Studies on Thyroid Hormones and some Biochemical Constituents of Follicular Fluid in Buffalo

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Abstract: Present study investigated the levels of some biochemical constituents and thyroid hormones in follicular fluid and serum and compared their concentrations in different size follicles of buffaloes. Buffalo ovaries with unknown reproductive status were collected from abattoir. The follicles visible on its surface were classified based on their diameter as small (<5 mm), medium (5-10 mm) and large (>10 mm) follicles. Twenty four samples from follicles along with blood samples of buffaloes belonging to respective category were collected. The follicular fluid and the serum samples were analyzed for total proteins, albumin, cholesterol, glucose and hormones tri-iodothyronine and thyroxine. The results of the present study revealed that, there was no specific trend observed in the levels of total proteins and albumin according to the size of the follicles but there was significant difference ($P<0.01$) in the levels of total proteins in follicular fluid and serum. The levels of total cholesterol showed increasing trend as the size of the follicle increases but the variations are not statistically significant. The serum total cholesterol concentration was significantly higher ($P< 0.01$) than that of follicular fluid. There was increasing trend of glucose concentration in the follicular fluid and also in the serum of respective buffalo with increase in the size of the follicle but the difference was non-significant. The serum glucose concentrations were significantly higher than the levels in the follicular fluid. There was no variation in the serum levels of triiodothyronine between buffalo bearing small and medium sized follicle while, highest serum level was observed in buffalo bearing large sized follicle. The significant ($P\leq0.01$) increase in the levels of T_3 in large sized follicle may attribute to the increase in the activity of monodeioniase enzyme. The follicular fluid thyroxine level showed increasing trend with the size of the follicle but the variation was not significant. There was no significant variation in the levels of T_4 in serum and follicular fluid in all the groups.

Keywords: Biochemical constituents, thyroxine (T_4), triiodothyronine (T_3), follicular fluid.

INTRODUCTION

Ovarian physiology is controlled by many exogenous and endogenous factors which include biochemical and endocrinological alterations occurring in follicular fluid during the oestrous cycle. Follicular fluid (FF) is part exudates of serum and is also partially composed of locally produced substances, which are related to metabolic activity of follicular cells. FF is viscous in nature is due to mucopolysaccharides and marked variations in color, from colorless to intense yellow, may be observed. Changes in follicular fluid may influence steriodogenesis, oocyte maturation, ovulation and transport of the oocyte and corpus luteum formation and function. Metabolic activity and blood-follicle barrier properties changes during the growth phase of the follicle and hence the different biochemical composition of the follicular fluid in different sized follicle could expect as reported by Alkalby *et al.*, [5]. The metabolites, ions, enzymatic characteristics and endocrine content of follicular fluid and oocyte development are highly correlated. Although recent development of ultrasonography enable researcher to monitor development of follicle in real time, follicular sample derived from abattoir continue to be cheap valuable material for investigating the biochemical content of the follicular fluid. Various reports suggest that follicular fluid proteins are derived from two sources: blood and surrounding somatic cell layers. As the research in follicular physiology advances, it is becoming clear that the fluid that bathes the granulosa and oocyte is not entirely exudates of serum. Follicular fluid contains several proteins, amino acids, sugars, enzymes, mucopolysaccharides, gonadotropins, vitamins and steroids. McNatty *et al*. [7] suggested utilization of follicular fluid as holding medium. Several studies have proved favorable effects of supplementing IVF medium with follicular fluid in pig. If follicular fluid could function as temporary environment for oocyte, researcher may someday be able to use this medium to preserve valuable genetic material for some time without damaging the oocyte. Thus the biochemical profile of follicular fluid in buffalo is a subject to practical importance.

MATERIALS & METHODS

Buffalo ovaries with unknown reproductive status were collected from Deonar Abattoir, Chembur, Mumbai, India during their evisceration. Pairs of ovary from each buffalo were collected in separate sterile plastic bags. The follicles visible on its surface were

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classified based on their diameter as small (less than 5 mm), medium (5 -10 mm) and large (more than 10 mm) follicles using digital vernier caliper. The fluids from these follicles were aspirated using tuberculin syringe. Twenty four samples each from small, medium and large sized follicles along with blood samples of buffaloes belonging to respective category were collected. Clear serum and follicular fluid was separated by centrifugation.

The follicular fluid and the serum samples were analyzed for total proteins, albumin, cholesterol, glucose, tri-iodothyronine and thyroxine. Total proteins, albumin, cholesterol, glucose were estimated using biochemical autoanalyser. Thyroid hormones were estimated by radioimmunoassay using the kits supplied by Board of Radioisotope Technology (BRIT), Bhabha Atomic Research Centre, Mumbai. Analysis of variance of the data was done according to Snedecor and Cochran [1] using Completely Randomized Design (CRD). Differences in means were tested using critical difference (CD) test.

RESULTS AND DISCUSSION

1. Total Proteins

As depicted in Figure **1** and Table **1** the mean values of total proteins in fluid of follicles and serum respectively, were: small size 6.18 ± 0.14, 7.02 ± 0.18; medium size 6.84 ± 0.20, 8.65 ± 0.40; large size 6.44 ± 0.16, 8.39 ± 0.22 g/dl. The levels of the total proteins concentration in follicular fluid found in present study are almost similar to the values reported by Shiny Joy [2] , Arshad *et al.* [3] in buffaloes and Leroy *et al.* [4] in dairy cows. The levels of total proteins in serum are almost similar to the levels reported by Alkalby *et al.* [5] in buffaloes. The lowest concentration (6.18 ± 0.14 g/dl) was observed in small sized follicle and highest (6.84 ± 0.20 g/dl) in medium sized follicle but the levels of total proteins did not significantly differ in medium and large sized follicles. Also there was no specific trend in the levels according to the size of the follicles. As regards to the relationship of the levels of total proteins in follicular fluid and in serum, there was the significant difference in the levels of total proteins in follicular fluid and serum. However Shiny Joy [2] and Jindal *et al*. [6] in their study in buffalo follicular fluid reported almost similar values in serum and follicular fluid. In present study, even though there was no specific trend observed in the levels of total proteins in the follicular fluid and serum, the concentration of total proteins in the serum has reflected on the levels of total proteins in the respective follicles. The levels of the total proteins concentration was significantly higher ($P < 0.01$) in the serum sample than respective follicular fluid.

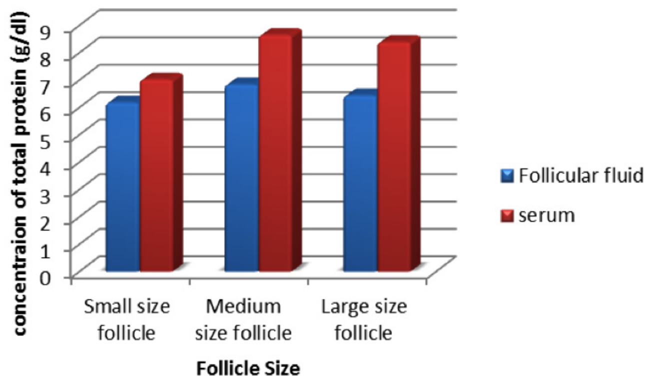

Figure 1: Profile of follicular fluid and serum total protein in buffalo.

2. Albumin

The mean values of albumin concentration in fluid of follicles and serum as depicted in Table **1** and Figure **2** respectively, were: small size 3.79 ± 0.11, 3.24 ± 0.12; medium size 4.24 ± 0.16, 3.74 ± 0.20; large size 2.93 ± 0.12, 2.70 ± 0.12 g/dl. The albumin concentrations in follicular fluid and serum in present study are almost similar to the levels reported by Shiny Joy [2] in buffalo. Lower levels of albumin in the large follicle are suggestive of actively developed follicle which needs amino acids and the ovary is one of the active tissues in catabolizing albumin. Also, the estrogen and water uptake relationship in growing follicle may dilute follicular protein concentration. The albumin concentration in the small, medium and large follicle was significantly higher ($P < 0.01$) than that of respective sera. The higher levels of albumin in follicular fluid than in the serum are also reported by Arshad *et al*. [3], McNatty *et al.* [7] and Ahmed [8] in buffaloes. The level of albumin in the serum has shown relation with the levels in respective follicular fluid i.e. low levels in serum corresponds to low levels of albumin in the respective follicular fluid. This indicates some relation with the levels of serum albumin with that of follicular fluid.

3. Total Cholesterol

As depicted in Figure **3** and Table **1** the mean total cholesterol concentration in fluid of follicles and serum respectively, were: small size 32.40 ± 2.10, 102.78 ± 4.94; medium size 34.40 ± 2.87, 99.74 ± 5.80; large size 38.97 ± 1.20, 102.53 ± 6.00 mg/dl. The serum

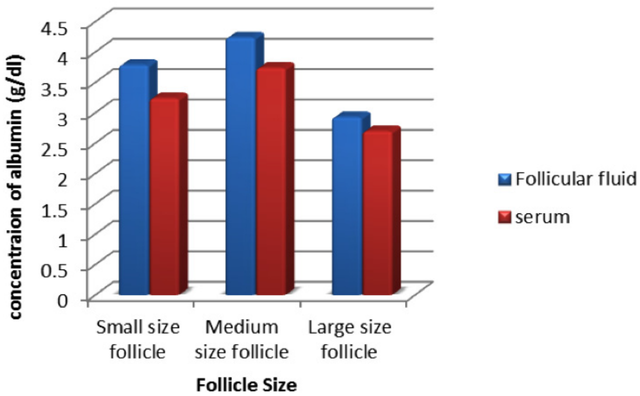

Figure 2: Profile of follicular fluid and serum albumin in buffalo.

levels of cholesterol are at par with the levels reported by Alkalby *et al*. [5] in buffalo. The levels of total cholesterol in follicle are almost similar with those reported by Khan *et al*. [9] in water buffalo and Abd Ellah *et al.* [10] during the oestrous cycle in buffalo. The levels total cholesterol showed increasing trend as the size of the follicle increases but the variations are not statistically significant. The observations that the total cholesterol concentration increased with the increase in the size of the follicle is in agreement with the findings of Leroy *et al.* [11] in dairy cows and Thakur *et al.* [12] in goats and Arosh *et al.* [13] in Bubaline follicular fluid. Higher cholesterol levels in large follicle reflect more influx of cholesterol from blood pool into follicular fluid for synthesis of follicular steroid hormones. Parmar and Mehta [14] further opined that endogenous synthesis of cholesterol in the follicle to meet the demand. Cholesterol in follicular fluid is derived from two sources, cellular synthesis from acetate and uptake from plasma lipoproteins. Higher total cholesterol concentration in large follicle can be explained by the increased permeability of the follicular wall in that follicle class, permitting the entrance of the large HDL fraction.

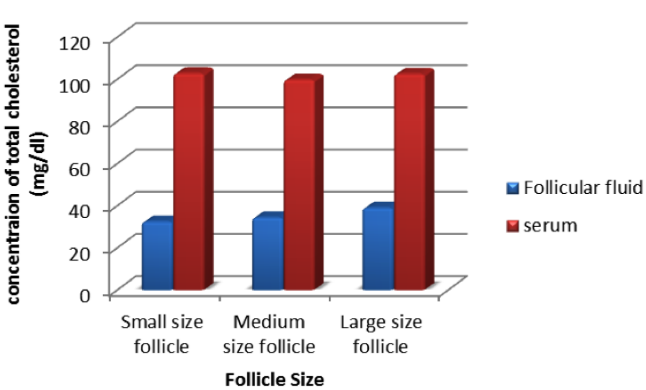

Figure 3: Profile of follicular fluid and serum total cholesterol in buffalo.

4. Glucose

The mean glucose concentration in fluid of follicles and serum respectively as depicted in Figure **4** and Table **1** were: small size 29.35 ± 2.92, 73.97 ± 3.70; medium size 33.88 ± 1.90, 75.15 ± 3.34; large size 36.39 ± 2.62, 78.09 ± 3.88 mg/dl. Follicular glucose levels are at par with the levels reported by Khan *et al.* [9] in buffalo. There was increasing trend of glucose concentration in the follicular fluid and also in the serum of respective buffalo with increase in the size of the follicle but the difference was non-significant. Similar trend was also reported by Arshad *et al*. [3], Nandi *et al.* [15], Abd El-Nasser *et al.* [16] in buffalo and Leroy *et al.* [11] in dairy cows and Thakur *et al.* [12] in goat. The results of the present study also indicated that the serum glucose concentrations were significantly higher than the levels in the follicular fluid. These findings are in agreement with the findings of Arshad *et al.* [3], Alkalby *et al.* [5], Jindal *et al.* [6] in buffaloes, Abd Ellah *et al.* [10] and Leroy *et al*. [11] in dairy cows. These findings imply that the principle source of follicular fluid glucose is blood and very little glucose is synthesized by granulosa cells of follicle. The present study revealed that the glucose concentrations were higher (even though non-significant) in large follicle than in small follicle.

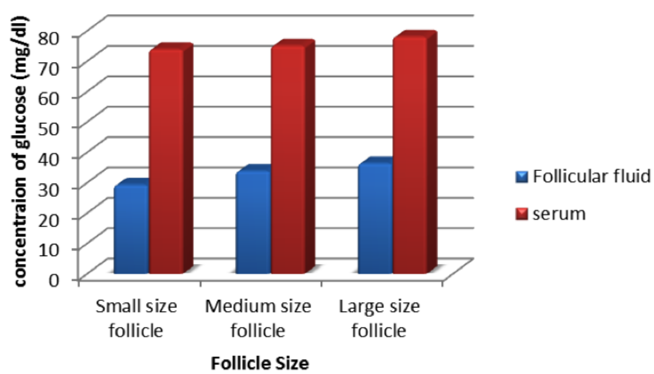

Figure 4: Profile of follicular fluid and serum glucose in buffalo.

5. Tri-iodothyronine

As depicted in Figure **5** and Table **1** the mean tri-iodothyronine concentration in fluid of follicles and serum respectively, were: small size 0.86 ± 0.05, 1.06 ± 0.08; medium size 0.75 ± 0.06, 1.04 ± 0.07; large size 1.14 ± 0.09, 1.58 ± 0.09 ng/dl. The significant ($P < 0.01$) highest level of 1.14 ± 0.09 ng/ml of T_3 is observed in large sized follicular fluid with no significant difference in the level of T_3 in small and medium sized follicular fluid. The levels of T_3 observed in our study are but lower than the values reported by Khan *et al.* [9] in preovulatory follicle (2.98 ± 1.43 ng/ml) and Arshad *et al.* [3] in small and large follicle (3.28 ± 0.30 ng/ml and 2.90 ± 0.26 ng/ml respectively) in buffalo. There was no variation in the serum levels of T_3 between buffalo bearing small and medium sized follicle. While highest serum level was observed in buffalo bearing large sized follicle. Though there was no specific trend observed in the levels of T_3 in follicular fluid according to size of the follicle, the significant higher concentration of T_3 in large follicle is contradictory to the findings of Alkalby *et al.* [5] in buffalo.

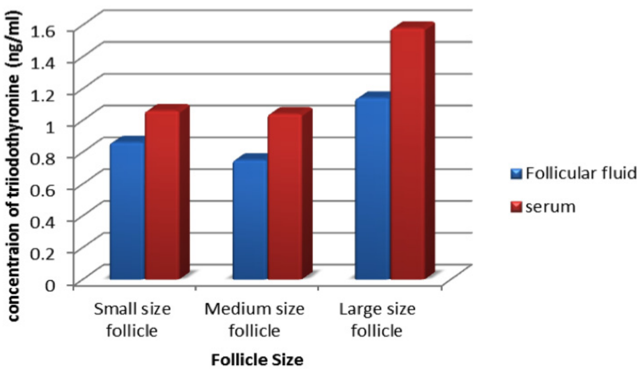

Figure 5: Profile of follicular fluid and serum triiodothyronine in buffalo.

6. Thyroxine

As depicted in Figure **6** and Table **1** the mean thyroxine concentration in fluid of follicles and serum respectively, were: small size 9.83 ± 2.01, 17.65 ± 3.60; medium size 12.46 ± 2.54, 21.49 ± 4.39; large size 12.64 ± 2.58, 17.84 ± 3.64 ng/ml. The levels of serum and follicular fluid T_4 observed in our study are lower than reported by Alkalby *et al.* [5] in buffalo and Khan *et al.* [9] in preovulatory follicles in buffalo. The follicular fluid thyroxine level showed increasing trend with the size of the follicle but the variation was not significant. These results are in agreement with the findings of Alkalby *et al.* [5] in buffalo in which they have reported that T_4 concentration was higher in large follicle than small follicle. In our study there was no significant variation in the levels of T_4 in serum and follicular fluid in all the groups. Ali *et al*. [17] in camel reported that, the animals having large follicles on their ovaries had significantly higher serum T_4 contents than those with small ovarian follicle.

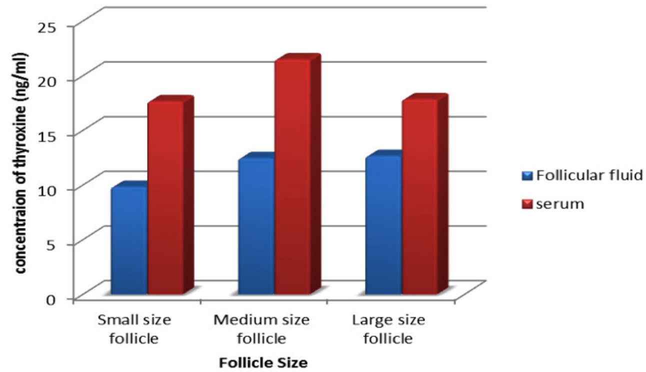

Figure 6: Profile of follicular fluid and serum thyroxine in buffalo.

Increased knowledge of metabolic and biochemical changes in relation to endocrine and structural

Table 1: Mean ± S.E of Biochemical Constituents and Thyroid Hormones of Follicular Fluid in Buffalo

Parameter	Small size follicle (<5mm)	Serum	Medium size follicle (5 – 10mm)	Serum	Large size follicle (>10mm)	Serum	C.D. Value
Total Protein (g/dl)	32.40^{b} ± 2.10	102.78^{a} ± 4.94	34.4^{b} ± 2.87	99.74^{a} ± 5.80	38.97^{b} ± 1.20	102.53^{a} ± 6.00	1%=0.96 5%=0.73
Albumin (g/dl)	3.79^{b} ± 0.11	3.24^{c} ± 0.12	4.24^{a} ± 0.16	3.74^{b} ± 0.20	2.93^{cd} ± 0.12	2.70^{d} ± 0.12	1%=0.52 5%=0.40
Total Cholesterol (mg/dl)	32.40^{b} ± 2.10	102.78^{a} ± 4.94	34.4^{b} ± 2.87	99.74^{a} ± 5.80	38.97^{b} ± 1.20	102.53^{a} ± 6.00	1%=15.64 5%=11.90
Glucose (mg/dl)	29.35^{b} ± 2.92	73.97^{a} ± 3.70	33.88^{b} ± 1.90	75.15^{a} ± 3.34	36.39^{b} ± 2.62	78.09^{a} ± 3.88	1% = 11.41 5% = 8.68
Tri-iodothyronine	0.86^{cd} ± 0.05	1.06^{bc} ± 0.08	0.75^{d} ± 0.06	1.04^{bc} ± 0.07	1.14^{b} ± 0.09	1.58^{a} ± 0.09	1%=27.65 5%=21.03
Thyroxine (ng/ml)	9.83 ± 2.01	17.65 ± 3.60	12.46 ± 2.54	21.49 ± 4.39	12.64 ± 2.58	17.84 ± 3.64	NS

Means with at least one common superscripts do not differ significantly (P<0.01). NS = Non significant.

alteration in growing and atretic follicles will lead to an understanding of the complexity of events surrounding the development of ovulatory follicle. If the genetic potential from *in-vitro* fertilization is to be maximized, thorough understanding of the basic biochemical changes ongoing during follicular development is required so that the optimal environment can be established for maturation of viable oocytes. It is documented that serum transudate, follicular fluid, also contain locally produced substances that share the metabolic activity together with the properties of blood-follicle barrier has been shown to change significantly during the growth phase of the follicle. Further studies were required for to determine the dynamics of other metabolites, enzymes, growth factors and minerals.

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