Studies on some Metabolites, Ionic and Hormonal Composition in Ovarian Follicular Fluid and Blood Serum in relation to size of the Follicle in Iraqi Buffaloes

دراسة بعض المركبات الأيضية والأيونية والهرمونية في السائل الجريبي المبيضي ومصل الدم وعلاقتهما بحجم الجريبة في الجاموس العراقي

H.M. Al-Rubaeae

Al-Furat Al-Awsat Technical University / Technical College - Al-Musaib

e-mail : drhashem48@yahoo.com

Abstract

The objective of this study was to determine the biochemical composition of follicular fluid obtained from different sized follicles and it's relationship with that of blood serum in Iraqi buffaloes. Ovaries of adult non-pregnancy and healthy buffaloes were collected from each buffalo at the time of slaughter. Follicular fluid was aspirated from small (3-10mm) and large (11-20mm) follicles and also serum separated and both stored at -20ºC for further analysis. The follicular fluid and blood serum sample were analyzed using commercial kits. The results showed that the follicular total protein concentration difference between small and large follicles was non-significant. The follicular glucose concentration was significantly increased ($p<0.05$) from small to large follicles. The follicular fluid concentration of total cholesterol and triglycerides, were significantly decreased ($p<0.05$) from small to large follicles. The blood serum concentrations of the total protein, glucose and total cholesterol were significantly higher ($p<0.05$) than follicular fluid in the small and large follicles, while blood serum triglycerides concentration was significantly lower ($p<0.05$) than follicular fluid in both classes of follicles. The follicular fluid calcium and sodium concentration were significantly increased with enlargement of follicular size. The follicular fluid potassium concentration of small follicles was significantly decreased than in large follicles, while no difference were found in concentration of magnesium between small and large follicles. The blood serum calcium, sodium and magnesium concentrations were significantly higher in comparison within follicular fluid, while the blood serum potassium concentration was significantly lower than follicular fluid in small and large follicles. The follicular fluid estradiol and progesterone concentrations were significantly increased with increased of follicular size while the blood serum estradiol and progesterone concentrations were significantly lower than follicular fluid in small and large follicles.

Key words: follicular fluid, metabolites, ions, hormones, serum, buffaloes.

المستخلص

هدف هذه الدراسة قياس المركبات الكيميوحيوية في السائل الجريبي الذي آخذ من جريبات مختلفة الاحجام بمصل الدم في الجاموس العراقي . جُمعت المبايض من جاموس بالغ وغير حامل وبحالة صحية سليمة ومن كل حيوان في وقت الذبح .سُحب السائل الجريبي من الجريبات الصغيرة (3-10 ملم) والكبيرة (11-20 ملم) وايضا فصل مصل الدم وخزن كلاهما بدرجة -20 درجة مئوية لحين التحليل .حللت عينات السائل الجريبي ومصل الدم باستعمال عدة تجارية . بينت النتائج اختلاف غير معنوي في تركيز البروتين الكلي في السائل الجريبي بين الجريبات الصغيرة والكبيرة . ارتفع معنوياً ($p<0.05$) تركيز الكلوكوز في السائل الجريبي من الجريبات الصغيرة الى الكبيرة .انخفض معنوياً($p<0.05$) تركيز الكوليستيرول الكلي و الدهون الثلاثية في السائل الجريبي من الجريبات الصغيرة الى الكبيرة. ارتفع معنوياً ($p<0.05$) تركيز البروتين الكلي والكلوكوز والكوليسترول الكلي في مصل الدم عن السائل الجريبي في الجريبات الصغيرة الى الكبيرة ، بينما انخفض معنوياً تركيز الدهون الثلاثية في مصل الدم عن السائل الجريبي في كلا الصنفين من الجريبات . ازداد معنوياً تركيز الكالسيوم والصوديوم في السائل الجريبي مع كبر حجم الجريبة .انخفض معنوياً تركيز البوتاسيوم في السائل الجريبي للجريبات الصغيرة عن الكبيرة ، بينما لايوجد اختلاف في تركيز المغنسيوم بين الجريبات الصغيرة والكبيرة. ارتفع معنوياً تراكيز الكالسيوم والصوديوم والمغنسيوم في مصل الدم مقارنة مع الموجود في السائل الجريبي ، بينما انخفض معنوياً تركيز البوتاسيوم في مصل الدم عن السائل الجريبي في الجريبات الصغيرة والكبيرة . ازداد معنوياً تركيز هرموني الموذق (estradiol) والحمل (progesterone) في السائل الجريبي مع زيادة حجم الجريبة ، بينما انخفض معنوياً تركيز هرموني الموذق والحمل في مصل الدم عن السائل الجريبي في الجريبات الصغيرة والكبيرة.

مفاتيح الكلمات : السائل الجريبي ، الايضيات ، الايونات ، الهرمونات ، مصل الدم ، الجاموس

Introduction

The water buffalo is an important livestock resource concentrated mostly in tropical and subtropical regions of the world (1). Buffalo is a multi-purpose species and contributed significantly to rural economy and dairy industry in many developing countries across the world (2). The reproductive physiology of buffalo is less understood, among which the knowledge of biochemical aspects of follicular fluid is the least. Follicular fluid originate mainly from the peripheral plasma by transudation across follicle basement lamina and accumulates in the antrum formed by the coalescence of small pocket of fluid (3).Therefore the composition of follicular fluid is similar, but not identical to that of the plasma (4). The ovarian follicular fluid provides suitable microenviroment for development, growth and maturation of the oocyte and is vital for maintenance of fertility in female (5).(6) demonstrated that the oocyte and granulosa cells grow and mature in a changing biochemical environment from small to large follicles. The metabolic activity of follicular cells together with the "barrier" properties of the follicular wall , is changing significantly during the growth phase of the follicles (7 , 8). Changes in biochemical constituents of blood are important indicators of physiological state of an animal (9). The principle source of follicular fluid glucose is blood and very little glucose, if any , is synthesized locally by the granulosa cells of follicles (10) . Blood glucose appears to be one of the key nutrients affecting ovarian activity in farm animals (11) .According to (7) the high correlation between total protein contents in follicular fluid and serum suggested that a substantial part of portion contents of follicular fluid originates from the serum. Cholesterol plays a significant role in the physiology of the ovary, as it is the precursor of steroid hormones secreted by this organ (12). Triglycerides might be the alternate sources of energy for cells in follicles (13). Biochemical metabolites concentration in the follicular fluid of the bovine ovary fluctuate considerably with the stage of cycle, follicle size and follicle status and presence of large follicles (14). Mineral are essential for growth and reproduction and are involved in a large number of digestive, physiological and biosynthetic process within the body (10).There was a highly significant positive correlation between the concentration of estradiol follicular size in the healthy cattle follicles (4). Several authors found that a high follicular fluid progesterone concentration was predictive of subsequent implantation and pregnancy (15,16). The aim of this present study was determine the values of metabolites (total protein, glucose, total cholesterol, and triglycerides), ions (calcium, potassium, sodium and magnesium) and hormones (estradiol and progesterone) in follicular fluid and blood serum in relation to follicular size in buffaloes.

Materials and methods

Collection of ovaries and processing of follicular fluid.

Two hundred and forty ovaries from 120 non-pregnant adult buffaloes in good health and with normal reproductive tracts upon macroscopical examination after slaughtered at abattoir of Province of Babil were collected during the period from January to December 2014. Those ovaries were immediately warped in plastic bags placed in an ice box and taken to the laboratory of department of technical animal production, Al-Musiab technical college within one hours after slaughter. In the laboratory the ovaries washed twice in 0.9% Nacl saline and than cleaned of any extraneous tissue. After that ,each ovary was examined for the presence of ovarian follicles . The diameters of the ovarian follicles for each female were measured with help of vernier calipers . Based on this ,the follicles were divided into two categories according to their diameter, small (3-10mm) and large (11-20mm). The follicular fluid from each follicle was collected from different sized follicles by aspiration using sterile disposable syringe fitted with 18-22 gauge needle. The follicular fluid obtained from small and large follicles from the same animal were pooled separately which yielded one sample for each sized follicle. The follicular fluid was then transferred to a 10 ml conical tube and allowed to settle for 20 minutes . The pooled follicular fluid from two different sized follicles were centrifuged at 3000 rpm for 15 minutes to remove the blood cells ,oocytes and granulosa cells, if any. Following centrifuged , the supernatant was collected ,fractioned into small tube and stored at -20˚C until used for further analysis.

Collection of blood samples

Jugular blood sample were collected aseptically from each buffalo into glass tube containing EDTA (ethylene diamine tetra acetic acid) as an anticoagulant, at the time of slaughter. Those tubes were placed in an icebox beside the ovaries and were carried to the laboratory. In the laboratory , those sample were centrifuged at 4000 rpm for 10 minutes to remove blood cells. Serum sample was separated and stored at- 20˚C for further investigation.

Biochemical analysis

The follicular fluid of small and large follicles and blood serum sample were analysed for various metabolites , ions and hormones concentrations. The concentration of each parameters in different groups was repeated at least three time. Metabolites such as (total protein, glucose, total cholesterol and triglycerides) ,ions (calcium, potassium sodium and magnesium) and hormones (estradiol and progesterone) were analysed using appropriate commercial kits. The concentration of glucose and total cholesterol were measured through colorimetric method by spectrophotometer (PD303-Germany) using commercial kits (Cromatest kits, Spain) at 505 and 490 nm absorbance respectively (17). Triglycerides and total protein were measured through colorimetric method commercial kits (Biolabo kits , France) at 500 and 550 nm absorbance respectively (18). Sodium and potassium concentration were estimated by colorimetric method using commercial kits (Stanbio kits, USA) and were measured photometrically at 546 and 580nm absorbance respectively (19). The concentrations of calcium and magnesium were evaluated spectrophotometrocally using commercially available kits (Biosystem kits ,Spain) at 600 and 520nm absorbance respectively (20). Follicular fluid and blood serum concentration of estradial and progesterone hormones were measured by microplate enzyme immuno assay method using commercial kits (Accu-bind kits ,USA) at 450nm absorbance (21). All measurement were carried out according to manufactures instructions.

Statistical analysis

The mean values ±sem for concentration of various biochemical composition of follicular fluid of small and large follicles and blood serum were computed. The variation in concentrations of various biochemical composition of follicular fluid and serum, the data were subjected to any way analysis of variance using completely randomized design (22) . The Duncan's multiple range test(23) was used to separate between significant means . Significance and non-significance of difference between mean values was determined at the 5% level of significance ($P<0.05$).

Results and Discussion

Total protein: The total protein concentration in follicular fluid of small and large follicles were 5.76± 0.79 and 6.07±0.92 gm/dl respectively (Table1). In present study the deference in total protein concentration between two follicular classes was non-significant.This indicates that the follicular contents of total protein do not change with increased in follicular growth, and also indicating that the total protein concentration may not have any specific bearing on the process of follicular development (11).These results in the present study were in agreement with the findings of (24), (10) and (25) in buffaloes, (6) and (13) in cattle, (26), (27) and (28) in camels, who reported that total protein concentration was relatively uniform throughout the follicular development. However, our results differed from those of (12) in buffaloes, and (7) and (29) in cattle, (30) in camels, and (31) in sheep and (32) and (33) in goats, Who reported a decrease in the total protein concentration as the follicles size increased. The serum concentration of total protein (7.92±0.97 gm/dl) was significantly higher than the concentration measured on small and large follicles. This results in agreement with(10) and (25) in buffaloes, (6) observed that serum contents of total protein were significantly higher than small and large follicles. According to(7), the high correlation total protein contents in follicular fluid and serum that substantial part of protein contents in follicular fluid originate from the serum.

Glucose: In the present study, follicular fluid concentration of glucose in large follicles (67.70±1.72mg/dl) were significantly higher ($P<0.05$) when compared with fluid from small follicles (43.47±0.28mg/dl) (Table1), These results were in agreement with previous reports in buffaloes (10) and (25), (6) and (13) in dairy cattle, (26) in camels, (34) in Sheep and (32) in goats. However, our results are contrast with finding of (35) in buffaloes and (30) in camels, who demonstrated that the glucose concentration was significantly lower ($P<0.05$) in large follicles compared with small follicles. The results showed also that the serum glucose concentration (89.02±3.20 mg/dl) was significantly higher ($P<0.05$) than follicular concentration (Table1). The same observation were previously reported on buffaloes (10) and(25). There is a possibility that the metabolism of glucose is less intensive in large follicles compared with small ones. Resulting in lower consumptions of glucose from fluid of large follicles (6). In large follicles relatively smaller number of granulosa cells consume glucose from a relatively large amount of follicular fluid (8). A further reason for this observation could be the increased permeability of the blood follicle barrier during follicular growth (36).

Table1: Mean (± SE) concentration of various metabolites in follicular fluid from different sized follicles and blood serum in Iraqi middle buffaloes

Follicles / Metabolites	Small follicles (3-10mm)	Large follicles (11-20mm)	Blood serum
Total protein (gm/dl)	5.76±0.79 B	6.07±0.92 B	7.92±0.97 A
Glucose (mg/dl)	43.47±0.28 C	67.70±1.72 B	89.02±3.20 A
Total Cholesterol (mg/dl)	70.25±6.8 B	58.80±8.0 C	118.59±5.32 A
Triglycerides (mg/dl)	32.56±3.40 A	24.77±2.52 C	27.33±2.20 B

Values with different letters within a row differ significantly ($P<0.05$)

Total cholesterol: Concerning the total cholesterol the results showed that the follicular fluid concentration of total cholesterol in small (70.25±6.80 mg/dl) follicles were significantly higher ($P<0.05$) than that in large (58.80±8.0mg/dl) follicles (Table1). In the present study, the total cholesterol concentration decreased with increased in follicular size, which was in agreement with findings of (12), (37), (3) and (35) in buffaloes, and (13) and (38) in cattle, and (30) and (26) in camels. Total cholesterol is consider the precursor of all steroid hormones, including estrogen and progesterone. Therefore the low concentration of total cholesterol in the large follicles indicates the biotransformation of cholesterol to sex steroids (30). The results showed that serum consternation of total cholesterol (118.59±5.32mg/dl) were significantly higher ($P<0.05$) than in small and large sized follicles. Total cholesterol in follicular fluid was in form of a constituent of high-density lipoprotein (HDL) (34). Therefore, the a vascular granulosa cells of the follicles totally depended on the total cholesterol from high-density lipoprotein, which was derived from the blood plasma by crossing the basement membrane of granulosa cells (33). However, the results of the present study differed from those reported in buffaloes (10,25), in cattle (6,39) in camels (27,28,40), in sheep (34), in goats (32,33). The discrepancy between these results may be due to differences in size and number of selected ovarian follicles used in each study, or this discrepancy is due to species differences or otherwise is not clear.

Triglycerides: Regarding the triglycerides the study showed that the follicular fluid concentration of triglycerides in small size follicles was 32.56±3.40 mg/dl, while for large sized follicles this value was 24.77±2.52mg/dl (Table1). Triglycerides are considered the storage form of lipids and their hydrolysis results in one molecule of glycerol and three molecules of fatty acid. Therefore, the decreased concentration of follicular fluid triglycerides with follicular development could be due to the alternate sources of energy for the cells in the follicles (6). Another reason for the high concentration of triglycerides in small follicles was that triglycerides didn't pass through the follicular membrane (41), and follicular triglycerides concentration were mainly a result of local metabolic processes (6). These results are in agreement to those reported for buffaloes by (25), and for cattle by(6)and (13), and for camels by(42),(26) and (28), and for sheep by(34). The results showed also that serum concentration of triglycerides (27.33±2.20mg/dl) were significantly lower ($P<0.05$) than in small and large sized follicles (Table1). A relatively stable concentration of triglycerides is maintained in the bovine ovarian follicles, concerning on increases in serum due to physiological status or diet (34).

Calcium: Concerning the calcium the results showed that the concentration of calcium significantly higher ($P<0.05$) in fluid from large follicles (7.69±1.29 mg/dl) than in fluid from small follicles (5.91±1.48 mg/dl), and there was a significant deference between serum and follicular fluid calcium concentration, the values being higher in the serum (9.91±1.75mg/dl) than in follicular fluid from either follicle class (Table2). The increase concentration of calcium in large follicles observed in the present study were in agreement to those reported for buffaloes by (44), (45), (46) and(47), in cattle(7), in camels (48), in sheep(34), in goats(49). Increase calcium concentration with follicular development had a role in steroidogenic capability of growing follicle and calcium played an important role in gonadotrophin regulation of ovarian steroidogenosis and ovulation (34). The calcium one of the macro-mineral which play a major role in reproductive, it's deficiency can effect fertility (50). The free calcium ions seem to be involved in the regulation of oocyte growth and meiosis restoration at the begining of oocyte maturation (51). However, our results differed from these of(10) and (3) in buffaloes, and (28) in camels, who reported there was no effect of follicular size on follicular calcium contents. This discrepancy between these results may be attributed to the deferences between species.

Potassium: In this study, the potassium concentration in small and large sized follicles classes in buffaloes were 10.83±1.70 and 6.67±1.89 mg/dl respectively (Table2). The higher concentration of potassium in small follicles compared to those in large ones in present study were in accordance with the reports those in buffaloes (10,45) in cattle (6,7), in camels (28) , in sheep (34) and in goats (49). the decreased concentration of follicular fluid potassium with follicular development could be due to the increased use of glucose by developing follicles a process that leeds to transfer of potassium ions from extracellular sites to intracellular sites (52). The serum concentration of potassium (5.02±1.40mg/dl) was significantly lower ($P<0.05$) than follicular fluid concentration (Table 2). Similar observation were made in dairy cows by(6) and in camels by (28). The significant higher concentration of follicular potassium relative to serum concentration and the absence of correlation indicating that potassium may be released locally in the follicular fluid (25).

Sodium: In the present study, follicular fluid of sodium concentration (51.61±1.20 mg/dl) in large follicles were significant higher ($P<0.05$) when compared with from small (46.27±2.02mg/dl) follicular (Table2). However serum sodium concentration (62.03±3.02mg/dl) was significantly higher than that in follicular fluid of small and large follicles (Table2). The results were in agreement with previous reports in buffaloes (45), in cattle (7), in camels (40) and (28), in sheep

(34), in goat (49), However our results are in contrast with the finding of (10) Who demonstrated that there was no effect of follicular size on follicular fluid sodium contents. Increased follicular fluid sodium concentration were related to follicles viability and were linked to the active follicular synthesis of estrogen (7). Enlargement of the follicle dimension with follicular growth was largely due to the movement of water from blood to antrum, a process that requires an osmotic gradient across the follicular wall. Thus, increased sodium concentration in large follicles could create an osmotic gradient across the follicular wall to facilitate osmosis (53).

Table2: Mean (± SE) consternations of various ions and hormones in follicular fluid from different sized follicles and blood serum in Iraqi buffaloes

Follicles	Small follicles (3-5mm)	Large follicles (11-20mm)	Blood serum
Ions			
Calcium (mg/dl)	5.91±1.48 C	7.69±1.29 B	9.91±1.75 A
Potassium (mg/dl)	10.83±1.70 A	6.67±1.89 B	5.02±1.40 C
Sodium (mg/dl)	46.27±2.02 C	51.61±1.20 B	62.03±3.02 A
Magnesium (mg/dl)	8.23±1.80 B	7.43±0.78 B	11.46±2.12 A
Hormones			
Estradiol 17-β (pg/ml)	338.50±13.57 B	715.50±31.70 A	66.24±10.27 C
Progesterone (ng/ml)	12.32±2.67 B	24.03±4.23 A	1.24±0.20 C

Values with different letters within a row differ significantly ($P<0.05$)

Magnesium: In present study indicated that the difference in follicular fluid magnesium concentration between small (8.23±1.80mg/dl) and large (7.43±0.78 mg/dl) follicles were non-significant, and there was significantly higher ($P<0.05$) concentration of magnesium in serum (11.46±2.12 mg/dl) as compared to follicular fluid from each sized follicles (Table2).The non-significant higher concentration of magnesium in small follicles compared to these in large ones in the present study were accordance with the reports those in buffaloes (45), (46) and (25), in cattle (7), in camels (28), in sheep (34) and in goats (49). The higher concentration of magnesium in the small follicles could help the mitosis of the follicular cells through the formation of the thrombin , a potent mitogen (54). Magnesium could substitute for calcium in through formation under low Ca/Mg ratio condition that exist in small follicles (34). As magnesium was antagonistic to calcium, the decreased magnesium with follicular development facilitated the calcium action in large follicles (46).

Estradiol 17- β: The results of the present study also indicated the concentration of estradiol 17-β was significantly lower ($P<0.05$) in follicular fluid (338.50±13.57 pg/ml) obtained from small follicles than those collected from large follicles (715.50±31.70pg/ml) (Table2). Table 2 depicts the serum concentration of estradiol 17-β (66.24±10.27pg/ml) were significantly lower ($P<0.05$) than is follicular fluid in small and large follicles . The follicular fluid concentration of large follicles were higher than serum (715.50±31.70pg/ml versus 66.24±10.27pg/ml). Therefore, large follicles are main source of serum estrogen. The estradiol 17-β alone has little effect on granulosa cells in maturing follicles, but its effect is important in initiating LH receptor expression and responsiveness (55), antrum formation (56) and prevention of atresia (57). The ovarian follicular fluid concentration are much higher than those in peripheral circulation (58, 59). A recent study on the follicular dynamics of silent estrus in buffaloes demonstrated smaller size and slower growth rate of the dominant ovulatory follicle associated with lessser concentration of estradiol (60) In agreement with our study in buffaloes (61), (44) and (35), in cattle , (62) and (63) in camels (64), in sheep (65) and (66), and in goats (67). These observations confirm our finding in the present study.

Progesterone: The higher concentration of progesterone reported in the present study in large (24.03±4.23ng/ml) compared to those in small follicles (12.32±2.67ng/ml), and also the results showed that the concentration of serum progesterone (1.24±0.20ng/ml) was significantly lower ($P<0.05$) than in follicular fluid obtained from all follicles categories (Table2). Progesterone which plays a key role in the regulation of the oestrous cycle. Several studies have reported on peripheral progesterone concentration during oestrous cycle of buffalo (68,69). The change in concentration of progesterone in blood during the oestrous cycle in buffaloes are similar to those in cattle, but the peak concentration is relatively less (70). (71) claimed that acyclic buffaloes showed a serum progesterone concentration can lesser than 0.5 ng/ml, whereas the concentration were greater than 0.5 ng/ml during all the phases of oesterous cycles. For assessing cycles ovarian activity and for early diagnosis of non-pregnant, progesterone concentration above 0.9 ng/ml are considered to be indicative of the presence of luteal function and those below 0.3 ng/ml are considered indicative of the absence of luteal function, with intermediate values considered inconclusive (72). the same observation were previously reported in buffaloes (44,73, 74), in cattle (62,75) and in sheep and goats (67).

Conclusion

In conclusion, based on these data we can infer that the concentrations of total protein, glucose, total cholesterol, calcium, sodium and magnesium were significantly higher in serum than that in follicular fluid. Follicular fluid concentration of triglycerides, potassium, estradiol 17-β and progesterone were significantly higher than serum concentrations. The metabolites, ions and hormonal concentrations in the follicular fluid fluctuate considerably with the follicle size. Follicular fluid composition provide a useful information about requirement for oocyte and follicular growth *in vitro* and may be indices a guide for cell culture and growth process.

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