Journal of Fertilization: In Vitro - IVF-Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology

Satheshkumar et al., JFIV Reprod Med Genet 2016, 4:3
DOI: 10.4172/2375-4508.1000187

Research Article

Effect of Physico-Biochemical Characteristics of Follicles on Quality and *In Vitro* Maturation of Bubaline Oocytes

S Satheshkumar[1]*, B Revathi Priya[1], K Brindha[1], A Roy[1] and K Kumanan[2]

[1]*Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai – 600 007, Tamil Nadu, India*
[2]*Tamil Nadu Veterinary and Animal Sciences University, Chennai- 600 051, Tamil Nadu, India*

Abstract

The physico-biochemical properties of follicles and its plausible effect on quality and maturational competency of oocytes during the different breeding periods of the year was studied in buffaloes (*Bubalus bubalis*). Ovaries were collected from sexually mature buffaloes slaughtered at abattoir during high breeding (December- February; HB) and low breeding (April-July; LB) periods of the year. The surface antral follicles were categorized as small (SF; ≤ 4 mm), medium (MF; 4-9 mm) and large (LF; >9 mm) follicles. The follicular fluid (FF) was aspirated from follicles of different categories and the recovered oocytes were assessed for their quality. The good quality oocytes were cultured *in vitro* to assess the maturational competency. The glucose (Glu), total proteins (TP) and triglycerides (Tgl) concentrations of FF were analysed and correlated with the oocyte quality and maturation capacity. Among the follicular categories, significantly (P<0.01) more percentage of good quality oocytes with high maturation rate was recovered from MFs and vice versa for oocytes from LFs in both the periods. The mean percentage of good quality oocytes and the mean percentage of oocytes that reached the MII stage was significantly (P<0.01) higher during HB than LB period. The Glu concentration increased as the follicle size increased, while the TP concentration remains similar in all follicular categories but the Tgl concentrations were significantly higher in SFs. In general, the concentrations of these three biochemical components, with significance to Tgl, were found to be increased in the FF during the LB period correlating with the deteriorated oocyte quality and developmental properties. This might mean that metabolic activities were less intensive resulting in lower consumption of these components by the follicular cells and oocytes during the LB than HB period leading to fall in fertility levels during the former period.

Keywords: Physico-biochemical properties of follicles; Quality and maturational competency of oocytes; Buffaloes

Introduction

Buffalo (*Bubalus bubalis*) is a significant livestock species with a socio-economic impact in South-Asian countries. There is a worldwide increasing interest in large-scale *in vitro* embryo production (IVEP) in buffaloes for faster propagation of superior germplasm and to enhance genetic progress through the maternal contribution [1]. Being reared in tropical and sub-tropical environments, the reproductive efficiency of buffalo is found to be lower when compared to crossbred cows [2]. Eventhough buffaloes are not seasonal breeders; a decline in reproductive activity is manifested by a reduced ovarian activity during the hot period when compared to the cold period of the year [3]. Inspite of the advantage of IVEP in producing more number of transferrable embryos, the above mentioned factors seem to compromise the outcome of the technology in this species. The influence of follicular wave characteristics on the ovarian activity of buffaloes was recorded previously [4]. However, the role of biochemical events occurring within the follicular micro-environment in determining the oocyte quality and maturational competency, a basis for fertility of the animal is less understood and needs to be studied.

Follicular fluid (FF), originating from secretory activity of granulosa and thecal cells and transfer of blood plasma constituents through the blood follicular barrier [5], forms the major component of micro-environment to which the cumulus oocyte complexes (COCs) are exposed during their developmental process. The biochemical characteristics of the FF may play a critical role in determining oocyte quality and their potential to achieve maturation and fertilization [6,7], a prerequisite for early embryonic development. Lipids, carbohydrates and amino acids are the three basic biochemical sources of energy and nutrition for COCs in FF [8,9]. It is reasonable to hypothesize that untoward seasonal changes in these biochemical constituents of the FF compromise the oocyte quality and its developing potential *in vivo*. The biochemical analysis of FF during different periods of the year may provide information on underlying seasonal metabolic changes that determine the physical and physiological characters of oocyte, and thus the fertility of the animal.

Hence, the objective of the present investigation was to study the concentrations of glucose (Glu), total proteins (TP) and triglycerides (Tgl) in buffalo FF during the high and low breeding periods of the year and its plausible effect on quality and maturational competency of oocytes.

Materials and Methods

Source and preparation of ovaries

Ovaries were collected from sexually mature buffaloes slaughtered at Chennai Corporation abattoir, Chennai, Tamilnadu and utilized for the study. The ovaries were transported in normal saline (0.9%) supplemented with 50 µg/ml gentamicin sulphate at 37°C in a thermos flask to the laboratory within 30 min of slaughter. The extra ovarian tissues were trimmed off and the ovaries were washed three times with tap water and five times with phosphate buffered saline to remove blood clots and superficial contamination. The study was conducted during

***Corresponding author:** S Satheshkumar, Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai – 600 007, Tamil Nadu, India, Tel: 04372-234 011; Fax: 91-4372-234 011; E-mail: drsatheshkumar6@rediffmail.com

Received June 21, 2016; **Accepted** July 05, 2016; **Published** July 12, 2016

Citation: Satheshkumar S, Revathi Priya B, Brindha K, Roy A, Kumanan K (2016) Effect of Physico-Biochemical Characteristics of Follicles on Quality and *In Vitro* Maturation of Bubaline Oocytes. JFIV Reprod Med Genet 4: 187. doi:10.4172/2375-4508.1000187

cold months (December- February) and hot months (April-July) of the year representing two groups *viz*., High breeding (HB; n=415 ovaries; 12 trials) and Low breeding (LB; n=354 ovaries; 12 trials) periods respectively.

Classification of surface antral follicles

The diameter of all the surface antral follicles were measured using a measuring scale and categorized into three types *viz*., small (SF; <4 mm), medium (MF; >4-9 mm) and large (LF; >9 mm) follicles [10]. These follicular categories were correspondingly related to recruited, developing and dominant stage follicles respectively [11].

Follicular count

The number of SFs, MFs and LFs were counted in individual ovaries and total antral follicular count (AFC) was arrived. The mean values of each category follicles and AFC were calculated accordingly.

Aspiration of follicular fluid

The FF from SFs, MFs and LFs (SFF, MFF and LFF respectively) were aspirated separately using a sterile hypodermic insulin syringe needle (18 G for LFs; 22 G needle for MFs and SFs) and dispensed in separate petri dishes. Mean FF volume per follicle (Total FF volume/No. of follicles) was determined for each follicle category separately.

The collected FF was screened under stereo zoom microscope (Nikon, Japan) and the COCs were recovered. After the oocyte recovery, the FF was transferred to 1.5 ml micro-centrifuge tube (Eppendorf, Germany) and centrifuged at 10,000 g for 10-15 min at 4°C. The supernatant FF of different follicular categories was aliquoted in separate micro-centrifuge tubes and stored at -20°C until further analysis. All the analyses were carried out separately for each follicular category.

Analysis of oocyte quality and maturational competency

Quality assessment of oocytes: The recovered COCs were graded based on their cumulus mass investment and homogeneity of ooplasm as Grade A, B, C, D and E [10]. A and B grade COCs were considered to be of good quality and selected for further maturation studies. The remaining oocytes (C, D and E grades) were categorized as poor quality oocytes. Mean percentage of good and poor quality oocytes were calculated separately for three follicular categories.

Assessment of maturation rate by *in vitro* culture of oocytes: *In vitro* maturation (IVM) media (Medium 199, sodium pyruvate 2.5 mM, fetal bovine serum 10%, follicular fluid 5%, porcine follicle stimulating hormone 0.5 µg/ml, luteinizing hormone 5.0 µg/ml, 17-ß estradiol 1.0 µg/ml and cysteamine 50 µM) was prepared freshly on the day of oocyte collection. Droplets (50 µl) of the media were placed in 35 mm petriplates and overlaid with sterile mineral oil and both the media and droplets were pre-equilibrated in CO_2 incubator for two hours before the oocytes were cultured. Good quality oocytes from each follicular category were washed in pre-equilibrated IVM medium and transferred into separate droplets (10-20 COCs per droplet) and subjected for maturation at 38.5°C in a humidified atmosphere with five percent CO_2 in air for 22 h.

After 22 h of incubation in IVM medium, all the oocytes were denuded of cumulus cells by repeated pipetting and assessed for their nuclear maturation using 4,6-diamidino-2-phenylindole (DAPI) stain (Millipore) as described by Fair et al. [12]. Stained oocytes were evaluated for nuclear status using an inverted microscope (Zeiss, Germany; 400X) equipped with epifluorescent illumination and filters giving maximum transmittance at 405 nm. The oocytes were classified as immature germinal vesicle (GV), partially matured germinal vesicle breakdown (GVBD) and completely matured metaphase II (MII) stages [13]. Mean percentage of oocytes that had attained different maturation stages was calculated for three follicular categories.

Biochemical analysis of follicular fluid

Aliquots of SFF, MFF and LFF samples were thawed at room temperature and subjected to biochemical analysis of Glu, TP and Tgl. The determination of metabolite levels in FF was done by colorimetric method [14] using appropriate photometric assays (Roche Diagnostics GmbH, Mannheim, Germany) and commercial kits (Agapee diagnostics, India). All analyses were performed at Centralized Clinical Laboratory, Madras Veterinary College, Chennai using clinical bio-chemistry Auto Analyzer (A15 Biosystems).

Statistical analysis

Data on follicular characteristics and biochemical parameters of different follicular categories during HB and LB periods were analysed by Student's t-test and by Analysis of Variance (ANOVA) with completely randomized design. The values in percentage were converted into arcsine radiance before they were subjected to one way analysis of variance. Biochemical parameters were correlated with quality of oocytes and their maturation stages. SPSS.10.0® software was used for analysis of data. Analysis of data was carried out as per Snedecor and Cochran [15].

Results

Follicular characteristics

The numbers of follicles per ovary and FF volume per follicle in each follicular category during the HB and LB periods were presented in Table 1. The number of SFs, MFs and LFs and AFC per ovary were non-significantly (P>0.05) higher during the HB when compared to LB. There was no significant difference in the FF volume aspirated from SFs and MFs between the periods, however the volume was significantly (P<0.05) more in LFs during the LB than HB.

Quality of oocytes

The percentage of good quality and poor quality oocytes recovered from each follicular category during HB and LB periods were presented in Tables 2 and 3. Among the different follicular categories, significantly (P<0.01) more percentage of good and poor quality oocytes were recovered from MFs and LFs respectively in both the periods. The mean percentage of good quality oocytes recovered during HB (65.40 ± 9.87%) was significantly (P<0.01) higher than recovered during LB (52.99 ± 8.80%) and vice versa for poor quality oocytes (34.60 ± 9.12 *vs.* 47.01 ± 10.27% respectively).

Maturation rate of oocytes

The maturation rate of oocytes subjected for IVM culture during HB and LB periods were presented in Tables 2 and 3. Perusal of data revealed that significantly (P<0.01) higher percentage oocytes recovered from MFs were found to be have reached MII stage than their counterparts from SFs and LFs. It was also found that significantly (P<0.01) more percentage of oocytes reached the MII stage during HB (42.82 ± 8.26%) than LB (24.16 ± 4.68%).

Biochemical parameters of follicular fluid

The Glu, TP and Tgl concentrations in FF of three categories of follicles in HB and LB periods were presented in Table 4 and Figure 1.

Glucose: The Glu concentration increased as the follicle size

Breeding period	No. of follicles/ovary (Mean ± SE)				FF volume/follicle (ml) (Mean ± SE)		
	SF	MF	LF	AFC	SFF	MFF	LFF
HB	3.13 ± 0.43^{a}	0.80 ± 0.05^{a}	0.34 ± 0.03^{a}	4.18 ± 0.18^{a}	0.03 ± 0.00^{a}	0.14 ± 0.01^{a}	0.34 ± 0.01^{a}
LB	2.37 ± 0.12^{a}	0.75 ± 0.09^{a}	0.30 ± 0.03^{a}	3.83 ± 0.20^{a}	0.04 ± 0.00^{a}	0.13 ± 0.01^{a}	0.47 ± 0.04^{b}
Significance	NS	NS	NS	NS	NS	NS	*

Values within columns with different superscripts differ significantly, *(P<0.05), NS: Not significant (P>0.05)

Table 1: Mean ± SE of follicular characteristics during different breeding periods.

Breeding period	Follicular category	Quality of oocytes (%) (Mean ± SE)		Maturation rate of oocytes (%) (Mean ± SE)		
		Good quality	Poor quality	GV	GVBD	MII
HB	SF	61.00 ± 12.30^{ab}	39.00 ± 5.86^{b}	3.51 ± 3.39^{a}	55.82 ± 11.13^{c}	40.67 ± 11.26^{b}
	MF	79.06 ± 5.46^{c}	20.94 ± 12.97^{a}	2.54 ± 1.01^{a}	36.54 ± 7.90^{ab}	60.91 ± 11.68^{c}
	LF	56.13 ± 9.87^{a}	43.87 ± 14.12^{b}	41.74 ± 12.73^{c}	33.71 ± 13.82^{a}	24.54 ± 4.54^{a}
	Significance	**	**	**	**	**
LB	SF	55.12 ± 6.72^{b}	44.88 ± 5.87^{ab}	37.81 ± 3.85^{c}	45.36 ± 4.39^{a}	16.83 ± 4.69^{ab}
	MF	60.69 ± 6.72^{b}	39.31 ± 8.46^{a}	18.62 ± 4.43^{a}	40.83 ± 6.18^{a}	40.55 ± 9.25^{c}
	LF	43.15 ± 12.92^{a}	56.85 ± 13.54^{c}	24.40 ± 2.82^{ab}	60.51 ± 7.41^{c}	15.09 ± 5.53^{a}
	Significance	**	**	**	**	**

Values within columns with different superscripts differ significantly, **(P<0.01)

Table 2: Mean ± SE of quality and maturation rate of oocytes recovered from different follicular categories.

Breeding period	Quality of oocytes (%) (Mean ± SE)		Maturation rate of oocytes (%) (Mean ± SE)		
	Good quality	Poor quality	GV	GVBD	MII
HB	65.40 ± 9.87^{b}	34.60 ± 9.12^{a}	15.40 ± 5.39^{a}	41.76 ± 10.13^{a}	42.82 ± 8.26^{b}
LB	52.99 ± 8.80^{a}	47.01 ± 10.27^{b}	26.94 ± 5.11^{b}	48.90 ± 6.90^{a}	24.16 ± 4.68^{a}
Significance	**	**	**	NS	**

Values within columns with different superscripts differ significantly, **(P<0.01)

Table 3: Mean ± SE of quality and maturation rate of oocytes recovered during different breeding periods.

Breeding period	Follicular category	Glucose (mg/dl) (Mean ± SE)	Total Protein (g/dl) (Mean ± SE)	Triglyceride (mg/dl) (Mean ± SE)
HB	SF	4.09 ± 0.69^{a}	7.23 ± 0.15^{a}	64.40 ± 4.60^{b}
	MF	8.86 ± 0.68^{a}	6.57 ± 0.09^{a}	39.80 ± 3.20^{a}
	LF	20.60 ± 0.96^{b}	6.14 ± 0.10^{a}	35.80 ± 5.10^{a}
	Significance	**	NS	**
LB	SF	6.78 ± 1.55^{a}	9.15 ± 0.45^{a}	101.05 ± 5.51^{c}
	MF	10.88 ± 1.95^{a}	8.87 ± 0.30^{a}	81.35 ± 5.47^{b}
	LF	25.09 ± 3.07^{b}	8.10 ± 0.23^{a}	64.97 ± 6.17^{a}
	Significance	**	NS	**

Values within columns with different superscripts differ significantly, **(P<0.01), NS: Not significant (P>0.05)

Table 4: Mean ± SE of biochemical parameters of follicular fluid in different follicular categories.

increased with a significantly (P<0.01) higher concentration in LFs than SFs and MFs, irrespective of the period of the study. Eventhough the Glu concentration was found to be higher in all the follicular categories during the LB there was no significant difference when compared with HB.

Total protein: There was no significant difference in TP concentration between the follicular categories. However, a non-significant increase in TP concentration was found in all the follicular categories during LB than HB period.

Triglycerides: The Tgl concentration in SFF was significantly (P<0.01) higher than that of MFF and LFF. In contrast to the Glu, the concentration of Tgl was found to be decreasing as the follicular size increased, irrespective of the periods. Comparison between the two periods of study revealed that concentration of Tgl was significantly (P<0.01) higher during LB than HB irrespective of the follicular category.

In general, the FF concentrations of all the biochemical metabolites studied were found to be higher during the LB than HB period.

Correlation between biochemical parameters and quality and maturation status of oocytes

The correlation between the biochemical parameters and quality of oocytes and their maturation status was presented in Tables 5 and 6. In SF, a significantly (P<0.05) positive correlation was found to exist between Glu concentration and poor quality oocytes. A highly significant (P<0.01) positive correlation was recorded between Glu, TP and Tgl concentrations and poor quality oocytes in LF category. A significant positive correlation was recorded between Glu, TP and Tgl concentrations and GV oocytes in MF and LF categories. In all the follicular categories, significantly (P<0.05) negative correlation exists between the Tgl concentration and MII stage oocytes.

Discussion

The numbers of SFs, MFs and LFs and AFC per ovary were higher during the HB than LB period indicating a better recruitment of follicles followed by better development and attainment of dominance

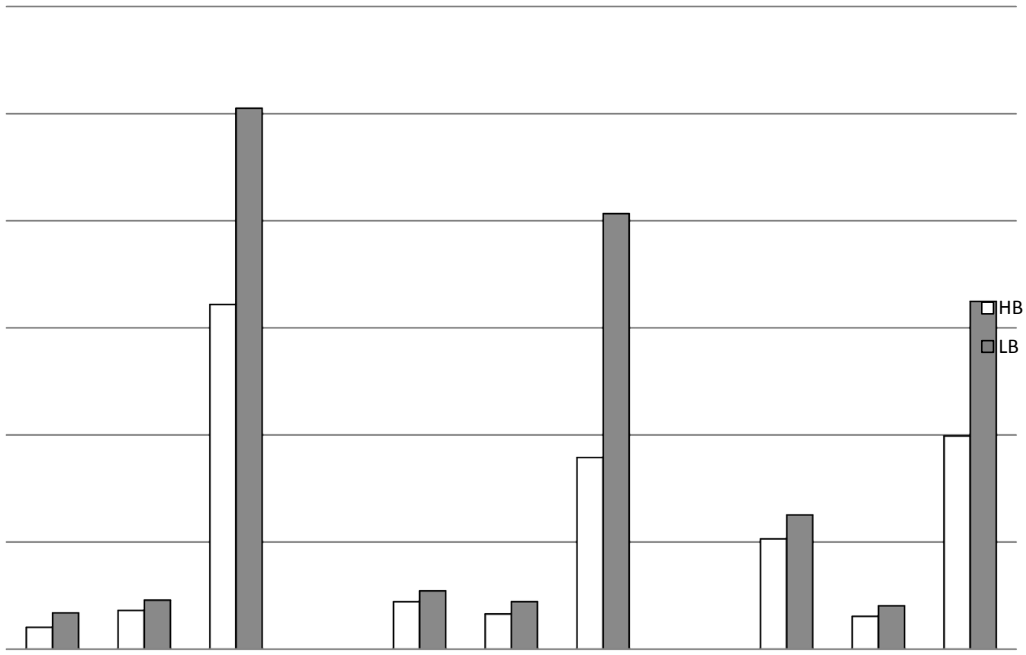

Figure 1: Biochemical parameters of follicular fluid during different breeding periods.

Follicular category	Biochemical parameter	Quality of oocytes	r value	Significance
SF	Glu	Good quality oocytes	0.1454	NS
		Poor quality oocytes	0.4383	*
	TP	Good quality oocytes	0.1565	NS
		Poor quality oocytes	0.4006	NS
	Tgl	Good quality oocytes	-0.0316	NS
		Poor quality oocytes	0.3692	NS
MF	Glu	Good quality oocytes	-0.0417	NS
		Poor quality oocytes	0.0200	NS
	TP	Good quality oocytes	-0.1267	NS
		Poor quality oocytes	0.1174	NS
	Tgl	Good quality oocytes	0.0199	NS
		Poor quality oocytes	0.2256	NS
LF	Glu	Good quality oocytes	-0.1213	NS
		Poor quality oocytes	0.5520	**
	TP	Good quality oocytes	0.0691	NS
		Poor quality oocytes	0.5839	**
	Tgl	Good quality oocytes	-0.0140	NS
		Poor quality oocytes	0.5408	**

*P<0.05, **P<0.01, NS: P>0.05

Table 5: Correlation between biochemical parameters and quality of oocytes in different follicular categories.

during the former period. It reflects a healthy follicular activity which might be one of the contributory factors for better fertility during the HB period. The results of the study corroborated with the findings of Manjunatha et al. [16] and Sadeghinezhad and Hasanzadeh [17], who confirmed seasonality in ovarian activity of buffaloes with more number of follicles in winter than in summer. They attributed the same for reduced growth rate and high degree of follicular atresia during the latter period.

To the best of our knowledge, this is the first report on FF volume per follicle (0.03-0.47 ml) in buffaloes. Interestingly the volume of LFF was significantly more during the LB than the HB period. The increased FF volume is the contributory factor for the increased size of the ovulatory follicle during the LB period (unpublished data). Similarly Summers et al. [18] reported a reduced fertility in cows having follicles with increased FF content. They correlated the increased FF volume in the bovine follicles with high androgen content indicative of poor steroidogenesis and they suggested that steroid excess in FF would result in reduced fertility through altered follicle development and oocyte maternal RNA abundance. However the factors contributing for increased volume of FF during the LB period in bubaline species has to be investigated.

Among the different follicular categories, significantly more

Follicular category	Biochemical parameter	Maturation status	r value	Significance
SF	Glu	GV	0.3097	NS
		MII	-0.1830	NS
	TP	GV	0.3984	NS
		MII	-0.1018	NS
	Tgl	GV	0.2402	NS
		MII	-0.4942	*
MF	Glu	GV	0.5192	*
		MII	0.2246	NS
	TP	GV	0.7820	**
		MII	0.1428	NS
	Tgl	GV	0.5210	*
		MII	-0.5070	*
LF	Glu	GV	0.4394	*
		MII	-0.0612	NS
	TP	GV	0.4425	*
		MII	0.3317	NS
	Tgl	GV	0.4606	*
		MII	-0.4295	*

*P<0.05, **P<0.01, NS: P>0.05

Table 6: Correlation between biochemical parameters and maturation status of oocytes after IVM culture.

percentage of oocytes recovered from MFs were of good quality and reached MII stage after IVM culture followed by oocytes from SFs, while LFs yielded poor quality oocytes with poor maturational capacity irrespective of the period of study. Similarly, Selvaraj et al. [19] and Amer et al. [20] also recorded that a great majority of the oocytes from MFs reached the MII stage and developed to blastocysts indicating perfect completion of cytoplasmic/nuclear maturation. The deterioration of oocyte quality in LFs (equivalent to dominant ovulatory follicles) might be a reflection of metabolic deficiencies within the follicular micro-environment. Vencato et al. [21] also reiterated that the biochemical composition of FF was influenced by follicular dimensions.

The Glu concentration increased as the follicle size increased in both the breeding periods in concurrence with the observations of Arshad et al. [22]. In the present study, the Glu concentration was higher in all the three follicular categories in LB than HB period. Statistically significant positive correlation between Glu concentration and poor quality and poor maturational capacity of oocytes might be indicating detrimental effect of intra-follicular hyperglycemic Glu levels on the health of the oocytes during the LB period [21]. A long-established tenet of COC energy metabolism was that the maturing COC used Glu for energy production and for numerous other cellular processes such as nucleic acid and purine synthesis, mucification and cellular homoeostasis [23,24]. Oocyte has a poor capacity to utilize Glu on itself and only cumulus cells metabolized the bulk of the Glu consumed by the COC to supply metabolic intermediates to the oocyte [25]. Taking into consideration of the above fact, the increased Glu concentration in LFF during the LB period might be attributed to poor utilization of Glu by the cumulus cells leading to accumulation of the metabolite in follicular micro-environment.

Contrary to the Glu levels, the concentrations of TP in different sized follicles were similar during both the breeding periods in accordance with the previous reports in ruminants [26,27]. However, similar to Glu, the TP concentrations were non-significantly higher during the LB than HB period. Thangavel and Nayeem [28] reported higher concentration of TP in atretic follicles than the normal follicles in buffaloes. In concurrence, our correlation study revealed an increase in poor quality and poor maturational capacity of oocytes in LF category as the TP concentrations increased in FF, indicative of increased atretic activities in larger dominant follicles especially during the LB period.

The Tgl which serve as an alternative energy source for the cells in follicular micro-environment [29] cannot pass through the blood-follicular barrier since they were transported primarily by the very low density lipoprotein fraction, which is too large to pass through this barrier [30]. So, the Tgl level at follicular micro-environment was mainly as a result of local metabolic processes [31]. Significantly higher concentration of Tgl in SFF than that of MFF and LFF, corroborating with the earlier reports [7], indicated that Tgl forms the major component of energy source during the initial recruitment phase, while Glu takes over in the later developmental phases of follicles in buffaloes [32]. On the contrary to other two components, Tgl concentration was significantly higher in FF during the LB period. In addition, a significant negative correlation recorded between Tgl concentrations and complete maturational capacity of oocytes further strengthened our views that Tgl concentrations might be having a higher impact on quality and maturational capacity of oocytes than the Glu and TP.

In general, the concentrations of these three biochemical components, with significance to Tgl, were found to be increased in the FF during the LB period correlating with the deteriorated oocyte quality and developmental properties. This could mean that metabolic activities were less intensive in the follicular micro-environment, resulting in lower consumption of these components by the follicular cells and/or oocytes [33] during the LB period than HB period.

It has been found chromatin modifiers that are essential for the establishment of heterochromatin marker H3K9me3 and DNA methylation, including HDAC1, SUV39H1, G9a, HP1, and Dnmt3a, play a key role during mammalian oogenesis [34]. As histone methyltranferases G9a together with DNA methyl transferases (DNMTs) are also essential for the maintenance of imprinted DNA methylation [35] and accurate expression of imprinted genes is crucial for the normal postnatal development in mammals [36], epigenetic mechanisms associated with the establishment and maintenance of DNA methylation at imprinted loci might be a novel direction for the assessment and study of gamete maturation in mammals.

In vivo, the relationship between the developing oocyte and the follicular micro-environment is a sensitive indicator of fertility. Being the collection procedures and culture conditions were similar for all the follicular categories, a significantly higher percentage of poor quality and poor maturation rate of oocytes recovered from LFs indicated major metabolic shortfalls in the final stages of the follicular journey towards the ovulation. Further, reduced utility of energy and nutritional sources, as determined by accumulation of major metabolites, might be reflecting the aberrations in certain key metabolic events of normal follicular and/or oocyte development [37], leading to low fertility syndrome in buffaloes during hot period of the year.

Acknowledgement

The work was carried out as a part of the research project entitled "Regulation of follicular dynamics in crossbred cows for augmenting fertility and superstimulation" funded by Department of Biotechnology (DBT), Government of India, Ministry of Science and Technology, New Delhi, India.

References

1. Zicarelli L (2002) Advanced reproductive technologies for improving buffalo production. 1st 440 Buffalo Symposium of Americas, Estacao das Docas, Belèm, Parà, Brazil.
2. Satheshkumar S, Palanisamy A, Rangasamy S, Kathiresan D, Devanathan TG, et al. (2011) Comparative analysis of follicular and luteal dynamics in oestrous cycles of buffaloes and crossbred cattle. Buffalo Bulletin 30: 148-156.
3. Singh J, Nanda AS, Adams GP (2000) The reproductive pattern and efficiency of female buffaloes. Anim Reprod Sci 60-61: 593-604.
4. Awasthi MK, Kavani FS, Siddiquee GM, Sarvaiya NP, Derashri HJ (2007) Is slow follicular growth the cause of silent estrus in water buffaloes? Anim Reprod Sci 99: 258-268.
5. Fortune JE (1994) Ovarian follicular growth and development in mammals. Biol Reprod 50: 225-232.
6. Nishimoto H, Matsutani R, Yamamoto S, Takahashi T, Hayashi KG, et al. (2006) Gene expression of glucose transporter (GLUT) 1, 3 and 4 in bovine follicle and corpus luteum. J Endocrinol 188: 111-119.
7. Ellah MRA, Hussein HA, Derar DR (2010) Ovarian follicular fluid constituents in relation to stage of estrus cycle and size of the follicle in buffalo. Vet World 3: 263-267.
8. Homa ST, Racowsky C, McGaughey RW (1986) Lipid analysis of immature pig oocytes. J Reprod Fertil 77: 425-434.
9. Sturmey RG, Reis A, Leese HJ, McEvoy TG (2009) Role of fatty acids in energy provision during oocyte maturation and early embryo development. Reprod Domest Anim 44 Suppl 3: 50-58.
10. Nandi S, Chauhan MS, Palta P (1998) Influence of cumulus cells and sperm concentration on cleavage rate and subsequent embryonic development of buffalo (*Bubalus bubalis*) oocytes matured and fertilized *in vitro*. Theriogenology 50: 1251-1262.
11. Ginther OJ (1993) A method for characterizing ultrasonically-derived follicular data in heifers. Theriogenology 39: 363-371.
12. Fair T, Hyttel P, Greve T (1995) Bovine oocyte diameter in relation to maturational competence and transcriptional activity. Mol Reprod Dev 42: 437-442.
13. Chohan KR, Hunter AG (2003) Meiotic competence of bovine fetal oocytes following *in vitro* maturation. Anim Reprod Sci 76: 43-51.
14. Nichol R, Hunter RH, Gardner DK, Leese HJ, Cooke GM (1992) Concentrations of energy substrates in oviductal fluid and blood plasma of pigs during the peri-ovulatory period. J Reprod Fertil 96: 699-707.
15. Snedecor GW, Cochran WG (1994) Statistical methods. (8th edition), Iowa State University Press, USA.
16. Manjunatha BM, Ravindra JP, Gupta PS, Devaraj M, Nandi S (2009) Effect of breeding season on *in vivo* oocyte recovery and embryo production in non-descriptive Indian river buffaloes (*Bubalus bubalis*). Anim Reprod Sci 111: 376-383.
17. Sadeghinezhad J, Hasanzadeh S (2010) Study of ovarian follicular development during summer and winter season in River Buffalo. Global Veterinaria 4: 582-586.
18. Summers AF, Pohlmeier WE, Sargent KM, Cole BD, Vinton RJ, et al. (2014) Altered theca and cumulus oocyte complex gene expression, follicular arrest and reduced fertility in cows with dominant follicle follicular fluid androgen excess. PLoS One 9: e110683.
19. Selvaraj R, Majumdar AC, Ansari MR (1992) Quality of oocytes from different sized follicles of buffalo ovaries obtained from slaughterhouse. Indian J Anim Sci 62: 434-435.
20. Amer HA, Hegab AO, Zaabal SM (2008) Effects of ovarian morphology on oocyte quantity and quality, granulosa cells, *in vitro* maturation and steroid hormone production in buffaloes. Anim Reprod 5: 55-62.
21. Arshad HM, Ahmad N, Zia-ur-Rahman, Samad HA, Akhtar N, et al. (2005) Studies on some biochemical constituents of ovarian follicular fluid and peripheral blood in buffaloes. Pakistan Vet J 25: 189-193.
22. Vencato Vencato J, Badon T, Bedin S, Cogo P, Simonato M, et al. (2014) Biochemical and fatty acids composition of water buffalo (*Bubalus bubalis*) follicular fluid. J Buff Sci 3: 82-88.
23. Moley KH, Chi MM, Knudson CM, Korsmeyer SJ, Mueckler MM (1998) Hyperglycemia induces apoptosis in pre-implantation embryos through cell death effector pathways. Nat Med 4: 1421-1424.
24. Sutton ML, Cetica PD, Beconi MT, Kind KL, Gilchrist RB, et al. (2003) Influence of oocyte-secreted factors and culture duration on the metabolic activity of bovine cumulus cell complexes. Reproduction 126: 27-34.
25. Sutton-McDowall ML, Gilchrist RB, Thompson JG (2010) The pivotal role of glucose metabolism in determining oocyte developmental competence. Reproduction 139: 685-695.
26. Biggers JD, Whittingham DG, Donahue RP (1967) The pattern of energy metabolism in the mouse oöcyte and zygote. Proc Natl Acad Sci U S A 58: 560-567.
27. Krishnan LM, Kumar VG, Ravindra JP, Ramesha KP (2005) Total protein concentration of ovarian follicular fluid in buffalo ovaries. Karnataka J Agric Sci 18: 777-779.
28. Albomohsen H, Mamouei M, Tabatabaei S, Fayazi J (2011) Metabolite composition variations of follicular fluid and blood serum in Iranian dromedary camels during the peak breeding season. J Anim Vet Adv 10: 327-331.
29. Thangavel A, Nayeem M (2004) Studies on certain biochemical profile of the buffalo follicular fluid. Indian Vet J 81: 25–27.
30. Kim JY, Kinoshita M, Ohnishi M, Fukui Y (2001) Lipid and fatty acid analysis of fresh and frozen-thawed immature and *in vitro* matured bovine oocytes. Reproduction 122: 131-138.
31. Grummer RR, Carroll DJ (1988) A review of lipoprotein cholesterol metabolism: Importance to ovarian function. J Anim Sci 66: 3160-3173.
32. Leroy JL, Vanholder T, Delanghe JR, Opsomer G, Van Soom A, et al. (2004) Metabolite and ionic composition of follicular fluid from different-sized follicles and their relationship to serum concentrations in dairy cows. Anim Reprod Sci 80: 201-211.
33. Nandi S, Kumar VG, Manjunatha BM, Ramesh HS, Gupta PSP (2008) Follicular fluid concentrations of glucose, lactate and pyruvate in buffalo and sheep and their effects on cultured oocytes, granulosa and cumulus cells. Theriogenology 69: 189-196.
34. Albomohsen H, Mamouei M, Tabatabaei S, Fayazi J (2011) Metabolite composition variations of follicular fluid and blood serum in Iranian Dromedary camels during the peak breeding season. J Anim Vet Adv 10: 327-331.
35. Russo V, Bernabo N, Giacinto D, Martelli A, Mauro A, et al. (2013) H3K9 trimethylation precedes DNA methylation during sheep oogenesis: HDAC, SUV39H, G9a, HP, and Dnmts are involved in these epigenetic events. J Histochem Cytochem 61: 75-89.
36. Surani MA (1998) Imprinting and the initiation of gene silencing in the germ line. Cell 93: 309-312.
37. Zhang T, Termanis A, Özkan B, Bao XX, Culley J, et al. (2016) G9a/GLP complex maintains imprinted DNA methylation in embryonic stem cells. Cell Rep 15: 77-85.