

FOLLICULAR DYNAMICS AND UTERINE STATUS AFTER SYNCHRONIZATION OF OVULATION IN EARLY POST-PARTURIENT EGYPTIAN BUFFALOES

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ABSTRACT

The present study aimed at instigating the buffalo ovarian and uterine response to ovsynch protocol when initiated 21 days postpartum (pp). Animals were arranged into a treated (n = 3) and a control (n = 3) group. The treated group was given the first dose of GnRH on day 0 (day 21 pp) followed by PGF2 alpha on day 7 and the second dose of GnRH on day 9. All animals were allowed a daily ultrasound examination from day 1 to day 9 and then every 12 h until detection of ovulation. The ovarian response of the treated group clearly showed the organized events of follicular growth ended by ovulation in one animal (33.3%) of the treated group; the changes in the small, medium, large and total follicle population and area did not differ significantly between the two groups; luteinization of the dominant follicle following the first GnRH injection in two animals (66.6%) indicated the reliability of the ovsynch strategy to improve the ovarian function; the uterine response was clearly evidenced by increasing the uterine wall homogeneity, decreasing the uterine lumen (less than 0.3 cm) and clearing contents by day 7 - 8 in the treated group. In conclusion, the ovsynch protocol has a beneficial effect on the ovarian function and uterine involution when applied 21 days pp in buffaloes.

Keyword: buffaloes, ovary, ovsynch, postpartum, ultrasound

INTRODUCTION

Control of the interval from parturition to subsequent conception is important to optimize the reproductive rate of a species. Buffaloes are characterized by their low reproductive efficiency as expressed by long calving interval; exceeding 500 days (Merty *et al.*, 1994), mostly due to the lowered ovarian activity (El-Wishy, 2007). In general, the ovarian response of buffaloes to stimulatory treatment seemed to be less than that reported in cattle (Totey *et al.*, 1991; Singh *et al.*, 2000). In buffaloes, the lowered availability of anovulatory follicle results in producing few and poor quality embryos (Madan *et al.*, 1996 and Misra, *et al.*, 1988).

In dairy cows, the postpartum anovulatory anestrus in dairy cows was attributed not only to a lack of follicular development, but also to the failure of a dominant follicle to ovulate (Roche *et al.*, 2000); usage of GnRH in a single injection causes an increase in the LH surge and ovulation during postpartum between days 10 - 18 in dairy (Schallenberger *et al.*, 1984) and days 21 - 31 in beef (Troxel and Kesler, 1984) cattle. A three injection schedule (GnRH-PGF2 α -GnRH),

named Ovsynch, was successfully implicated for synchronization of ovulation in cattle (Pursley *et al.* (1995) and buffaloes (Paul and Prakash, 2005).

In the mean time of approving that the ovsynch protocol effectively induces ovulation in dairy cows as early as 21 days postpartum (Amaya-Montoya *et al.*, 2007), there is no available information on its usage during an early postpartum stage in buffaloes. The present study was designed to evaluate the ovarian response and uterine changes following synchronization of ovulation by ovsynch protocol applied earlier after calving as a point of economic value in promoting the productive and reproductive potentials of buffaloes.

MATERIALS AND METHODS

The present study was carried out on a total number of six newly parturient, lactating buffalo cows, kept in the Educational Farm, Faculty of Veterinary Medicine, Benha University during the period from April to August 2009. All animals were housed in a free-stall barn, offered 4 kg of mixed ration for lactation (consisting of cotton seed cack, line seed cack, yellow corn, bran, molasses, lime and NaCl) once daily, and suckled twice daily (0600 and 1800 h), and the average milk yield was approximately 2 - 3 kg/day.

All animals were examined ultrasonographically before the start of treatment for identifying the state of ovulation and presence of corpus luteum on the ovary. Animals were randomly allocated in two nearly similar groups for treatment and control, each of which was three buffalo cows.

Animals in the treated group (n = 3)

were treated with the ovsynch protocol (Figure 1) according to Pursley *et al.* (1995); all animals received 20 µg i.m. injection of Buserelin acetate as GnRH analogue (Receptal®, Intervet International B.V.; equal 5 ml) on day 0 (day 21 PP) followed by 500 µg i.m. injection of synthetic PGF₂α; Cloprostenol (Estrumate®; Schering-Plough Animal health, equal 2 ml) on day 7 then by second i.m. injection of 20 µg GnRH analogue on day 9. In the control group, all animals were injected by saline comparable to the dose and time of the hormonal treatment.

By using transrectal ultrasonography (PieMedical 240, 6-8 MHz linear array probe), ovarian morphology was monitored daily starting from the day before the first to the second GnRH injection thence after twice daily (08.00 and 20.00 h) till occurrence of ovulation and re-examined after 10 days to confirm ovulation and occurrence of the CL. To analyze the changes in follicular dynamics after GnRH treatment, all antral follicles (≥ 2 mm) were counted and measured. The observed follicles were classified into small (< 0.5 cm), medium (0.5 - 1.0 cm) and large (≥ 1.0 cm) sizes. Diameters and volumes (cm³) of luteal structures and cavities were determined. The transverse diameter of the anterior 1/3 section of both uterine horns and the progression of uterine involution were evaluated. Animals were observed twice daily for at least 30 minutes before milking by an experienced person to detect signs of estrus and to be bred naturally (Figure 2).

The obtained data was tabulated and statistically computed, where appropriate, by the linear regression analysis using a Microsoft Excel computer program according to Awasthi *et al.* (2006).

RESULTS

1. Ovarian findings

1.1. Follicular dynamics

As shown in Figures (3, 4, 5 and 6), the changes in the number and area of small, medium and large follicular size (<0.5 cm, 0.5-1.0 cm and >1.0 cm diameter, respectively) and whole follicular population in the treated and control groups did not show any significant variation along the experiment period. Moreover, the characteristics of follicular waves (Table 1) showed a similar pattern in the two groups except for the diameter of the dominant follicle at the first GnRH treatment, which was significantly larger prior to luteinization (1.38 ± 0.12 Vs 0.98 ± 0.06 cm, $P < 0.05$).

1.2. Ovulatory response

As shown from Figures 7 and 8, there was an occurrence of luteinization of the dominant follicle (LF) after the first GnRH injection in two out of three treated animals (66.67%) and failure of ovulation or CL development. Luteinization of the largest follicle was evident ultrasonographically in two responding buffaloes by thickening in the wall (1.05 cm width) and increasing the echogenicity of the follicular wall one day after the first GnRH treatment, reaching its maximum diameter (2.69 cm) on day 2; regression of the luteal structure started by decreasing the echogenicity, the diameter and collapsing the cavity area from day 3, and became ultrasonographically difficult to detect by day 5-6 before PGF₂α treatment. Out of three treated animals, there was one buffalo cow showed (33.3%) and ovulation 24 - 36 h after the second GnRH injection.

2. Uterine findings

The dorsal uterine diameter (Figure 9A)

did not differ between the treated ($y = -0.05x + 2.90$, $R^2 = 0.69$) and control ($y = -0.06x + 2.97$, $R^2 = 0.82$) groups except on day 1 where it showed a significant decrease ($p < 0.001$) in the treated group. The ventral diameter of the uterus (Figure 9B) showed a significant ($p < 0.05$) decrease on day 4 and 7, followed by a significant ($p < 0.05$) increase on day 9 in the treated group; both groups were negatively correlated with days post-treatment ($y = -0.07x + 3.52$, $R^2 = 0.78$ and $y = -0.08x + 3.75$, $R^2 = 0.81$, respect.). The cranial diameter (Figure 9C) showed a significant increase on day 8 and 9; both treated and control groups were negatively correlated with days post-treatment ($y = -0.07x + 3.99$, $R^2 = 0.81$ and $y = -0.06x + 3.88$, $R^2 = 0.76$, respect.). The transverse diameter in the treated group (Figure 10A) showed significant ($P < 0.05$) decrease on day 1, 3, and 10, but significant ($P < 0.05$) increase on day 9 when compared to that in the control group ($y = -0.11x + 6.28$, $R^2 = 0.75$ and $y = -0.15x + 6.71$, $R^2 = 0.87$, respect.). The uterine lumen (Figure 10B) revealed highly significant ($P < 0.05$) decrease along the days of the experiment in the treated group when compared with control ($y = -0.02x + 0.45$, $R^2 = 0.84$ and $y = -0.02x + 0.59$, $R^2 = 0.73$, respect.).

DISCUSSION

The present study revealed that the ovsynch protocol applied at an early postpartum period (day 21) precisely synchronized ovulation within 24 - 36 h after the second-GnRH treatment in 1/3 (33.3%) buffaloes and induced early clearance of uterine secretion as evidenced by reducing the uterine lumen diameter. However, the changes in the total follicular population and/or area were not significantly different between the treated and the

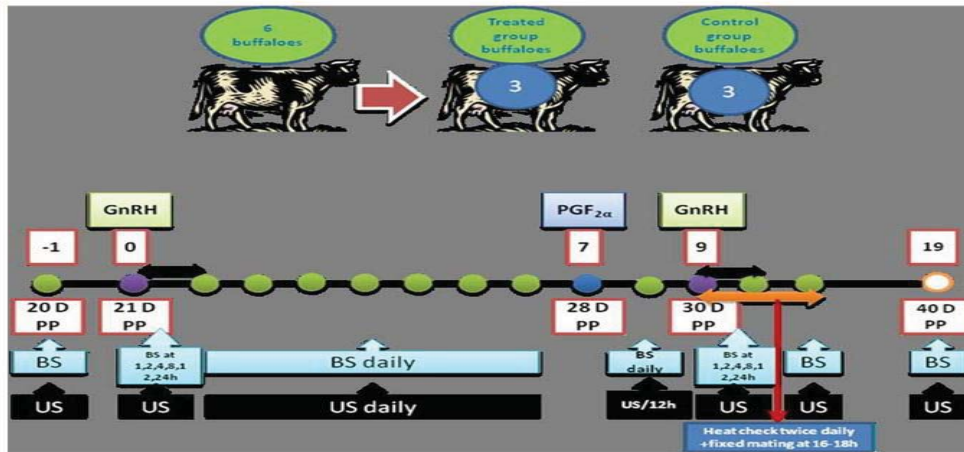


Figure 1. Diagrammatic scheme of ovsynch regimen and protocol of work. US: ultrasound examination; PP: postpartum; GnRH: gonadotrophin releasing hormone; PGF_{2α}: prostaglandin F_{2α}.

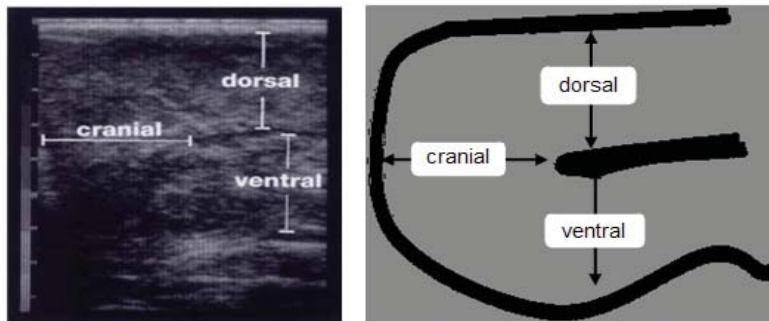


Figure 2. Ultrasonographic measuring of the dorsal, cranial, and ventral diameters of a uterine horn.

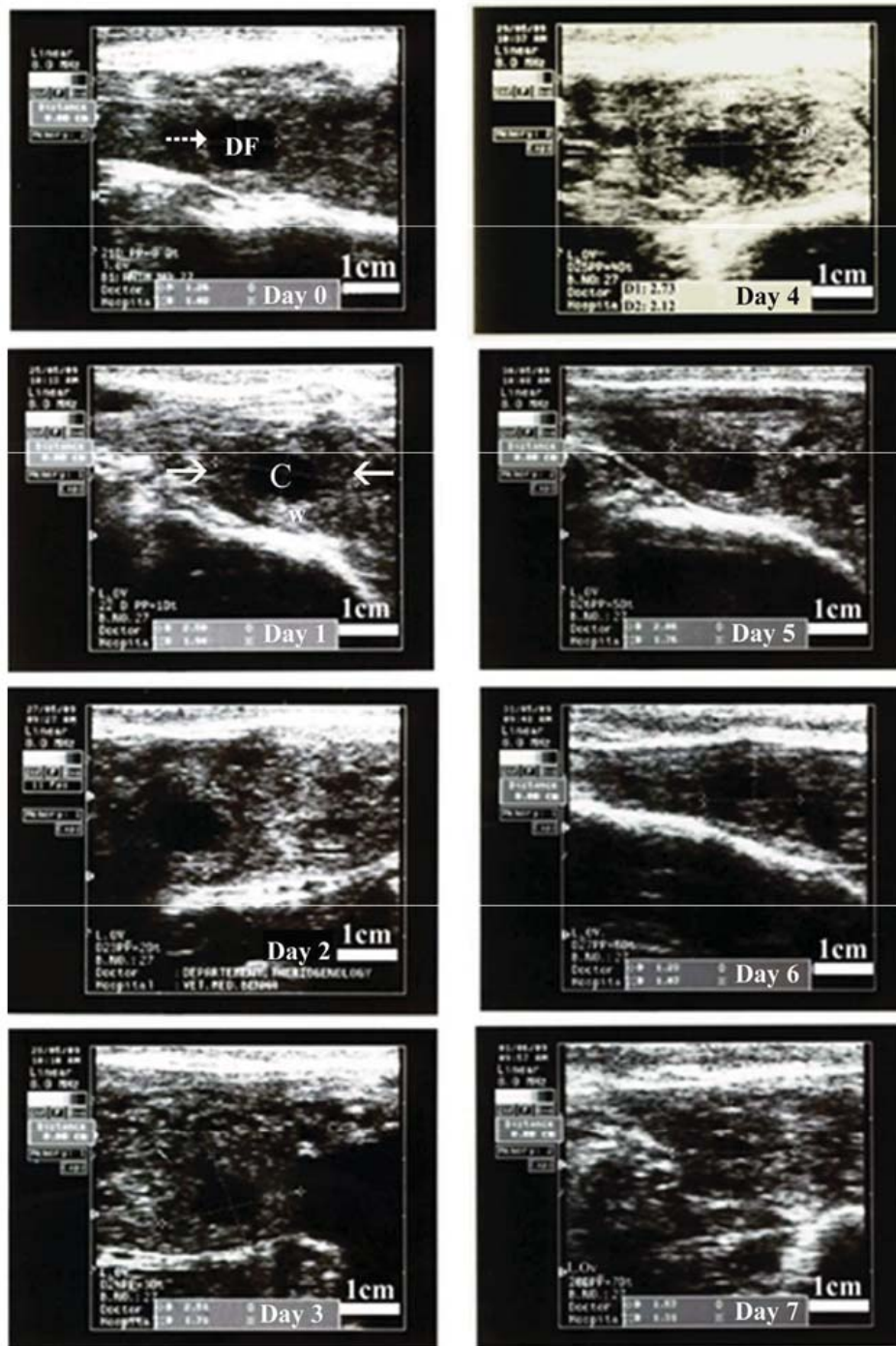


Figure 3. Changes in the characteristics of small follicle population (< 0.5 cm) in the buffaloes treated and control groups following ovsynch (GnRH-PGF2 α -GnRH) protocol started on day 21 days postpartum. Neither follicular number (A) nor area (B) differed significantly between the two groups during the monitored period. Values presented were means.

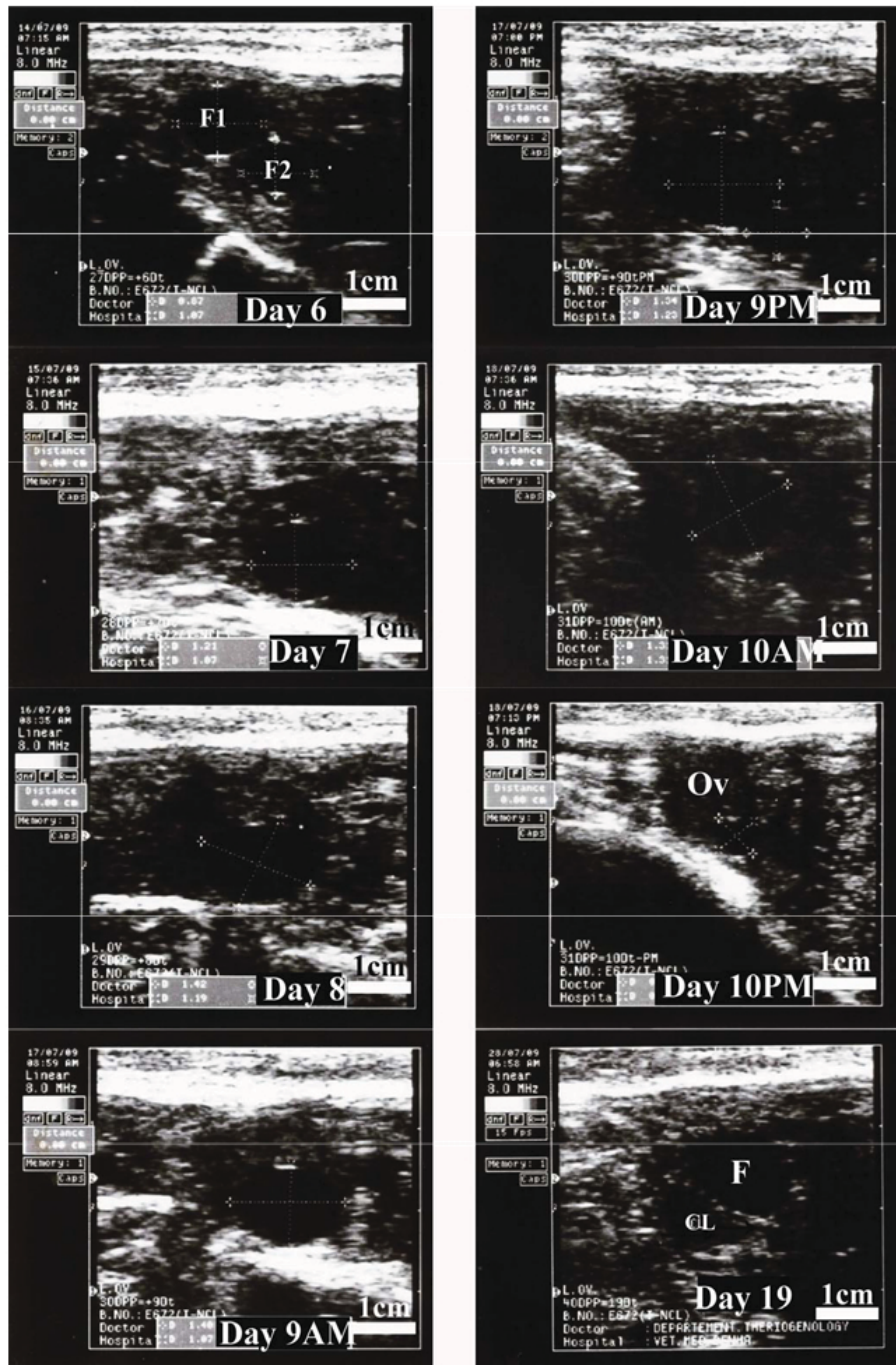


Figure 4. Changes in the characteristics of medium follicle population (0.5 - 1.0 cm) in the buffaloes treated and control groups following ovsynch (GnRH-PGF2 α -GnRH) protocol started on day 21 days postpartum. Neither follicular number (A) nor area (B) differed significantly between the two groups during the monitored period. Values presented were means.

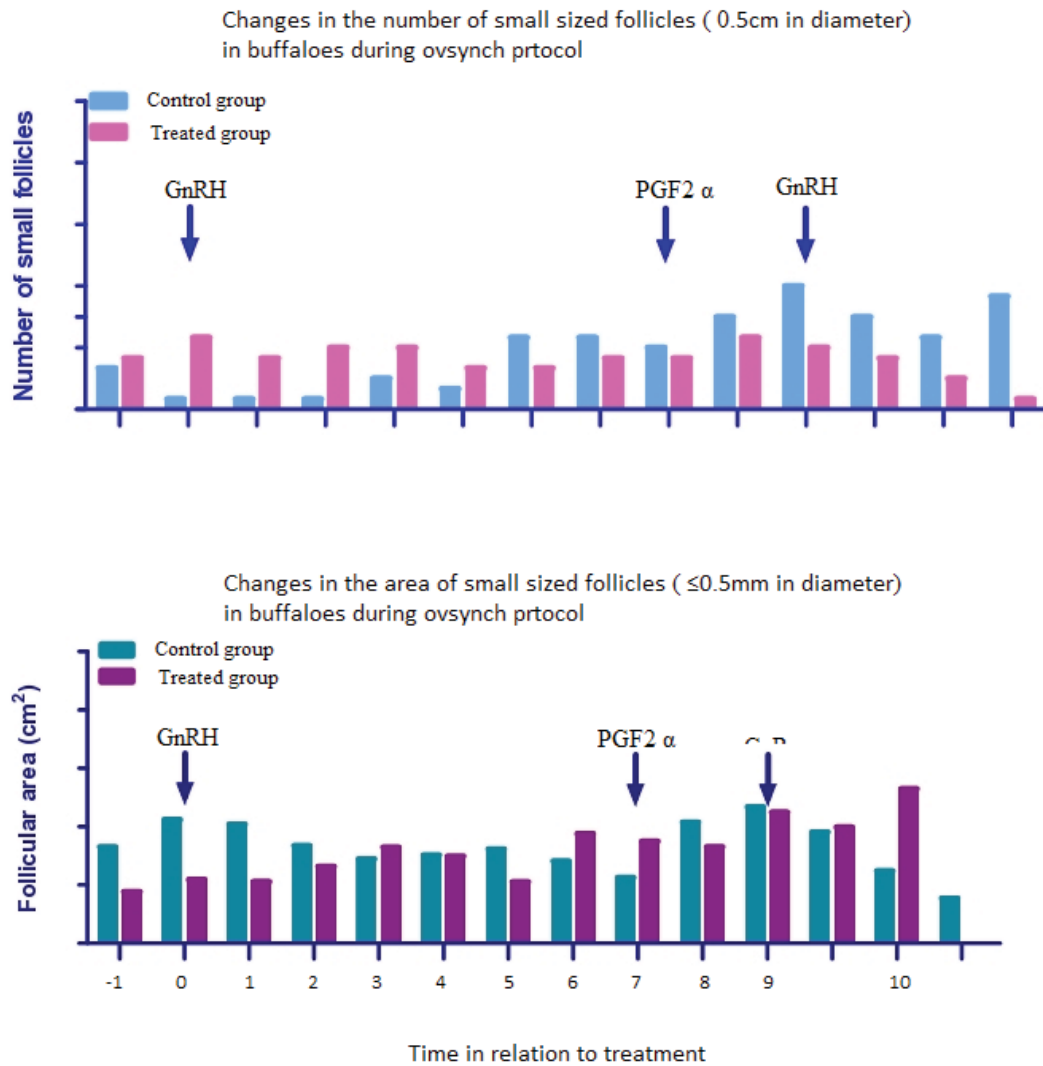


Figure 5. Changes in the characteristics of large follicle population (> 1.0 cm) in the buffaloes treated and control groups following ovsynch (GnRH-PGF2 α -GnRH) protocol started on day 21 days postpartum. Neither follicular number (A) nor area (B) differed significantly between the two groups during the monitored period. Values presented were means.

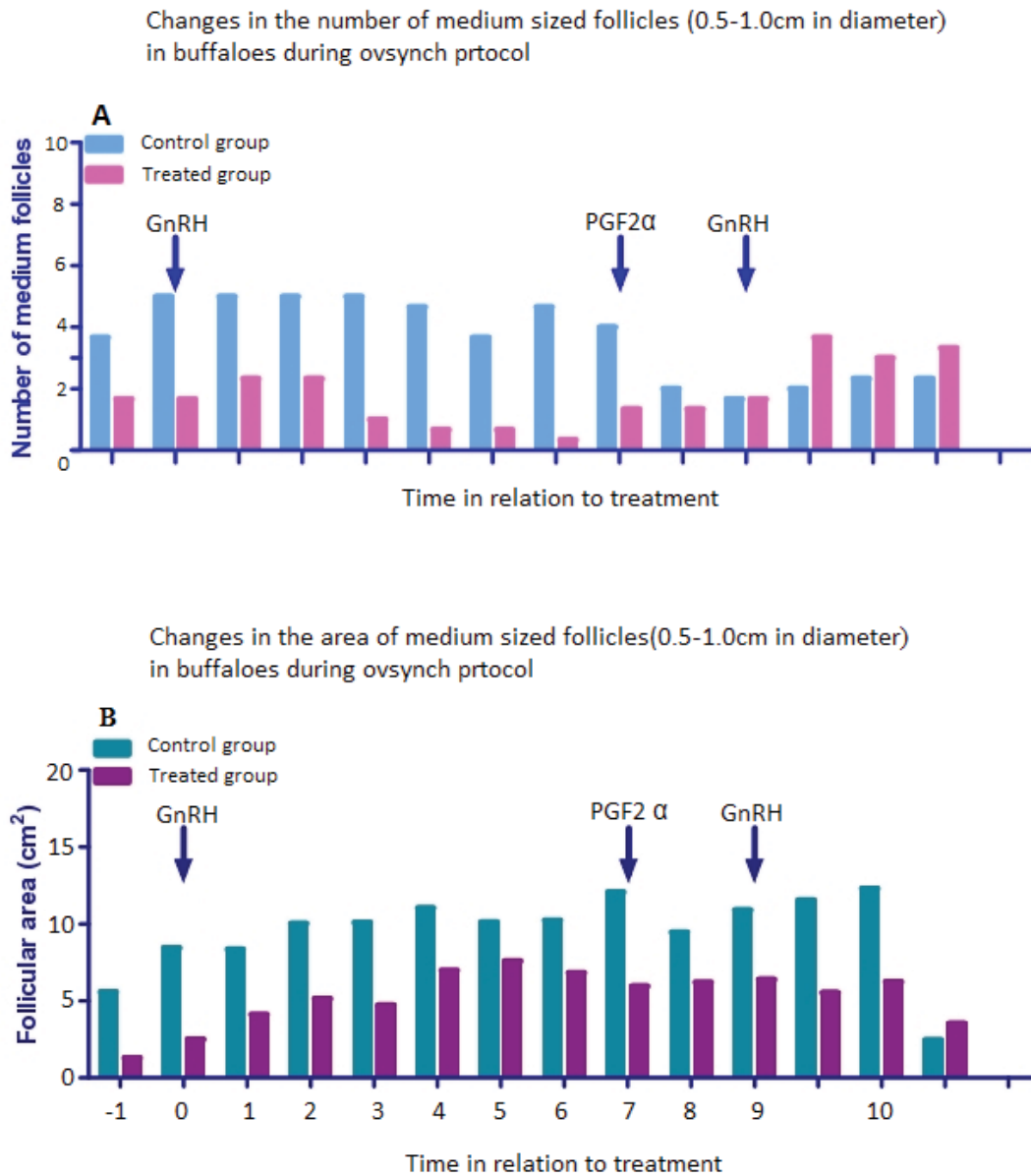


Figure 6. Changes in the characteristics of total follicle population (> 0.2cm) in the buffaloes treated and control groups following ovsynch (GnRH-PGF2 α -GnRH) protocol started on day 21 days postpartum. Neither follicular number (A) nor area (B) differed significantly between the two groups during the monitored period. Values presented were means.

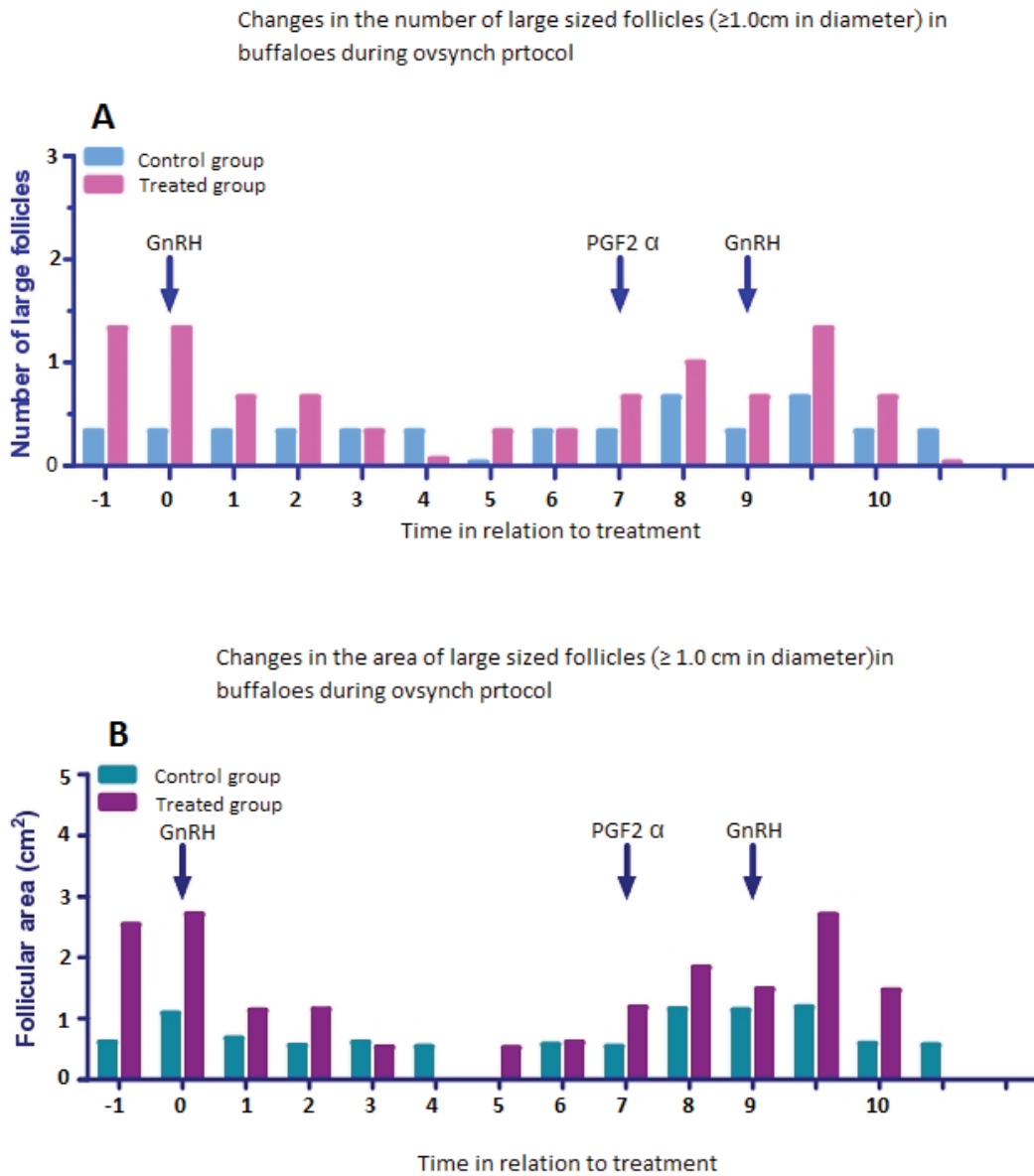


Figure 7. Representative patterns of follicular growth and regression on the ovary of buffaloes treated with ovsynch protocol 21 days postpartum. The largest follicle in the treated group showed a unique pattern of growth that ended by ovulation in one buffalo while regressed in the other two animals. Dotted and dashed lines refer to timing of GnRH and PGF2 α treatment, respectively. Lut: luteinization; Ov: ovulation.

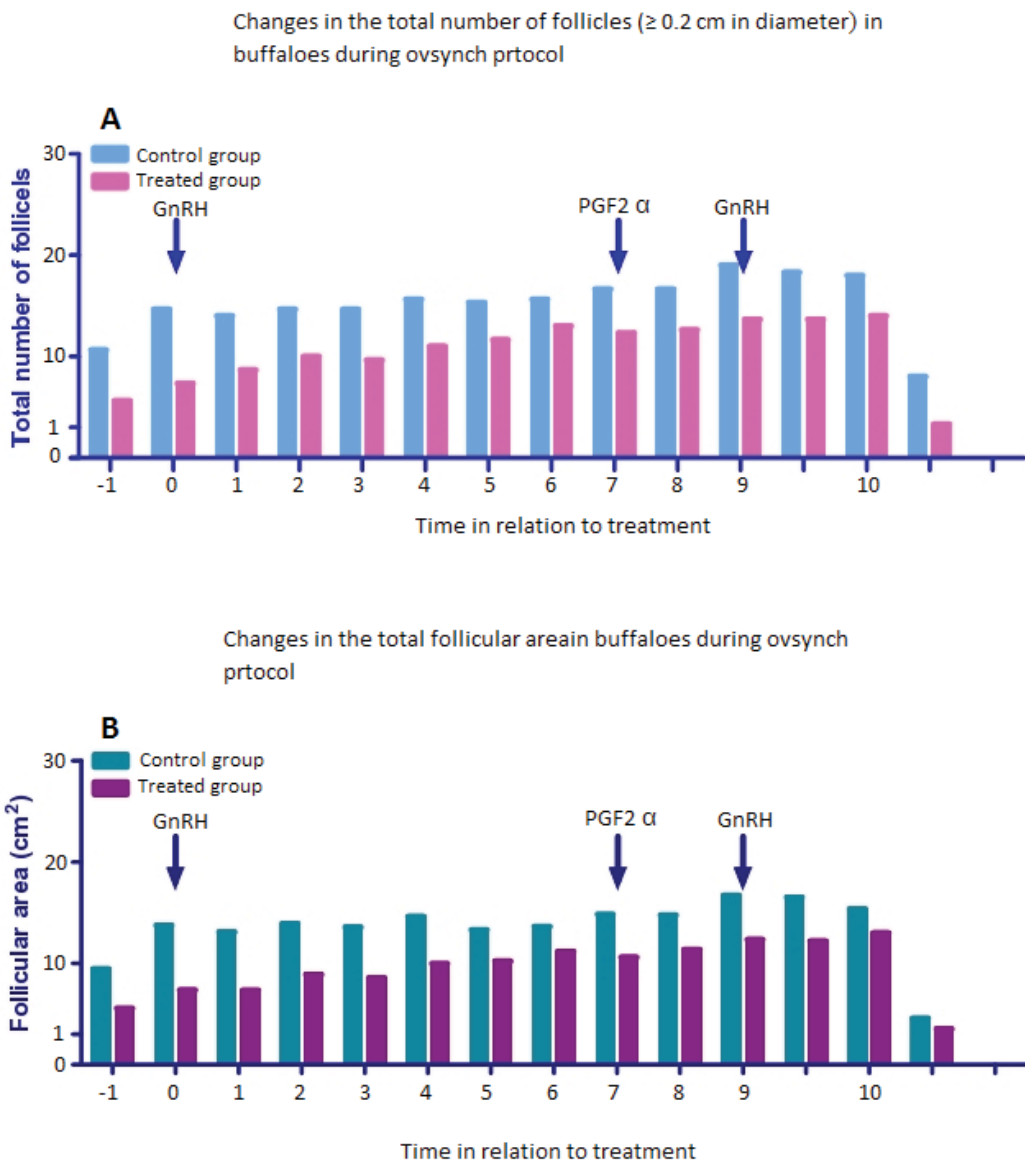


Figure 8. The pattern of induced luteal tissue development and regression on the ovary of treated buffaloes with ovsynch protocol 21days postpartum. The luteinization of dominant follicle started on Day 1. The luteal tissue volume showed a rapid decrease per unit of time as compared with luteal tissue diameter.

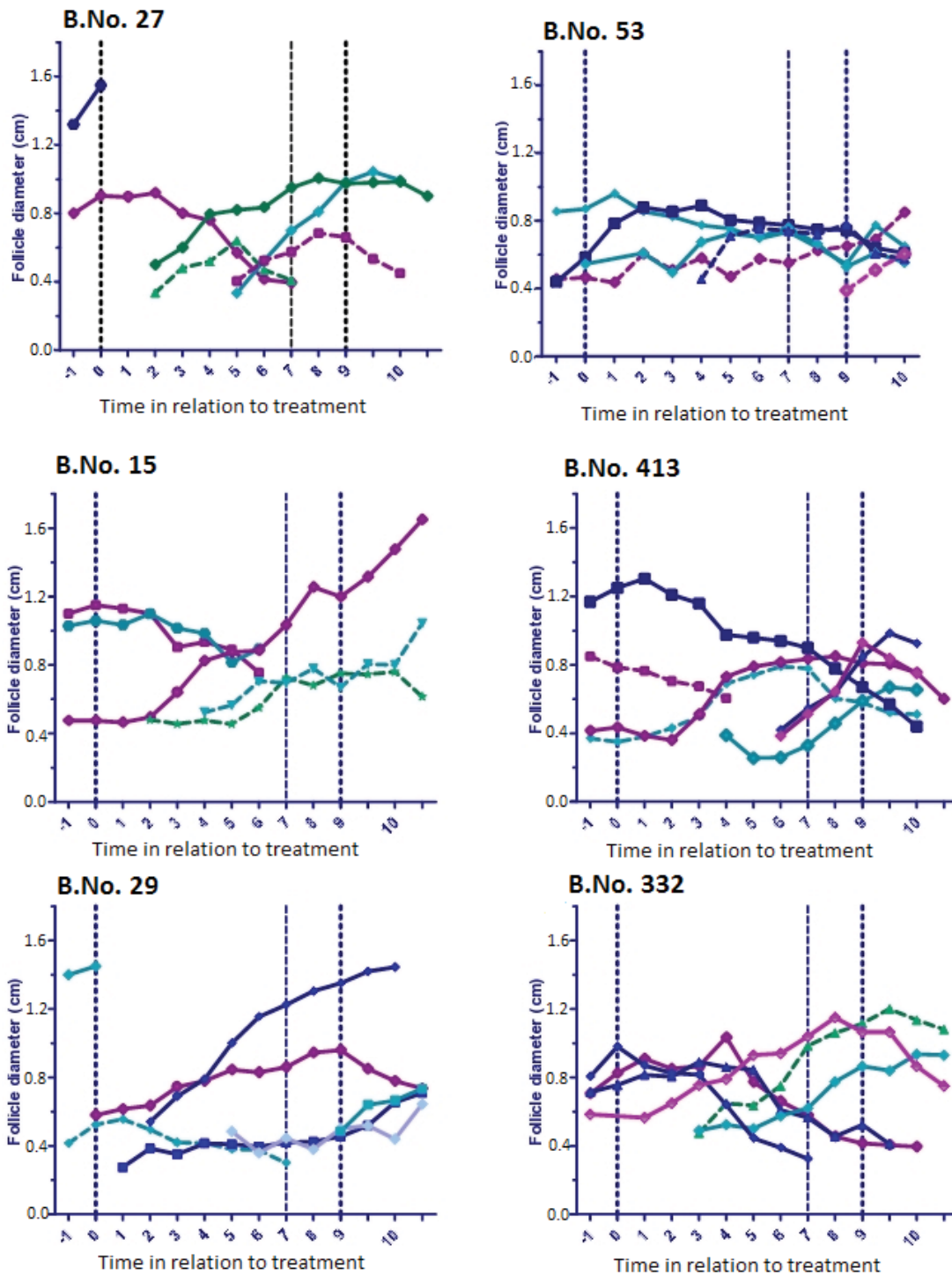


Figure 9. A representative ultrasonographic pictures of the uterus on Day 0, 2, 7, 10 in the treated (right side panel) and control (left side panel) buffaloes with ovsynch protocol started on Day 21 postpartum. The homogeneity of the uterine wall increased, while the lumen diameter and echogenicity decreased in the treated group compared with control. The arrow refers to the detectable uterine lumen in the control group on Day 10.

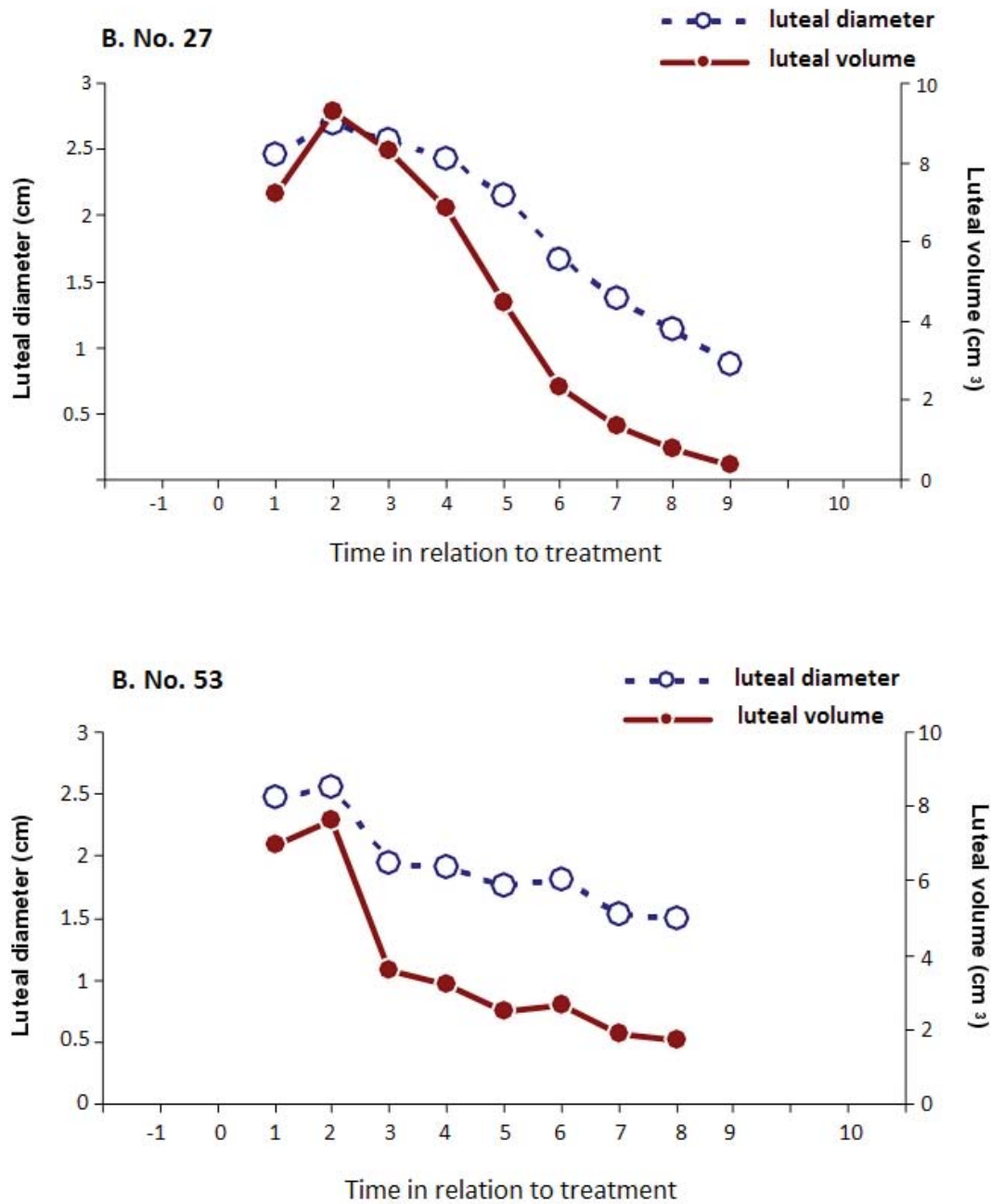


Figure 10. Changes in the uterine dorsal (A), cranial (B) and ventral diameter of the uterine wall in the treated and control groups treated with ovsynch protocol started on Day 21 postpartum. The cranial and ventral diameter of uterine horn (s) increased significantly in the treated group due to the increase in vascularity accompanying the onset of estrus. Values presented are mean \pm SEM. * $P < 0.05$, ** $P < 0.001$.

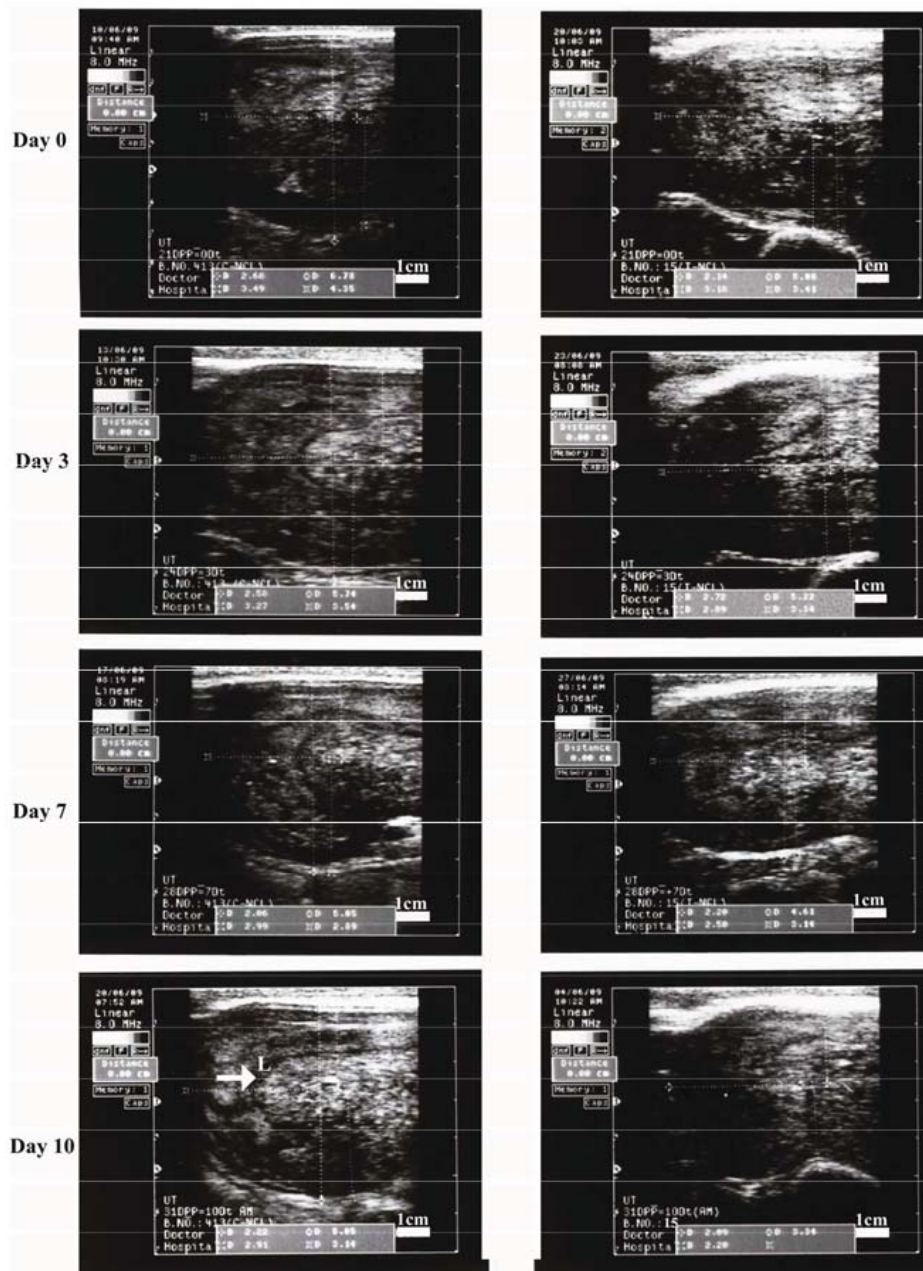


Figure 11. Changes in the transverse uterine diameter (A) and lumen (B) in the treated and control groups treated with ovsynch protocol started on Day 21 postpartum. If the diameter taken as a guide to confirm the uterine involution (< 3 cm), it did not complete neither in treated nor in control groups. If the lumen diameter used as a guide (< 0.3 cm), the uterus of treated animals involuted by Day 7 (i.e. 28 days PP). The uterine lumen showed a progressive and significant decrease in the treated animals compared with the control group. Values presented are mean \pm SEM. * $P < 0.05$, ** $P < 0.001$.

Table 1. Characteristics (Mean \pm S.E.M) of the ovarian waves in Egyptian Buffaloes treated with ovsynch during early postpartum period.

Parameters	Treated group		Control group		P-value	
	Mean	\pm SEM	Mean	\pm SEM	F-test	t-test
Diameter of dominant at start of treatment (cm)	1.38a	0.12	0.98b	0.06	0.360	0.039
Max. follicle diameter after treatment (cm)	1.38	0.18	1.01	0.10	0.462	0.139
Growth rate of the dominant follicle (mm/d)	1.49	0.07	1.65	0.17	0.243	0.453
Length of growth phase (days)	8.00	1.53	5.67	0.67	0.320	0.234
Range	5 – 10 days		5 – 7 days			
Day of emergence of the dominant follicle	2.33	1.45	2.67	2.03	0.679	0.900
Range	2 – 5 days		1 – 6 days			
Beginning diameter (cm)	0.45	0.06	0.43	0.03	0.316	0.813
Day of maximum diam. of the dominant follicle	9.33	0.33	6.33	1.45	0.100	0.114
Range	9 – 10 days		4 – 9 days			
Day of emergence of the 1st wave after treatment	2.33	0.33	4.33	0.88	0.250	0.101
Range	2 – 3 days		3 – 6 days			
Linear growth rate of the dominant follicle	0.40	0.09	0.29	0.19	0.339	0.637
max diameter of 2nd largest follicle	1.00	0.02	0.88	0.16	0.044	0.520
Day of deviation	6.67	1.20	4.33	0.67	0.471	0.165
Range	5 – 9 days		3 – 5 days			

control groups. The poor ovarian response in the treated group to the first GnRH basically might be attributed either to the little population of FSH-dependant follicles, the low LH secretion from pituitary in response to GnRH injection or the low GnRH receptors in pituitary gland. This finding came in association with some previous reports (Dufour and Roy, 1985; Lucy *et al.*, 1991; Pursley *et al.*, 1995; Walters *et al.*, 2008) indicating that the high rates of ovulation after GnRH injection are due to the presence of a potentially ovulatory follicle (> 9.0 mm); the early postpartum period is characterized by reduction in the number of small sized follicles as the number of days postpartum increases (Lucy *et al.*, 1991; El-Wishy, 2007).

The present results revealed luteinization of the dominant follicle following the first GnRH injection in the treated group, a finding which emphasized occurrence of ovulation in response to GnRH treatment during the postpartum period for 60% buffaloes (Baruselli *et al.*, 2003) and 85% cattle (Wiltbank, 1997). Follicle luteinization was detected in 37.5% non-cyclic buffaloes synchronized by ovsynch 96-118 days postpartum (Ali and Fahmy, 2007).

The present study indicated occurrence of ovulation in one case of the treated group (33.3%), 24-36 h after the second GnRH injection. This finding was consistent with that reported earlier in cows (Demüral *et al.*, 2006) and buffaloes (Warriach *et al.*, 2008) assuming that ovsynch protocol for estrus synchronization has potential application for improvement of fertility in anestrus buffaloes during early postpartum period even during extreme summer months through suppression of prolactin secretion (Roy and Prakash, 2009).

Following GnRH injection, low intensity of estrus was detected, a finding which came in accordance with some previous studies

(Pattabiraman *et al.*, 1986; Barkawi *et al.* (1995) indicating the presence of poor signs of heat in GnRH treated buffaloes.

The present study showed fluctuation in thickness of the uterus, but was negatively correlated with the day postpartum. The treated group showed a prominent clearance of the uterine lumen as indicated from the noticeable decrease of the uterine lumen echogenicity and diameter comparable to the control one. This might be attributed to the increased ovarian estradiol secretion by large follicle(s) that has a local effect to increase the rate of uterine involution (Sheldon and Dobson, 2000; Sheldon *et al.*, 2003). The ultrasonographic pattern of decreasing the uterine diameter by increasing time after parturition came in accordance with some previous studies (Okano and Tomizuka, 1987; Tian and Noakes, 1991; Kamimura *et al.*, 1993; Sheldon *et al.*, 2003). Besides, the transverse uterine diameter recorded in the present study for the treated and control groups was inconsistent with previous reports in buffaloes (Usmani *et al.*, 2001; Lohan *et al.*, 2004; Khasatiya *et al.*, 2006).

From the present study, it can be concluded that the ovsynch protocol is potentially able to improve the ovarian and uterine function when applied during early postpartum in buffaloes, a finding which needs to be confirmed in further study on a bigger number of post-parturient buffalo cows.

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