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Determine the effect of ovarian and non ovarian factors on follicular population in murrah buffalo using Ultrasound graphy technique

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Abstract
The aim of this study was to investigate the effect of age, body condition score (BCS), CL, pregnancy status and ovary localization on follicular population in Murrah buffalos using Ultrasound graphy technique. 160 female Murrah buffaloes (80 pregnant and 80 empty) with average age of 2- 11 years were used. All animals were maintained in a semi intensive system. The results obtained revealed that, ovaries of Murrah buffaloes with BCS 4 have higher number of follicles than those with BCS 3.The ovaries from pregnant animals have more number of medium size follicles as compared to those of non-pregnant buffaloes. The right ovaries in Murrah buffaloes have lower number of large follicles than left ovaries.

Keywords: Buffalo, ovarian, non ovarian factors, follicular population

1. Introduction
Ultrasound graphy technique is simple, non invasive imaging technique without side effect and has been used for various functions such as a tool in buffalo reproductive management (Giuseppina, 2012) [4]. Gyan *et al.* (2017) [6] reported that this technique has helped in diagnosis of various ovary and uterus diseases such as ovarian cyst and tumors, in addition to helped in predicting estrus in dairy animals after prostaglandin administration. Presicce *et al* (2003) [12] registrated that the ovarian follicular dynamics and follicular growth in buffalo was similar to that observed in cattle and was characterized by waves of follicular growth and regression. Ultrasound graphy has proved to be a valuable tool in assessing the status of ovarian structures such as follicle and corpus luteum in cyclic and non-cyclic buffaloes. For this purpose the current study was designed to investigate the influence of related factors such as age, body condition score (BCS), CL, pregnancy status and ovary localization on follicular population in Murrah buffalos using Ultrasound graphy technique.

2. Materials and methods
2.1Study area
The present research was conducted in year 2018 – 2019 at Central Institute for Research on Buffalo, Hisar Haryana, India, located between Latitude: 29°09′14″ N Longitude: 75°43′22″ E and Elevation above sea level: 216 m.

2.2 Experimental animals
The study was conducted at buffalo's farm in Central Institute for Research on Buffaloes (CIRB) during period from September, 2018 to January, 2019. 160 female Murrah buffaloes with average age of 2- 11 years were used.
The animals were maintained in a semi intensive system of housing at CIRB animal farm and were fed balance ration consisting of green fodder, wheat straw and concentration with specific mineral mixture developed by the institute.

2.3 Determination of animal's Body Condition Scoring
Animal body condition scoring was determined according to that method described by (Anitha *et al.*, 2011) [1] and classified animals from 1-5 score.

2.4 Transrectal Ultrasound graphy examination

Ultrasound graphy was performed by using a portable real-time B-mode transrectal ultrasound scanner (PVF -738F, TOSHIBA, SSA220 Japan) with an intra operative 7.0 MHz micro convex transducer. Each ovary was scanned several planes by maneuvering the transducer along it surface to ovary identity the ovarian structures. Positions and sizes of follicles (≥ 3mm) were measured after freezing the image using inbuilt calipers. However, follicles < 3mm were counted by seeing the image on the ultrasound screen appears as black circular structure surrounded by echogenic ovarian tissues. Ovary sizes, follicular population, localization of CL and uterus structure were examined and the diagnosis was made on basis of echogenicity of images. The pregnancy was confirmed by the presence of a visible fetus on viewing screen of the ultrasound machine which was irregularly shaped echogenic structure surrounded by non echogenic black color image in uterus (Sharma *et al*., 2012) [14].

3. Statistical Analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) Version 18. The analysis of variance and Duncan's test statistics were used to analyze appropriate data sets. Differences were significant at $P<0.05$ (Kouamo *et al*., 2014) [8, 9].

4. Results

4.1 Effect of non ovarian factors on follicular population

The Effect of non ovarian factors on follicular population was presented in Table 1. The number of small follicles in animals having BCS 4 (7.35±0.69) were significantly higher ($P<0.05$) as compared to animals having BCS 3 (4.47±0.64). However, no significant difference was observed between medium and large follicles in animals having different BCS. The total number of follicle per ovary was significantly greater ($P<0.05$) in animals having BCS 4 (9.85±0.74) as compared to BCS 3 animals 6.78±0.69. This effect was owing to more number of small follicles in the group. On the other hand, no significant different ($P<0.05$) was observed between the age of animal and the follicular population. Number of medium follicles was significantly higher ($P<0.05$) in pregnant animals when compared with non-pregnant cyclic animals. However, there was no significant different between the number of small follicles, large follicles and total number of follicle in pregnant and non-pregnant animals.

4.2 Effect of ovarian factors on follicular population

The effect of ovarian factors on follicular population was presented in Table 2. The ovary having CL had more number of medium size follicles (1.95±0.17) as compared to ovary without CL (1.39±0.18)and difference was significant ($P<0.05$). There was no significant difference in the total number of follicles present in both ovaries. Right ovary was having significantly lower ($P<0.05$) number of large follicles than left ovary (0.28±0.04 vs.0.43±0.05). This decrease has no effect on the total number of follicle per ovary. While, no significant observed between numbers of small follicle, large follicle and ovary localization at the present study.

Table 1: Means (± SE) values of BCS, age and pregnancy status

factors		NO	Number of follicles			Total of follicle / ovary
			Small	Medium	Large	
BCS	Thin (1-2)	0	0.0	0.0	0.0	0.0
	Good (3)	72	4.47±0.64a	1.64±0.16	0.67±0.07	6.78±0.69a
	V. Good (4)	88	7.35±0.69b	1.77±0.18	0.73±0.08	9.85±0.74b
	Fat(5)	0	0.0	0.0	0.0	0.0
	P < 0.05	-	0.003	0.594	0.587	0.003
Age	2 - 5	123	5-55±0.56	1.63±0.14	0.66±0.06	7.85±0.58
	6 - 12	37	7.11±1.02	1.92±0.28	0.84±0.14	9.86±1.14
	P < 0.05	-	0.180	0.333	0.166	0.102
Pregnancy status	pregnant	80	5.79±0.72	1.96±0.17b	0.73±0.08	8.48±0.80
	empty	80	5.70±0.65	1.48±0.19a	0.71±0.07	7.89±0.66
	P < 0.05	-	0.928	0.051	0.911	0.572

a,b,c In each column different letters (a, b) indicated significant difference between group ($p<0.05$). N=number of buffalo cow NO = Number SE = Standard Error BCS = Body Condition Score v= vary

Table 2: Means (± SE) values of CL and ovary localization

factors		NO	Number of follicles			Total of follicle / ovary
			Small	Medium	Large	
CL	Present	80	5.81±0.73	1.95±0.17^b	0.73±0.09	8.49±0.80
	Absent	80	5.76±0.65	1.39±0.18^a	0.74±0.08	7.89±0.66
	P<0.05	-	0.959	0.024	0.912	0.564
Ovary Localization	Right	160	3.06±0.26	0.81±0.08	0.28±0.04^a	4.15±0.27
	Left	160	2.72±0.26	0.91±0.09	0.43±0.05^b	4.05±0.29
	P<0.05	-	0.361	0.446	0.013	0.801

a,b,c In each column different letters (a, b) indicated significant difference between group ($p<0.05$). N=number of buffalo cow, NO = Number, SE = Standard Error, BCS = Body Condition Score

5. Discussion

The present study demonstrated that the average number of small follicles were significantly higher ($P<0.003$)in animals that had BCS 4.0 as compared to those having BCS 3.0. However, there was no significant difference in average number of medium and large follicles between these two groups. The results were similar to that found in cow (Kouamo *et al*., 2014) [8, 9]. This has been attributed to low Blood concentration of growth hormones such as Insulin like grow factor -1 (IGF-1). High plasma levels of IGF-1 resulting from improved nutrition, increases the sensitivity of granulosa cells to FSH stimulation (O'Callaghan and Boland, 1999) [10].

Ryan *et al.* (1994) [13] also found a relationship between the blood concentration of IGF-1 and fat or thin animals presented low concentrations of IGF-1. On the other hand the effect of age on follicular population showed no significant different between animals. May be this related to most of animals their ages between 2-5 years were heifers and have BCS grade 3. The results are similar to that reported in young steer and heifer's cow (Breier *et al.*, 1988; Granger *et al.*, 1989) [2, 5].The feed restriction causes a decrease in hepatic concentration of IGF-I and lead to deceleration of follicular growth.

This study also demonstrated that the pregnancy status in buffaloes did not affect the follicular population. Further, the ovary bearing Corpus luteum has more number of medium size follicles as compared to ovary without CL. This might be due to more blood supply in the ovary and continuous follicular growth waves every 8 to 10 days without dominance during pregnancy despite the continuous production of progesterone (Ginther *et al.*, 1989) [3]. The corpus luteum has a negative influence for the growth follicles larger than approximately 7mm after day 21 or 22 of pregnancy (Pierson and Ginther, 1989) [11]. The left ovary has significantly greater number of large follicles than right ovary. The presence corpus luteum in the ovary may contribute to unfavorable condition for follicular growth. As a result, follicle regressed and led to lower COCs recovered from ovaries with a presence of corpus luteum (Hafez and Hafez, 2000) [7].

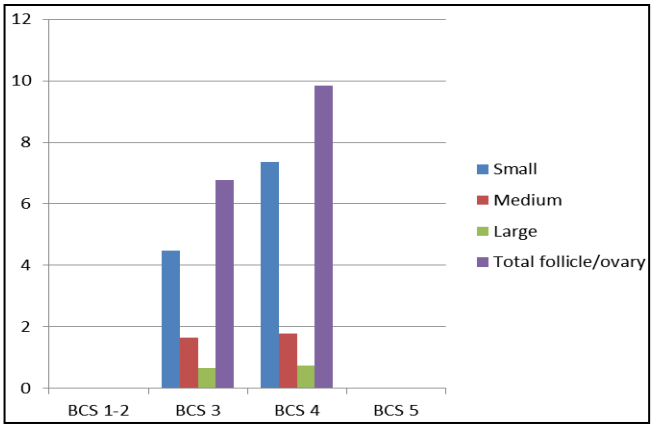

Fig 1: Relationship between BCS and follicular population in Murrah buffaloes

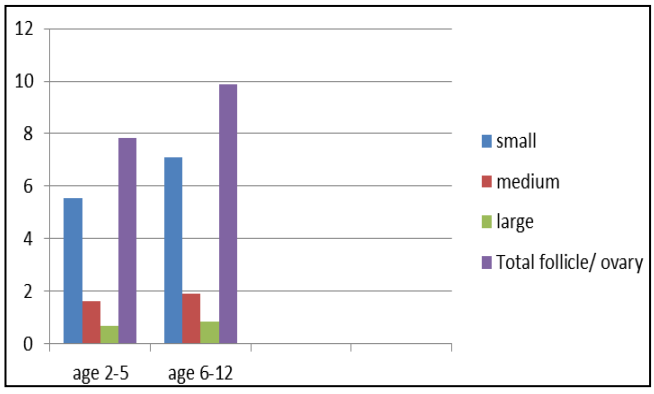

Fig 2: Relationship between Age and follicular population in Murrah buffaloes

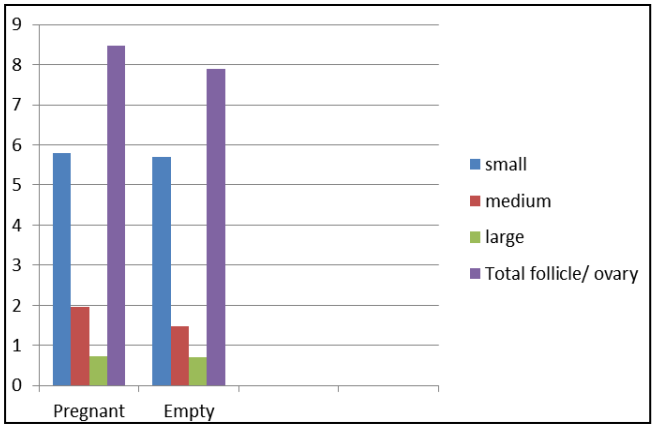

Fig 3: Relationship between pregnancy status and follicular population in Murrah buffaloes

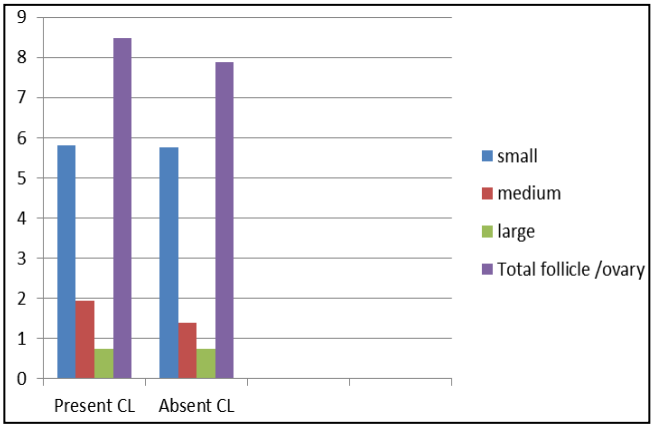

Fig 4: Relationship between present or absent of CL and follicular population in Murrah buffaloes

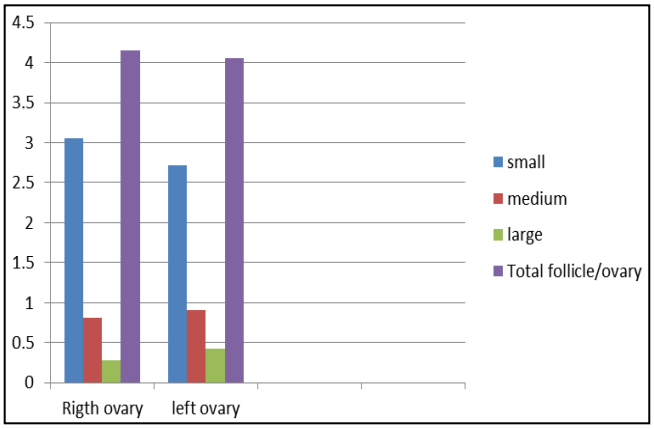

Fig 5: Relationship between ovary localization and follicular population in Murrah buffaloes

6. Conclusion

This study revealed that the ovaries of Murrah buffaloes with BCS 4 have higher number of follicles than those with BCS 3.The ovaries from pregnant animals have more number of medium size follicles as compared to those of non-pregnant buffaloes. The right ovaries in Murrah buffaloes have lower number of large follicles than left ovaries. Future studies are require to study any seasonal variation if any, between different BCS, age, pregnancy status, presence or absence of CL and ovary localization.

7. Acknowledgements
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