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Association Between Glucose, Cholesterol and Total protein of Ovarian Follicular Fluid and Follicle Size in Different Seasons of Iraqi Sheep

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Abstract The present study was undertaken at Al-Qadisiyah slaughter house located in Al-Diwaniyah City, throughout the period from (June –August 2023) and (Dec. – Feb. 2024) to evaluate certain biochemical components(Glucose. Cholesterol and Total Protein) in the follicular fluid of Iraqi Sheep. A total of (240)ovaries were collected from (120) adult non-pregnant ewes(72 samples of follicular fluids for each size, 12samples/month), age ranging between (2 to 3) years old in a good health with normal genitalia tracts. The diameter of ovarian follicles were measured using calipers, the follicles were distributed into three categories: small (<3mm), medium (3-5mm) and large (6-10 mm), follicular fluid aspirated per follicle size within the same ewe and centrifuged at 4000 rpm for 10 min. the supernatant was collected to use for determine concentration of glucose, Cholesterol and total protein in the ovarian follicular fluid. The results shown that season of year and size of follicle affected significantly($P<0.01$) on glucose and cholesterol concentration, while total protein concentration did not influenced by season and follicle size, in addition, there was a highly significant differences($P<0.01$) recorded among sized follicles within the seasons. However, the concentrations of glucose, cholesterol and total protein in the follicular fluid were decreased as the follicle sizes increased.

Keywords: Sheep, Follicular fluid, Follicle ,Biochemical Components.

Introduction Sheep are considered one of the major economically important livestock in Iraq . Playing an important role in providing food source . They provide with different types of products such as meat, milk and wool (1) .The follicular fluid is a complex dynamic biological fluid that surrounds the developing oocyte. It is produced in growing antral follicles and contains a variety of molecules, such as proteins, steroid hormones, polysaccharides, metabolites, reactive oxygen species, and antioxidants (2). It is forms the biochemical environment of the oocyte before ovulation (3) The constituents of follicular fluid are regarded as a regulating factors in follicular development steroid genesis (4). The concentrations of metabolites(glucose, total protein and cholesterol) may be decrease or increase in different sizes of follicles related with different seasons (5,6,7),There are several studies performed on follicular fluid content such as biochemical components (glucose, cholesterol, total proteins, albumin, globulin and steroids) in camel (8) , cattle (9), and goats (10). Although biochemical parameters in buffalo (11) and goat (12) are available, the information's on biochemical analysis of follicular fluid of ewes are either little or limited, so the present study was performed to evaluate the association between some biochemical components and follicle size in different seasons of year in Iraqi Sheep.

Materials and Methods

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Ethical approval

The study was approved by the Committee for Research Ethics at the College of Vetewrinary Medicine, University of Al-Qadisiyah, Iraq.

A total of (240)ovaries were collected from (120) adult non-pregnant ewes(72 samples of follicular fluids for each size , 12samples/month) throughout the period from (June –August 2023) and (Dec. – Feb. 2024), age ranging between (2 to 3) years old in a good health with normal genitalia tract, slaughtered at Al-Qadisiyah slaughter house located in Al-Diwaniyah city were used in this study. Ovaries were transported in box contain cold normal saline, to the lab of the college of veterinary medicine within 2-4 hours, the diameters of the ovarian follicles were measured using calipers, the follicles were distributed into three categories; small (<3mm), medium (3-5mm) and large (6-10 mm). Follicular fluid was aspirated by a sterile disposable 1ml insulin syringe gauge (27×1/2mm) ,the follicular fluid transferred to Eppendorf tube and allowed to settle for 15 min, the fluid was centrifuged at 4000 rpm for 10 min. the supernatant was collected for analysis for biochemical components, the measurement of parameters was carried out according the instructions of manufacturers kits company (Beckman Coulter Company / USA.) using a full automated Clinical chemistry analyzer (AU480 Clinical Chemistry System (Beckman Coulter Company / USA).

Statistical analysis

Data of the present study was expressed as Means ± SE and the statistical analysis was done by using SPSS (Statistical package for social science for Windows version 16.0. (13) using one way ANOVA , followed by Duncan's multiple range test (14) to compare the significant differences among means of the different parameters. Differences were considered to be significant at (P<0.05).

Results

► Glucose Concentration

The overall mean of glucose concentration in follicular fluid was(18.510±0.381 mg/dl),the results of the current study presented in Table(1) demonstrated that season of year and follicle size were affected significantly(P<0.01) on glucose concentration of follicular fluid. However the highest mean value of glucose concentration have been shown during winter(23.755±0.310 mg/dl)and the lowest mean value was(13.264±0.159 mg/dl) during summer. On the other hand there was a highly significant(P<0.01) differences among different sizes of follicle, being medium follicle(3-5mm) exceeded other follicles in glucose concentration(19.998±0.920 mg/dl),also there was a highly significant differences(P<0.01) recorded among sized follicles within the seasons. The level of glucose observed in follicular fluid of medium follicles was significantly difference (p<0.01) from follicular fluid of small and large size in both seasons, it was lower in summer(11,842±0.082mg/dl) and higher(28.155±0.095mg/dl) in winter season .

Table -**1**- Influence of season of year and follicle size on concentration of glucose(mg/dl) of follicular fluid in Iraqi sheep

Follicle size	Summer (June, July, August)		Winter (Dec., January , Feb.)		Total (No.)	Overall Means ± SE
	Sample of Follicular Fluids No.	eans ± SE	Sample of Follicular Fluids No.	Means ± SE		
3mm<	36	18.545^{c} ±0.0484	36	27.542^{a} ±0.561	72	23.043^{a} ±0.578
3-5mm	36	19.465^{b} ±0.098	36	12.142^{e} ±0.116	72	15.803^{c} ±0.419
6-10mm	36	18.597^{c} ±0.26	36	14.980^{d} ±0.103	72	16.788^{b} ±0.247
Overall means of seasons	36	18.869^{a} ±0.101	36	18.221^{b} ±0.643	72	18.545 ±0.352

a ,b ,c ,d ,e ,f Means in the same column or row with different superscripts differ significantly(P<0.01).

► Cholesterol concentration

The overall mean of cholesterol concentration in follicular fluid was (18.545±0.352 mg/dl)(Table.2),the influence of season of year on cholesterol concentration was highly significant ((P<0.01),being the highest mean value of cholesterol was(18.869±0.101 mg/dl) during summer and the lowest mean value was (18.221±0.693 mg/dl) during winter, also there were a highly significant(P<0.01) differences among different sizes of follicle being, the small follicle(<3mm) was recorded the highest level of cholesterol (23.043±0.578 mg/dl) and medium follicle(3-5mm) registered the lowest value(15.803±0.419mg/dl).,in addition, it was observed that differences in follicular fluid concentrations of cholesterol among different sized follicles within both seasons were statistically significant (P<0.01). However the follicular fluid of

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small follicle in winter season superior other follicles in the level of cholesterol(27.542±0.561mg/dl).

Table 1: Data across seasons

Follicle size	Summer (June, July, August)		Winter (Dec., January, Feb.)		Total (No.)	Overall Means ± SE
	Sample of Follicular Fluids No.	Means ± SE	Sample of Follicular Fluids No.	Means ± SE		
<3mm	36	18.545^{c} ±0.0484	36	27.542^{a} ±0.561	72	23.043^{a} ±0.578
3-5mm	36	19.465^{b} ±0.098	36	12.142^{e} ±0.116	72	15.803^{c} ±0.419
6-10mm	36	18.597^{c} ±0.26	36	14.980^{d} ±0.103	72	16.788^{b} ±0.247
Overall means of seasons	36	18.869^{a} ±0.101	36	18.221^{b} ±0.643	72	18.545 ±0.352

a ,b ,c ,d Means in the same column or row with different superscripts differ significantly (P<0.01).

► Total Protein Concentration

The overall mean of total protein concentration in follicular fluid was (6.225±0.206 g/dl),the results presented in Table -3- showed that season of the year had no significant effect on total protein concentration, however the highest mean value of the total protein was recorded during winter (6.428±0.069 g/dl) and the lowest mean value during summer season (6.023±0.406g/dl) , also there was non-significant differences among different sizes of follicle , it was observed that the level of total protein decreases as the follicular size increases where the highest mean value was (6.917±0.595g/dl) for small follicles(<3mm) and the lowest mean value was (5.520±0.107 g/dl) for large follicles(6-10mm). On the other hand the level of total protein within summer season observed in follicular fluid of small follicles differed significantly(p<0.05)from follicular fluid of large follicle(7.340±1.181g/dl)vs.(4.720±0.088g/dl) .

Table-**3**- Influence of season of year and follicle size on concentration of total protein(g/dl) of follicular fluid in Iraqi sheep

Follicle size		Summer (June, July, August)	Winter (Dec., January , Feb.)		Total (No.)	Overall Means ± SE
	Sample of Follicular Fluids No.	Means ± SE	Sample of Follicular Fluids No.	Means ± SE		
<3mm	36	7.340a ±1.181	36	6.495a ±0.171	72	6.917a ±0.595
3-5mm	36	6.010ab ±0.142	36	6.470a ±0.87	72	6.240a ±0.087
6-10mm	36	4.720b ±0.088	36	6.427a ±0.077	72	5.520a ±0.107
Overall means of seasons	36	6.023a ±0.406	36	6.428a ±0.069	72	6.225 0.206±

a ,b Means in the same column or row with different superscripts differ significantly((P<0.05).

Discussion

Glucose is the main energy substrate in the ovary, and a sufficient supply of it is necessary to sustain the ovarian activity(15). It is plays an important role through anaerobic pathway, leading to formation of lactate (16). It has a key role in processes of metabolism in the ovaries, Consisting from the glycolysis in the granulose cells (17).In the present study we observed that concentration of glucose in follicular fluid of large follicular size was significantly lower (P<0.01) as compared with other follicles. The possibility that glucose metabolism is less intensive in large follicles compared with small ones, resulting in a lower consumption of glucose from follicular fluid and in a reduced secretion of lactate into the follicular fluid (18) . our results are in accordance with the previous studies (8,19) in camel (20) in buffaloes, (21,22) in sheep. However, other studies in Iraq have found that the concentration of glucose in follicular fluid was increased as the follicular size increased (23) in buffaloes, (24,25) in sheep , (26) in goats. The concentration of glucose may be decrease or increase in different sizes of follicles associated with different seasons (5,7).also

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we observed a significant effect of season on glucose concentrations. This result is in agreement with those stated by (22,24) in sheep who found that the glucose concentration changed significantly between the seasons. Glucose is a universal fuel for the energy metabolism and biological synthesis pathways of all animal cell types (27). Glucose has been reported to be an essential energy substrate for maturation of bovine oocytes (28,29). .

Cholesterol: Cholesterol in follicular fluid derived from two sources, cellular synthesis from acetate and uptake from plasma lipoprotein, Cholesterol plays a significant role in the physiology of ovary as it is the precursor for steroid hormone synthesis and the follicular fluid contained only high-density lipoprotein, therefore the avascular granulosa cells of the follicles totally depended on the cholesterol from high-density lipoprotein, which was derived from the blood plasma by crossing the basement membrane of granulosa cells (12).The results of the current study demonstrated that the cholesterol concentrations in small follicles were significantly higher ($P<0.01$) than in large follicles. Similar results were obtained by (29,23,30) in buffaloes, (10,18,31,32) in cows and (26) in goats.

The decreased cholesterol concentrations in large follicles might be attributed to the conversion of cholesterol to steroid hormones, estrogen and progesterone during steroidogenesis.. However, our results differed from those of (33) in buffaloes, (21,34) in sheep. Who found that Cholesterol concentration in relation to size of follicle increased significantly ($P<0.05$) as follicle diameter increased. In addition, the results obtained in the current study showed that there were a significant seasonal variations in cholesterol concentration between seasons. These results were in agreement with those reported by (22,24) in sheep, (35,36,37,38) in camels. .

Total protein: in the present study we observed that total protein concentration in the follicular fluid was higher in small follicle as compared to other follicles but the differences in follicular fluid concentration of total protein between different sized follicles were statistically non- significant. The possible reason might be the follicular contents of total protein did not change with increased in follicles growth, and it may not have any specific bearing on the process of follicular development. (33).

The concentration of total protein in ovarian follicular fluid indicating that it may not have a specific impact on the process of follicular development (32,39). Similar results obtained by (18) , in cows, (12,21,25) in sheep , (26) in goats, (30) in buffaloes .who reported a decrease in the total protein concentration as the follicle size increased.However, our results differed from that demonstrated by, (20 , 40) in buffaloes, (31)in cows. On the other hand, we observed non-significant effect of season on total protein concentrations .this result was in agreement with the findings observed by (22,24) in sheep.

Conclusion:

It could be concluded that glucose and cholesterol concentrations of ovarian follicular fluid decrease or increase in different sizes of follicles associated with different seasons of year, while total protein concentration did not correlated to sizes of follicles or season of year in this study.

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Conflict of Interest

The authors declare that there is no conflict of interest in publishing this paper.

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