SHORT COMMUNICATION

Comparison of Estradiol Cypionate, Estradiol Benzoate and Gonadotropin-Releasing Hormone in Fixed-Time Artificial Insemination in Multiparous Murrah Buffaloes

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Abstract

The present study was aimed to evaluate the efficiency of estradiol cypionate, estradiol benzoate and gonadotropin-releasing hormone during fixed-time artificial insemination (FTAI) protocols in multiparous Murrah buffaloes. A total of 35 buffaloes were used between September and November 2021 and subjected to a synchronization protocol. On day 0, insertion of an intravaginal progesterone device (CIDR, 1.38 g) was made with intramuscular (i/m) injection of estradiol benzoate (EB, 2 mg); On day 9: CIDR removed with i/m injections of Dinoprost (12.5 mg) and eCG (400 IU), and then buffaloes were randomized into three treatment group: 1) EB 1 mg on day 10 (n=13). 2) estradiol cypionate (EC) 1 mg on day 9 (n=12), and 3) Buserelin acetate (GnRH) 0.0105 mg on day 10 (n=10). The estrus signs observed during the FTAI (56 h after CIDR withdrawal) and conception rate at 90 days after FTAI were recorded. The conception rates were 61.5, 58.3 and 60.0% for EB, EC and GnRH treatment groups, respectively. There was no significant difference between the treatment groups for conception rate and signs of estrus. Therefore, it was concluded that all three treatments can be efficiently used during estrus synchronization protocol with FTAI in multiparous Murrah buffaloes.

Keywords: Buffalo farm, Conception, Estrus synchronization, Heat signs, Pregnancy

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Introduction

Estradiol is a lipophilic steroid hormone that passively diffuses through the plasma membrane into the cytoplasm and binds the nuclear steroid receptors (Palmisano *et al.,* 2018). In buffalo and other species, it is synthesized mainly in the dominant follicles in the ovaries but other tissues can also produce it, such as the placenta, adrenal cortex, testes, brain and others (Acconcia *et al.,* 2018). Estrogens have several functions in reproductive physiology such as estrus expression, secondary sexual characteristics, induction of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) release (Hull and Harvey, 2014). Estradiol and its esters are often used to promote pharmacological control of the estrous cycle (Andrade *et al.,* 2012). These estrogens produce different circulation profiles of estradiol, probably due to differences in the esterification of the molecule (Sier *et al.,* 2017). Estradiol benzoate has been widely used as an ovulation inducer when administered 24 h after progesterone withdrawal. However, its replacement by a hormonal analogue, called estradiol cypionate, applied concomitantly with progesterone withdrawal has been evaluated (Khasatiya *et al.,* 2017; Weiller *et al.,* 2021). Estradiol cypionate (EC), as an ovulation inducer, is an alternative that makes it possible to carry out FTAI protocols, with reducing one visit, labour and minimizing the animal›s handling stress. Remaining systemically bioavailable for a longer time, EC administered together with the removal of the progesterone implant maintains plasma levels sufficient to stimulate the LH surge after the drop-in progesterone levels (P4) (Weiller *et al.,* 2021). Ovulation synchronization programs have already been used frequently in bovines (Dhami *et al.,* 2015). However, in bubaline, the work is scant and inconsistent. Thus, the objective of this study was to evaluate the efficiency of

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estradiol cypionate, estradiol benzoate and gonadotropin-releasing hormone during estrus synchronization with FTAI protocols in multiparous Murrah buffaloes.

Materials and Methods

The experiment was conducted at Khasa Murrah Buffalo Farm, Hisar, Haryana, India. The predominant climate in the region is semi-arid subtropical. The multiparous Murrah buffaloes, aged between 4-9 years, more than 90 days in milk with a body condition score (BCS) of 2.5-4.0 were used. A total of 35 buffaloes, which showed no clinical signs of infectious or metabolic disease, and changes in the genital organs at the gynaecological examination between September and November 2021 were subjected to a synchronization protocol. On day 0 (D0), insertion of an intravaginal progesterone device (CIDR, 1.38 g) was made with intramuscular (i/m) injection of estradiol benzoate (EB, 2 mg); on day 9 (D9): CIDR was removed and i/m injections of Dinoprost ($PGF_2\alpha$, 12.5 µg) and eCG (400 IU) were given. The buffaloes were then randomized into three treatments, *viz.,* 1) estradiol benzoate (EB) 1 mg, i/m on day 10 (13 buffaloes), 2) estradiol cypionate (EC) 1 mg, i/m on day 9 (12 buffaloes), and 3) Buserelin acetate (GnRH, 0.0105 mg) on day 10 (10 buffaloes) (Table 1). All drugs were administered between 08:00 a.m. to 9:00 a.m. on the respective days. The fixed-time artificial insemination (FTAI) was done 56 h after the removal of the intravaginal P4 implant. In non-return cases, the pregnancy was diagnosed by rectal palpation 90 days after FTAI.

Table 1: Drugs used during estrus synchronization in multiparous Murrah buffaloes

Brand name	*Composition*	*Dose and route of administration*	*Manufacturer*
Eazi breed CIDR 1380	Progesterone 1.38 g/ insert	1 Insert, Intravaginal	Zoetis
Pregheat	Estradiol benzoate 1 mg/ mL	2 ml, 1 mL, IM	Virbac
Folligon	1000 I.U. PMSG-eCG/Vial	400 IU, IM	MSD
Lutalyse	Dinoprost 5 mg/ mL	2.5 mL, IM	Zoetis
Receptal	Buserelin acetate 0.0042 mg/ mL	2.5 mL, IM	MSD
Depo-Estradiol	Estradiol cypionate 5 mg/mL	1 mL, IM	Pfizer

The conception rate and estrus sign data were analyzed using the chi-square test ($\chi 2$). The data were considered significant if $p<0.05$.

Results and Discussion

Buffalo is seasonal polyestrous species and its reproductive efficiency is affected by environmental conditions. Therefore, our study was conducted during the breeding season of buffalo. Moreover, in this study, the EC and EB were injected on days 9 and 10, respectively, during estrus synchronization protocol. It was due to the pharmacokinetic properties of EB. It has a shorter half-life and earlier induction of LH peak than EC (Martínez *et al.,* 2007). In our study, the signs of estrus expression, particularly vulval swelling, mucus discharge and uterine tone, did not differ significantly between the three groups of estrus synchronization (Table 2). These results of estrus expression were supported by previous studies (Kumar *et al.,* 2016; Satish *et al.,* 2019; Weiller *et al.,* 2021). In buffalo, uterine tone evaluation is a reliable positive sign of estrus (Manohar *et al.,* 2014). In our study, all the estrus signs reflected that all the three (EC, EB and GnRH) ovulation inducers showed a positive effect.

Table 2: Clinical Signs of induced estrus and Conception rate (%) in Murrah buffaloes subjected to estradiol benzoate (EB), estradiol cypionate (EC) and GnRH treatment during CIDR based estrus synchronization protocol

Parameters	Treatment groups		
	EB	EC	GnRH
No. of buffaloes	13	12	10
Vulva swollen (%)	100	100	100
Mucus discharge (%)	53.8	50.0	50.0
Uterine tone (%)	92.3	100	100
No. of pregnant buffaloes	8	7	6
Conception rate (%)	61.5	58.3	60.0

The overall conception rate in this study was 60.0% (21/35) and there was no significant difference between the three treatment groups (Table 2). Our results were supported well by Satish *et al.* (2019) using GnRH on the same farm and Weiller *et al. (*2021) using EC and EB during the estrus synchronization protocol. However, de Carvalho *et al.* (2020) observed a lower conception rate of 45.5 and 50.0 % with EB and EC, respectively, during unfavorable breeding season. Previous studies revealed that the estradiol with progesterone (E2+CIDR) based estrus synchronization protocol is better than others in buffalo during the breeding or non-breeding seasons. The E2+CIDR protocol is responsible for the large diameter of the dominant follicle and timely ovulation (Bhat *et al.,* 2015; de Carvalho *et al.,* 2020).

Conclusion

The present findings of non-significant variations in estrus signs and conception rates following use of EB, EC and GnRH treatment in a CIDR based estrus induction/synchronization protocol in buffaloes suggested that all three treatments can be efficiently used in estrus synchronization protocol with FTAI during breeding season in multiparous Murrah buffaloes.

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