

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.611.421>

Comparative Study of Follicular Turn Over in Cyclic and Non-Cyclic Murrah Buffaloes through 2D Trans-Rectal Ultrasonography

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ABSTRACT

The present research was based on study of follicular dynamics in cyclic (n=6) and non-cyclic (n=6) Murrah buffaloes by using 2D trans-rectal ultrasonography from day 1 to 21 of study period. During each examination, number of small (<4mm), medium (4-8mm) and large follicles (>8mm) were assessed along with diameter of medium and large follicles. Moreover, diameter of corpus luteum was also studied daily in cyclic animals. The number of small follicles was significantly (P<0.05) higher on 6th day (2.75±0.25) of cycle as compared to number of small follicles on days 4, 9, 14, 16 and 18-21 of study in cyclic animals. The number of small follicles in non-cyclic buffaloes did not differ significantly (P<0.05) on day 3 (2.00±0.00), 10 (2.00±0.00) and 20 (2.00±0.58) of observations but these were significantly higher than other days of study. Significantly higher number of medium size follicles was recorded on 6th day (4.50±1.50) as compared to day 18 and 20 of study in cyclic animals. But there was no variation in non-cyclic animals throughout the study. The number of large follicles on day 3 (1.80±0.20) versus days 15, 16 and 18-20 of study varies significantly (P<0.05) while on other days there was non-significant variation. The number of large follicles in cyclic animals varied non-significantly throughout the study. The diameter of medium sized follicles in cyclic animals on day 5 (6.77±0.42) was significantly higher than other days of cycle except days 19 and 20 of the cycle. However, the diameter of medium follicles on 1st day (7.18±.45mm) of the study was significantly higher than follicles of days 7, 11-13, 17-19 and 21 of study in non-cyclic animals. The diameter of largest follicles recorded between 11.45±2.35mm and 14.12±1.40mm in cyclic and non-cyclic animals, respectively. The large size follicle diameter on 21st day of study was significantly (P<0.05) higher than the diameter of 6th day while on other days there was non-significant variation in non-cyclic animals. In cyclic buffaloes, CL appeared on 5th day of cycle and attained maximum size on 14th day (16.43±.40mm) and then declined. From the present study, it was concluded that the selected non-cyclic animals were not in true anestrus condition as there was follicles development on ovaries that reaches to preovulatory size but not ovulated that may be due to irregular gonadotrophin synthesis or hormonal imbalance and managemental errors.

Keywords

2D ultrasonography,
Corpus luteum,
Follicle, Murrah
buffaloes.

Article Info

Accepted:

26 September 2017

Available Online:

10 November 2017

Introduction

In recent years, the capabilities of ultrasound imaging have increased enormously. The application of real time B-mode ultrasonography in bovine reproduction has grown rapidly in the last decade. As ultrasonography is non-invasive technique, therefore, repeated examinations of an animal's reproductive system can be performed without impairing its breeding potential and fetal development. Real time ultrasonographic examination has allowed the monitoring of individual follicle on a daily basis (Fricke, 2002). Ultrasonography has helped in the diagnosis and differentiation of various types of ovarian cyst (Reeves, *et al.*, 1984; Edmondson, *et al.* 1986; Farin, *et al.*, 1990) and tumours (Kahn and Ludlow, 1989). Ultrasonography also helped in predicting estrus in dairy animals after prostaglandin administration (Smith, *et al.*, 1998). B-mode ultrasonography has a central role in the characterization of ovarian follicular dynamics in the different domestic species and in the development of several protocols to control ovarian function for assisted reproductive technologies (ARTs) such as timed artificial insemination (Adams *et al.*, 2008).

The use of ultrasonography to observe ovarian activity has been reviewed in great detail (Pierson and Ginther, 1988 and Beal *et al.*, 1992). The structures like ovarian follicles, cysts and corpora lutea (CL) have been previously identified by real-time ultrasonography (Kastelic *et al.*, 1990; Beal *et al.*, 1992 and Singh *et al.*, 1997) in cattle. Fricke (2002) reported that follicles are fluid-filled structures which absorb ultrasound waves and are displayed as black on the screen (anechoic or non-echogenic). In contrast, the CL contains varying degrees of dense cells, which reflect the ultrasound waves and result in a gray image on the

screen. Linear-array transducers of 5.0 and 7.5 MHz frequency ranges are most commonly used in cattle, and most of the veterinary ultrasound machines are compatible with probes of different frequencies. Depth of tissue penetration of sound waves and image resolution is dependent upon and inversely related to the frequency of the transducer (Fricke, 2002). An ultrasound machine equipped with a 5.0 MHz transducer is most useful for bovine practitioners conducting routine reproductive examinations; however, small ovarian structures such as developing follicles are best imaged with a 7.5 MHz transducer. The current study has revealed that ultrasonography is a useful tool in Murrah buffaloes to study follicular dynamics. It was further observed that this technique was easily applicable without any risk to developing dam. Ultrasonography has proved to be a valuable tool in assessing the status of ovarian structures e. g, follicle and corpus luteum in cyclic and non-cyclic buffaloes. For this purpose, trans-rectal ultrasonography was carried out in the buffaloes at a definite interval to assess the nature of cyclicity in buffaloes.

Materials and Methods

Location

The present study was conducted on 12 Murrah buffaloes at Buffalo Farm, Department of Livestock Production Management (LPM), College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana).

Experimental plan

All the animals selected for study were grouped into two having six animals in each: (a) non-cyclic (n=6) and (b) Cyclic (n=6). The

acyclicity of animals was confirmed on the basis of farm record and per rectal examination at 10 days apart.

Ultrasound Machines and Transducers

The 2D B-mode of ultrasonography was applied for characteristics of follicle and corpus luteum throughout estrous cycle of cyclic and for 21 days in non-cyclic animals. Ultrasonography was conducted using Sono Scape S6, portable digital and Toshiba Nemio-XG 3D ultrasound machines. A linear array trans-rectal-transducer having frequency between 5.0 to 7.5 MHz was utilized for 2D ultrasonography for characteristics of follicles and corpus luteum.

Ultrasonographic examination of cyclic and non-cyclic buffalo

For this, animals were restrained properly without use of any tranquilizing agents; faecal material was removed from the rectum and ovaries were located. Ultrasound jelly was applied over the surface of transducer before its insertion into the rectum. The transducer was moved along the dorsal surface of the reproductive tract and then it was moved laterally to examine the ovaries. Ovaries were scanned by visualizing the structures in each ovary by sliding the transducer from medial to the lateral aspect of the ovary. During each examination, number and diameter of follicles and presence of CL were recorded to facilitate sequential evaluation of follicles turnover. All measurements were made using the built-in, on-screen calipers. Diameters of follicles >4mm were recorded. All ultrasonographic examinations were performed by the same operator.

Observations

The following parameters were recorded in cyclic and non-cyclic animal groups

Number of small, medium and large size ovarian follicles

Total number of ovarian follicles

Diameter of medium and large size ovarian follicles

Diameter of corpus luteum in cyclic buffaloes

Analysis and interpretation of data

The ultrasound images recorded in the machine were reviewed in the scanner itself to re-examine the images in detail. The data collected were statistically analyzed by "ANOVA" for finding out average, standard deviation, standard error using computerized SPSS 16.0 software program. The measurements were depicted in the form of tables.

Results and Discussion

In the present study, the number of small, medium and large follicles varied between 1.00 ± 0.00 to 2.75 ± 0.25 , 2.17 ± 0.40 to 4.50 ± 1.50 , 1.00 ± 0.00 to 1.83 ± 0.48 (Table 1) and 1.00 ± 0.00 to 2.00 ± 0.58 , 1.33 ± 0.33 to 2.40 ± 0.60 and 1.00 ± 0.00 to 1.80 ± 0.20 (Table 3) in cyclic and non-cyclic animals, respectively. There was non-significant difference ($P < 0.05$) in number of medium and large size follicles between cyclic and non-cyclic buffaloes. Similarly, the diameter of medium and large follicles varied between 4.97 ± 0.29 to 6.77 ± 0.42 mm, 9.20 ± 0.95 to 11.45 ± 2.35 mm (Table 2) and 4.79 ± 0.21 to 6.11 ± 0.40 mm, 10.21 ± 0.64 to 14.12 ± 1.40 mm (Table 4), respectively in cyclic and non-cyclic animals. There was non-significant difference ($P < 0.05$) in diameter of large size follicles between cyclic and non-cyclic buffaloes. The diameter of largest follicles ranged between 11.45 ± 2.35 mm (Table 2) and 14.12 ± 1.40 mm (Table 4) in cyclic and non-

cyclic animals, respectively. There was significant difference ($P<0.05$) in diameter of largest follicles between cyclic and non-cyclic buffaloes.

The mean values of small number of follicles in cyclic animals varied between 1.00 ± 0.00 to 2.75 ± 0.25 (Table 1). In our study, the number of small follicles was significantly ($P<0.05$) higher on 6th day (2.75 ± 0.25) of cycle as compared to number of small follicles on days 4, 9, 14, 16 and 18-21 of study. The number of small follicles was varied non-significantly ($P<0.05$) on other days of study (Table 1). The mean number of medium size follicles in cyclic animals varied between 2.17 ± 0.40 to 4.50 ± 1.50 . In our study, significantly higher number of medium size follicles was recorded on 6th day (4.50 ± 1.50) as compared to day 18 and 20 of estrous cycle but there was non-significant difference on all other days of study (Table 1). The mean number of large follicles in cyclic animals varied between 1.00 ± 0.00 to 1.83 ± 0.48 . The number of large follicles in cyclic animals varied non-significantly ($P<0.05$) throughout the days of scanning (Table 1).

The mean diameter of medium size follicles in cyclic Murrah buffaloes varied between 4.97 ± 0.29 to 6.77 ± 0.42 mm (Table 2). The diameter of the follicle on day 5 (6.77 ± 0.42) of cycle was significantly higher than other days of cycle except day 19 and 20 of cycle (Table 2). The mean diameter of large size follicles in cyclic Murrah buffaloes varied between 9.20 ± 0.95 to 11.45 ± 2.35 mm (Table 2). Large size follicles diameter varies non-significantly throughout days of study (Table 2).

The mean number of small follicles in non-cyclic animals varied between 1.00 ± 0.00 to 2.00 ± 0.58 (Table 3). The number of small follicles in non-cyclic animals did not differ significantly ($P<0.05$) on day 3 (2.00 ± 0.00),

10 (2.00 ± 0.00) and 20 (2.00 ± 0.58) of observations but these were significantly higher than other days of study (Table 3). The mean number of medium follicles in non-cyclic animals varied between 1.33 ± 0.33 to 2.50 ± 0.87 (Table 3). The number of medium follicles in non-cyclic animals varies non-significantly between days 1-21 of study (Table 3). The number (Mean \pm SE) of large follicles in non-cyclic Murrah buffaloes varied between 1.00 ± 0.00 to 1.80 ± 0.20 (Table 3). The number of large follicles on day 3 versus days 15, 16 and 18-20 of study were statistically significant ($P<0.05$) while on other days there was non-significant variation (Table 3).

The mean diameter of medium size follicles in non-cyclic Murrah buffaloes varied between 4.79 ± 0.21 to 7.18 ± 0.45 mm (Table 4). The diameter of follicles on 1st day (7.18 ± 0.45 mm) of the study was significantly higher than the diameter of medium size follicles on days 7, 11-13, 17-19 and 21 of ultrasonographic examinations while on other days there was non-significant variation (Table 4). The mean diameter of large size follicles in non-cyclic animals varied between 10.21 ± 0.64 to 14.12 ± 1.40 mm. The large size follicle diameter on 21st day of study was significantly ($P<0.05$) higher than the diameter of 6th day while on other days there was non-significant variation (Table 4).

In non-cyclic and cyclic animals, the total number of follicles varied between 2.83 ± 0.31 to 4.83 ± 0.48 and 4.00 ± 0.45 to 5.67 ± 0.80 respectively on different days of study (Table 5).

Total number of follicles was statistically non-significant ($P<0.05$) in cyclic and non-cyclic animals on different days of study (Table 5). However, total number of follicles was recorded more in cyclic Murrah buffaloes as compare to non-cyclic Murrah buffaloes.

Diameter of corpus luteum in cyclic buffaloes

The mean \pm SE diameter of CL varied between 9.36 ± 0.54 to 16.43 ± 0.40 mm in cyclic Murrah buffaloes (Table 6). The diameter of CL was significantly higher on day 14 as compare to day 5 and 21 of estrous cycle.

In general, more or less there was progressive increment in diameter of CL from day 6 to 14 (Table 6). The mean value of CL diameter was significantly ($P<0.05$) lower on day 5 (9.36 ± 0.54 mm) than all other days of study (Table 6).

Ovarian dynamics in cyclic buffaloes

Trans-rectal ultrasonography with linear array transducer having frequency of 5- 7.5 MHz was found useful for imaging follicle of different sizes. The follicles were counted, measured and categorized into small (<4 mm), medium (4-8mm) and large (>8mm) as per previous studies (Dutt *et al.*, 2014). It was not possible to measure the follicles having diameter less than 4 mm, therefore their numbers were counted only. The earlier studies using ultrasound revealed that follicular wave begins with emergence of a group of small antral follicles just before the day of ovulation (Ginther *et al.*, 1996).

A second wave of growth emerges approximately day 10 after ovulation and for three-wave cycles, an additional wave emerges at day 16 after ovulation. For both the two and three-wave cycles, the ovulatory follicle arises from the final wave in cattle (Ginther *et al.*, 1996). Aslan *et al.*, (2000) observed the difference in detection of follicles by ultrasound and rectal palpation, opined that ultrasound was more effective at identifying follicles greater than 10 mm in diameter than rectal palpation. Follicles with

10 to 15 mm in diameter were detected in 90% of cases using ultrasonography versus 62% of the cases using rectal palpation. Follicles greater than 15 mm were detected with 100% accuracy by both ultrasonography and rectal palpation. Manual diagnosis of follicles <10 mm is generally inaccurate and ultrasound offers the possibility to diagnose the follicles <5 mm (Hanzen *et al.*, 2000). In present study, follicles around 4mm were detected trans-rectally through real time 2D ultrasonography.

The mean number of small follicles in cyclic animals varied between 1.00 ± 0.00 to 2.75 ± 0.25 . In current investigation, the maximum number of small follicles was recorded on 6th and 17th day of cycle indicating initiation of new follicular wave. Differences between follicles number during the cycle were due to the emergence of small follicles (beginning of wave) and their growth and regression. It is similar to study done in Egyptian buffaloes (Baruselli *et al.*, 1997). Manik *et al.*, (1999) reported no significant difference in number of small follicles in control animals on various days of estrous cycle in cyclic buffaloes. Akshey *et al.*, (2005) observed that there was no difference in cyclic Murrah buffaloes in the number of small follicles between day 3 and day 5. Yilmaz *et al.*, (2014) reported that the number of follicles <5 mm in diameter showed no significant difference throughout the estrous cycle in water buffaloes.

In present study, significantly higher number of medium size follicles was recorded on 6th day (4.50 ± 1.50) as compared to day 18 and 20 of estrous cycle but there was non-significant difference on all other days of study. Barkawi *et al.*, (2009) observed maximum number of follicles that had diameters ≥ 4 mm was detected between days 3 and 7 of the estrous cycle in both normal and uterine pathology associated in Egyptian buffaloes.

Table.1 Number (Mean ± SE) of small (<4mm), medium (4-8mm) and large ovarian follicles (>8mm) in cyclic Murrah buffaloes on various days of estrous cycle

Days of estrous cycle	Number of small follicles	Number of medium follicles	Number of large follicles
1	1.33±0.33 ^{abcde}	3.50±0.64 ^{ab}	1.00±0.00
2	2.00±0.70 ^{bcde}	3.80±0.37 ^{ab}	1.33±0.33
3	2.00±0.44 ^{de}	4.20±0.80 ^{ab}	1.50±0.50
4	1.00±0.00 ^{ab}	4.20±0.58 ^{ab}	1.40±0.24
5	1.75±0.48 ^{abcde}	3.00±0.91 ^{ab}	1.40±0.24
6	2.75±0.25 ^e	4.50±1.50 ^b	1.50±0.22
7	2.00±0.58 ^{bcde}	3.40±0.68 ^{ab}	1.67±0.33
8	1.50±0.22 ^{cde}	3.20±0.49 ^{ab}	1.00±0.00
9	1.00±0.00 ^{ab}	2.50±1.50 ^{ab}	1.00±0.00
10	1.67±0.33 ^{abcde}	3.00±0.84 ^{ab}	1.60±0.24
11	1.50±0.29 ^{abcde}	3.00±0.58 ^{ab}	1.33±0.21
12	1.25±0.25 ^{abcde}	2.50±0.56 ^{ab}	1.33±0.21
13	1.25±0.25 ^{abcde}	2.83±0.48 ^{ab}	1.50±0.22
14	1.50±0.50 ^{abcd}	3.00±0.55 ^{ab}	1.17±0.31
15	1.40±0.24 ^{abcde}	3.20±0.20 ^{ab}	1.40±0.40
16	1.50±0.50 ^{abcd}	3.75±0.48 ^{ab}	1.50±0.29
17	2.00±0.00 ^{abcde}	2.60±0.51 ^{ab}	1.20±0.20
18	1.00±0.00 ^{ab}	2.33±0.49 ^a	1.50±0.22
19	1.00±0.00 ^{ab}	2.67±0.42 ^{ab}	1.83±0.48
20	1.00±0.00 ^{ab}	2.17±0.40 ^a	1.67±0.21
21	1.00±0.00 ^{ab}	2.50±0.22 ^{ab}	1.40±0.24

Note: Mean values with different superscripts (a, b, c, d, e) differ significantly (P<0.05)
Mean values without superscripts did not differ significantly (P<0.05)

Table.2 Diameter (Mean ± SE) of medium (4-8mm) and large (>8mm) size follicles in cyclic Murrah buffaloes on various days of estrous cycle

Days of estrous cycle	Diameter of medium follicles(mm)	Diameter of large follicles (mm)
1	5.46±0.42 ^{ab}	9.20±0.95
2	4.97±0.29 ^a	9.72±0.87
3	5.39±0.12 ^{ab}	9.85±0.15
4	5.56±0.35 ^{abc}	9.40±0.48
5	6.77±0.42 ^d	9.52±0.52
6	5.75±0.15 ^{abc}	9.60±0.27
7	5.79±0.27 ^{abc}	9.63±0.37
8	5.08±0.30 ^a	9.60±0.82
9	5.68±0.20 ^{abc}	11.45±2.35
10	5.21±0.23 ^{ab}	10.85±0.83
11	5.18±0.39 ^{ab}	10.68±0.85
12	5.45±0.22 ^{ab}	9.91±0.53
13	5.10±0.25 ^a	10.43±0.88
14	5.37±0.17 ^{ab}	10.14±0.61
15	5.25±0.23 ^{ab}	10.47±0.23
16	5.47±0.38 ^{ab}	10.34±0.27
17	5.74±0.23 ^{abc}	10.65±0.45
18	5.30±0.32 ^{ab}	9.33±0.37
19	6.50±0.28 ^{cd}	9.45±0.48
20	6.21±0.22 ^{bcd}	10.12±0.53
21	5.82±0.27 ^{abc}	10.14±0.59

Note: Mean values with different superscripts (a, b, c, d) differ significantly (P<0.05)
Mean values without superscripts did not differ significantly (P>0.05)

Table.3 Number (Mean ± SE) of small (<4mm), medium (4-8mm) and large (>8mm) follicles in non-cyclic Murrah buffaloes on various days of ultrasonographic examinations

Days of ultrasonographic examinations	Number of small follicles	Number of medium size follicle	Number of large follicles
1	1.00±0.00 ^a	1.80±0.49 ^a	1.60±0.24 ^{ab}
2	1.00±0.00 ^a	2.50±0.29 ^a	1.40±0.24 ^{ab}
3	2.00±0.00 ^b	1.50±0.50 ^a	1.80±0.20 ^b
4	1.00±0.00 ^a	1.60±0.24 ^a	1.20±0.20 ^{ab}
5	1.00±0.00 ^a	1.83±0.65 ^a	1.17±0.17 ^{ab}
6	1.50±0.50 ^a	2.00±0.52 ^a	1.50±0.34 ^{ab}
7	1.00±0.00 ^a	2.17±0.40 ^a	1.33±0.21 ^{ab}
8	1.00±0.00 ^a	1.33±0.21 ^a	1.50±0.22 ^{ab}
9	1.00±0.00 ^a	1.50±0.22 ^a	1.60±0.24 ^{ab}
10	2.00±0.00 ^b	1.75±0.25 ^a	1.50±0.22 ^{ab}
11	1.00±0.00 ^a	2.50±0.87 ^a	1.25±0.25 ^{ab}
12	1.00±0.00 ^a	1.60±0.24 ^a	1.33±0.21 ^{ab}
13	1.50±0.28 ^a	2.00±0.63 ^a	1.50±0.29 ^{ab}
14	1.00±0.00 ^a	2.40±0.60 ^a	1.33±0.33 ^{ab}
15	1.33±0.33 ^a	2.33±0.56 ^a	1.00±0.00 ^a
16	1.50±0.50 ^a	1.83±0.40 ^a	1.00±0.00 ^a
17	1.00±0.00 ^a	1.60±0.40 ^a	1.17±0.17 ^{ab}
18	1.00±0.00 ^a	1.33±0.33 ^a	1.00±0.00 ^a
19	1.00±0.00 ^a	1.40±0.24 ^a	1.00±0.00 ^a
20	2.00±0.58 ^b	1.60±0.40 ^a	1.00±0.00 ^a
21	1.50±0.50 ^a	1.50±0.29 ^a	1.17±0.167 ^{ab}

Note: Mean values with superscripts (a, b) differ significantly (P<0.05)

Table.4 Diameter (Mean ± SE) of medium (4-8mm) and large (>8mm) size follicles in non-cyclic Murrah buffaloes on various days of ultrasonographic examinations

Days of ultrasonographic examinations	Diameter of medium Size (mm)	Diameter of large size follicle large (mm)
1	7.18±0.45 ^b	11.08±1.19 ^{ab}
2	6.01±0.32 ^{ab}	11.40±1.11 ^{ab}
3	5.42±0.63 ^a	12.04±0.64 ^{ab}
4	5.91±0.54 ^{ab}	10.92±1.02 ^{ab}
5	6.12±0.39 ^{ab}	10.97±1.37 ^{ab}
6	5.83±0.52 ^{ab}	10.21±0.64 ^a
7	5.41±0.18 ^a	11.05±0.67 ^{ab}
8	6.02±0.29 ^{ab}	11.02±0.72 ^{ab}
9	5.69±0.39 ^{ab}	11.58±0.96 ^{ab}
10	5.94±0.37 ^{ab}	11.89±0.72 ^{ab}
11	5.12±0.24 ^a	12.60±0.73 ^{ab}
12	5.38±0.25 ^a	12.07±0.95 ^{ab}
13	4.79±0.21 ^a	12.31±0.70 ^{ab}
14	5.79±0.61 ^{ab}	11.30±0.96 ^{ab}
15	6.08±0.45 ^{ab}	13.58±0.54 ^{ab}
16	5.90±0.55 ^{ab}	12.37±01.31 ^{ab}
17	5.33±0.63 ^a	12.64±1.01 ^{ab}
18	5.29±0.51 ^a	12.35±1.42 ^{ab}
19	4.98±0.31 ^a	13.12±0.95 ^{ab}
20	5.84±0.59 ^{ab}	12.90±0.44 ^{ab}
21	5.31±0.71 ^a	14.12±1.40 ^b

Note: Mean values with different superscripts (a, b) differ significantly (P<0.05)

Table.5 Number (Mean \pm SE) of total follicles in non-cyclic and cyclic Murrah buffaloes on various days of ultrasonographic examinations

Day of cycle	Non-cyclic	cyclic
1	3.83 \pm 0.48	4.83 \pm 0.17
2	3.67 \pm 0.42	5.17 \pm 0.80
3	3.17 \pm 0.478	5.67 \pm 0.80
4	2.83 \pm 0.31	4.50 \pm 0.62
5	3.33 \pm 0.61	4.33 \pm 0.56
6	4.83 \pm 0.48	4.5 \pm 0.62
7	3.83 \pm 0.54	4.83 \pm 0.31
8	3.5 \pm 0.43	5.00 \pm 0.45
9	4.17 \pm 0.31	4.67 \pm 0.62
10	2.83 \pm 0.17	4.67 \pm 0.42
11	2.83 \pm 0.54	5.33 \pm 0.33
12	3.33 \pm 0.42	4.67 \pm 0.33
13	4.33 \pm 0.71	5.17 \pm 0.40
14	3.33 \pm 0.49	4.67 \pm 0.56
15	3.67 \pm 0.56	4.83 \pm 0.40
16	3.5 \pm 0.56	4.00 \pm 0.68
17	3.67 \pm 0.33	4.33 \pm 0.49
18	3 \pm 0.45	4.17 \pm 0.54
19	3.5 \pm 0.34	4.33 \pm 0.49
20	3.33 \pm 0.33	4.00 \pm 0.45
21	2.83 \pm 0.31	4.33 \pm 0.33

Table.6 Diameter (Mean \pm SE) of corpus luteum in cyclic Murrah buffaloes on various days of estrous cycle

Days of estrous cycle	Diameter (mm)
5	9.36 \pm 0.54 ^a
6	12.84 \pm 0.44 ^{bc}
7	13.79 \pm 0.24 ^{bcd}
8	13.54 \pm 0.75 ^{bcd}
9	14.44 \pm 1.09 ^{cde}
10	13.61 \pm .87 ^{bcd}
11	15.28 \pm 0.62 ^{de}
12	14.96 \pm 0.72 ^{de}
13	14.36 \pm 0.66 ^{cde}
14	16.43 \pm 0.40 ^e
15	14.16 \pm 0.72 ^{de}
16	13.52 \pm 0.92 ^{bcd}
17	12.72 \pm 0.77 ^{bc}
18	12.5 \pm 0.47 ^{bc}
19	12.44 \pm 0.42 ^{bc}
20	12.12 \pm 0.41 ^{bc}
21	11.2 \pm 0.33 ^b

Note: Mean values with different superscripts (a, b, c, d, e) differ significantly (P<0.05)

Further they reported no significant difference in number of medium size follicles recorded on various days of estrous cycle compared to day 1 (days first post ovulation). Akshey *et al.*, (2005) observed that there was no difference in cyclic Murrah buffaloes in the number of medium follicles between day 3 and day 5.

The mean number of large follicles in cyclic animals varied non-significantly. No significant difference in number and diameter of the large follicles was observed on various days of estrous cycle. Akshey *et al.*, (2005) reported that the number of large follicles was significantly higher at day 5 which is contrary to our findings in cyclic Murrah buffaloes. It may be due to breed difference. Barkawi *et al.*, (2009) observed no significant difference in number of large follicles recorded on various days of estrous cycle compared to day 1 (day first post ovulation) in Egyptian buffaloes.

The mean diameter of medium size follicles in cyclic animals was variable during estrous cycle. The significant increase in diameter of medium follicles was recorded on day 5 as compared to other days except day 19 and 20 of the estrous cycle. Yilmaz *et al.*, (2014) reported that follicles between 5 and 8 mm in diameter were fewer during late diestrus and follicular phase in water buffaloes.

The mean diameter of large size follicles in cyclic Murrah buffaloes was variable with maximum size of 11.45 ± 2.35 mm but there was non-significant difference throughout the study. During estrous cycles in cattle dominant follicles reach a maximum diameter of approximately 10–20 mm (Fortune *et al.*, 1988; Savio *et al.*, 1988 and Ginther *et al.*, 1989). Barkawi *et al.*, (2009) observed maximum diameter of dominant follicle that was insignificantly greater than the other dominant follicles that observed throughout the estrous cycle in Egyptian buffaloes.

Yilmaz *et al.*, (2014) reported that follicles between 8-12 mm in diameter were less during metestrus and follicular phase. They further reported that follicles at least 12 mm in diameter were more numerous in follicular phase in water buffaloes. Ginther *et al.*, (1989) reported that the sub-dominant follicles reach maximum diameters of approximately 8 mm in cyclic cattle. Contrary to our finding, Presicce *et al.*, (2005) measured the largest size follicle in first postpartum ovulation as 13.5 ± 0.8 and 14.1 ± 0.4 mm in primiparous and pluriparous buffaloes, respectively which is higher than present study finding.

The CL is a transient endocrine gland formed after ovulation of follicle. In present investigation, the diameter of corpora lutea ranged between 9.36 ± 0.54 to 16.43 ± 0.40 mm in cyclic animals. A non-significant increase in diameter of CL was observed from day 6 to 13. The mean value of CL diameter was significantly lowest on day 5 indicating immaturity of CL and highest value was recorded on day 14 indicating maturity of CL. Barkawi *et al.*, (2009) observed maximum diameter of CL (15 ± 0.04 mm) on 7th day of cycle which did not differ significantly in both normal and in Egyptian buffaloes with uterine pathology. Vecchioa *et al.*, (2012) measured the diameter of the CL on day 5 and 10 after AI in buffaloes as 17 ± 0.0 vs. 17.0 ± 0.0 mm and 17 ± 0.0 vs. 21.0 ± 0.0 mm, respectively in non-pregnant vs. pregnant. They reported diameter of CL on day 15 was 17 ± 0.0 mm vs. 19.0 ± 0.0 mm and which subsequently attained the size as 15 ± 0.0 mm vs. 21.0 ± 0.0 mm (non-pregnant vs pregnant) on day 20. Kayacik *et al.*, (2005) measured the size of the CL from the beginning of the second day post ovulation and recorded its maximum size on days 6, 9, and 10 in short, normal and long cycles, respectively in cows. Honparkhe *et al.*, (2004) measured the mean ultrasonic size of CL at day 9 between

10.7±0.28 to 12.0±0.13mm, which further reached the size ranging between 8.8±0.11 to 9.5±0.23mm on day 16 and these values are lying in the range of present investigation. Rakesh *et al.*, (2013) measured the dimension of CL that increased gradually with the advancement of stage of estrous cycle and peak values were observed between 12 and 16 days of estrous cycle. Alejandro *et al.*, (2014) measured the maximum luteal diameter as 19.58±4.16 mm and 17.74±3.32 mm respectively in Murrah buffaloes and buffalo heifers on day 15 and 17 of the estrous cycle, respectively which is quite higher than present findings.

Ovarian dynamics in non-cyclic buffaloes

In present investigation, the mean number of small follicles in non-cyclic Murrah buffaloes was variable. The highest number of follicles was observed on day 3, 10 and 20 of study as compared to other days. Lucy *et al.*, (1991) and El-Wishy (2007) reported that the early postpartum period is characterized by reduction in the number of small sized follicles. In contrast to our study, Dutt *et al.*, (2014) reported the mean number of small follicle in a range between 3.17±0.17 to 4.17±0.48 without any significance in non-cyclic Murrah buffaloes.

In present study, the mean number of medium sized follicles in non-cyclic Murrah buffaloes varied non-significantly with maximum number on day 11 of the study period. Dutt *et al.*, (2014) reported the mean number of medium follicles in non-cyclic buffaloes were in a range of 3.17±0.65 to 4.33±0.76 under field conditions. This difference may either be due to difference in season of study or managerial practices.

In present study, the mean number of large follicles in non-cyclic Murrah buffaloes varied. The maximum number of follicles was

recorded on day 3 (1.80±0.20) of the cycle compared to day 15, 16 and 18-20 (1.00±0.00 on all these days) of study. Dutt *et al.*, (2014) reported the mean number of large follicles within a range between 2.33±0.21 to 3.00±0.26 in non-cyclic buffaloes.

In current study, the mean diameter of medium and large sized follicles in non-cyclic Murrah buffaloes varied non-significantly. The maximum diameter of medium follicles (7.18±.45) was recorded on 1st day and maximum diameter (14.12±1.40) of large follicles was recorded on 21st day of the study. Kumar *et al.*, (2012), Kandiel *et al.*, (2013) and Dutt *et al.*, (2014) reported that the mean values of follicular diameter of medium sized follicles varied from 4.21±0.02 to 4.48±0.12 mm in non-cyclic buffaloes. Dutt *et al.*, (2014) found that the follicular diameter of large size follicles varies between 8.46±0.16 to 9.07±0.30mm in non-cyclic buffaloes under field conditions. They further concluded that the mean number of small, medium, large and total follicles did not differ significantly between and within the control and in group under estrus induction treatment prior to estrus induction treatment indicating emergence of constant number of follicles at any day during anoestrus condition. This is contrary to present study which may be due to differences in managerial condition at farm.

The mean number of total follicles in cyclic Murrah buffaloes varied between 4.00±0.45 to 5.67±0.80. No statistical difference was observed during the cyclic period. Similarly, Akshey *et al.*, (2005) observed that there was no significant difference in the number of total follicles between day 3 and 5 in cyclic Murrah buffaloes. In present investigation, the mean values of total number of follicles varied from 2.83±0.31 to 4.83±0.48 in non-cyclic Murrah buffaloes during different days of examinations. The number of follicles was

comparatively higher in cyclic as compare to non-cyclic Murrah buffaloes. Kumar *et al.*, (2012) reported that average number of follicles on both ovaries ranged between 7.50 ± 0.50 to 9.33 ± 1.69 within groups and the maximum size of dominant follicle in their study was 13.03 ± 0.89 mm in non-cyclic buffaloes. Kachiwal *et al.*, (2012) opined that average size of follicle on the day of heat (15.25 ± 1.28 mm) is greater as compared post breeding (12.00 ± 0.82 mm) in Kundhi buffaloes. These variations may be due to breed difference.

Although the total number of follicles in cyclic animals was higher in comparison to non-cyclic animals but as per our findings, the non-cyclic animal was not in true anestrus condition, as there was follicles growth on ovaries. There was follicular development in non-cyclic animals but no CL formation i.e. ovulation failure occurs in these animals. It might be possible that these acyclic animals may come in estrus but was not diagnosed due to decreased heat intensity, short duration of estrus or unobserved heat signs. Anovulation may be due to irregularities in gonadotrophin synthesis or hormonal imbalance in these non-cyclic animals. Thus, it was concluded that, in these types of animals, induction of cyclicality is indicated by using different hormonal regimens like Ovsynch or Progesterone based protocols.

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How to cite this article:

Gyan Singh, R.K. Chandolia, Ravi Dutt, Anil Saini, Jasmer Dalal and Malik, R.K. 2017. Comparative Study of Follicular Turn Over in Cyclic and Non-Cyclic Murrah Buffaloes through 2D Trans-Rectal Ultrasonography. *Int.J.Curr.Microbiol.App.Sci.* 6(11): 3590-3602. doi: <https://doi.org/10.20546/ijcmas.2017.611.421>