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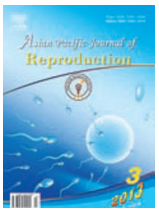

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Treatment of anestrous Nili-Ravi buffaloes using eCG and CIDR protocols

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ABSTRACT

Objective: To determine the effect of equine chorionic gonadotropin (eCG) and controlled internal drug releasing (CIDR) on estrus response, ovulation and pregnancy rate in Nili-Ravi buffaloes under field condition. **Methods:** Twenty anestrous buffaloes with more than 150 days postpartum were used in this study. To confirm anestrous condition, ovarian status and serum progesterone concentration were determined before the start of study. Buffaloes were randomly divided into eCG (n=10) and CIDR (n=10) groups. eCG group were treated with eCG (Chronogest 1000 I.U. i.m.), while CIDR group received CIDR devices for 7 days. All buffaloes in CIDR group were injected PGF2 α (0.25 ug/mL; 2 mL; i.m.) one day before CIDR removal. Buffaloes were observed for estrus visually and were inseminated with frozen-thawed semen at detected estrus. Ovulation was confirmed by presence of CL 12 day post AI. Pregnancy diagnosis was done 50 days post AI. **Results:** Estrus response, ovulation rate and pregnancy rate were similar (P>0.05) among eCG and CIDR groups (90% *vs*. 80%; 78% *vs*. 75% and 50% *vs*. 60% respectively). **Conclusion:** It is concluded that both CIDR and eCG protocols are effective and promising remedies for the anestrous buffaloes under commercial as well as under field conditions.

1. Introduction

The buffalo has great contribution to livestock sector of Pakistan. The buffalo is usually reared for the purpose of milk and meat, which produces about 70 percent of the total milk and 50 percent total red meat[1]. In Pakistan, buffalo production is favored by farmers and consumers due to higher milk price and better nutritional constituents than cow milk. The buffalo rearing is practiced by livestock farmers in riverine areas and its popularity is similar in arid and semi-arid areas. Its popularity is owing to low inputs, excellent utilization poorer quality roughages, better adaptation to harsher environments and more resistant to several bovine tropical diseases than cattle[2].

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Although buffalo exhibit many of the known reproductive disorders and have delayed onset of puberty, poor estrus expression, longer post-partum ovarian quiescence, and most importantly lowered conception rate particularly when bred artificially[2]. It is a general observation that large number of buffaloes is culled or slaughtered every year because of anestrous. But anestrous is still big dilemma in buffalo reproduction which are associated with lower peaks of FSH and LH, and inherently suboptimal functioning of hypothalamus-pituitary-gonadal axis[3]. This condition of anestrous exaggerated in arid and semi-arid area due to unavailability of wallowing ponds to combat the heat stress during the summer months[4].

The strategy of estrus synchronization has been employed to resolve problem of anestrous in buffaloes but their results are variable. It has been observed that progesterone based synchronization protocol provided optimal fertility rate in anestrous buffaloes. These protocols have been combined with different gonadotropins to achieve the maximum results [5-8]. Similarly, equine chorionic gonadotropin (eCG) is widely used in estrus synchronization programs for small and large ruminants

industry due to its cost-effectiveness. Because of its direct action over folliculogenesis, it has been experimented to treat the anestrous in buffaloes[9,10]. But the most of previous studies have been practiced in well managed dairy farm where better conditions, environmental and nutrition factors played a major impact response to estrus synchronization treatment and conception rate[7]. However under village condition where variable body condition score, age, stage of lactation and level of nutrition might be the factors which influence the use of synchronization protocols. The application of more simplified protocols could be more beneficial way to combat the anestrous in buffaloes under field conditions. Therefore, present study was conducted to compare the single equine chorionic gonadotropin (eCG) injection and progesterone-based (CIDR) protocol on estrous expression and pregnancy rate in anestrous buffaloes under field conditions.

2. Materials and methods

2.1. Animals

The study was conducted in small herds in the periphery of District Islamabad during the low breeding season (April-July 2012). The study population was comprised of twenty multiparous buffaloes with more than 150 days in milk, moderate body condition scores (2.5-3.5), 4-6 years age and 400-550 kg weight. Rectal palpation examination and previous calving history revealed normality of involved buffaloes. Anestrous condition was determined by no palpable CL on ovaries and absence of estrus signs after calving. Moreover, blood samples were collected from the buffaloes before the start of trial to measure the progesterone concentration as an index of ovarian activity.

2.2. Treatment

Buffaloes were allocated into two treatment groups; CIDR (n=10) and eCG (n=10). Controlled internal drug releasing (CIDR) 1.38 g progesterone CIDR; Eazibreed ®; Inter Ag, Hamilton, New Zealand) devices were inserted in buffaloes intravaginally for 7 days. On day 6, animals were injected the lutolyic dose of PGF2α (Cyclomate, Starlab, Lahore, Pakistan; 2 mL; i.m.). CIDRs were removed on day 7. In eCG group, buffaloes were treated with equine chorionic gonadotropin (Chronogest-PMSG; 1000 I.U. Fatro® 4 mL, i.m.) once at the start of trial.

2.3. Estrus detection and AI

Estrus behavior was observed by the owner in treated buffaloes for 96 hours after the removal of CIDR. But in eCG treated animals estrus was detected for a week after injection. After showing the typical signs of estrus, buffaloes were inseminated with frozen thawed semen by a trained single technician.

2.4. Ovulation and pregnancy diagnosis

Ovulation rate was determined through ultrasonography after 10-12 days after insemination that was based on presence of corpus luteum on the surface of the ovary. Pregnancy diagnosis was performed after 45-50 of insemination through ultrasonography.

2.5. Progesterone analysis

Two blood samplings were done before the trial (11 days apart) through venepuncture from the experimental buffaloes. The blood samples were centrifuged within 2 h after collection and serum was stored at - 4 ℃ until assayed. Serum progesterone concentrations were determined through solid phase competitive ELISA by using commercially available kit (Bio Check, Inc. USA, Lot # RN-29786). The sensitivity of the progesterone ELISA assay was 0.3 ng/mL. Animals with <1 ng/mL serum progesterone concentrations in samples (low-low) classified anestrous. Likewise, when the samples with concentrations of plasma progesterone >1 ng/mL (high-low) considered as sub-estrous.

2.6. Statistical analysis

All data were analyzed with a statistical software program (SPSS Version 13.0 for Windows; SAS Institute, Cary, North Carolina, USA). Estrus, ovulation, and pregnancy rate were compared among CIDR, eCG and control group using *chi*-square test. Duration to estrus between the groups was compared using *t*-test. A probability level of (P<0.05) was considered significant.

3. Results

The results of progesterone analysis showed that 75% buffaloes were suffering from true anestrous state with < 1 ng/mL progesterone in blood before the trail. Whereas, 25% buffaloes were in sub-estrous condition.

The effect of CIDR and eCG on estrus response, ovulation and pregnancy rate in anestrous buffaloes is presented in Table 1. The results showed that the estrus response did not differ (P>0.05) between CIDR and eCG groups. The mean interval to estrus from CIDR removal and eCG injection to estrus did not vary (P>0.05) but it was synchronous in CIDR as compared to eCG group. Similar ovulation (average 75%) and pregnancy rate (average 55%) (P>0.05) was observed in CIDR and eCG treated buffaloes.

Table 1
Comparison of CIDR and eCG protocols on estrus response, ovulation and pregnancy rate in anestrous buffaloes.

	eCG (n=10)	CIDR (n=10)
Estrus response (%)	9/10 (90)	8/10 (80)
Interval to estrus (hrs.)	67.5±8.7	43.5±2.2
Ovulation rate (%)	7/9 (78)	6/8 (75)
Pregnancy rate (%)	5/10 (50)	6/10 (60)

4. Discussion

The present study described the comparison of two different estrus synchronization protocols of eCG and CIDR based on different reproductive events in Nili-Ravi anestrous buffaloes. Previous reports are in consistent regarding the effect of both protocols on estrus response in cows and buffaloes[7-13]. These hormonal interventions have been used previously to induce estrus and ovulation in farm animals by stimulating the maturation of Graafian follicles, either directly as in case of PMSG or indirectly by CIDR inducing a surge in release of LH surge [14,15]. In the present study, we found a non-significant effect on estrus response between eCG and CIDR groups which varied from 80%-90%. The previous studies regarding the use of CIDR and eCG are in agreement to the successful estrus induction but some authors reported that eCG do not have any significant effect on estrus response in beef cattle[16] and sheep[17]. In the present study, it has been found that the CIDR induced the estrus within 42 hrs after its removal with tight synchrony as compared to eCG where it 72 hrs following the injection. But Zaabel *et al*.,[12] found great variability in use of CIDR alone however, in combination with GnRH[12] or EB[8] induce more synchrony. Fu *et al*.,[11] found the variability in estrus onset by using different doses of PMSG in Chinese Holstein cows which is similar to our report.

Ovulation and pregnancy rate were similar across the treatment groups. The average ovulation and pregnancy rate was 75% and 55% treatment groups. Similar results were obtained when eCG was used in anestrous buffaloes[9,10] and cows[11]. Likewise, a number of studies using CIDR have been conducted in anestrous buffaloes which described the similar results[7,8,12,13]. These results clearly showed that there is positive effect of eCG and CIDR on estrus subsequently ovulation and pregnancy in anestrous buffaloes. However effect of these treatments on non-pregnant animals return to first estrus and fertility has not been determined so this phenomenon needs to be explored in future studies.

In conclusion, eCG and CIDR devices are equally effective to induce estrus with optimal pregnancy rate in anestrous buffaloes. It is implied that these regimens has great potential in fertility improvement in anestrous buffaloes under field conditions.

Conflict of interest statement

We declare that we have no conflict of interest statement.

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