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Impact of buserelin acetate or hCG administration on day 12 post-ovulation on subsequent luteal profile and conception rate in buffalo (*Bubalus bubalis*)

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ABSTRACT

The present study investigated the impact of gonadotropic hormone administration on day 12 post-ovulation on subsequent luteal profile and conception rate in buffaloes. All the buffaloes ($n = 48$) were estrus synchronized by a synthetic analogue of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), administered 11 days apart, followed by insemination during mid to late estrus. To examine the effect of mid-luteal phase hormonal treatment, buffaloes were randomly divided into control (normal saline, $n = 14$), d12-BA (buserelin acetate, 20 μg, $n = 17$) and d12-hCG (hCG, 3000 IU, $n = 17$) groups. Ovaries were scanned on the day of induced estrus to measure the preovulatory follicle (POF) diameter and on days 5, 12, 16 and 21 post-ovulation to examine the alterations in corpus luteum (CL) diameter. On the day of each sonography, blood samples were collected for the estimation of plasma progesterone. In treatment groups, luteal profile (CL diameter and plasma progesterone) on day 16–21 post-ovulation was better ($P < 0.05$) as well as first service conception rate was higher (52.9% in each treatment group *vs.* 28.6%, $P > 0.05$) compared to controls. All the pregnant buffaloes exhibited higher ($P < 0.05$) plasma progesterone on various post-ovulation days than their respective non-pregnant counterparts. Treatment-induced accessory corpus luteum (ACL) formation was observed in 58.8 per cent and 70.6 per cent buffaloes of d12-BA and d12-hCG group, respectively, that also had higher ($P < 0.05$) plasma progesterone compared to controls. Compared to the spontaneous CL, the diameter of ACL was less ($P < 0.05$) in the treatment groups. In conclusion, buserelin acetate and hCG administration on day 12 post-ovulation leads to accessory CL formation, improves luteal profile and consequently increases conception rate in buffaloes.

1. Introduction

Embryonic death is a major contributor towards repeat breeding in dairy animals (Diskin and Morris, 2008). The losses due to early embryonic (<25 days after fertilization) and late embryonic (between days 25 and 45 of gestation) mortality can be as high as 20–44 and 8–17 per cent, respectively (Humblot, 2001).

A delicate balance between luteolytic and antiluteolytic mechanisms is required for the successful establishment

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of pregnancy. Antiluteolytic mechanisms are orchestrated by the conceptus to change endometrial function and, ultimately, block luteolysis (Binelli et al., 2009). The opportunity to suppress luteolytic mechanisms through hormonal approaches during the maternal recognition of pregnancy is a possible strategy for improving the conception rates in dairy animals. Administration of gonadotropin releasing hormone (GnRH; Campanile et al., 2008b; Rastegarnia et al., 2004) and human chorionic gonadotropin (hCG; Campanile et al., 2007; Santos et al., 2001) were employed to prevent luteal insufficiency-induced early loss of embryos. Treatment with GnRH or hCG during mid-luteal phase improves luteal functions through accessory corpus luteum (ACL) formation as well as by decreasing circulating estradiol (Kerbler et al., 1997; Mann and Lamming, 2001).

In fact, low plasma progesterone during the early luteal phase is associated with poor embryo survival (Diskin and Morris, 2008). Progesterone is required not only to maintain a suitable uterine environment but also to facilitate the elongation of conceptus and, consequently, the secretion of adequate interferone-tau (IFN-τ; Mann, 2002). In cattle, IFN-τ suppresses estradiol and oxytocin receptors, attenuates endometrial secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), extends the life span of CL (Kim et al., 2003), and hence, provides more time to conceptus to establish. The beneficial impact of progesterone supplementation on conception rate was demonstrated in buffaloes (Kumar et al., 2003), whereas, the positive impact of gonadotropic hormone (buserelin acetate or hCG) administration during the mid-luteal phase on post-treatment luteal profile and subsequent conception rate is yet to be established in buffaloes. Hence, the objective of present study was to determine the impact of buserelin acetate (BA), or hCG administration on day 12 post-ovulation on subsequent luteal profile (CL diameter and plasma progesterone), formation of accessory CL and conception rate in buffalos.

2. Materials and methods

2.1. Animals and management

The study was conducted on 48 reproductively normal lactating Murrah buffaloes. The selected buffaloes (body weight: ~400–500 kg, body condition score: 4–5, parity: 2–4, days in milk: 104.8 ± 1.8 days) were regular cycling and free from any apparent pathological problem of genital tract. Buffaloes were housed under semi-loose housing system and were fed chaffed green fodder, wheat straw, concentrate (maize or wheat 60%, groundnut cake 25%, wheat bran 10%, rice bran 4% and common salt 1%), mineral mixture and drinking water *ad libitum*. Lactating buffaloes were milked twice a day, morning (04.00 am) and evening (03.00 pm).

2.2. Estrus synchronization and artificial insemination (AI)

The buffaloes were estrous synchronized using synthetic $PGF_{2\alpha}$ analogue (500 μg, Cloprostenol Sodium, Vetmate™, Vetcare, Bangluru, India) injections administered (i.m.) 11 days apart. The onset of estrous was observed by a teaser bull paraded twice daily starting 48 h after the second $PGF_{2\alpha}$ injection. Artificial insemination (AI) with frozen thawed semen were carried out by the same inseminator during mid to late estrus and thereafter at 24 h interval (Fig. 1), if the dominant ovulatory follicle was detectable at transrectal ultrasonography.

2.3. Groups

Following AI and ovulation, the buffaloes were randomly divided in three groups *viz.*, control (normal saline 5 ml, $n = 14$), d12-BA (buserelin acetate 20 μg, $n = 17$) and d12-hCG (hCG 3000 IU, $n = 17$) group. The hormonal treatments were administered (i.m.) on day 12 post-ovulation.

2.4. Ultrasonography and blood sampling

Transrectal ovarian ultrasonography was carried out using a battery operated B-mode ultrasound scanner (Agroscan AL, ECM, Angouleme, France) equipped with inbuilt interchangeable 5/7.5 MHz linear-array rectal transducer (ALR 575 probe, ECM, Angouleme, France). Scanning was carried out at 24 h intervals starting from the onset of estrus till ovulation and thereafter on days 5, 12, 16 and 21 post-ovulation (Fig. 1). Ovaries were systematically scanned and images were recorded in the recorder and on a diagram of the ovary by carefully sketching the size, and relative location of all follicles and visible CL (Ghuman et al., 2010). Optimal scan images were frozen and the size of the follicles/CL was determined by measurement of the largest and smallest diameter of the follicles/CL and thereafter, average diameter calculated. All measurements were made using the built-in, on-screen callipers. A follicle which disappeared after the end of estrus was considered as the ovulatory follicle. The ovulatory follicle diameter was, retrospectively, the diameter recorded on the day just before the day of disappearance (ovulation) of follicle. Day of ovulation (day 0) was the last day when the follicle was found intact before disappearing at subsequent examination about 24 h later (Ghuman et al., 2010). On the day of treatment (day 12 post-ovulation), diameter of dominant follicle was recorded and thereafter, on day 16 it was confirmed for the formation of accessory corpus luteum (ACL). Pregnancy was confirmed on day 40 post-ovulation by ultrasonographic examination.

Blood samples were collected (10 ml) from the jugular vein in heparinized vaccutainer vials after each ultrasonography (Fig. 1). Plasma was separated immediately after blood collection by centrifugation at 1500 × *g* for 15 min at 4 °C and plasma aliquots were stored at −20 °C until analysis.

2.5. Hormone assays

Plasma progesterone was assayed with a solid-phase radioimmunoassay using antisera raised in our laboratory (Ghuman et al., 2009). Sensitivity of the assay was 0.1 ng/ml; intra- and inter-assay variation coefficients were 6.2% and 9.5%, respectively.