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Article

Correlation of Metabolic and Ionic Components of Follicular Fluid with Estrogen Concentration

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Abstract: This study was aimed to assess the correlation between the metabolic and ionic composition of ovarian follicular fluid and its relationship to the concentration of estrogen and prolactin hormones as the follicle size changes in the local female cows. Ovaries were collected (80 ovaries) from non- pregnant 40 adult females cow at Hilla slaughterhouse at Babil province. The gathered ovaries were transported to the laboratory within two hours. Follicular fluid was pulled from small (2-5 mm) follicles, medium (6-10 mm), and large (11-20 mm) follicles, then it stored at 5 ° C until assayed. Follicular fluid samples were analyzed for metabolites composition of (cholesterol, total protein, and glucose), and ionic composition (calcium, phosphorus, copper, magnesium, zinc) ,and hormone concentration (estrogen and prolactin). The results of the study showed that the total protein and glucose concentrations were increased significantly(p> 05.0) with increased of follicle size. The study also showed the calcium, copper, and phosphorus concentration were increased significantly(p> 05.0) with changes of follicular size, as well as the estrogen concentration was significantly increased (p > 05.0) With increased follicular size. A significantly (p> 05.0) correlation was found between both magnesium, zinc ions, and estrogen hormone.

Keywords: follicular fluid, follicular size, ionic, Estrogen

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1. Introduction

Follicular fluid is a complicated mixture composed of serum and secretions that were created from granular cells and derived from plasma into the follicular cavity [1]. Follicular and oocyte were grown and matured in a biochemical condition, which correlated with the change in the follicular size from small to large. In addition, all materials that have been found in the follicular fluid has a relationship with oocyte maturity [2]. Follicular fluid also has different functions such as keeping the oocyte division in steady-state, oocyte protection from decomposing during the ovulation process and affects the maturity and fertility of the oocyte [3]. The constituents of follicular fluid change throughout the growth state [4], and there is a relationship between follicular size and follicular fluid [5].

Furthermore, the follicular fluid contains metabolic constituents (total protein, and cholesterol), ions (Magnesium, and Sodium), hormones (estrogen), lipids, inhibition, and growth factors [6]. These compounds have a direct effect on the maturity and fertilization of the oocyte [7]. Cholesterol considered as a raw material used for manufacturing the steroid hormones [8]. While Glucose affects the completeness and final maturity of the cow oocytes as well as after fertilization and growth to the macrophage stage [9]. When the average protein content in the food increased, the concentration of amino acids in the follicular fluid will increase, and this affects the growth and follicles differentiation as well as oocyte production numbers for each follicle. Moreover, their effect on the development stages and differentiation of oocyte synthesis [10]. Ions level and their concentration within the follicular fluid are not constant due to their contents change as a result of changing in the size of follicles and vital operations [11]. Ions stimulate enzymes and hormones system [12] . Mineral enzymes, which minerals considered as an essential component, are very important for steroid hormones synthesis (estrogen and progesterone) [13]. Some of the ionic constituents of the follicular fluid (Calcium and Copper) their concentrations increase with increasing the size of the granule. The matured follicles contain a high concentration of steroids compared to the secondary follicles [14]. Investigation of follicular fluid components provides a clear picture of follicle and oocyte essential requirements for the continued growth and maturity so it can be easy to oocytes maturate and fertility in the laboratory (in-vitro). According to the above information, this study aimed to determine the relationship between metabolic components (Cholesterol, glucose, total protein), ions (Calcium, phosphorus, Copper, and Magnesium) and the concentration of estrogen in the follicular fluid.

2. Materials and Methods

2.1. Samples collection and follicular fluid

This study was carried out in the laboratories of the Technical animal production department/Technical college of Mussaib, during the time from April 2018 until June/2019. Eighty ovaries were collected form forty non-pregnant cows, which were slaughtered during the breeding season in Babil province. Selected cows were in good health and with normal reproductive tracts during the examination. Collected ovaries then were warped in plastic bags that contain natural physiological salt solution (NaCl) with a concentration of 0.9% and sited in an icebox for farther investigation within two hours after slaughtering. The reproductive tracts were washed with natural cold salt solution and left to dry and then were cleaned from any extraneous tissue that might be present on the ovaries. After that, ovarian follicles for each female were measured using Vernier calipers. Based on the above measurements, follicles were divided into three groups regarding their diameter, small (2-5 mm), medium (6-10 mm), and large (11-20 mm). Follicular fluid was pulled for each follicle using sterile disposable syringes of (1, 5, 10 mL) in size and fitted with (23-29) gauge needle [15]. Follicular fluid contents were collected from each species and animal individually and then mixed with the follicular fluid that taken from the follicles of the matching group in the same day (for every collection process) and put in 10 mL test tube (Centrifuge tube) and allowed to settle down for 15 minutes. Next, the pooled follicular fluid from three different sized follicles was centrifuged with 3000 rpm for 10 min. and floating follicular fluid was pulled using a sterile pipette and keep it at -5 C until analysis.

2.2. Biochemical Analysis

Samples of the follicular fluid were analyzed to quantify the metabolic, ionic, and hormonal constituents. The concentrations of total protein, glucose, and cholesterol were measured using Spectrophotometer (PD303-, Germany) with a commercial kit (Bio labo kit, France) at 564 and 490 nm, respectively. Ionic components were measured by techniques according to company kits instructions, (Kit, Spain Cromates) for Mg and Cu with wavelength 520 nm, (RANDOX-Kit England) for Ca and P with wavelength 570 nm, while Zinc ions were measured using (Spectrmm Kit –England) with wavelength 520 nm. Besides that, Estrogen hormone was measured by (AccuBind, USA) kit using Enzyme-Linked Immune Sorbent Assay (ELISA) (Metertch- Germany) with 620 nm wavelength, on the other hand, (Biotech) kit was used for prolactin hormone measurement with wavelength 450 nm.

2.3. Statistical Analysis

Data were analyzed using SPSS version 23.0 [16]. Comparing the means among different parameters was achieved using an independent sample t-test (2-tailed). All the results are detailed as mean ± standard deviation of mean and considered statistically significant when p £ 0.05 [17].

3. Results and Discussion

For metabolic constituents, The studying results exhibited that the concentration of cholesterol in large size of follicles (6.78 ± 2.70 mg/dL) was higher in comparison with a small size of follicles (3.48 ± 2.91) with no significant differences for all sizes as shown in Table (1). Usually, vascular granulocytes for ovarian follicles need cholesterol as they grow up and reproduce. Therefore, cholesterol is withdrawn from the follicular fluid. As a result, it will decrease in the small size of follicles. when the size of the follicles increased, their follicular cells proliferate and start to release cholesterol into the follicular fluid and use it for fat hormones/adipose manufacturing. (Estrogen and progesterone) [18].

Table (1) also shows a significant increase in the total protein concentration as the follicles size increased. In contrast, its concentration in follicular fluid of small follicles was (44.84 ± 0.10 mg/dL), and its concentration increased in ovarian follicular fluid to (53.71 ± 0.12 and 61.195 ± 0.13) mg/dL for both medium and large follicles respectively. The rising in total protein concentration for the large follicles may be attributed to the increasing production of fat hormones/adipose, which required to bind with proteins to transport these hormones [19].

For the glucose component, the outcome results showed, glucose concentration levels were significantly elevated as the size of follicles increased (37.52±1.15, 50.84±2.03, and 70.69±2.50) mg/dL, respectively. This significant increase in the glucose concentration with the increase of follicles size might be due to the metabolism of glucose in the large follicles is less in comparison with small size [20], as well as less consumption by the granule cells in large follicles, this results in agreement with [21].

Table 1. Metabolic compounds concentration for different ovarian follicles size

Metabolic compounds	Small follicle (2-5)mm	Medium follicle (6-10)mm	Large follicle (11-20)mm	P Value ≤0.05
Total cholesterol (mg/dL)	3.48±2.91	4.97±2.81	6.78±2.70	NS
Total protein (mg/dL)	44.84±0.10	53.71±0.12	61.195±0.13	Sig.
Glucose (mg/dL)	37.52±1.15	50.84±2.03	70.69±2.50	Sig.

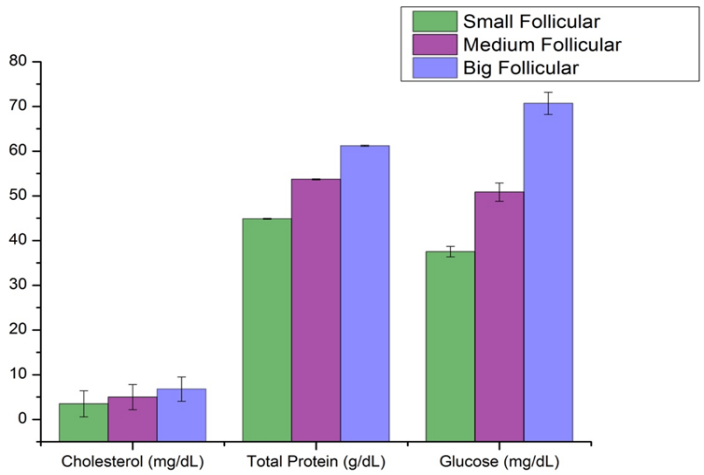

Figure 1. cholesterol, total protein, and glucose concentrations in different follicular sizes

For the ionic compounds, the obtained results showed, calcium concentration in the ovarian follicular fluid significantly increased as the size of the follicles increased (Table 2, Figure 2) which reaches in large size follicles (9.18±0.25) mg/dL, and (6.09±0.30, 7.49±0.35) mg/dL for medium and small sizes of follicles respectively. This trend in calcium concentration is due to the vital role of calcium in the synthesis of steroid hormones like estrogen. Estrogen concentration level increase as the follicles size increase for that reason, it needs to draw more calcium concentration from blood to follicular fluid as the size of the follicles increased [22] .

Moreover, the results demonstrated that there is a significant decrease in phosphorous concentration as the size of follicles increased (8.70±0.84, 6.24±0.33, and 2.72±0.62 mg/dL) for small, medium and large size of follicles respectively. This might be due to the crucial acts for phosphorous ions in metabolic activity for cell and energy production [23]. Table 2 (figure 2) also demonstrated that there is no significant change in Magnesium concentration as the size of the follicles increas , Magnesium may assist in the filamentous division of follicular cells during thrombin formation [24] .

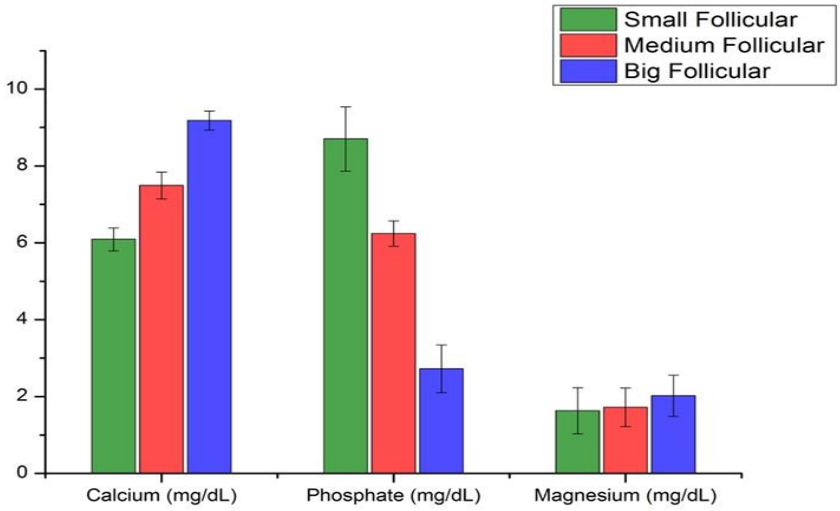

Figure 2. calcium, phosphate, and magnesium concentrations in different follicular sizes

Furthermore, Table 2, Figure 3, showed a significant elevation in Copper ion concentration from small to medium and large follicles with 75±0.09, 198±0.13, and 225±0.32, respectively. Our explanation for this elevation is due to the vital role for copper in the material metabolic process as well as it works as a structural component for some important enzymes and leads to increase the activity of sexual hormones [25] . While for zinc ion, the outcome results indicated that there is a significant decreasing as the size of follicles increased (Table 2, Figure 3). Our finding agrees with [26] . zinc ion considered as the basic raw material for the formation of elements in lager follicles as well as it is so important for sexual maturity and reproductive ability.

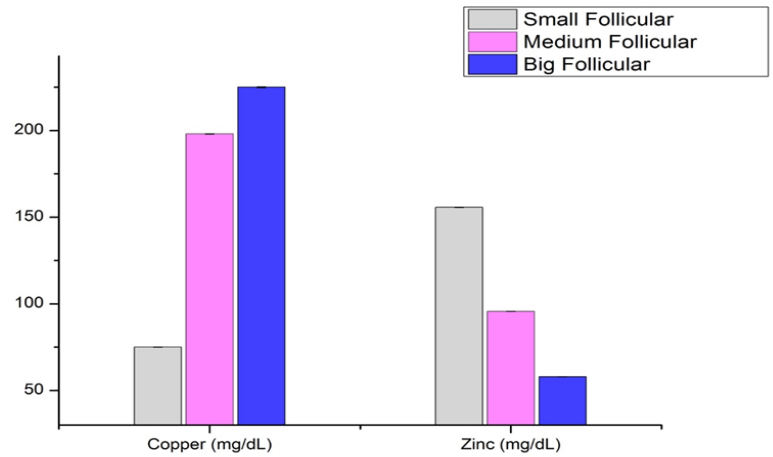

Figure 3. Copper and zinc concentrations in different follicular sizes

Table 2. Ionic constituents in different sizes of ovarian follicles

Ionic compounds	Small follicle (2-5)mm	Medium follicle (6-10)mm	Large follicle (11-20)mm	P Value ≤0.05
Calcium (mg/dL)	6.09±0.30	7.49±0.35	9.18±0.25	Sig.
phosphor (mg/dL)	8.70±0.84	6.24±0.33	2.72±0.62	Sig.
copper (mg/dL)	75±0.09	198±0.13	225±0.32	Sig.
Magnesium (mg/dL)	1.63±0.60	1.72±0.50	2.02±0.54	NS

Zinc (mg/dL)	155.6±0.14	95.67±0.07	57.84±0.05	Sig.

For hormonal constituents, table 3. presented a significant increase in estrogen concentration as the follicle size increased (258.21±3.21, 343.32±3.83, and 589.12±3.66) for small, medium, and large respectively. The theca cells convert cholesterol in to testosterone under LH influence, which later gets converted in to estradiole in granullosa cells under the effect of FSH [27]. While for the prolactin hormone, the obtained results showed that there are no significant differences between all follicle sizes. Prolactin hormone inhibits the growth of ovarian follicles and ovulation; as a result, this leads to infertility and lower estrogen hormone levels [28].

Table 3. hormonal constituent concentrations in different sizes of ovarian follicles

Hormonal constituents pg/ml	Small follicle (2-5)mm	Medium follicle (6-10)mm	Large follicle (11-20)mm	P Value ≤0.05
Estrogen	258.21±3.21	343.32±3.83	589.12±3.66	Sig
Prolactin	2.77±2.62	2.21±1.24	1.77±1.12	Ns

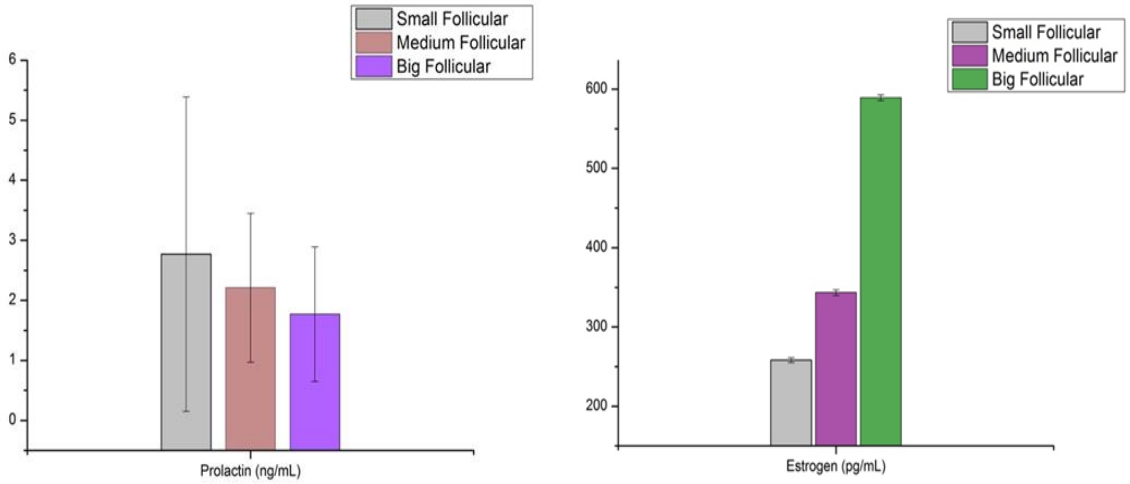

Figure 4. prolactin and Estrogen hormones concentrations in different follicular sizes

Table (4) showed a negative correlation (not significant) (-0.24) between total cholesterol and estrogen hormone. Cholesterol considered as a precursor for fat hormones by Hydo-xylation starting pathway, which gives Pregnenolone and then oxidized to give progesterone that oxidized to androgen hormone and estrogen as well [29]. Moreover, Table (4) exhibits that there is a positive correlation (non- significant) between total protein and estrogen hormone (0.35). Protein usually contained binding protein (Albumin) that transfers hormones and fat proteins, which are considered a source for free cholesterol that entered in the production of estrogen and progesterone hormones [30]. Correlation between glucose and estrogen hormone was negative, non-significant, with(- 0.26). It was well known that glucose is vital for the biological activity of hormones [31, 32].

The table also shows a positive and non-significant association between calcium and estrogen with (0.47). Calcium depends on a mechanism for vital participation in the manufacture of steroid hormones from the adrenal gland in Pregnenolone and ovary [33}.

There is a non- significant negative association between phosphorus and estrogen hormone with (-0.30), phosphorous is one of the constituents of nucleic acids and some proteins; also it considered as energy transfer element and metabolism of phospholipids, and a major part of many coenzymes such as CAMP and for that reason has the most important effect on reproduction [34, 35]. A positive (non-significant) correlation for copper and estrogen hormone (0.42) was obtained while for magnesium and zinc with estrogen hormone the results showed there were significant positive associations reaches 0.57, and 0.55 respectively, as shown in below table. Early embryonic death, resorption of embryo and increased necrosis of the placenta are common symptoms for copper deficiency [36]. Same thing for zinc which plays a vital role in reproductive hormones and immune system furthermore, zinc is known to be essential for sexual maturity and many other things [37; 38]. Moreover, table (4), showed a non-significant negative (-0.36) correlation between prolactin and estrogen hormones, it is well known that prolactin hormone regulates LH secretion and the former has a positive direct action with FSH hormone in the Estradiole-17 synthesis [39].

Table 4. correlation of metabolic and ionic components with Estrogen hormone

Parameters corelation	Correlation Coefficient (r)	p-Value
Total cholesterol and Estrogen	-0.24	Ns
Total protein and Estrogen	0.35	Ns
Glucose and Estrogen	-0.26	Ns
Calcium and Estrogen	0.47	Ns
Phosphorus and Estrogen	-0.3	Ns
Copper and Estrogen	0.42	Ns
Magnesium and Estrogen	*0.57	Sig.
Zinc and Estrogen	*0.55	Sig.
Prolactin and Estrogen	-0.36	Ns

$P \leq 0.05$

4. Conclusion

This study exhibited that the follicular fluid components and their concentrations varied according to the stage of development of follicle. And these components are used as a guide for the essential info in the maturation of follicular cells and oocytes Outside the animal's body (in vitro).

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