South African Journal of Animal Science 2022, 52 (No. 4)

Addition of equine chorionic gonadotropin in controlled internal drug-release-based synchronization improves reproductive performance of Nili-Ravi buffalo in the sub-tropics

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(Submitted 31 January 2022; Accepted 30 May 2022; Published 14 October 2022)

Abstract

This study evaluated the effect of equine chorionic gonadotropin (eCG) and factors such as body condition score (BCS), cyclicity, and breeding season in a controlled internal drug release (CIDR)-based oestrus synchronization protocol on ovarian follicle dynamics, oestrus expression, ovulation rate, and pregnancy per artificial insemination (AI) (P/AI) in Nili-Ravi buffalo. Adult buffaloes (n = 88), 6.69 ± 1.17 years, parity 3.36 ± 0.97, and BCS 2.76 ± 0.45, were randomly administered eCG or saline, concurrent with prostaglandin F2α (PGF2α) treatment on Day 6 in a CIDR synchronization protocol during the breeding season and low breeding season. Follicular dynamics and oestrus expression were recorded from eCG administration until ovulation. Fixed-time artificial insemination (FTAI) was performed at 48- and 60-hour intervals after CIDR withdrawal. On Day 35 post-AI, pregnancy was diagnosed with ultrasonography. There was no significant difference in the size of the ovulatory follicle between eCG-treated buffaloes (ETB) and saline-treated buffaloes (STB). The growth rate of the dominant follicle, oestrus response and intensity, ovulation, and P/AI were higher (P <0.05) in eCG-treated buffaloes. It was concluded that eCG improved the growth rate of the dominant follicle, oestrus expression, and P/AI in a CIDR-based FTAI programme in Nili-Ravi buffalo. The deleterious effects of poor BCS, acyclicity, and low breeding season could be reduced with a progesterone-based fixed time AI protocol with the addition of eCG.

Keywords: buffalo, controlled internal drug release, equine chorionic gonadotropin, productive efficiency
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Introduction

Buffaloes play a big role in food security and sustainable agricultural economy in Asian countries (Ahmad & Arshad, 2020). They have a significantly longer productive life (16 years old) and have the ability to convert low-grade roughages, and produce more calves, milk, and meat than other cattle. Buffaloes are tolerant to harsh climates and several bovine tropical diseases (Gordon, 1996; Jainudeen, 2002; Wanapat & Kang, 2013). Despite several merits, buffalo reproduction is impaired by the factors such as poor ovarian reserve, inadequate oestrus expression, imprecise timing of insemination, and seasonal pattern of breeding and calving (Singh et al., 2000; Madan & Parkash, 2007). Moreover, improper oestrus detection (30–40%), a wide range of oestrus duration (4–64 h), ovulation time, and poor pregnancy outcomes are major contributors to the low adaptability of AI in buffalo (Qayyum *et al.*, 2018).

The advances in controlled breeding programmes have allowed issues with progesterone F2α and eCG in buffaloes to be circumvented (Carvalho *et al.*, 2013; Khan *et al.*, 2018; Monteiro et al., 2018) during the breeding and low breeding seasons. The use of eCG in FTAI and oestrus and ovulation synchronization programmes to improve reproductive responses is trending in dairy cattle (Garcia Isperito *et al.*, 2012; Patron-Collantes *et al.*, 2017; Prata *et al.*, 2017; Randi *et al.*, 2018).

It has been established that eCG prolongs follicle stimulating hormone (FSH)- and luteinizing hormone (LH)-like functions, which trigger oestradiol and progesterone levels (Núñez-Olivera *et al.*, 2020) and ultimately

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v52i4.05

improve follicular growth and ovulation rate in beef cows (Sales *et al.*, 2011). Subsequently, the enhanced competency of the emerging corpus luteum, which increases progesterone (P4) concentration, has a beneficial effect on the development and survival of the embryo (Kenyon *et al.*, 2012). Equine chorionic gonadotropin improved fertility in dairy cows with reduced or compromised LH release, and in acyclic buffaloes during the early postpartum period in summer (Garcia-Ispierto *et al.*, 2012; Garcia-Ispierto *et al.*, 2013; De Rensis & Lopez-Gatius, 2014) and in cows with poor BCS (Souza *et al.*, 2009). In tropical conditions, eCG improves the efficacy of the FTAI programme in buffaloes during the low breeding season (Murugavel *et al.*, 2009; Carvalho *et al.*, 2013). However, fewer studies have investigated the effect of eCG and associated factors such as BCS, cyclicity, and breeding season on buffalo fertility.

Therefore, this experiment was designed to determine the effect of eCG, BCS, cyclicity, and breeding season on follicular dynamics, oestrus expression, ovulation rate, and pregnancy by AI in Nili-Ravi buffaloes assigned to CIDR synchronization with FTAI.

Materials and Methods

All procedures in this experiment were approved by the Ethics Committee for the Care and Use of Experimental Animals at the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan (Reference No. DR/512). Adult buffaloes (n = 88) with no palpable abnormality were raised on seasonal fodder in free-stall housing facilities and were milked twice a day. The mean age and weight of buffaloes were 6.69 ± 1.17 years and 458.80 ± 44.39 kg, respectively, with parities of 3.36 ± 0.97 and BCS of 2.76 ± 0.45. Before the initiation of the experiment, the BCS was recorded visually on a scale of 1 to 5 (1 = emaciated, 5 = obese) (Ayres *et al., 2*009). This study was conducted in the breeding season (September–February) and low breeding season (March–August) (Warriach *et al., 2*008). All buffaloes were fitted with CIDR (1.38 g progesterone, Pfizer Co, USA) on a random stage of the oestrus cycle (Day 0), and this was removed on day 7. Two millilitres of PGF2α (d-cloprostenol 0.150 mg) (Dalmazine, Fatro®, Ozzano Emilia Italy) was administered intramuscularly (IM) on Day 6. All buffaloes were randomly allocated to two groups, i.e., the eCG-treated buffaloes (ETB) (n = 43), and the saline-treated buffaloes (STB) (n = 45) during the breeding and low breeding seasons. Equine chorionic gonadotropin-treated buffaloes received 1000 IU of ECG (CHRONO-GEST PMSG, Intervet®, Holland) (4 ml, IM), whereas STB received normal saline on Day 6 (Figure 1).

All buffaloes were monitored for oestrus expression twice a day (morning and evening) for thirty minutes after CIDR removal up to the first FTAI. Oestrus response was estimated as the total buffaloes treated divided by the buffaloes in heat. Buffaloes showing oestrus signs were scored for oestrus intensity followed by FTAI. Oestrus intensity was graded on a scale 1–5 (1 = poor, 5 = excellent), based on behavioural signs (Yousuf *et al.*, 2015) (Table 1). Fixed-time artificial insemination was performed at 48 and 60 hours after CIDR withdrawal with semen (35 million sperm per 0.5 ml straw) with a minimum of 40% post-thaw motility from the same bull, administered by a single, experienced AI technician.

Table 1 Oestrus intensity score based on behavioural signs in buffalo

Oestrus intensity score	Grade	Characteristics
1	Poor	No uterine tone with no behavioural signs
2	Satisfactory	Mild uterine tone, slight mucus discharge, some restlessness
3	Good	Intermediate uterine tone, mucus discharge, restlessness, nervousness
4	Very good	Good tone, stand to be mounted, vulvar swelling, thick mucus discharge, restlessness
5	Excellent	High tone, stand to be mounted, thick mucus discharge, restless

Oestrus intensity score was recorded at the time of FTAI based on the last two days' behavioural activity

The entire reproductive tract was scanned with a B-Mode ultrasound console (Honda HS-1500) with a 7.5 MHz linear transducer at the start of the experiment. Cyclic or acyclic status was confirmed based on the presence or absence of the corpus luteum, which was confirmed by ovarian ultrasonography conducted twice at 10-day intervals before the start of the synchronization protocol. A subset of buffaloes was scanned every 12 hours after eCG administration (Day 6) until ovulation to measure the size of the dominant follicle, the follicle growth rate, and ovulation rate and time. Ovulation was confirmed by the absence of the large dominant follicle that was present during previous ultrasound scanning (Sá Filho *et al.*, 2010).

Ovulation was further confirmed by visualizing the corpus luteum on the ovary seven days post FTAI. Pregnancy was diagnosed by ultrasonography on Days 35–40 post FTAI and was declared positive after the amniotic membrane, anechoic amniotic fluid, and heartbeat of the conceptus were observed.

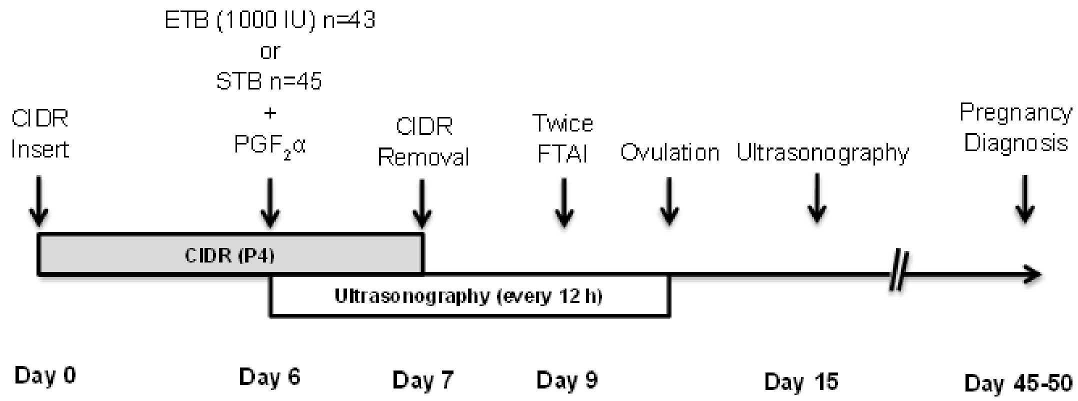

Figure 1 Timeline for buffaloes treated with equine chorionic gonadotropin (n = 43) and buffaloes treated with (n = 45) as a control group in Nili-Ravi buffalo synchronized by controlled internal drug release
CDIR, controlled internal drug release; DF, dominant follicle; eCG, equine chorionic gonadotropin; ETB, eCG-treated buffaloes; FTAI, fixed time artificial insemination; STB, saline-treated buffaloes

The normality of the data was determined with Kolmogorov-Smirnov and Shapiro-Wilk tests. The effects of treatment, BCS, cyclicity, and breeding season on oestrus response, ovulation rate, and P/AI were analysed using chi-square tests. Oestrus intensity was compared by non-parametric analysis with the Mann-Whitney test. The size and growth rate of the dominant follicle and the ovulation time were compared using Student's *t*-test. Statistical analysis was performed using SAS Enterprise Guide (version 4.2, SAS Inst. Inc., Cary, NC, USA). A probability level of P <0.05 was considered significant.

Results and Discussion

The diameter of the dominant follicle did not differ (*P* >0.05) on days 6, 7, and 9 and just before ovulation because of eCG. Ovulation occurred earlier (70.0 ± 2.0 h vs. 77.5 ± 2.3 h) (*P* <0.05), and oestrus response was greater (100% vs. 91%) (*P* <0.05) in ETB than in STB. Similarly, oestrus intensity was better (*P* <0.05), ovulation rate tended to be higher (P = 0.18) (Table 2), and P/AI was greater (*P* <0.0.5) in ETB than in STB (Figure 2).

Table 2 Effect of equine chorionic gonadotropin at the time of prostaglandin F2α administration on follicular dynamics, oestrus expression,* and ovulation rate in Nili-Ravi buffalo synchronized by controlled internal drug release (mean ± s.e.m.)

Variable	**Treatment**		***P*-value**
	ETB	**STB**	
Buffaloes (n)	41	34	
Diameter of DF on Day 6 (mm)	8.2 ± 0.4	8.2 ± 0.4	0.94
Diameter of DF on Day 7 (mm)	9.7 ± 0.4	9.2 ± 0.4	0.37
Diameter of DF on Day 9 (mm)	13.1 ± 0.4	12.4 ± 0.4	0.29
Pre-ovulatory follicle size (mm)	14.8 ± 0.3	14.5 ± 0.5	0.54
Growth rate of the ovulatory follicle from the time of eCG treatment until FTAI (mm/day)	1.8 ± 0.0	1.4 ± 0.1	0.01
Interval between device removal and ovulation (h)	70.9 ± 2.0	77.5 ± 2.3	0.03
Oestrus response (%)	100 (43/43)	91 (41/45)	0.04
Oestrus intensity **	3.2 ± 0.1 (n=43)	2.4 ± 0.1 (n=45)	0.00
Ovulation rate (%)	82.9 (34/41)	69.4 (26/37)	0.18

CDIR, controlled internal drug release; DF, dominant follicle; eCG, equine chorionic gonadotropin; ETB, eCG-treated buffaloes; FTAI, fixed time artificial insemination; STB, saline-treated buffaloes
*Oestrus expression (oestrus response and oestrus intensity assessed at the time of FTAI)
**Oestrus intensity score was given from 1 to 5; 1 = poor and 5 = excellent

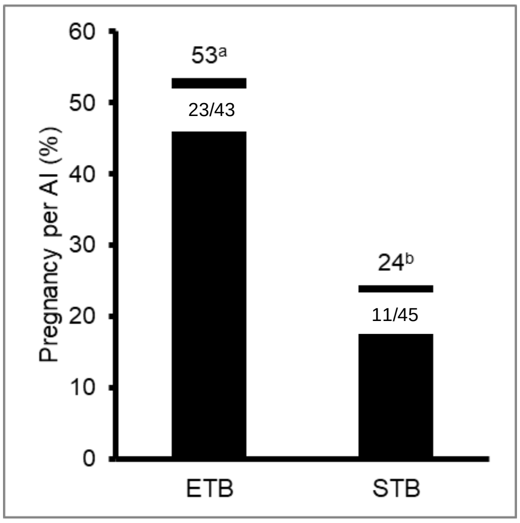

Figure 2 Effect of equine chorionic gonadotropin on pregnancy per AI in controlled internal drug release synchronized Nili-Ravi buffalo. Values with different superscripts between groups differ significantly (*P* <0.05); AI, artificial insemination; ETB, eCG-treated buffaloes; STB, saline-treated buffaloes

The oestrus response did not differ (*P* >0.05) as a result of body condition or eCG. The ovulation rate was greater in eCG-treated buffaloes with higher BCS compared to those with a lower BCS (100% vs. 65%; *P* <0.05). P/AI was greater due to eCG in buffaloes with lower BCS compared with those with higher BCS (40% vs. 12%; *P* <0.05). P/AI was greater (41% vs. 12%) (*P* <0.05) in buffalo without eCG that had a higher BCS (Table 3).

Table 3 Effect of body condition and eCG on oestrus response, ovulation rate, and pregnancy per AI in Nili-Ravi buffalo* synchronized by controlled internal drug release

Variables	BCS > 2.75		BCS < 2.75	
	ETB	STB	ETB	STB
Oestrus response (%)	100 (23/23)^{a}	91.6 (11/12)^{aA}	100 (20/20)^{a}	90.9 (30/33)^{aA}
Ovulation rate (%)	100 (21/21)^{aA}	78.5 (11/14)^{bA}	65 (13/20)^{aB}	65 (15/23)^{aA}
Pregnancy per AI (%)	65 (15/23)^{aA}	41 (5/12)^{aA}	40 (8/20)^{aA}	12 (4/33)^{bB}

Values with different superscripts within rows differ significantly (*P* < 0.05)
Values with small letter superscript are compared within the BCS
Values with capital letter superscript are compared across BCS. Contrast ETB vs. ETB, STB vs. STB
* BCS, body condition score 1–5: 1 = emaciated, 5 = obese
AI, artificial insemination; eCG, equine chorionic gonadotropin; ETB, equine chorionic gonadotropin-treated buffaloes; STB, saline-treated buffaloes

The oestrus response did not differ because of cyclicity or eCG (P >0.05). The ovulation rate did not differ because of eCG in cyclic buffaloes (93% vs. 81%) (*P* >0.05), whereas the ovulation rate was greater because of eCG in acyclic buffaloes (78% vs. 65%) (*P* <0.05). The ovulation rate did not differ (*P* >0.05) because of ovarian cyclicity in cyclic or acyclic buffaloes. P/AI did not differ because of eCG in cyclic buffaloes (77% vs. 42%) (*P* >0.05). Furthermore, P/AI was higher because of eCG in acyclic buffaloes (43% vs. 21%) (*P* <0.05) but did not differ because of ovarian cyclicity in ETB (*P* >0.05), although it was higher because of ovarian cyclicity in STB (*P* <0.05) (Table 4).

Table 4 Effect of ovarian cyclicity and equine chorionic gonadotropin on oestrus response, ovulation rate, and pregnancy per AI in Nili-Ravi buffalo synchronized by controlled internal drug release

Variables	Cyclic		Acyclic	
	ETB	STB	ETB	STB
Oestrus response (%)	100 (13/13)^{aA}	83.3 (10/12)^{aA}	100 (30/30)^{aA}	93.9 (31/33)^{aA}
Ovulation rate (%)	93 (13/14)^{aA}	81 (9/11)^{aA}	78 (21/27)^{aA}	65 (17/26)^{bA}
Pregnancy per AI (%)	77 (10/13)^{aA}	42 (5/12)^{aB}	43 (13/30)^{aA}	21 (7/33)^{bA}

Values with different superscripts within rows differ significantly ($P < 0.05$)
Values with small letter superscripts are compared within ovarian cyclicity
Values with capital letter superscripts are compared across ovarian cyclicity. Contrast ETB vs. ETB, STB vs. STB; AI, artificial insemination; ETB, equine chorionic gonadotropin-treated buffaloes; STB, saline-treated buffaloes; Cyclicity was described based on the presence or absence of signs at the time of controlled internal drug release insertion

Oestrus response did not differ significantly because of breeding season or eCG. The ovulation rate was better because of eCG during breeding and the low breeding season. Moreover, the ovulation rate did not differ significantly because of the breeding season in both groups. P/AI was higher because of eCG during the breeding and low breeding season. However, it did not differ significantly in both groups (Table 5).

Table 5 Effect of breeding season and equine chorionic gonadotropin on the oestrus response, ovulation rate, and pregnancy per artificial insemination in controlled internal drug release synchronized Nili-Ravi buffalo

	Breeding season		Low breeding season	
	ETB	STB	ETB	STB
Oestrus response	100 (16/16)^{aA}	83.3 (15/18)^{aA}	100 (25/25)^{a}	94.7 (18/19)^{aA}
Ovulation rate (%)	87.5 (14/16)^{aA}	72.2 (13/18)^{bA}	80 (20/25)^{aA}	68.4 (13/19)^{bA}
Pregnancy per AI (%)	62.5 (10/16)^{aA}	27.7 (5/18)^{bA}	48.1 (13/27)^{aA}	22.2 (6/27)^{bA}

Values with different superscripts within rows differ significantly ($P < 0.05$)
Values with small letter superscripts are compared within 'breeding season'
Values with capital letter superscripts are compared across breeding season. Contrast ETB vs. ETB, STB vs. STB; ETB, equine chorionic gonadotropin-treated buffaloes; STB, saline-treated buffaloes; Breeding season (September–February), low breeding (March–August)

This study demonstrated useful practical knowledge about the effect of eCG, BCS, cyclicity, and season on oestrus behaviour, follicular dynamics, and P/AI in CIDR-synchronized buffaloes. The addition of eCG enhanced the growth rate of the ovulatory follicles. These findings are similar to studies on beef cows treated with eCG (Sá Filho *et al.*, 2010). Inadequate levels of FSH and LH were linked to anoestrus in buffalo under tropical and sub-tropical conditions (Murugavel *et al.*, 2009). This indicates that eCG addition is helpful for optimal functioning of the hypothalamus-pituitary-gonadal axis in buffaloes. Equine chorionic gonadotropin has an FSH-like effect in the bovine and increases the size of the follicle, which results in increased production of oestrogen from the granulosa cells and enhances oestrus behaviour in buffaloes.

The ovulation rate was better because of eCG following FTAI. These outcomes are consistent with studies in buffalos, beef heifers, and lactating cows (Murugavel *et al.*, 2009; Sales *et al.*, 2011; Garcia-Ispierto *et al.*, 2012). During the non-breeding season, Carvalho *et al.* (2013) reported that the addition of eCG to a P4-based FTAI protocol increased the percentage of ovulating buffaloes (66% vs. 44%) (P = 0.05). Furthermore, in the current study, eCG induced early ovulation (71 h) after CIDR removal because of its ovulation-modulating potential (Bryan *et al.*, 2013). This promising effect of eCG might have improved the P/AI because of more competent oocytes from the buffaloes.

The percentage of buffaloes that exhibited oestrus was higher in ETB than in STB in the current study. Similar results were reported in *Bos taurus* beef cows subjected to progesterone-based FTAI protocols (Pessoa *et al., 2*016). Moreover, the oestrus intensity was greater because of eCG treatment in ETB than in STB in the present study. There is a positive relationship between high oestrus behaviour score and blood oestradiol levels in dairy cows (Lyimo *et al.*, 2000). Wanapat & Kang (2013) reported that elevated levels of oestradiol improve fertilization because of better sperm transport. Similarly, the beef cows that displayed standing oestrus before FTAI had higher preovulatory concentrations of oestradiol and higher fertility compared with animals that did not display this behaviour (Perry *et al.*, 2008). Therefore, it can be speculated that better oestrus behaviour due to eCG administration may have improved the fertility of the buffaloes subjected to the FTAI protocol in the present study.

Similarly, eCG improved P/AI, which is consistent with findings in buffaloes, *Bos indicus* cows/heifers, and dairy cows treated with eCG (Murugavel *et al.*, 2009; Sales *et al.*, 2011; Bryan *et al.*, 2013; Rodrigues *et* *al.*, 2013). However, eCG did not improve fertility in Mediterranean buffalo and Holstein cows (Pulley *et al.*, 2013). Improved fertility because of eCG in the current study could be because increased growth of the dominant follicle with pronounced oestrus behaviour resulted in timely ovulation and a higher ovulation rate.

In the present experiment, eCG does not affect the ovulation rate in buffaloes with poor BCS. These findings contradict a study on beef cows (Sales *et al.*, 2011) but might be because of the comparatively low number of animals. Of interest, the ovulation rate was higher in ETB with a higher BCS. In the current study, eCG improved P/AI compared with non-treated buffaloes with a poor BCS. These outcomes are consistent with the studies on *Bos indicus* beef and Holstein cows (Sales *et al.*, 2001; Souza *et al.*, 2009). Dairy animals with low BCS have a lower concentration of P4 during dioestrus (Santos *et al.*, 2001) and it might mean that animals with a poor BCS have greater P4 metabolism or improper CL function.

In several studies, eCG has been shown to increase the plasma P4 concentration in *Bos indicus* cows/heifers, Hereford beef cows, and buffaloes (Sa Filho *et al.*, 2010; Carvalho *et al.*, 2013; Tortorella *et al.*, 2013; Rodrigues *et al.*, 2013). Therefore, it is speculated that increased fertility because of eCG in buffaloes with a lower BCS could be because of higher P4 concentrations. Thus, the beneficial effects of eCG include a better ovulation rate, follicular or oocyte function, or higher E2 concentration, which cannot be neglected in terms of improving fertility in buffaloes with a lower BCS.

The ovulation rate and P/AI in acyclic buffaloes were higher in the ETB group following P4-based FTAI. These findings are consistent with a study in buffaloes (Murugavel *et al.*, 2009). In another study, eCG enhanced the conception rate in anovular dairy cows following P4-based FTAI protocols (Garcia-Ispierto *et al.*, 2013). Barreiros *et al.* (2014) commented that eCG was beneficial in improving the fertility of anoestrus cows. These findings support the concept of using eCG as a tool for improving fertility in acyclic buffaloes. Similarly, eCG enhanced the ovulation rate and P/AI during the breeding and low breeding seasons. The addition of eCG improved fertility during the low breeding season to equal the fertility achieved during the breeding season in buffaloes. These findings concur with a study on buffalo during the non-breeding season (Carvalho *et al.*, 2013). This indicates that eCG could also be a tool for improving fertility during the low breeding season in buffaloes.

Conclusion

In conclusion, eCG treatment upon CIDR removal improved oestrus expression, follicle growth rate, ovulation, and P/AI in Nili-Ravi buffaloes exposed to an FTAI protocol. Equine chorionic gonadotropin is helpful in enhancing fertility in acyclic buffaloes with a lower BCS, during the breeding and low breeding seasons.

Acknowledgments

The authors thank Prof. Dr. Melih Aksoy and Dr. Ejaz Ahmad (Adnan Menderes University, Ayden, Turkey) for providing eCG hormone.

Author's contribution

NA and MIN conceived the idea, planned the study, and wrote the manuscripts. MID, MH, AH, UR performed the research. MA and MRY contributed to data arrangement and analysis.

Conflict of interest declaration

The authors declare that there is no conflict of interest.

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