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Follicular dynamics, hormonal and biochemical profile across seasons in buffaloes

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ABSTRACT

The present study was conducted to deduce the alteration in follicular dynamics, hormonal and biochemical profile across seasons in buffaloes. In this study, 14 cyclic buffaloes were selected for assessing the follicular dynamics, hormonal and biochemical profile, 7 each during summer and winter. Higher ambient temperature was observed in summer as compared to winter. Nonsignificant difference across seasons with respect to growth rate of follicles and duration of follicular waves was observed. Serum progesterone (P4) showed no significant difference across seasons but differed significantly with respect to days of the estrous cycle in both seasons. Cortisol level did differ significantly from day 4 to day 16 of estrous cycle across seasons. Follicular hormones, viz. estradiol and P4 were significantly higher irrespective of seasons as compared to peripheral level. Follicular biochemical parameters, viz. cholesterol, total protein significantly differed between serum and follicular fluid during summer. Similarly during winter, follicular glucose was higher as compared to serum. In conclusion, season has a significant effect on peripheral cortisol with nonsignificant alterations in follicular dynamics, follicular hormones (E2 and P4) and biochemical milieu across seasons in cyclic buffaloes.

Key words: Buffalo, Cyclicity, Follicular constituents, Follicular dynamics, Seasonality

Season has a major effect in controlling the rhythmicity of buffalo reproduction by partaking direct and indirect effect on reproductive system (Rao and Pandey 1983). Secretion of reproductive hormones is altered by seasonality in buffaloes (Khan *et al.* 2012) and these seasonal alterations in endocrine function obstruct the oviductal and uterine environments (Wolfenson 1997). In buffaloes, heat stress has a pronounced effect on the estrus behavior by reducing the length and estrus intensity (Rao and Pandey 1983). Marai and Haeeb (2010) reported that higher peripheral cortisol during summer in buffaloes blocks E2-induced sexual behavior during summer (Rao and Pandey 1983). In addition to peripheral microenvironment, follicular microenvironment is considered as a reliable indicator for follicular selection, growth and dominance in cattle. Follicular environment has a predominant role in influencing steroidogenesis, oocyte maturation ovulation and corpus luteum formation eventually maintaining pregnancy (Beg and Ginther 2006). Follicular development depends not only on the locally secreted hormones and growth factors, but also on the stimulus from higher center i.e. hypothalamo-pituitary axis, which is directly or indirectly controlled by environment temperature (Khodaei-Motlagh *et al.* 2011). Seasonality alters follicular growth, steroid secretion and follicular gene expression (Wolfenson *et al.* 1997) and studies in buffalo indicated that follicular biochemical composition varies with follicular size, estrous cycle stage and with cyclicity in buffaloes as well (BakiAcar *et al.* 2013). Follicular E2: P4, regarded as an important indicator for follicular health (Varughese *et al.* 2014), varies with reproductive cyclicity. Furthermore, the synthesis of follicular steroid hormones is dependent upon the presence and involvement of several biochemical components, viz. protein, glucose and cholesterol (Alkalby *et al.* 2012).

Above mentioned reports are based on slaughterhouse investigations and pertinent to alteration in biochemical and hormonal components in peripheral circulation and follicular fluid during acyclicity in buffaloes. Nonetheless, these investigations fail to depict the real-time follicular dynamics along with peripheral hormonal and biochemical profile vis–à–vis along with follicular constituents' alteration during different seasons in cyclic buffaloes. Since reports on the alterations in follicular microenvironment along with peripheral alterations during reproductive cyclicity in buffalo across seasons are scanty, the present study was designed to test the hypothesis of alteration, if any, in the follicular dynamics along with peripheral hormonal and biochemical profile vis–à–vis follicular microenvironment during different seasons in cyclic buffaloes.

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MATERIALS AND METHODS

Study location and selection of animals: The study was conducted at ICAR-Central Institute for Research on Buffaloes farm, Hisar, located at 212 m above mean sea level, latitude 29.17 north and longitude 75.72 east. This study was conducted in cyclic pluriparous Murrah buffaloes (age 4.5–6.5 years; body condition score >3.0) with 7 animals each during summer and winter. Buffaloes, used in this study, weighed between 400–550 kg and were cycling regularly as monitored by ultrasonography at 10 days interval. All animals were managed under semi-intensive system and were fed with *ad lib.* green fodder, wheat straw (2–2.5 kg), concentrate feed, mineral mixture. The study was conducted during summer (May to August) and winter (October to February). Environment temperature varied between 20 to 46°C in summer and 20 to 1.5 °C during winter. All the experimental procedures were carried with the approval of Institutional Animal Ethical Committee (IAEC).

Environment parameters: The climatological data during summer and winter of the study period is shown in Table 1. Daily temperature, relative humidity and wind velocity observations were obtained from agricultural meteorological division, CCS Haryana Agricultural University, Hisar, India.

Table 1. Climatological data during different seasons of the study period

Season	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Wind velocity (m/sec)
Winter	24.48±3.08	10.42±3.46	71.25±8.39	2.97±0.21
Summer	37.67±1.76	25.67±1.02	60.00±8.27	6.25±0.85

Ovarian activity monitoring: Ultrasonography was carried out by a single operator using a B mode ultrasound scanner (Toshiba, SSA 220, JustVision, Japan) equipped with an intraoperative 7.5 MHz microconvex transducer 10 days prior to the start of the study in both seasons to monitor the cyclicity of the animals. Ovarian activity was examined by transrectal ultrasonographic examinations on alternate day in all buffaloes pre- and post-estrus period i.e. from day –10 to day 18 (day 0: day of estrus). Estrus was adjudged by the estrus signs (vaginal discharge, frequent urination and vulval tumification), size of regressing corpus luteum, presence of pre-ovulatory follicle and days interval from previous ovulation. Ovulation was determined by the disappearance of a large follicle and subsequent appearance of a corresponding corpus luteum in the same ovary in the same location. Based on the size of corpus luteum and days from ovulation the stage of estrous cycle was calculated retrospectively. Each ovary was scanned in several planes by maneuvering the transducer along the ovarian surface to identify its cyclic structures. Based on the emergence and disappearance of the dominant follicle, follicular growth rate and length of follicular waves were determined.

Sampling of follicular fluid: Pre-ovulatory follicular fluid samples were collected under epidural anesthesia (xylocaine 2%, Astra IDL, India; 6 ml) as described by Pieterse *et al.* (1988). Follicles were aspirated under vacuum pressure (50 mm Hg, Vacuum Pump, K-MAR-5100, Cook IVF Co. Australia) using 18 G needle attached to attached to transvaginal transducer (7.5 MHz, Esaote, Aquila Vet). The contents of the follicles with a diameter of >10 mm were aspirated in 15mL testube with 0.1% heparin and stored at –20°C for hormone (P4 and E2) and biochemical analyses.

Blood sampling, hormone and biochemical analysis: Blood (10 ml) was collected weekly during summer and winter on day –8, –2, 4, 10 and 16 of the estrous cycle considering day 0 as day of estrus. In addition, blood was collected during pre-ovulatory follicular sampling during different seasons in BD vacutainer® serum tubes and collected blood was centrifuged at 3,000 rpm for 15 min. Serum was collected and stored at –20°C for hormone (P4 and cortisol) estimation during summer and winter. Hormone (cortisol, E2 and P4) analysis was done with bovine specific ELISA following kit's protocol (Cusa Biotech. Co. Ltd., China). The sensitivity of P4, cortisol and E2 assays' were 0.12 ng/mL, 0.049 ng/mL and 0.75 pg/mL, respectively. The intra– and inter–assay coefficient of variation for hormones (P4, cortisol and E2) assays' were <10 and <12%, respectively. Serum and follicular biochemical parameters, viz. total protein, cholesterol, triglycerides and glucose were analyzed in automatic biochemistry analyzer (Coralyzer200, Tulip Diagnostics. Pvt. Ltd, India) following kit protocols (Coral Clinical Systems Pvt. Ltd, India).

Statistical analyses: Analyses were carried out using SAS 9.3 software using repeated measure ANOVA using General Linear Methods (GLM) procedure was done using to determine the effect of season on follicular dynamics, hormones concentration in serum and follicular fluid followed by Tukey's *post hoc* test. Season-wise Pearson's correlation coefficients were derived to observe the relationship among the hormones. All results shown, were regarded significant at $P<0.05$.

RESULTS AND DISCUSSION

The higher ambient temperature observed during summer is in conjunction with earlier reports (Singh *et al.* 2013). Follicular characteristics, viz. length of follicular wave, first wave and pre-ovulatory follicle diameter and their growth rate showed no difference across seasons, which was in contrast to Rohilla *et al.* (2005) (Table 2). Variation might be attributed to the duration and the severity of environmental exposure. Though, alterations in follicular characteristics and dynamics were not found in cyclic buffaloes across seasons, derangement of follicular dynamics with persistent dominant follicle with anovulation was documented in acyclic buffaloes (Rohilla *et al.* 2005). In addition, decrease in nutritional status also act as causal factor (Qureshi 2009), but this is unlikely as all study

buffaloes were under uniform feeding management. From these findings, it is evident that exposure of cycling buffaloes to environmental stress does not suppress the overall pattern of follicular dynamics being concordant to Wolfenson *et al.* (1997). Effect of season on peripheral cortisol was significant (P<0.01) across seasons, but not observed with days. Difference across days was observed in P4 attributing to the cyclic changes in corpus luteum during estrous cycle (Baki Acar *et al.* 2013). Similarly, serum cortisol showed a significant difference (P<0.01) in cyclic buffaloes on day 4, 10 and 16 of estrous cycle across seasons, being low during winter (P<0.05) as reported in buffaloes (Singh *et al.* 2013). This study ascertains that seasonal cortisol variation is the response to physiological acclimation (Marai *et al.* 2010) by the activation of higher centres i.e. hypothalamus triggered by skin thermo receptors. But, these results are in contrast with Torres-Junior *et al.* (2008) in cattle, who reported no change in serum cortisol post exposure to heat stress. Furthermore in winter, serum cortisol followed a decreasing trend as compared to summer. Likewise, comparison of hormonal parameters across different days of estrous cycle during summer and winter revealed that P4 during summer differed significantly between day –8 as compared to day –2, 4 and 10 of estrous cycle. Similarly during winter, P4 differed significantly between day 2 as compared to day 4 and 16. This difference is contributed to the cyclic changes of corpus luteum secreting P4. Cortisol showed difference between day 8 and day –2, 4, 10 and 16 of the estrous cycle during winter. The reason for this difference across days of the estrous cycle during winter needs to be investigated. Moreover, it has been shown that cortisol is significantly altered during chronic heat exposure as compared to acute heat stressed animals contributed by increased thermotolerance and intrinsic cellular resistance to elevated temperature (Torres-Junior *et al.* 2008).

Hormone (E2 and P4) profile of pre-ovulatory follicle vis-à-vis serum in cyclic buffaloes across seasons is shown in Table 3. Irrespective of seasons, follicular E2 concentration was significantly (P<0.05) higher than P4 in follicles. A significant difference (P<0.01) in P4 levels between the serum and follicle across seasons was observed. Nonsignificant correlation existed between serum and follicular P4 concentration across seasons (Table 4). Higher follicular E2 and P4 levels than serum across seasons and higher E2: P4 ratio was deduced during both seasons

Table 2. Follicular dynamics during summer and winter in cyclic buffaloes

Parameter	Season	
	Summer (N=7)	Winter (N=7)
Growth rate of follicles (I wave) (mm)	0.98±0.11	0.85±0.19
Growth rate of follicles (II wave) (mm/day)	0.86±0.21	0.93±0.10
Diameter of largest follicle of I wave (mm)	10±1.20	11±0.95
Diameter of pre-ovulatory follicle (mm)	14.96±2.03	13.16±0.33
Duration of I wave (days)	10.79±0. 34	11.57±0.73
Duration of II wave (days)	10.55±0.54	10.24±0.10
Follicular fluid (mL)	0.70±0.01	0.90±0.05

Values expressed in mean±SE.

Table 3. Hormone (E2 and P4) profile of pre-ovulatory follicle *vis-à-vis* serum in cyclic buffaloes during summer and winter

Parameter	Season			
	Summer (N=7)		Winter (N=7)	
	Serum	Follicle	Serum	Follicle
Estradiol (ng/mL)	0.21±0.045^{a}	10.73±2.22^{b}	0.41±0.041^{a}	6.70±0.30^{c}
Progesterone (ng/mL)	0.43±0.08^{a}	5.73±0.44^{b}	0.45±0.10^{a}	5.64±0.56^{b}
Follicular estradiol: progesterone ratio	1.88		1.19	

Values expressed in mean±SE; values in a row with different superscripts differ significantly (P<0.01).

Table 4. Correlation coefficient, r (P value) of serum and follicular hormones during summer and winter

Parameter	Summer progesterone		Winter progesterone	
	Serum	Follicle	Serum	Follicle
Progesterone serum	-	-0.32 (0.4700)	-	0.14 (0.7517)
Estradiol follicle	-0.54 (0.2077)	0.55 (0.1970)	-0.03 (0.9431)	0.59 (0.1624)

Table 5. Biochemical profile in pre-ovulatory follicle vis-à-vis serum in cyclic buffaloes during summer and winter

Parameter	Season			
	Summer (N=7)		Winter (N=7)	
	Serum	Follicle	Serum	Follicle
Glucose (mg/dL)	69.0± 6.22^{a}	65.57± 4.16^{a}	56.83± 3.63^{a}	76.42± 3.25^{b}
Total protein (g/dL)	6.78± 0.69^{a}	4.47± 0.21^{b}	6.43± 0.52^{a}	6.26± 0.36^{a}
Cholesterol (mg/dL)	77.28± 14.03^{a}	42.37± 5.94^{b}	84.60± 15.43^{a}	82.36± 10.10^{a}
Triglyceride (mg/dL)	36.05± 5.68	35.33± 1.73	38.98± 1.03	41.57± 1.48

Values expressed in mean±SE; values in a row of each biochemical parameter with different superscripts differ significantly (P<0.05).

depicting progressive follicular growth. Likewise, higher follicular E2 and P4 were in conformity to Aller *et al.* (2013). In addition, earlier study by Wolfenson *et al.* (1997) showed a decrease in E2 concentration depending on the duration and severity of heat stress exposure. Furthermore, it can be substantiated that an alteration of steroid concentration in follicular fluid was noticed after chronic heat exposure rather than acute heat stress. The present findings were in accordance with Paula *et al.* (2008) with respect to follicular fluid steroid profile, besides no correlation between the serum and follicular E2 and P4 across seasons, which was in discordant with Baki Acar *et al.* (2013).

Biochemical parameters, viz. total protein, cholesterol, triglycerides (TG) and glucose in pre-ovulatory follicle and serum during estrus are shown in Table 5. Follicular biochemical profile, viz. glucose, TG and protein were in agreement with AbdEllah *et al.* (2010). Nonetheless, lower serum glucose was deduced in winter is in contrast with Leroy *et al.* (2004). Though higher glucose concentration in large follicle can be speculated due to the low metabolism of glucose in large follicles or/and higher influx of glucose from peripheral circulation into follicles which needs further investigations. Furthermore, high follicular glucose might be due to higher reserves of glucose derived from blood and increased permeability of the blood–follicle barrier during folliculogenesis (Leroy *et al.* 2004). During summer, serum total protein (6.78±0.69 g/dL) and follicle (4.47±0.21 g/dL), were within the physiological range in buffaloes (BakiAcar *et al.* 2013) but, was in contrast from Arshad *et al.*(2005) which could be due to the change in the nutritional status across seasons (Qureshi 2009). Higher peripheral cholesterol concentration than follicle during summer was in agreement with Arshad *et al.* (2005). But, no such difference was found during winter which was in contrast with Alkalby *et al.* (2012). Furthermore, serum cholesterol observed was lower than earlier report (Arshad *et al.* 2005), but was in conjunction with Baki Acar *et al.* (2013). This could be due to variation in nutritional status and/or biotransformation of cholesterol to steroid hormones; but the present study fails to affirm the existence of such phenomenon during winter. Nonetheless, it can be speculated that lower follicular protein, might be due to lower follicular protein arising due to reduced transport of blood lipoprotein across the blood–follicle barrier. This study affirms that lower follicular fluid protein decreases synergistically with follicular cholesterol thereby affecting ovarian cyclicity. Triglyceride showed no significant difference between serum and follicular compartments across seasons, but was higher as compared to previous study (Baki Acar *et al.* 2013). But, significant difference between serum and follicular TG was reported by Leroy *et al.* (2004) where lower follicular TG was observed. This can be attributed to species difference, physiological and health status of the follicles under study, besides follicular TG are considered as the alternate source of energy for follicular local metabolic processes implicated for progressive follicular growth and maturation which correlate the study findings.

In summary, serum P4 showed no variation across seasons, but a significant difference was seen across days of estrous cycle during summer and winter. Likewise, peripheral cortisol showed a significant difference across days and seasons with lower peripheral cortisol during winter than summer. Follicular E2 and P4 levels were significantly higher irrespective of seasons as compared to serum levels and follicular biochemical parameters between serum and follicular fluid differed significantly with respect to glucose, total protein and cholesterol. From the present study, it is evident that there is variation in peripheral cortisol, with non-significant change in follicular dynamics and hormonal milieu across seasons in cyclic Murrah buffaloes.

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