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Ultrasonographic ovarian biometry, follicular count and grading in Pandharpuri buffalo (*Bubalus Bubalis*)

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Abstract
The study was carried out from June 2021 to October 2021. Ultrasonographic ovarian biometry, follicular count, and grading in twenty-eight Pandharpuri Buffaloes were done. The results showed that the average length of the right ovary (26.951 ± 0.828 mm) was significantly (P<0.05) higher as compared to the left ovary (23.955 ± 0.799 mm). Nonsignificant differences were observed in the average width of the right (20.338 ± 0.743 mm) and left (18.801 ± 0.498 mm) ovaries of Pandharpuri buffaloes. The right ovaries were more active than the left ovaries. A total of 300 follicles were observed on 48 ovaries of Pandharpuri buffaloes through ultrasonography. The mean visible follicular count was 6.25 per buffalo. The follicles were graded as small, medium, and large based on their diameter. The number of small-sized follicles was found to be significantly (p<0.01) higher (5.00 ± 0.22) as compared to large (0.45 ± 0.08) and medium (1.04 ± 0.13) sized follicles.

Keywords: Ovarian biometry, follicular count, follicular grading, Pandharpuri buffalo

Introduction
According to the 20th livestock census in 2019, India ranks first for the buffalo population (109.85 million) and contributes immensely to the total milk production in the country. Buffaloes are gaining popularity due to their high milk fat percentage compared to other farm animals. Among various breeds, Pandharpuri buffalo is a unique breed known for high milk production and fat percentage. Western Maharashtra is the predominant home tract for these buffaloes. These buffaloes are well known for excellent body weight gain, and disease resistance power. The interest in Pandharpuri buffalo breeding has increased in the last two decades. However, farmers face problems in buffalo productivity due to their low reproductive efficiency. Therefore, it is necessary to study the ovarian dimensions and functional structures so that the knowledge generated can be used in research on this unique breed. Recent literature showed that a significantly less follicular population is present in the buffalo ovaries. Furthermore, the scientific literature related to ovarian structures and follicular dynamics is very scanty though some efforts have been made by a few researchers (Patel *et al.*, 2009, Allah *et al.*, 2013, Di Francesco *et al.*, 2012) [13, 1, 5]. The information related to the Pandharpuri breed of buffalo is also minimal, and therefore, the present study was done to observe the ovarian dimensions, follicular count, and grading.

Materials and methods
The research was conducted to observe the ultrasonographic ovarian dimensions, follicular count, and grading in twenty-eight Pandharpuri Buffaloes (*Bubalus Bubalis*) weighing between 450 to 600 kg with a good body condition score of more than 3.5. By well lubricated gloved hand, rectal palpation of internal genitalia of the individual buffalo was performed to rule out any anatomical abnormalities of genitalia and ovarian defects. Careful examination of the reproductive organs was made to evaluate the internal organ. The animals suffering from chronic or metabolic diseases were excluded from this study. The experimental buffaloes were provided with balanced nutrition before the study. Deworming has been carried out with bolus Ivermectin. These buffaloes were vaccinated against Foot and Mouth Disease (FMD), Hemorrhagic Septicemia (HS), and Black Quarter (BQ) diseases. Buffaloes were maintained under a loose housing system. All the buffaloes were hand milked twice daily. They were fed with green fodder (Lucerne, Dashrath, Jowar, etc.), dry fodder, concentrate, salt, mineral mixture. Drinking water was available for these buffaloes *ad libitum.* All the buffaloes were

subjected to ovarian examinations using an ultrasound machine having a linear probe with a 5 to 7.5 MHz frequency. These buffaloes were restrained in a suitable holding chute for restricting the movement. Ovarian length and width were measured by the ultrasound machine and a comparison was made. Follicles on each ovary were counted as those observables on the ovarian surface with the help of ultrasound machine. Ovarian follicles were identified as black (anechoic) circular shapes on the ultrasound screen.

Statistical analysis

The data collected were analyzed as per standard methods described by (Snedecor and Cochran, 1981). Values were presented as mean ± SE.

Results and Discussion

Ovarian length and width in Pandharpuri buffaloes

In the present study, a total of fifty-six left and right ovaries (twenty-eight each) were examined through ultrasonography to know the average length and width in Pandharpuri buffaloes. Careful ultrasonographic examinations revealed that the average length of the right ovary (26.951 ± 0.828 mm) was significantly (P<0.05) higher as compared to the left ovary (23.955 ± 0.799 mm) in Pandharpuri buffaloes. Nonsignificant differences were observed in the average width of the right (20.338 ± 0.743 mm) and left (18.801 ± 0.498 mm) ovaries of Pandharpuri buffaloes. This study showed that right ovaries were more active than left ovaries. This finding is in accordance with Khandoker *et al.* (2011) [6], who reported a significantly (P<0.05) higher length (23.2 mm ± 0.06 mm) of the right ovary as compared to the left (21.4 ± 0.05mm) ovary in buffaloes. Further, they observed no significant differences in the width of the left (16.3 ± 0.06 mm) and right ovaries (16.0 ± 0.05 mm) of buffaloes, similar to the present study's findings. However, the average length and width were less than the present study, which may be due to the differences in the operator's observations. Furthermore, functional structures are extracellular materials within the ovary responsible for the differences in length and width of ovaries in buffaloes. Similar to the present study, no significant difference in the width of the left (21.00 ± 0.1mm) and right ovary (20.00 ± 0.1mm) was also observed by Carvalho *et al.* (2005) [4] in Murrah buffalo. Razzaque *et al.* (2008) [14] reported lesser average length (22.1 ± 0.13 mm) and width (13.9 ± 0.03 mm) of cycling ovaries compared to non-cycling Nagpuri buffalo ovaries (24.5 ± 0.07mm Length and 15.2 ± 0.04 mm width). This may be due to functional structures present on the ovary. Leal *et al.* (2007) [9] reported 22.7 cm ovarian mean length in buffalo species, which is less than the present study. Patel *et al.* (2009) [13] observed that the mean length and width of the left ovaries were 31.00 ± 5.00, 13.50 ± 0.50 mm respectively. In buffaloes, the corresponding values for right ovaries were 27.50 ±1.50, 25.00 ± 2.00, respectively. Numerical differences in the width of the left and right ovary in the present study and other previous studies may be due to breed differences. The present study's findings and earlier findings of the workers indicated that the buffaloes' right ovaries possess a longer length than the left one; however, no significant difference was observed in the width of ovaries. The results of average length (mm) and width (mm) of the right and left ovaries of Pandharpuri buffaloes are summarized in Table 1

Table 1: Mean (± S.E.) for ovarian length and width

Ovary	Length (mm)	Width (mm)
Right	26.951^{a} ± 0.828	20.338 ± 0.743
Left	23.955^{b} ± 0.799	18.801 ± 0.498
SD	4.520	3.390
T - Statistic	2.601	1.713
T - Table (p<0.05)	2.005*	2.005
T - Table (p<0.01)	2.670	2.670

* Significant at p<0.05

Means bearing different superscripts within the column differed significantly (p<0.05)

Ovarian follicular count and grading in Pandharpuri buffaloes

In the present study, a total of 300 follicles were observed on 48 ovaries of Pandharpuri buffaloes through ultrasonography. The mean visible follicular count was 6.25 per buffalo. Almost similar results were recorded by Amer *et al.* (2008) [2], who recorded 6.8 follicles in buffalo ovaries. Kumar *et al.* (1997) [8], Manjunatha *et al.* (2008) [12], Aziz *et al.* (2012) [3] and Makwana *et al.* (2012) [10] reported a lower average number (5.20 ± 0.97, 4.8 ± 0.2, 4.03 and 3.36 ± 0.08, respectively) of follicles in buffalo ovaries. However, compared to the present study, Liang *et al.* (2007) reported a higher average number (7.69 ± 0.33) of follicles in buffalo ovaries. This may be due to breed differences.

The follicles were graded as small, medium, and large based on their diameter. In four sessions, the grading was done for follicles of both ovaries from six buffaloes. The statistical analysis of the data indicated a significant difference among different grades of follicles. The number of small-sized follicles was found to be significantly (p<0.01) higher (5.00 ± 0.22) as compared to large (0.45 ± 0.08) and medium (1.04 ± 0.13) sized follicles. The results of the present findings are in agreement with the findings of Razzaque *et al.* (2008) [14], who found that the average number of small follicles (3.30 ± 0.30) was higher than medium (1.77 ± 0.25) and large follicles (91.22 ± 0.22) in cycling buffaloes. They also noted similar observations for was small (3.30±0.30), medium (1.77 ± 0.25) and large (1.22 ± 0.22) follicles in non-cycling buffaloes. Manik *et al.* (2003) [11] reported no significant difference in the number of small, medium and large follicles in cattle. This may be due to species variation. Konrad *et al.* (2017) [7] reported that the average number of medium-sized follicles was higher than small and large follicles in buffaloes. This may be due to the difference in follicular dynamics of these buffaloes. The results of follicular count and gradings are given in Table 2.

Table 2: Follicular Count and Grading

Buffalo Number	Follicles Available		
	Small (<3mm)	Medium (3-5 mm)	Large (>5mm)
1	4.25	1.53	0.58
2	5.75	1.04	0.38
3	5.00	1.15	0.57
4	5.25	0.57	0.33
5	5.25	0.90	0.16
6	4.50	1.06	0.68
Mean ± S.E.	5.00^{c} ± 0.22	1.04^{b} ± 0.13	0.45^{a} ± 0.08
S. D.	0.55	0.31	0.19
P value	0.000		

Based on the findings recorded in this study, the following conclusions were drawn.

1. The average length and width of the right ovary were significantly more than the left ovary in Pandharpuri buffaloes.
2. The number of small-sized follicles was significantly higher than large and medium-sized follicles. The mean visible follicular count was 6.25 per Pandharpuri buffalo.

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