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Effect of Season on some Biochemical Components of The Ovarian Follicular Fluid in Local Iraqi Ewes

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ABSTRACT

This study was conducted in the technical college of Mussaib/ department of Animal Production Techniques to investigate the effect of season on some follicular fluid biochemical parameters in the ovaries of the-*local Iraqi ewes* . Fifty ovaries were collected from Karbala and Najaf butcheries for two seasons: the first season (August 2021) and the second season (January 2022). Moreover, collected follicles were divided according to their sizes into three groups (first group < 3 mm), (second group 3-5 mm), and (third group > 5mm) The results showed a significant increase ($P \leq 0.01$) for protein, glucose, cholesterol, and sodium, and a significant increase ($P \leq 0.05$) for calcium in the first season compared to the second season, and the significant increase was ($P \leq 0.05$) for potassium in the second season compared to the first season. As for the follicles sizes the result exhibited, there were highly significant differences in the size of the follicles for the first group (< 3 mm) $P \leq 0.01$, as it reached in the first season 8 (32.00%) and in the second season 13 (52.00%) and also found highly significant differences in the size of the follicles for the second group (3-5 mm) 11 (44.00%) in the first season and 6 (24.00%) in the second season ($P \leq 0.01$). for the follicles of the third group (> 5mm), there were no significant differences, as it reached 6 (24.00%) in the first season and 6 (24.00%) in the second season. The groups also differed significantly within the same season. The highly significant increase for the second group was ($P \leq 0.01$) compared to the rest of the groups within the first season, while in the second season, the high significant increase was for the first group ($P \leq 0.01$) compared to the rest of the groups.

Keywords: Follicles, season, Iraqi local sheep, Ionic, and metabolic components,

الخلاصة

أجريت التجربة في الكلية االتقنية المسيب / مختبر الفسلجة التابع لقسم تقنيات الانتاج الحيواني لقياس الصفات الكيموحيوية في مبايض الاغنام المحلية العراقية وجمعت لهذه الدراسة (50) جهاز تناسلي أنثوي للأغنام المحلية من مجزرة كربلاء والنجف لموسمين: الموسم الاول (أب) للعام2021والموسم الثاني (كانون الثاني) للعام2022. أظهرت النتائج وجود زيادة معنوية ($P \leq 0.01$) للبروتين والكولوكوز والكولسترول والصوديوم ومعنوية($P \leq 0.05$) للكالسيوم في الموسم الاول مقارنة بالموسم الثاني, وكانت الزيادة عالية المعنوية ($P \leq 0.05$) للبوتاسيوم في الموسم الثاني مقارنة بالموسم الاول .اما بالنسبة للمقارنة بين للفئات الحجمية وجود فروقات معنوية عالية في حجم الجريبات للفئة الاولى عند مستوى معنوية($P \leq 0.01$) اذ بلغ في الموسم الاول 8 (32.00%) وفي الموسم الثاني 13 (52.00%) وأيضا وجد فروقات عالية المعنوية في حجم الجريبات للفئة الثانية اذ بلغ في الموسم الاول 11 (44.00%) وفي الموسم الثاني 6 (24.00%) عند نفس مستوى المعنوية اما في الجريبات الفئة الثالثة فلم تكن هناك فروقات معنوية اذ بلغ في الموسم الاول 6 (24.00%) وفي الموسم الثاني 6 (24.00%).كما أختلفت الفئات معنويا ضمن الموسم الواحد، فقد كانت الزيادة عالية المعنوية للفئة الثانية ($P \leq 0.01$) مقارنة ببقية الفئات ضمن الموسم الاول,اما في الموسم الثاني فقد كانت الزيادة عالية المعنوية ($P \leq 0.01$) للفئة الاولى مقارنة ببقية الفئات.

الكلمات المفتاحية : الجريبة، الموسم، الاغنام العراقية المحلية ، الايونات، والمركبات الايضية

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1. INTRODUCTION

The Primary follicle is considered the basic reproductive unit in the ovary of females and the storage of these follicles is fixed and non-renewable after birth it is surrounded by one layer of epithelial cells. During its growth stages, the follicle passes through the primary, secondary , and mature follicles [1]. Follicular fluid is produced locally during the metabolic activity of the follicle cells ,and part of it is filtered from the blood serum and its formation begins in the developing follicle [2]. The follicular fluid contains ionic components such as sodium, magnesium, calcium, chlorine, phosphorous, hormones and metabolic compounds such as glucose, triglycerides, protein and total cholesterol [3]. Our study aimed to know the effect of season on some biochemical parameters of ovarian follicular fluid in local Iraqi ewes.

2. MATERIALS AND METHODS

Fifty genitals of slaughtered ewes were collected, 25 for each season from different regions in Najaf and Karbala provinces. The follicular fluid was withdrawn from each follicle using sterile medical syringes with a volume of 1/2ml, and kept in different tubes (2 ml) regarding the follicle sizes at a temperature of 5º C until the analysis was performed [4] Metabolic, ionic analysis and folliculometry were conducted in the reproductive physiology lab / Animal Production Techniques Department at Al- Mussaib Technical College for the period from August 2021 to January 2022. Kits from Human company (Germany) were used to evaluate the level of metabolic components (cholesterol, glucose, protein, cholesterol and triglycerides) as well as ionic components (Sodium, potassium and calcium). In addition**,** Visible follicles on the surface of the ovary were measured starting with those with a diameter of 1 mm and up, and the outer diameter of the follicle was measured using the Vernia and the follicles were classified according to these measurements into three groups (first group < 3 mm), (second group 3-5 mm), and (third group > 5mm) [3]. The obtained data was analyzed using SPSS V. 24, (IBM USA) software. Student t- test with P-value ≤ 0.05 was used for data analysis.

3. RESULTS AND DISCUSSION

3.1 Metabolic Compounds

3.1.1: Glucose and protein

The obtained results showed (table 1)that there are highly significant differences ($P \leq 0.01$) for total protein in the first season(August) (7.99 ±0.21g/ dL) and in the second season (.89 ±0.10 g/ dL), while for glucose there are also high significance differences ($P \leq 0.01$) in the two seasons ,as it reached for the first season (8.84 ±0.43mmol/L) and second season (2.73 ± 0.11mmol/L) [5,6] indicated that the increase in proteins in the follicles during the first season (the reproductive season) is expected. Several studies indicated the presence of many growths of follicles as a result of reproductive activity and the high level of hormones during the reproductive

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season. The results also showed that the percentage of small follicles in the ovaries of the first season was %32.00 which is less than in the second season (52.00%) as shown in Table 1. However, this may not be the general trend, since the period during which the ovaries were collected is few and confined to a few days of August the first season ,(which is the month closest to the reproductive season ,and the increase in the size of the follicles may have the main causes for partially compensated of the lack of protein in the small follicles. The main sources of glucose in the follicular fluid comes from filtration of blood serum, while the second source of it comes from synthesis by follicular cells ,especially granulosa cells [7]. Glucose plays important role in ovarian metabolism because it is the main source of energy in the ovary through its metabolism by an anaerobic pathway and formed the lactate [8]. Furthermore, since the first season in this study is the closest to the reproductive season, it is expected to get accelerated growth for follicles during this period which led to an increase in glucose concentration compared to the second season [9]. mentioned that the significant superiority of glucose concentration with the increase in the size of the follicle may be attributed to its metabolism and consumption by the follicle cells whose number increases with the development of the follicle size when compared with the medium and small follicles. When the follicles grow, the barriers between them and the blood become more permeable, and therefore glucose is filtered in large quantities into the follicular fluid from the blood serum [10]. The results of this study are in agreement with the above as well as with [10,7] in their study on goats, they noticed an increase in glucose concentration with the increase in the size of the follicle, and it agrees with [11, 12]

Table1. effect of the season in the level of glucose and total protein of the ovarian follicular fluid in local Iraqi ewes

Periods	Mean ± Standard Deviation	
	Glucose (mmol/L)	Total Protein (g/dl)
First Season (August)	8.84 ±0.43	7.99± 0.21
Second Season(January)	2.73 ±0.11	5.89 ±0.10
Sig. level	**	**
**P ≤ 0.01		

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3-1-2: Triglycerides and cholesterol.

The results in table 2 indicated that there were no significant differences in triglycerides between the first season and the second season. As for cholesterol, a highly significant ($P<0.01$) was found in the first season (1.62 ±0.06 mmol/L) and in the second season (0.962 ±0.03 mmol/L). In general, total cholesterol in the follicular fluid is derived from two sources, the first source is from acetate which is produced by the follicular cells, especially granulosa cells, and the second from lipoproteins filtered from the blood serum [13]. The follicular fluid contains only high-density lipoprotein (HDL),therefore, the vascular granulosa cells of the follicles depend on the cholesterol filtered from these lipids derived from the blood plasma through their passage through the basement membrane of the granulosa cells, and they do not depend on the molecules of low-density lipoproteins (LDL) because they have large molecules in size that cannot pass through the barriers that separate the blood and the follicle [14]. The low level of total cholesterol in the follicular fluid of small follicles may be due to the increased consumption of it in the proliferation, growth and development of granulosa cells. Therefore, it is withdrawn from the follicular fluid into the cells, and its quantity decreases ,and when the growth of the follicles is completed, their proliferation decreases and they begin to excrete cholesterol in the fluid. Cholesterol is considered as precursor for the synthesis of steroid hormones [2] .The results of our study agree with some published studies, including the study of [2] in sheep**,** [15] in cows, and with [16] in local buffaloes, as they showed an increase in the level of total cholesterol with an increase in the size of the follicle.

Table2 . effect of the season in the level of triglycerides and cholesterol of the ovarian follicular fluid in local Iraqi ewes

Periods	Mean ± Standard Deviation	
	Triglycerides (mmol/L)`	Cholesterol (mmol/L)
First Season (August)	0.984 ±0.10	1.62 ±0.06
Second Season(January)	0.782 ±0.05	0.962 ± 0.03
Sig. level	NS	**
**P ≤ 0.01 ,(NS :not significant).		

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3-2: Ionic Compounds

The results in Table 3 showed that there were significant differences in the level ($P \leq 0.01$) for sodium ions, as it reached in the first season (213.56 ±5.17 mmol/L) and in the second season (147.17±4.27 mmol/L). Also, significant differences were found for potassium ion ($P \leq 0.01$) for the two seasons, as it reached in the first one (2.11 ±0.09 mmol / L) (and in the second 6.28 ±0.19 mmol/L). Moreover, a significant improvement in calcium ions was found in the first season (10.04±1.12mmol/L) and in the second season (7.79±0.10mmol/L). This result is a regular reflection of the reproductive activity in the reproductive season closest to the first season .(The significant increase in sodium ions in this season is due to the increase in vesicular growth in this period [17,7]. For the second season, (a period of decrease in reproductive activity) ,there is decreasing in follicular growth ,which reflected the decreasein the concentration. of sodium ions.

The increasein sodium concentration in large follicles is related to the production of estrogen hormone, which production increases with the increase in the size of the follicle due to the ability of this hormone to retain the sodium ion inside the cell [18] .These results are in agreement with the study by [3,2] on sheep. It also agrees with [19]. In cow, with [20] in cattle, and with [21] in camels. As for Potassium the decrease in the rate of potassium ion level with the growth and development of the size of the follicle in the first season may be due to the increased consumption of glucose for energy for the development and growth of the cells of the follicle ,and this effort leads to the transfer of potassium ion from outside the cell (from the follicular fluid to the inside of the cell) in the follicle [22]. Regarding the calcium ion, the results indicated that the level significantly increased in the first season (10.04 ± 1.12 mmol/L) in comparison with the second season (7.79± 0.1 mmol/L) . it is well known that calcium ion plays an important role in the formation of estrogen and regulate gonadotropic hormones for the process of lipogenesis in the ovary and ovulation [18]. The free calcium ion plays a role in regulating oocyte development and determining meiosis at the beginning of follicle and oocyte maturation [23].

Table3 effect of the season in the level of sodium, potassium and calcium ions of the ovarian follicular fluid in local Iraqi ewes

Periods	Mean ± Standard Deviation		
	Sodium (mmol/L)	Potassium (mmol/L)	Calcium (mmol/L)
First Season (August)	213.56 ± 5.17	2.11 ±0.09	10.04 ±1.12
Second Season(January)	147.17 ±4.27	6.28 ±0.19	7.79 ±0.10
Sig. level	**	**	*
**P ≤ 0.01, * P ≤ 0.05			

3-3:The size of the follicles

The results shown in Table 4 indicated that there are highly significant differences in the size of the follicles for the first group (<3 mm) (P ≤ 0.01), as it reached in the first season 8 (32%) and in the second season 13 (52.00%). Also found highly significant differences in the size of the follicles for the second group (3-5mm) as it reached in the first season 11(44 %) and in the second season 6 (24%) , ,but in the follicles of the third group (more than 5 mm) there were no significant differences, as it reached 6(24%) in the first season and the second season 6(24%). From these results, it indicated that the relationship of the size of the follicles to the season is not completely clear (lack of clarity of the seasonality of reproductive in local Iraqi sheep or due to the small sample size in this study) . At a time when the small follicles in the first season were less than they are in the second season, the relationship was reversed in the medium-sized follicles, and then the differences in large follicles vanished, and the abundance of small follicle growths in the second season compared to the first season may be due to the decline in reproductive performance in this cold period of the year and led to a decrease in the medium-sized follicles due to the lack of opportunity to obtain nutrients due to competition between the small follicles compared to the first season .

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Table4: Percentage distribution of the studied sample according to the follicles sizes for the two seasons

Groups	First the season (August) the number(%)	The second season (January) the number(%)	P-value
First group (<3 mm)	(%32.00) 8	(%52.00) 13	0.0084 **
Second group (3-5)	(%44.00)11	(%24.00) 6	0.0084 **
Third group (>5mm)	(%24.00) 6	(%24.00) 6	N 1.00
Total	25	25	---
P-value	0.0087 **	0.0052 **	---

**P ≤ 0.01 ,(NS :Not significant.)

4. CONCLUSIONS

The effect of metabolic components in different seasons, increased in the first season (August) and decreased in the second season (January), ionic compounds also increased in the first season(August), except for potassium, which decreased in the season compared to the second season (January), the seasons also did not differ in the size of mature follicles, but differed in the smaller size groups.

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Conflict of interests.

There are non-conflicts of interest.

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