induced increase in the AFC in GI (eCG=1000UI) compared with G2 (eCG=500IU) and both G3 and G4 (non-eCG-treated) on Day 11 may explain the highest CR in G1 compared with other groups. These results came in agreement with Furukawa et al. (2020) and Mossa et al. (2012) who reported that cows with high AFC had higher pregnancy rates. Also, in accordance with our results, Cushman et al. (2009) recorded higher pregnancy rates in heifers with high vs those with low AFC. Moreover, the largest diameter of the LF in G1 in comparison with G2 and G3 ($P>0.05$) and G4 ($P<0.05$) explains the highest CR in G1 in comparison with other groups. Butler et al. (2011) reported that the larger the size of the DF, the more likely a heifer to ovulate after GnRH treatment (administrated on Day 13 in the current study) to synchronize ovulation. It was assumed that the increase in serum E2 level due to higher AFC (Furukawa et al., 2020) and larger DF (Rodrigues et al., 2013) in G1 vs other groups is conductive to higher estrous induction rate and ovulation rate with subsequent higher CR. On the other hand, Evans et al. (2012) attributed the lower fertility in cows with low AFC to effects on oocyte quality and endometrium receptivity. Nonetheless, Baruselli et al. (2004) found that the eCG-treatment at the time of CIDR removal resulted in higher serum P4 level in the diestrous of the following cycle, as the situation in Day 13 post-breeding in the present study, as a result of the formation of bigger CL from lager follicle which favors higher pregnancy rate.

Conclusion

It could be concluded that the incorporation of eCG especially at a dose of 1000 at the time of CIDR removal in P4-based-GnRH treatment regime for induction and synchronization of puberty in buffalo-heifer improves antral follicles count and increase LF diameter in a manner such conductive to higher conception rate.

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.

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