



# Synchronization of follicular wave emergence following ultrasound-guided transvaginal follicle ablation or estradiol-17 $\beta$ administration in water buffalo (*Bubalus bubalis*)



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## ABSTRACT

The aim of this study was to assess the synchrony in follicular wave emergence and subsequent ovulation following dominant follicle ablation or estradiol-17 $\beta$  administration. Six cycling Murrah buffaloes were sequentially allotted to three groups, that is, control, follicular ablation, and estradiol-17 $\beta$  groups. For the control group, buffaloes at random stages of estrous cycle were examined daily by transrectal ultrasonography for 14 days and the day of wave emergence was recorded. Following induced luteolysis and ovulation (Day 0), these buffaloes were included in the ablation group. All follicles (>5 mm) were ablated on Day 3 or 5 or 7 ( $n=2$  each day). Seven days after the ablation, these buffaloes were administered prostaglandin F<sub>2 $\alpha$</sub>  to induce luteolysis and ovulation. Following this, buffaloes were included in the estradiol treatment group with estradiol administered on similar days as for ablation in the ablation group. Luteolysis was induced nine days after the estradiol injection. All animals of the treatment groups were subjected to transrectal ultrasound and blood samplings daily from treatment to induced ovulation. The follicular waves emerged significantly earlier ( $P=0.001$ ) in both the ablation ( $2.1 \pm 0.79$  days) and estradiol ( $4.0 \pm 0.25$  days) treatment groups than the control group ( $8.3 \pm 0.88$  days). The deviation from mean day of ovulation was greater ( $P=0.02$ ) for the control group buffaloes ( $1.66 \pm 0.3$  day) than those of the treatment groups (ablation,  $0.76 \pm 0.2$  and estradiol,  $0.58 \pm 0.2$  day). In conclusion, both ablation and estradiol resulted in synchronous emergence of a new follicular wave irrespective of stage at which the treatment was given, with greater synchrony of ovulations in water buffalo.

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## 1. Introduction

Water buffalo is a predominant species in south and south-east Asian countries for dairy and meat industries. Despite contributing 10% of the world's milk

production, the productivity of buffalo is significantly hampered by reproductive factors viz. late attainment of puberty, poor estrus expression, seasonality, and post-partum anoestrous (Singh et al., 2000; Barile, 2005). Several attempts have been made to develop controlled breeding techniques by utilizing hormone-based estrous and ovulation synchronization protocols with varying degrees of success (Brito et al., 2002; de Araujo Berber et al., 2002; Neglia et al., 2003b; Rastegarnia et al., 2004; Paul and Prakash, 2005; De Rensis and Lopez-Gatius, 2007).

The success of ovulation synchronization treatment is largely dependent on the status of follicle and CL (Corpus luteum) at the time of initiation of treatment (Brito et al., 2002; De Rensis et al., 2005). A synchronous wave emergence prior to ovulation synchronization protocols provides better ovulation synchrony that allows fixed-time artificial insemination without the need to detect estrus in cattle (Brogliatti et al., 1998; Bo et al., 2006). The follicular wave emergence can be synchronized by physical or hormonal methods. The physical method involves transvaginal ultrasound-guided follicle ablation (removal) of all follicles  $\geq 5$  mm, regardless of stage of the estrous cycle (Bergfelt et al., 1994; Brogliatti et al., 1998; Garcia and Salaheddine, 1998; Martinez et al., 2000). In cattle, follicular ablation removes the suppressive effects of estradiol and inhibin (secreted by larger follicles) on FSH (Follicle stimulating hormone) (Evans et al., 2002) and therefore results in a rise in plasma FSH concentration (Bergfelt et al., 1994). The hormonal methods involve administration of estradiol and GnRH (Deyo et al., 2001; Bo et al., 2002; Mapletoft et al., 2002; Kim et al., 2005; Siqueira et al., 2009). An exogenously administered estradiol exerts an inhibitory effect on gonadotrophin secretion thus leading to follicular suppression. Irrespective of the technique used and the stage of the estrous cycle, occurrence of the estrous a new FSH surge leads to emergence of a new follicular wave one day after ablation and four days after estradiol administration in cattle (Bergfelt et al., 1994; Colazo et al., 2003; Siqueira et al., 2009).

Transvaginal ultrasound-guided follicular aspiration has been used for oocyte retrieval in buffalo (Neglia et al., 2003a; Gupta et al., 2006), but not for follicular wave synchronization. The incorporation of an estradiol treatment has been documented for various superovulatory protocols in buffaloes (Uoc et al., 1992; Nguyen et al., 1997). However, it is not known if the use of an estradiol would result in synchronization of wave emergence in buffaloes. Therefore, the present study was designed to employ ultrasound-guided transvaginal follicle aspiration as a non-hormonal physical method and administration of estradiol-17 $\beta$  as a hormonal method to test the following hypotheses: (1) Ablation of all follicles  $\geq 5$  mm in antral diameter or exogenous injection of estradiol-17 $\beta$  will induce synchronous emergence of a new follicular wave irrespective of the status of dominant follicle and follicular wave at the time of treatment. (2) Synchrony of follicular wave emergence will result in ovulation synchrony. To ensure that treatment periods covered all phases of dominant follicle, we characterized the follicular dynamics of the first (anovulatory) wave in this breed (Murrah) of water buffaloes (e.g., day of selection, growth phase, static phase, regressing phase of

the dominant, and largest subordinate follicle) before start of the experiment.

## 2. Materials and methods

### 2.1. Animals

The experiment was carried out on six normal cycling Murrah buffaloes (nulliparous to pluriparous, non-lactating and weighing 400–500 kg) maintained in a semi-loose housing system with ad lib. supply of water and standard feeding schedule. All buffaloes had a body condition score of 3–4 (scale of 1–5) were subjected to the three treatments (control, follicular ablation, and estradiol treatment) in succession (i.e., from July to September) without any gap. The geographical location of experiment place was at 30.9°N 75.85°E with humid subtropical climate.

### 2.2. Ultrasonography and transvaginal ultrasound-guided follicular ablation

All buffaloes were examined daily by transrectal ultrasonography using B-mode Aloka SSD 500 scanner equipped with a 7.5-MHz side-fire linear array transducer (Aloka Medical Ltd., Tokyo, Japan) to record the location, size, and number of follicles, and size of corpora lutea beginning from the start till the end of experiment. Sketches of left and right ovary were drawn daily and the dataset was used to characterize the wave emergence, growth, and regression patterns of the dominant and subordinate follicles, time of selection, and ovulation (Ginther et al., 1997). The follicular ablation was carried out by transvaginal ultrasound-guided aspiration of ovarian follicles (Bergfelt et al., 1994) using a B-mode scanner (Aloka SSD 500) equipped with 5.0-MHz end-fire sector array transducer. Properly restrained buffaloes were subjected to caudal epidural anesthesia using 5–10 ml of 2% Xylocaine (Inj. Lignocaine, AstraZeneca Pharma India Ltd, Karnataka, India) followed by cleaning of perineal area. An 18-gauge 50-cm-long, sterile needle (COVA needle, Misawa Medical Industry, Japan) attached to a 10-cc syringe was advanced through the vaginal wall into the antrum of follicle to aspirate the follicular fluid. This procedure was repeated for all follicles  $\geq 5$  mm in both ovaries.

### 2.3. Definitions

Follicle ablation was defined as collapse of the antral follicle following evacuation of follicular contents. A follicular wave was defined as the synchronous growth of group of follicles ( $\geq 4$  mm in diameter), which was followed by selection and continuous growth of the dominant follicle (Ginther et al., 1989b). The day of follicular wave emergence following ablation was retrospectively determined as the day when the dominant follicle was first identified at 4–5 mm in diameter (Baruselli et al., 1997). The day of selection of dominant follicle was defined as the day when difference in diameters of the dominant and subordinate follicles was first detected to be  $>2$  mm. The growth rates of dominant and subordinate follicles were calculated by subtracting the diameter of the follicle at first detection

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