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Is slow follicular growth the cause of silent estrus in water buffaloes?

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Abstract

The present experiment was conducted to study the growth profile of the ovulatory follicle in relation to the expression of estrus following administration of $PGF_{2\alpha}$ to subestrus buffaloes. After detection of a mature corpus luteum by examination per rectum, confirmed by ultrasound scanning, subestrus buffaloes ($n = 20$) were treated (Day 0) with single dose of Dinoprost tromethamin (25 mg, i.m.). Blood samples were collected at 0, 24 and 48 h after treatment for estimation of plasma progesterone concentration. Growth profile of the ovulatory follicle was monitored daily through ultrasound scanning starting from Day 0 until ovulation and the regression profile of CL was monitored at 0, 24 and 48 h of treatment. Estrus was detected by exposure to a fertile buffalo bull three times a day until expression of overt estrus or ovulation. Behavioral estrus was recorded in 14 animals and 6 animals ovulated silently. Sixteen animals including six animals with silent estrus ovulated from the dominant follicle present at treatment (Group A) and remaining four animals ovulated from the dominant follicle of succeeding follicular wave (Group B). The intervals from treatment to estrus (6.5 ± 0.25 versus 3.2 ± 0.27 days, $P < 0.001$) and treatment to ovulation (7.5 ± 0.25 versus 5.4 ± 0.46 days, $P < 0.005$) were significantly longer in animals of Group B compared with animals of Group A. Significant differences were observed in growth profile of the ovulatory follicle between animals of Groups A and B with respect to size of the follicle on Day 0 (9.8 ± 0.7 versus 5.3 ± 0.45 mm, $P < 0.001$), daily growth rate (0.97 ± 0.07 versus 1.6 ± 0.2 mm/day, $P < 0.01$) and increase in diameter (4.1 ± 0.6 versus

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7.8 ± 0.7 mm, $P < 0.01$). The animals with silent estrus (subgroup A-2) had significantly smaller diameter of the ovulatory follicle on Day 0 (7.7 ± 0.4 versus 11.0 ± 0.7 mm, $P < 0.005$), its daily growth rate was significantly slower (0.7 ± 0.02 versus 1.1 ± 0.1 mm/day, $P < 0.01$) and they recorded significantly longer interval from treatment to ovulation (7.3 ± 0.56 versus 4.2 ± 0.27 days, $P < 0.001$) compared with the animals that showed overt estrus (subgroup A-1). The corpus luteum area (CL area) and plasma progesterone (P_4) concentration declined continuously from 0 to 48 h after $PGF_{2\alpha}$ treatment in the animals of both the Groups A and B. Non-significant differences were observed in mean CL area and plasma P_4 concentration at 0, 24 and 48 h post-treatment between animals of Groups A and B and also between animals of subgroups A-1 and A-2. The small size and the slow growth rate of the ovulatory follicle were identified as the possible cause of silent estrus in subestrus buffaloes after $PGF_{2\alpha}$ treatment.

Keywords: Water buffaloes; Subestrus; Follicular growth; Prostaglandin; Progesterone; CL area

1. Introduction

Subestrus or silent estrus constitutes the single largest problem affecting the reproductive efficiency in water buffaloes thereby increasing the inter-calving period and loss in milk production (Awasthi et al., 1998). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or its analogue induces luteolysis and has been successfully used for the induction of estrus and ovulation (Singh et al., 1979; Pant and Singh, 1991; Awasthi et al., 1998) and for improving reproductive efficiency in subestrus buffaloes (Bruce et al., 1988).

Several reports have concluded that the interval from prostaglandin treatment to luteolysis is similar when prostaglandin is given to cattle with a mature corpus luteum, regardless of day of treatment, as indicated by profiles of plasma P_4 concentration (King et al., 1982; Macmillan and Henderson, 1984; Stevenson et al., 1984). However, the time interval from treatment to estrus response has been inconsistent following treatment of subestrus buffaloes with $PGF_{2\alpha}$ (Dhoble and Gupta, 1987). Failure of prostaglandin treated subestrus buffaloes to show overt signs of estrus, together with the wide variation in time interval in induced estrus, are the major constraints to the broader adoption of AI for genetic improvement of buffaloes (Singh et al., 2000).

Ultrasonography can be used to study the regression profile of corpus luteum and growth profile of the ovulatory follicle during the period from prostaglandin treatment to clinical response in animals with a responsive CL. In perspective, present work was designed to study the growth profile of the ovulatory follicle, regression profile of CL and plasma P_4 profile in subestrus buffaloes following administration of $PGF_{2\alpha}$ and to explore the possible relationship between the findings of ultrasonographic observations and the response to the treatment.

2. Materials and methods

2.1. Animals and treatment

Twenty lactating subestrus buffaloes of the Mehsana breed, 5–8 years of age and weighing 480–640 kg with an average lactational yield of 1850 kg were selected at the Livestock Research Station, SD Agricultural University, Sardar Krushinagar, Gujarat (India) for present study. Buffaloes were observed three times per day for behavioral estrus from soon after parturition. Animals that did not show estrus, but that had a corpus luteum palpable per rectum 8 weeks after parturition were selected as subestrus animals for use in the experiment. Postpartum interval to treatment

varied between 60 and 96 days in these animals. The animals were stall-fed and provided with balanced ration, mineral supplementation and seasonal green fodder. They were suckled followed by hand milking twice per day. The Livestock Research Station is located in north Gujarat at 24°19′ latitude north and 72°19′ longitudes east at an elevation of 152.52 m above mean sea level. The climate of the region is tropical and semi-arid with moderate rainfall. The winters are extremely cold and dry, while summers are hot and dry. The experiment was conducted during breeding season from September to January with average minimum and maximum ambient temperatures of 7.4–24.9 °C and 25.6–36.5 °C, respectively and relative humidity from 66.0 to 93.3% in the morning and 16.4–61.3% in the evening. After detection of a mature corpus luteum by examination per rectum, confirmed by ultrasound scanning, these animals were randomly treated (Day 0) at unknown stages of CL development with a single dose of dinoprost (Lutalyse; Upjohn, 25 mg i.m.) to induce the estrus. Treated animals were closely monitored for expression of behavioral estrus through heat detection with fertile buffalo bull thrice daily; each session lasting for 1 h in the morning, afternoon and the late evening and animals were considered in estrus only if they accepted bull mounting. Secondary signs of estrus, viz. congestion of vaginal mucus membrane and presence of estrual mucus were detected through visual examination and uterine tonicity was assessed by examination per rectum in the animals that did not show behavioral estrus.

2.2. Ovarian ultrasonography

The ovaries of treated animals were scanned using a real-time B-mode ultrasound scanner (Sigma-110 Master-Vetson, Kontron Medical, SAS, France) equipped with a 6.5 MHz convex linear array transducer designed for intra-rectal placement. The growth and regression profile of the largest and the second largest follicles were recorded once daily each morning from the beginning of treatment till detection of ovulation and the regression profile of CL area was monitored at 0, 24 and 48 h of treatment. The ovarian maps were drawn to record the diameter and relative position of follicle ($\geq$ 4 mm) and the size of CL during each examination. When the follicle being scanned was not spherical, the diameter was estimated by averaging two measurements taken at 90° to each other. Ovulation was defined as disappearance of previously identified follicle ($\geq$ 10 mm) from one ultrasound examination to the next (Nasser et al., 1993). Desired images were frozen on the screen and the follicular diameter and the length and width of CL were measured using a built in caliper system. Corpus luteum area (CLA) was calculated using a formula (CLA = CL length × 0.5 × CL width × 0.5 × 3.14 (Kastelic et al., 1990a)). Hard copy (sonogram) was taken using videographic thermal printer (Sony, UP-895 MD, Sony Corporation, Japan).

2.3. Blood sampling

Blood samples were collected at 0, 24 and 48 h after treatment for estimation of plasma P_4. Approximately 10 ml of blood was collected from the jugular vein into heparinized tubes; the plasma was separated out immediately after collection of blood by centrifugation of samples for 10 min at 3000 × g. Plasma samples were stored frozen at −20 °C until analyzed. One or two drops of 0.01% merthiolate (thiomersal) was added as preservative.

2.4. Progesterone assay

Progesterone (P_4) concentration was estimated in plasma samples using a radioimmunoassay (RIA) kit (Immunotech-SA, Marsielle Cedex, France) employing standard technique (Kubasic

et al., 1984). The sensitivity of the assay was 0.1 ng/ml. Intra-assay coefficient of variation was 5.4%, while inter-assay variation was 9.1%. Cross-reactivity of the antibody with progesterone, 17α-dihydroprogesterone and 20α-hydroxyprogesterone was 100, 0.13 and 0.96%, respectively. Luteal regression was defined to have occurred when plasma P_4 concentration was < 1.0 ng/ml 48 h after treatment (Brito et al., 2002).

2.5. Statistical analysis

Pearson's correlation coefficients were calculated between plasma progesterone concentration and CLA at 0, 24 and 48 h after treatment of all the animals. To analyze the ovarian dynamics, CLA and plasma P_4 concentration treated animals were divided in to two groups, viz. A and B based on pattern of ovulation. Animals of Group A ($n = 16$) ovulated from the dominant follicle present at treatment, while animals of Group B ($n = 4$) ovulated from the dominant follicle of succeeding follicular wave. Animals of Group A were further divided into two subgroups according to their behavioral response to treatment. In animals of subgroup A-1 ($n = 10$) ovulation was accompanied with behavioral signs of estrus, while animals of subgroup A-2 ($n = 6$) ovulated silently without showing behavioral estrus. The rate of growth and regression of the dominant follicle was calculated by linear regression analysis and regression coefficient was calculated. Growth rate of the ovulatory follicle was then compared between animals of Groups A and B using unequal completely randomized design. The same test was applied to compare the growth rate of ovulatory follicle between animals of subgroups A-1 and A-2. Diameters of the largest and the second largest follicle were compared within the same group using a paired *t*-test. Least square analysis of variance using two-way classification with interaction was used to compare the diameters of the largest and the second largest follicle between two groups of animal. Student's *t*-test was used to compare the plasma P_4 concentration, CLA, interval from treatment to estrus and to ovulation, ovulatory follicle characteristics including increase in diameter from treatment to ovulation and maximum diameter between animals of two different groups (Snedecor and Cochran, 1986).

3. Results

All buffaloes had an ultrasonographically visible mature corpus luteum with plasma P_4 concentration > 1.0 ng/ml, when prostaglandin was administered. The response to $PGF_{2\alpha}$ treatment with respect to expression of estrus, interval from treatment to ovulation and growth profile of the ovulatory follicle is presented in Table 1. Out of 16 animals of Group A, 10 animals showed signs of behavioral estrus (subgroup A-1) within 4 days of treatment and remaining 6 animals ovulated silently (subgroup A-2). However, there were presence of uterine tonicity, estrual mucus and hyperaemia of vaginal mucus membrane suggestive of these animal (subgroup A-2) being in estrus. All animals of Group B expressed estrus within 6–8 days after treatment; thus, total 14 out of 20 animals showed behavioral estrus.

The intervals from treatment to estrus (6.5 ± 0.25 versus 3.2 ± 0.27 days, $P < 0.001$) and from treatment to ovulation (7.5 ± 0.25 versus 5.4 ± 0.46 days, $P < 0.005$) were significantly greater in animals of Group B than that of Group A. Likewise, significant differences were observed in growth profile of the ovulatory follicle between animals of Groups A and B with respect to size of the follicle on Day 0 (9.8 ± 0.7 versus 5.3 ± 0.45 mm, $P < 0.001$), daily growth rate (0.97 ± 0.07 versus 1.6 ± 0.2 mm/day, $P < 0.01$) and increase in diameter (4.1 ± 0.6 versus 7.8 ± 0.7 mm, $P < 0.01$). However, non-significant difference was recorded in maximum diameter of the follicle prior to ovulation between two groups (Table 1).

Table 1
The interval between administration of prostaglandin and estrus or ovulation and growth profile of the ovulatory follicle in subestrus Mehsana buffaloes ovulating the dominant follicle present at treatment or the dominant follicle of the succeeding follicular wave

Characteristics	Group A	Group B	*P*-value
Interval			
Treatment to estrus (days)	3.2 ± 0.27 ($n = 10$)	6.5 ± 0.25 ($n = 4$)	<0.001
Treatment to ovulation (days)	5.4 ± 0.46 ($n = 16$)	7.5 ± 0.25 ($n = 4$)	<0.005
Ovulatory follicle			
Diameter on Day 0 (mm)	9.8 ± 0.7	5.3 ± 0.45	<0.001
Growth rate (mm/day)	0.97 ± 0.07	1.6 ± 0.2	<0.01
Increase in diameter (mm)	4.1 ± 0.6	7.8 ± 0.7	<0.01
Maximum diameter (mm)	13.9 ± 0.6	13.2 ± 0.3	NS

Group A: Ovulated from the dominant follicle present at treatment; Group B: Ovulated from dominant follicle of succeeding follicular wave.

There were time interaction effects on the diameter of the largest follicle ($P < 0.05$) and the second largest follicle ($P < 0.01$) within and between the groups. The diameters of the largest and the second largest follicles were not different among groups until 48 h after treatment, when animals of group A had greater size ($P < 0.05$) of the largest follicle compared with those of Group B, while the second largest follicle was greater ($P < 0.01$) in Group B than in Group A. In Group B, the diameter of the largest follicle was greater at 24 h ($P < 0.01$) and at 48 h ($P < 0.05$) after treatment than the second largest follicle. Then the size difference between the first and the second largest follicle disappeared on Days 3–5 after treatment (Fig. 1). However, the second largest follicle was greater ($P < 0.01$) in size on Day 6 prior to ovulation, while the first largest follicle significantly ($P < 0.01$) decreased in size.

The interval from treatment to ovulation was significantly longer in animals of subgroup A-2 compared with animals of subgroup A-1 (7.3 ± 0.56 versus 4.2 ± 0.27 days, $P < 0.001$

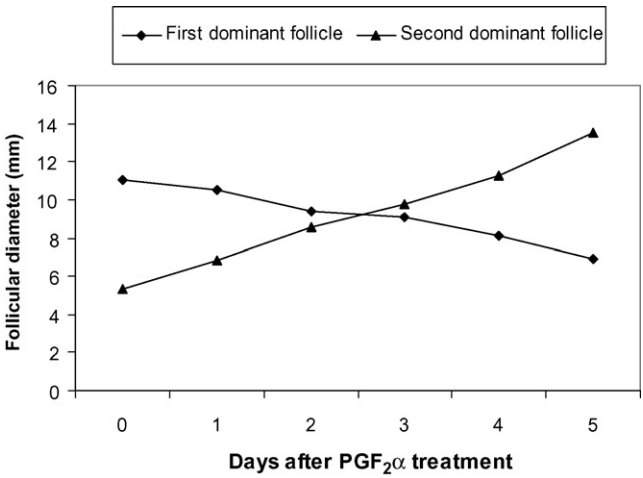

Fig. 1. Growth and regression profiles of the first and second dominant follicles in Mehsana buffaloes ovulating from the dominant follicle of succeeding follicular wave.

Table 2
The interval between administration of prostaglandin and estrus or ovulation and growth profile of the ovulatory follicle in subestrus Mehsana buffaloes with overt or silent estrus

Characteristics	Subgroup A-1	Subgroup A-2	*P*-value
Interval			
Treatment to estrus (days)	3.2 ± 0.27 ($n = 10$)	–	–
Treatment to ovulation (days)	4.2 ± 0.27 ($n = 10$)	7. 3 ± 0.56 ($n = 6$)	<0.001
Ovulatory follicle			
Diameter on Day 0 (mm)	11.0 ± 0.7	7.7 ± 0.4	<0.005
Growth rate (mm/day)	1.1 ± 0.1	0.7 ± 0.02	<0.01
Increase in diameter (mm)	3.5 ± 0.9	5.2 ± 0.9	NS
Maximum diameter (mm)	14.5 ± 0.8	12.8 ± 0.6	NS

Subgroup A-1: Animals with overt estrus ovulating from the dominant present at treatment; subgroup A-2: Animals with silent estrus ovulating from the dominant follicle present treatment.

(Table 2)). Similarly, the diameter of the ovulatory follicle was significantly smaller (7.7 ± 0.4 versus 11.0 ± 0.7 mm, $P < 0.005$) in size on Day 0 and daily growth rate of the ovulatory follicle was significantly slower (0.7 ± 0.02 versus 1.1 ± 0.1 mm/day, $P < 0.01$) in animals of subgroup A-2 than in animals of subgroup A-1 although ovulation occurred in animals of both subgroups from the dominant follicle of follicular wave present at treatment. However, non-significant difference was recorded in the size of the ovulatory follicle on the day prior to ovulation between animals of subgroups A-1 and A-2 (Table 2).

Overall mean CL area was recorded to be 2.02 cm^2 and the mean progesterone concentration was 2.66 ng/ml prior to treatment, which decreased to 0.87 cm^2 and 0.22 ng/ml, respectively, at 48 h of treatment (Fig. 2). Pearson's correlation coefficients for plasma P_4 concentration and CLA were 0.46 ($P < 0.05$), 0.14 ($P > 0.05$) and 0.21 ($P > 0.05$) at 0, 24 and 48 h after treatment, respectively. The CL area and plasma P_4 concentration declined continuously from 0 to 48 h after treatment in animals of both the Groups A and B. However, plasma P_4 concentration declined at an increasing rate from 0 to 24 h in a curvilinear fashion (Fig. 2). Then plasma P_4 concentration

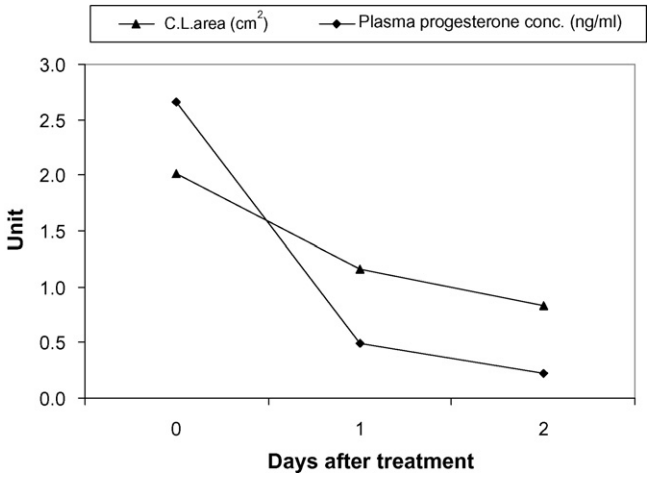

Fig. 2. Regression profiles of CL area and plasma progesterone concentration following prostaglandin treatment in subestrus Mehsana buffaloes.

Table 3
The corpus luteum area and plasma progesterone concentration following administration of prostaglandin in subestrus Mehsana buffaloes ovulating from the dominant follicle present at treatment or from the dominant follicle of the succeeding follicular wave

Subgroup	CL Area (cm^2)			Progesterone concentration (ng/ml)		
	0 h	24 h	48 h	0 h	24 h	48 h
A ($n=16$)	2.1 ± 0.2	1.2 ± 0.1	0.9 ± 0.1	2.3 ± 0.2	0.4 ± 0.1	0.2 ± 0.03
B ($n=4$)	1.9 ± 0.1	1.2 ± 0.2	0.7 ± 0.4	2.8 ± 0.3	0.8 ± 0.3	0.3 ± 0.1
P-value	NS	NS	NS	NS	NS	NS

Subgroup A: Ovulated from the dominant follicle present at treatment; subgroup B: Ovulated from dominant follicle of succeeding follicular wave.

Table 4
The corpus luteum area and plasma progesterone concentration following administration of prostaglandin in subestrus Mehsana buffaloes with overt or silent estrus

Group	CL Area (cm^2)			Progesterone concentration (ng/ml)		
	0 h	24 h	48 h	0 h	24 h	48 h
A-1 ($n=10$)	2.1 ± 0.3	1.2 ± 0.2	1.0 ± 0.1	2.0 ± 0.3	0.4 ± 0.1	0.2 ± 0.04
A-2 ($n=6$)	1.9 ± 0.2	1.1 ± 0.2	0.7 ± 0.1	2.7 ± 0.1	0.4 ± 0.02	0.2 ± 0.05
P-value	NS	NS	NS	NS	NS	NS

Group A-1: Animals with overt estrus ovulating from the dominant present at treatment; Group A-2: Animals with silent estrus ovulating from the dominant follicle present treatment.

declined in a linear fashion from 24 to 48 h after treatment similar to that of CL area, the size of which regressed at a steady rate from 0 to 48 h after treatment. There were non-significant differences in mean CL area and mean plasma P_4 concentration at 0, 24 and 48 h between animals of Groups A and B (Table 3) and also between animals of subgroups A-1 and A-2 (Table 4).

4. Discussion

The growth profile of the ovulatory follicle, regression profile of CLA and plasma P_4 profile were studied in subestrus water buffaloes after $PGF_{2\alpha}$ treatment. The luteolysis was complete by 24 h of treatment in all the treated animals as evident from the present finding that all animals had their plasma P_4 concentration < 1.0 ng/ml at 24 h suggesting that the ultrasound scanning of ovary is a reliable technique to identify the functional CL (Smith et al., 1998).

Six animals ovulated silently without showing behavioral signs of estrus after $PGF_{2\alpha}$ treatment in present study. The present finding approximates with earlier reports in subestrus buffaloes (Pant and Singh, 1991; Awasthi et al., 1998; Kharche and Srivastava, 2001) and in cyclic buffaloes (Manik et al., 2002; Brito et al., 2002). Non-significant differences were recorded in plasma P_4 concentration and CL area profiles between the animals with overt estrus (subgroup A-1) and silent estrus (subgroup A-2) in present study. Similar observations were reported in cyclic Murrah-cross buffaloes with respect to decline in plasma P_4 concentration and CL area after $PGF_{2\alpha}$ treatment (Brito et al., 2002). However, some authors reported that although plasma P_4 concentrations decrease in treated animals that fail to show estrus after $PGF_{2\alpha}$ treatment, the

rate of decline is comparatively slower and plasma P_4 concentrations are usually higher than in animals that responded to treatment (Dhaliwal et al., 1988; El-Belely et al., 1995).

In general, plasma P_4 concentrations prior to treatment were lower than some previous reports (Chauhan et al., 1982; Choahn, 1998; Brito et al., 2002) but similar to others (Williams et al., 1986; Bruce et al., 1988; El-Belely et al., 1995). The mean CLA before treatment was similar to previous report in *Bubalus bubalis* (Brito et al., 2002); however, it was smaller than reported in *Bos taurus* cattle (Kastelic et al., 1990a). The plasma P_4 concentration was < 1.0 ng/ml at 24 h after $PGF_{2\alpha}$ treatment in all the treated animals. The present observation corroborates well with previous reports that the interval from prostaglandin treatment to luteolysis is similar when prostaglandin is administered to cattle with a mature corpus luteum, regardless of day of treatment as indicated by profiles of plasma P_4 (King et al., 1982; Macmillan and Henderson, 1984; Stevenson et al., 1984). The correlation coefficient between plasma P_4 level and CLA decreased after $PGF_{2\alpha}$ treatment, the plasma P_4 concentration declined at an increasing rate than the regression of CLA during first 24 h, indicating that functional changes precede the morphological changes during luteolysis. Similar observation has been reported in cyclic and bred but non-pregnant heifers (Kastelic et al., 1990a), cyclic cows (Assey et al., 1993) and cyclic buffaloes (Brito et al., 2002).

All animals including those that did not show overt estrus revealed complete luteolysis by 24 h of treatment in present study suggesting that animals were treated during responsive stage of CL. The possibility of the incidence of ovulation without expression of signs of estrus is greater among the cows when treated with $PGF_{2\alpha}$ during early stage of diestrus (Macmillan, 1978) and this phenomenon occurs most frequently among cows treated at 5–7 days of estrous cycle. A newly formed CL is resistant to $PGF_{2\alpha}$ treatment, but its sensitivity to luteolytic effect of $PGF_{2\alpha}$ increases with time. Cows that lack sufficient numbers of sensitive luteal cells to respond to $PGF_{2\alpha}$ treatment will have partial luteolysis (Berardinelli and Adair, 1989) whereby signs of estrus might be least or absent in cattle treated during early stages of diestrus (Macmillan and Henderson, 1984). It seems possible that under partial luteolysis rate of decline in plasma P_4 concentration may be slow and animal may ovulate despite plasma P_4 concentration being remain higher. The partial luteolysis has been suggested to be the cause of silent estrus in buffaloes after $PGF_{2\alpha}$ treatment (Dhaliwal et al., 1988; El-Belely et al., 1995). Despite complete luteolysis by 24 h of treatment, six animals did not show overt estrus in present study suggesting that the partial luteolysis may be one of the causes of silent estrus in buffaloes after $PGF_{2\alpha}$ treatment but other factors are probably also involved.

The size of the ovulatory follicle was significantly smaller on Day 0 at treatment in animals with silent estrus (subgroup A-2) than that of animals with overt estrus (subgroup A-1), although animals of both subgroups ovulated from the dominant follicle present at treatment. Moreover, the growth rate of the ovulatory follicle was significantly slower in animals with silent estrus than that of animals with overt estrus. Probably, dominant follicles of these animals (subgroup A-2) were at the early stages of growth phase on Day 0. Nevertheless, these dominant follicles were expected to grow at a faster rate than they actually recorded in the presence of declining plasma P_4 level similar to that in animals, which showed overt estrus. Contrary to present finding, Brito et al. (2002) could not find difference in growth profiles of ovulatory follicle between animals with overt estrus and silent estrus though these animals ovulated from the largest follicle present at $PGF_{2\alpha}$ treatment.

During the follicular development, increase in LH pulsatility associated with a dramatic increase in LH receptors in granulosa cells of dominant follicle stimulate its terminal development up to preovualtory stage (Savio et al., 1993). Recent studies showed that locally produced factors; predominantly the insulin-like growth factors (IGFs) have a modulating effect on terminal

follicular development (Webb et al., 1999). It is suggested that LH may contribute to increased concentrations of bioavailable IGFs in dominant follicle by regulating the synthesis and proteolysis of IGF binding proteins. In addition, the higher intrafollicular concentrations of inhibin in dominant follicle might enhance LH-stimulated androgen production by theca cells, thus contributing to increased estradiol-17β synthesis by granulosa cells resulting in sustained terminal growth and maturation of dominant follicle (Bodensteiner et al., 1996; Ginther et al., 2001).

It seems possible that the dominant follicle in animals with silent estrus grew slowly either due to lower availability of LH to developing follicle affecting the bioavailability of various growth factors needed for terminal growth of dominant follicle or the synthesis of growth factors and/or their binding proteins might have been affected. As the expression of estrus behavior is dependent on amount of estrogen produced by granulosa cells, it may be possible that estradiol production might have affected which in turn depends on androgen production under influence of LH from theca cells. Nevertheless, the slow growth rate of the ovulatory follicle after $PGF_{2\alpha}$ treatment may be used as predictor of silent estrus in subestrus buffaloes. However, further research is needed to identify the factor(s) affecting the growth rate of the ovulatory follicle resulting in silent estrus in subestrus buffaloes after $PGF_{2\alpha}$ treatment.

The dominant follicle of first follicular wave during the growth or early static phase is capable of further growth and ovulation when luteolysis is induced with $PGF_{2\alpha}$ in cattle (Kastelic et al., 1990b; Kastelic and Ginther, 1991). However, when the dominant follicle of first follicular wave is in late static phase and a new follicular wave has been emerged, the first dominant follicle regresses and the dominant follicle of second follicular wave becomes the ovulatory follicle. Similar observations were recorded in cyclic water buffaloes after $PGF_{2\alpha}$ treatment (Brito et al., 2002). In present study, treatment with $PGF_{2\alpha}$ was employed at an unknown stage of the estrous cycle and the diameters of the largest and second largest follicles present in the ovary were recorded and analyzed. In the animals that ovulated from the largest follicle present at treatment (Group A), this follicle continuously increased in diameter to become the ovulatory follicle, while the second largest follicle continuously regressed, indicating that the largest follicle was the dominant follicle and the second largest follicle was the subordinate follicle of ovarian follicular wave present before treatment. In the animals, which ovulated from the second largest follicle (Group B), the largest follicle continuously regressed, while the second largest follicle continuously grew to become the ovulatory follicle. Therefore, the largest follicle was the dominant follicle of follicular wave present at the time of treatment, but was in its late static or early regression phase; it regressed and the dominant follicle of succeeding follicular wave grew until ovulation occurred.

When the largest follicle present at treatment became the ovulatory follicle (Group A), it exhibited slower growth rate, a smaller increase in diameter and a non-significantly greater maximum diameter prior to ovulation than the ovulatory follicle of succeeding follicular wave (Group B). On the other hand, longer intervals from treatment to estrus and from treatment to ovulation were recorded among the animals that ovulated from the second largest follicle despite its faster growth rate before ovulation. These results are in agreement with earlier reports in cyclic cattle (Kastelic et al., 1990b; Kastelic and Ginther, 1991) and buffaloes (Brito et al., 2002), which recorded differences in interval from treatment to ovulation and in characteristics of the ovulatory follicle in animals that ovulated from existing or succeeding follicular wave after induced luteolysis. The ovulatory follicle of succeeding follicular wave had to undergo considerable growth prior to ovulation compared with the ovulatory follicle present at treatment and this was consistent with its faster growth rate and longer interval from treatment to ovulation in present study. A similar observation has been reported in cyclic cattle (Kastelic and Ginther, 1991). Therefore, it may be

suggested that the status of future ovulatory follicle with respect to its developmental stage at the time of $PGF_{2\alpha}$ treatment is the regulatory factor that affect the time interval from $PGF_{2\alpha}$ treatment to estrus and ovulation in subestrus buffaloes.

In conclusion, the small size and the slow growth rate of the ovulatory follicle were associated with silent estrus in subestrus buffaloes after $PGF_{2\alpha}$ treatment. It may be possible to anticipate the occurrence of silent estrus in advance, based on ultrasonographic study on the initial size and subsequent growth rate of the ovulatory follicle after $PGF_{2\alpha}$ treatment in such animals.

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