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# POSTPARTUM OVARIAN ACTIVITY OF MEHSANA BUFFALOES WITH NORMAL AND ABNORMAL PARTURITIONS

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## ABSTRACT

Early return of ovarian cyclical activities is a pre-requisite for subsequent fertility. These events occur in all postpartum animals irrespective of periparturient disease, however, reproductive disorders being multifaceted impair reproductive performance by altering follicular activities. Therefore, the present investigation was planned to study ovarian activities in normal and abnormal parturition of Mehsana buffaloes. The ultrasonographically monitoring of postpartum ovarian activity between day 5$^{th}$ and 45$^{th}$ in normal (n=25) and abnormal (n=10) parturition of Mehsana buffaloes was carried out. Further, these buffaloes were also sub-grouped on the basis of cyclicity and conception to know the difference in ovarian activities. The results of study revealed significantly larger emergence diameter and diameter of dominant follicle in normally parturited buffaloes whereas follicular atresia was frequently observed in abnormal parturited buffaloes and non-cyclic as well as non-conceived groups of buffaloes.

**Keywords:** Abnormal parturition, Buffalo, Normal parturition, Postpartum ovarian activity

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The period immediately after calving is also very important in medium-low yielding buffaloes because of its vast influence on reproductive performance. Normal uterine involution and the reestablishment of the ovarian function postpartum are crucial to obtain short calving to conception interval that is required to optimize milk and calf production. After parturition, the dominant follicle is selected around 10–12 days after calving (Beam and Butler, 1997). Early return of ovarian cyclical activity is accepted to be beneficial for subsequent fertility (Darwash *et al.*, 1997). In buffaloes, the establishment of a new pregnancy within 90 days post-partum (PP) is one of the biggest challenges (El-Wishy, 2007). The strong positive association exists between early commencement of postpartum ovulatory cycles and subsequent pregnancy (Galvao *et al.*, 2010) has focused research attention on the regulation and re-initiation of ovarian activity and ovulatory cycles in early lactation prior to the breeding period. These events occur in all postpartum cows irrespective of periparturient disease, environment or dietary deficiencies. However, reproductive disorders being multifaceted impairs reproductive performance by altering follicular activities. Looking to these facts infertility should be evaluated accordingly. Therefore, study focused on ovarian activities will surely be a beneficial for the buffaloes reproduction as the buffaloes are major source of milk in India.

## MATERIALS AND METHODS

The present experiment was intended to study the ovarian activities in both normally and abnormally parturited Mehsana buffaloes between May-2016 and May-2017. The Mehsana buffaloes with normal parturition (without any assistance) and abnormal parturition (buffaloes suffered with either maternal or fetal type dystocia) were selected from Livestock Research Station, Sardar Krushinagar Dantiwada Agricultural University, Sardar Krushinagar as well as from the clinical cases reported at Dr. V.M. Jhala Clinical Complex, College of Veterinary Science & Animal Husbandry, Sardar Krushinagar Dantiwada Agricultural University, Deesa and from milk shed area of Banaskantha district for present study.

Total 35 Mehsana buffaloes were selected and subsequently divided into two groups, i.e., group I (n=25) were normally parturited healthy Mehsana buffaloes irrespective of parity. Whereas, group II (n=10) included 10 Mehsana buffaloes suffered from dystocia to act as an abnormal parturition group for the study. Further, buffaloes of groups I and II were clubbed and divided into two groups based on postpartum cyclicity, *viz.*, cyclic group (n=26) and non-cyclic group (n=9). The cyclic buffaloes (n=26) were further sub-divided as conceived (n=19) and non-conceived (n=7) subgroups.

The ovarian activity of each buffalo was examined ultrasonographically (10-5MHz, Sonosite, Titan Ltd. Hitchin, United Kingdom) on 5$^{th}$, 10$^{th}$, 15$^{th}$, 20$^{th}$, 25$^{th}$, 30$^{th}$, 35$^{th}$, 40$^{th}$ and 45$^{th}$ day postpartum. The transducer was inserted per-rectally and placed on the postpartum ovaries to measure the size of follicles. The ovarian

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ultrasonographic scanning was accomplished in several planes to identify all the follicles. These follicles were followed, and follicle continuously increasing in size was considered dominant follicle (DF), and the same was followed up to ovulation. The various observations related to ovarian activity *viz*., emergence diameter of follicles (mm), diameter of the dominant follicle (DF) (mm), time taken by DF to attain dominance (days), growth rate (mm/day), ovulation (day) and corpus luteum (mm) were recorded. The statistical difference between groups was tested by t test at 1% significance.

## RESULTS AND DISCUSSION

**Ovarian activity:** The ultrasonography was performed to assess postpartum ovarian activity to understand the ovarian follicle dynamics of normally and abnormally parturited Mehsana buffaloes (Table 1 & Fig. 1). The ultrasonography was successfully used to evaluate follicular fate (Awasthi *et al.*, 2006). The ovarian activity was judged by recruitment of follicles, their growth and selection followed by either ovulation or atresia, and corpus luteum. All of these events were observed from day 5 to 45 postpartum at an interval of five days in both normally and abnormally parturited buffaloes. El-Wishy (2007) reported that follicular activity is resumed early (15–30 days) in the postpartum period in buffaloes. The resumption of follicular activities in different breeds of buffaloes were studied within forty days postpartum (Usmani *et al.*, 1985; Ingawale *et al.*, 2012), while the mean duration for postpartum ovarian activity in buffaloes was also reported for more than forty days (Khasatiya *et al.*, 2005).

**Emergence diameter of dominant follicle:** The mean emergence diameter of the dominant follicle in group I (6.48 ± 0.32 mm) was significantly ($p<0.01$) larger than group II (4.81 ± 0.50 mm). The emergence diameter differed non-significantly with diameter being larger in non-cyclic (6.77 ± 0.59 mm) than cyclic (5.74 ± 0.33 mm) postpartum Mehsana buffaloes. The average follicular emergence diameter of conceived subgroup (5.67 ± 0.41 mm) differed non-significantly from the non-conceived subgroup (5.90 ± 0.61 mm). The observed emergence diameter of the dominant follicle was 6.48 ± 0.32 mm, ranging from 3.70 to 8.70 mm in normally parturited buffaloes. Similarly, Kulick *et al.* (1999) and Ginther (2000) reported that slightly larger follicular emergence diameter of the two largest follicles (8.3 ± 0.2 and 7.8 ± 0.2 mm; and 8.5 and 7.7 mm, respectively) at the end of selection from a recruited follicular pool in cows. They also orated that deviation in size, which was characterized by the continued growth of the largest follicle to become the dominant follicle and reduced or terminated growth of remaining follicles to become subordinate follicles. Conversely, Khare (2004) and Nair (2008) in Mehsana buffaloes reported a lesser diameter of emerging follicles than present findings in normally parturited Mehsana buffaloes. The emergence diameter of the dominant follicle in abnormally parturited Mehsana buffaloes understudy was significantly ($p<0.01$) smaller than normally parturited buffaloes. Correspondingly, Sheldon *et al.* (2002) reported that cows with uterine disease have smaller follicular diameters with the explanation of spontaneous bacterial contamination of the postpartum uterus influenced the ovarian location for dominant follicle selection. Similarly, Mateus *et al.* (2002) and Hanafi *et al.* (2008) also reported disturbed ovarian activity in animals with postpartum uterine infections. The emergence diameter in postpartum regular cyclic and non-cyclic buffaloes as well as conceived and non-conceived buffaloes showed non-significant difference. However, Khasatiya *et al.* (2005) observed the significant early resumption of postpartum ovarian activity in fertile Surti buffaloes as compared to infertile buffaloes. Awasthi *et al.* (2006) in suckled water buffaloes showed one wave follicular pattern as a normal phenomenon with growth, regression, and regrowth, culminating in ovulation. Carruthers *et al.* (1980) reported that following parturition, there is an early resumption of sequential but transient FSH increase of 2-3 days duration in cows which results in the emergence of the first postpartum follicular wave subsequent decline in FSH results in selection of dominant follicle. At the hypothalamus and pituitary level, the oestradiol-induced preovulatory LH surge is blunted when bacterial endotoxin is infused into the uterus or administered intravenously (Peter *et al.*, 1989; Battaglia *et al.*, 1997). In notion to Sheldon *et al.* (2002, 2008), the uterine infection does not appear to affect peripheral plasma FSH concentration profiles or ovarian follicle wave emergence in cows.

**Diameter of dominant follicle:** The maximum detected diameter of the follicle during the sequential ultrasonographic examination was considered the diameter of dominant follicle (Table 1 and Fig. 1). The observed diameter of dominant follicle in normally parturited buffaloes (13.00 ± 0.44 mm) was significantly ($p<0.01$) larger than abnormally parturited Mehsana buffaloes (10.43 ± 0.64 mm) of the present study. One buffalo from group II developed a cystic ovary and the size of the cyst size was 36.80 mm with the luteinized wall (3.55 mm) on day 30 postpartum followed by a gradual reduction in the size of the cyst. The postpartum cyclic buffaloes (n=25) and non-cyclic buffaloes (n=9) had no

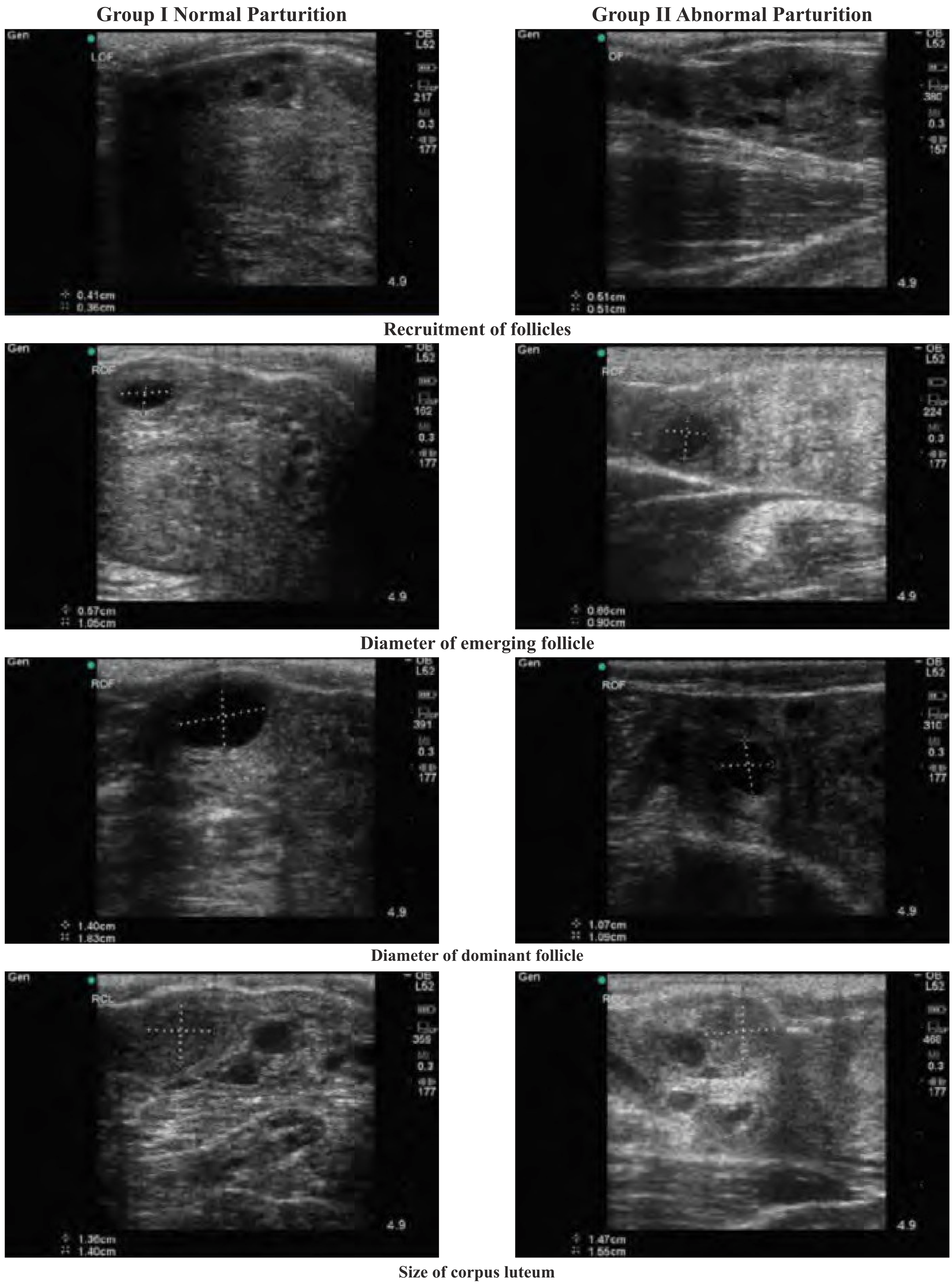


Fig. 1. The postpartum follicular characteristics in normal and abnormal parturition of Mehsana buffaloes

**Table 1. The postpartum ovarian activities in different groups of Mehsana buffaloes.**

| Particulars | Group I | Group II | Group I and II pooled | | Cyclic Group | |
|---|---|---|---|---|---|---|
| | Normal Parturition | Abnormal Parturition | Cyclic Group | Non-Cyclic Group | Conceived Sub-group | Non-Conceived Sub-group |
| Emergence Diameter of DF (mm) (Mean ± SE) | $6.48 \pm 0.32_p$<br>(3.7-8.7)<br>(n=25) | $4.81 \pm 0.50_q$<br>(2.90-7.50)<br>(n=10) | 5.74 ± 0.33<br>(2.90-8.70)<br>(n=26) | 6.77 ± 0.59<br>(2.90-8.70)<br>(n=9) | 5.67 ± 0.41<br>(2.90-8.50)<br>(n=19) | 5.90 ± 0.61<br>(4.20-8.70)<br>(n=7) |
| Diameter of D.F. (Maximum) (mm) (Mean ± SE) | $13.00 \pm 0.44_p$<br>(9.40-16.10)<br>(n=25) | $10.43 \pm 0.64_q$<br>(8.70-14.20)<br>(n=9) | 12.22 ± 0.48<br>(8.90-16.10)<br>(n=25) | 12.60 ± 0.85<br>(8.70-15.90)<br>(n=9) | 12.12 ± 0.58<br>(8.90-16.10)<br>(n=18) | 12.46 ± 0.90<br>(9.80-15.90)<br>(n=7) |
| Time to Attain Dominance (Days) (Mean ± SE) | 14.80 ± 1.02<br>(5.00-25)<br>(n=25) | 14.44 ± 0.56<br>(10.00-15.00)<br>(n=9) | 14.60 ± 0.99<br>(5.00-25.00)<br>(n=25) | 15.00 ± 0.83<br>(10.00-20.00)<br>(n=9) | 14.17 ± 1.16<br>(5.00-25.00)<br>(n=18) | 15.71 ± 2.02<br>(10.00-25.00)<br>(n=7) |
| Growth Rate of D.F.(mm/day)(Mean ± SE) | 0.48 ± 0.05<br>(0.19-1.56)<br>(n=25) | 0.39 ± 0.04<br>(0.26-0.62)<br>(n=9) | 0.48 ± 0.05<br>(0.19-1.56)<br>(n=25) | 0.39 ± 0.02<br>(0.25-0.51)<br>(n=9) | 0.50 ± 0.07<br>(0.19-1.56)<br>(n=18) | 0.44 ± 0.<br>(0.23-0.82)<br>(n=7) |
| Postpartum Ovulation Between days: 20-25 Days | 2 | 2 | 2 | 2 | 2 | - |
| 25-30 Days | 6 | 1 | 6 | 1 | 5 | 1 |
| 30-35 Days | 7 | 2 | 6 | 3 | 5 | 1 |
| 35-40 Days | 2 | - | 2 | - | 2 | - |
| 40-45 Days | 2 | - | 2 | - | 1 | 1 |
| >45 Days | 2 | - | 3 | - | 1 | 2 |
| Overall | 21 (84.00%) | 5 (50.00%) | 21 (80.77%) | 6 (66.66%) | 16 (84.21%) | 5 (71.43%) |
| Follicular Atresia | 4 (16.00%) | 4 (40.00%) | 5 (19.23%) | 3 (33.33%) | 3 (15.79%) | 2 (28.57%) |
| Follicular Cyst | - | 1 (10.00%) | - | - | - | - |
| Maximum Size of CL (mm) (Mean ± SE) | 12.92 ± 0.90<br>(7.10-19.30)<br>(n=19) | 12.24 ± 1.11<br>(9.40-15.10)<br>(n=5) | 12.98 ± 0.88<br>(7.20-19.30)<br>(n=18) | 12.16 ± 1.48<br>(7.10-17.30)<br>(n=6) | 13.14 ± 1.04<br>(7.20-19.30)<br>(n=15) | 12.20 ± 1.27<br>(10.60-14.70)<br>(n=3) |
| Maximum size of CL detected on: $25^{th}$ Day | - | 1 | 1 | - | 1 | - |
| $35^{th}$ Day | 2 | 1 | 2 | 1 | 1 | 1 |
| $40^{th}$ Day | 11 | 2 | 8 | 5 | 7 | 1 |
| $45^{th}$ Day | 6 | 1 | 7 | - | 6 | 1 |

**Note:** Figures in parenthesis indicates range; DF = Dominant follicle; n= number of animals; Subscripts row wise = p & q ($p<0.01$)

significant difference in the mean diameter of dominant follicle (12.22 ± 0.48 and 12.60 ± 0.85 mm, respectively). The average diameter of dominant follicle was 12.12 ± 0.58 and 12.46 ± 0.90 mm in conceived (n=18) and non-conceived (n=7) subgroups of Mehsana buffaloes. Correspondingly, Sheldon *et al.* (2002) reported a smaller diameter of dominant follicles in cows with a uterine infection. Though uterine infection does not appear to affect peripheral plasma FSH profiles (Sheldon *et al.*, 2008), yet in present findings, there was a significant difference between dominant follicular diameter of normally and abnormally parturited buffaloes. The observation of Nair (2008) and Yindee *et al.* (2011) in buffaloes for a diameter of dominant follicle was lower as compared to that observed during the present study in normal postpartum Mehsana buffaloes, while Khare (2004) reported a larger diameter of dominant follicle in postpartum Mehsana buffaloes. Presicce (2004) reported 11.0 mm and 13.8 mm diameter of dominant follicle in Italian water buffalo heifers and buffaloes, which were in accordance to diameter of dominant follicle found in the present study. Yotov and Atanasov (2013) noted that parity affects the size of medium and large follicles. They observed size of the medium and large follicle as 7.98 ± 0.94 and 13.1 ± 0.96 mm in multiparous, whereas 6.42 ± 0.40 and 10.12 ± 0.72 mm in primiparous Bulgarian Murrah buffaloes, respectively. However, the observed diameter of dominant follicle in postpartum regular cyclic and conceived Mehsana buffaloes was non-significantly different compared to non-cyclic and non-conceived subgroups of Mehsana buffaloes. These findings are suggestive of no relation of diameter of dominant follicle with postpartum cyclicity and conception.

**Time taken to attain dominance:** The time to attain the dominance by emerged follicle differed non-significantly between group I and II, cyclic and non-cyclic group and conceived and non-conceived subgroups of Mehsana buffaloes (Table 1 and Fig. 1). A significantly less period

(5.33±0.47 days) for a selected follicle to become dominant was reported by Khare (2004) compared to the present study in normally and abnormally parturited Mehsana buffaloes. The more prolonged calving to follicular dominance interval was reported for high postpartum bacterial score category cows (10.30±0.60 days) than standard bacterial score cows (8.80±0.30 days) due to increased bacterial contamination associated with fewer dominant follicles (Sheldon *et al.*, 2002). The selected follicle in cyclic and non-cyclic (14.60 ± 0.99 and 15.00 ± 0.83 days, respectively) as well as conceived and non-conceived (14.17±1.16 and 15.71±2.02 days, respectively) Mehsana buffaloes reached to dominant state with a non-significant difference. Although, the time taken by emerged follicle to get the dominant stage was more in non-cyclic and non-conceived buffaloes compared to cyclic and conceived Mehsana buffaloes. The possible clarification for this in the outlook of Satheshkumar *et al.* (2011), the dominant follicle (DF) of anovulatory waves reached the maximum size earlier and remained in the static phase for a significantly ($P < 0.05$) greater number of days (2.0-2.2 days) than in crossbred cows (0.67-1.67 days), which indicated an early loss of LH receptors in the dominant follicle (DF) of buffaloes.

**Follicular growth rate:** The follicular growth rate differed non-significantly in group I (0.48±0.05 mm/day) compared to group II (0.39±0.04 mm/day) Mehsana buffaloes. The progression rate of follicle to dominant size was slower in abnormal parturition than normal parturition of Mehsana buffaloes. The follicular growth rate gets slower in the presence of bacterial contamination and uterine diseases in cows (Sheldon *et al.*, 2002). The faster follicular growth rate during normal postpartum in Mehsana buffaloes (1.70±0.51 mm/day) was reported by Khare (2004). Identically, Awasthi *et al.* (2006) found faster follicular growth in suckled Mehsana buffalo with a two-wave cycle (1.17±0.33 mm/day). Opposite to these findings, the progression in follicular size monitored by Awasthi *et al.* (2006) with one-wave cycle (0.32±0.01 mm/day) was slower than results of present study. The emerged follicle grew comparatively at a non-significant slower rate in non-cyclic and non-conceived groups (0.39 ±0.02 and 0.44±0.07 mm/day) in the present study in view to cyclic and conceived groups (0.48±0.05 and 0.50±0.07 mm/day) of Mehsana buffaloes. These observations corroborated with the findings of Satheshkumar *et al.* (2011). They stated that a smaller diameter and slow growth rate of the ovulatory follicle might cause sub-estrus and smaller CL in Murrah graded buffaloes. A slower follicular growth rate (0.33±0.01 mm/day) in postpartum anestrous buffaloes was reported earlier (Nair, 2008). Although, faster follicular growth following estrus synchronization protocol was also observed in buffaloes with two-wave cycles (Presicce, 2004).

**Ovulation:** The most dominant follicles ovulated between $20^{th}$ to $45^{th}$ day postpartum in eutocic group I and $20^{th}$ to $35^{th}$ day postpartum in dystocic group II Mehsana buffaloes. At a glance, it reflects that dystocic buffaloes ovulated in narrow window of time contrary to eutocic buffaloes. Similarly, Khare (2004) in buffaloes reported the mean duration of first postpartum ovulation coinciding with the range revealed during the present study in normally parturited postpartum Mehsana buffaloes. However, Arya and Madan (2001) in Murrah buffaloes reported earlier time of postpartum ovulation. Contrarily, Perera *et al.* (1981) reported requirement of longer duration for postpartum ovulation in buffaloes. Usmani (1992) reported average interval from parturition to first ovulation as 44.94±2.77 days in contra-lateral ovary and 59.24±4.19 days for buffaloes with postpartum ovulation in the ipsilateral ovaries. Qureshi and Ahmad (2008) found mean post-partum ovulation interval in Nili-Ravi buffaloes as 59.37 ± 4.76 days, ranging from 24 to 150 days. Risco *et al.* (1994) reported that cows with retained fetal membrane (24.20±3.70 days) exhibited earlier ($p>0.05$) ovulation than normal cows (29.00±3.70 days), whereas, El-Malky *et al.* (2010) reported significantly late ovulation in buffaloes suffered with retention of placenta (93.60 days) than normally parturited buffaloes (49.90 days). Sheldon *et al.* (2002) reported that cows with uterine disease had mean interval from calving to ovulation as 15.70±0.70 days. Risco *et al.* (1994) reported cows with milk fever had first postpartum ovulation at an average of 30.80±3.10 days, while control cows had an average of 20.40±3.30 days ($p<0.05$). Usmani *et al.* (2001) found a similar mean interval to first postpartum ovulation in infected and normal Nili-Ravi buffaloes calved normally. The postpartum dominant follicles ovulate maximally and minimal follicular atresia was noted in normal parturition (19/25, 76.00% and 4/25, 16.00%, respectively) when compared with abnormal parturition (5/10, 50.00% and 4/10, 40.00%, respectively) in postpartum Mehsana buffaloes in this study. Peter *et al.* (1989) and Battaglia *et al.* (1997) found that oestradiol-induced preovulatory LH surge is blunted when bacterial endotoxin is infused into the uterus or administered intravenously. This may be the probable reason for fewer ovulations and higher follicular atresia noted in an abnormally parturited group of buffaloes that might have more bacterial uterine infection than normally parturited buffaloes during postpartum. In confirmation to this, cows with uterine disease were less likely to ovulate the first postpartum dominant follicle (8%

vs. 40%, $P < 0.05$, E. Scarr, unpublished data) than normal animals (Sheldon *et al.*, 2008). The per cent ovulation in postpartum cyclic and non-cyclic buffaloes had very marginal difference, whereas, per cent atretic follicles were very high in postpartum non-cyclic buffaloes (33.33% vs. 19.23%) up to 45th day postpartum. The postpartum anovulatory anoestrus may be due to failure of a dominant follicle (DF) to ovulate. The more ovulation and lesser follicular atresia were noted in conceived subgroups (16/19 and 3/19) than non-conceived subgroup (5/7 and 2/7) of postpartum Mehsana buffaloes during the present investigation up to day 45 postpartum.

**Corpus luteum (CL):** The non-significant differences in the size of CL were detected between respective subgroups. The corpus luteum was detected between 25th to 45th day in normal parturition and 25th to 35th day in abnormal parturition group in this study (Table 1 and Fig. 1). Usmani *et al.* (1985) observed formation of 1st corpus luteum on day 23.80±1.70 after calving in Nili-Ravi buffaloes as indicated by plasma progesterone concentration (>1.5 ng/ml), however, only 32% of these corpora lutea were palpable. The maximum buffaloes in present study had largest size of CL on fortieth day postpartum in both groups (Table 1). Further, the maximum size of CL was detected in normal parturition than abnormal parturition of Mehsana buffaloes during postpartum. Similarly, Usmani *et al.* (2001) found that the life span of corpus luteum formed after first ovulation was shorter (11 days) in Nili-Ravi buffaloes with subclinical uterine infection than that of a normal estrous cycle (15 to 17 days).

## CONCLUSIONS

The abnormal parturition reduces the emergence diameter of dominant follicle with greater atresia of dominant follicle that adversely affects postpartum fertility; non-cyclic and non-conceived buffaloes reflect greater emergence diameter of dominant follicle, with slower growth rate, larger diameter of dominant follicle and more atresia of dominant follicle. Such, studies are lacking on Indian buffaloes. The findings of present work are quite useful in planning further research in this vital area of puerperal period and predicting subsequent fertility.

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