STUDY ON SOME HORMONAL AND BIOCHEMICAL CONSTITUENTS OF FOLLICULAR FLUID AND BLOOD PLASMA IN BUFFALOES

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ABSTRACT

Ovarian physiology is controlled by many exogenous and endogenous factors including the endocrinological and biochemical alteration that occur in the follicular fluid during the estrus cycle. the aim of this study was to determine and compare the concentrations of some hormonal and biochemical composition in peripheral circulation and follicular fluid of buffaloes .For this purpose ,ovaries collected from adult buffaloes immediately after slaughter, and blood samples where also collected from these buffaloes before slaughter in Basra abattoir.

Samples were classified into two groups according to the follicle diameter, small (4-8 mm) and large (> 8 mm). The follicular fluid and plasma samples were analyzed for biochemical composition using commercial kits.

The results showed that small follicles had significant higher ($p<0.05$) , T3, glucose, and cholesterol concentrations than large follicles while no differences were found in concentrations of TSH between both groups .The concentrations of ,T4, progesterone, estrogen, and total protein were significantly higher ($P< 0.05$) in large follicles than small follicles.

The results of the present study also indicated that the TSH, T4, glucose, total protein and cholesterol concentrations in the blood plasma were significantly higher

($p<0.05$) than follicular fluid in both groups. There were no significant differences in concentrations of T3, and progesterone between blood plasma and follicles.

INTRODUCTION

Follicular fluid (FF)Is in part exudates of serum and is also partially composed of locally produced substances, which are related to the metabolic activity of the follicular cells (1).Follicular fluid is an a vascular compartment with in the mammalian ovary, separated from the perifollicular stroma by the follicular wall that constitutes "blood-follicle barrier"(2) changes in (FF) may influence steroidogenesis, oocyte maturation, ovulation and transport of the oocyte to the oviduct as well as the preparation of the follicular for subsequent corpus luteum formation and function(3). Metabolic activity and blood-follicle barrier properties change during the growth phase of the follicle and hence a different biochemical composition of the FF indifferent size follicles could expected (4). As the oocyte and granulosa cells grow and mature in a biochemical environment that changes from small to large follicles ,the metabolite, ion and enzymatic characteristics of FF and follicle or oocyte development are highly correlated (5) Although recent development of ultrasonography enables researches to monitor development of follicles in real time, follicular samples derived from abattoir-harvested ovaries continue to be cheap and valuable materials for investigating the biochemical natures of follicular fluid(6).The biochemical method utilizes the ratio of estradiol and progesterone in FF as criterion to determine follicular health(7)(8). The synthesis of steroid hormones by the developing follicle is dependent upon the presence and activation of several key proteins (9)(10).

Although gonadotropins play a major role in the regulation of follicle function(11),also other factors such as thyroid hormones have been evaluated as potential regulators in ovarian steroidogenesis(12)(13). protein in human follicular fluid originate from plasma or produced by follicular structures (14) and the cholesterol is the precursor of steroid hormones. Consequently, the concentration of total protein and cholesterol may indicate follicular and ovarian functions. Glucose was crucial energy substrate for ovarian activity (15)(16).The ovaries of the cow are the major source of oestrogens,androgens and progestin found in peripheral blood (17).Hormonal concentrations in the follicular fluid of the bovine ovary fluctuate considerably with the

stage of the cycle follicular size and status(8).The aims of this study was to determine and compare the concentrations of some hormonal and biochemical composition in peripheral and follicular fluid of buffaloes in Basra governorate.

MATERIALS AND METHODS

Collection of blood samples:

About 10ml of blood samples were collected from the jugular vein of each buffalo cow before the time of slaughter in a test tube containing EDTA (ethylene diamine tetra acetic acid) as anticoagulant.

These tubes were placed in icebox and carried to the laboratory of Veterinary Medicine College, these samples were centrifuged at 5000rpm for 5 minute, the plasma was separated and stored at -20c for further analysis.

Collection of ovaries;

Pairs of ovaries from 30 mature, nonpregnant buffalo cows were collected after slaughter in the winter season, 2009 from Basra abattoir. the ovaries were wrapped in plastic sheet, placed in icebox and taken to the laboratory within 90 minutes after slaughter .the ovaries washed with normal saline, each ovary was examined for the presence of follicles and the diameter of ovaries follicles present on each ovary was measured by using vernier calipers .these follicles classified into two groups according to their diameter, small (4-8 mm) and large (> 8 mm). Then the follicular fluid was aspirated using disposable sterilized syringe. The follicular fluid samples were centrifuged at 5000rpm for 5 minutes, decanted, and stored at -20c until analysis.

Hormonal analysis:

Used T3, T4 and TSH enzyme immunoassay test kit catalogs number:

125-300 Total triiodothyronine product code:

225-300 Total thyroxin product code:

325-300 Thyrotropin:

All from monobind inc. Lake forest, CA92630, USA

Progesterone Enzyme Immunoassay Test KIT Catalog Number: BC-11

Estrogen (E2) Enzyme Immunoassay Test KIT Catalog Number: BC-1111

Biochemical analysis:

Estimation serum blood glucose Used kit (liquid glucose GOD-PAP) from (Randox) company.

Total cholesterol determination kit (cholesterol CHOD-PAP. Biocon Diagnostic hek8, Germany).

Total protein determination using special kit (Human / Total protein liquicolor, Germany).

RESULTS

Table 1: Concentrations of some hormonal and biochemical Constituents in follicular fluid and plasma in buffaloes (Mean ± SE)

constituents	Small follicles	Large follicles	Blood
T3 (*gn*/ml)	1.63±0.I6 a	1.18±0.07 b	1.67±0.15 a
T4 (µg/dl)	7.91±0.75 c	22.28±2.05 b	49.15±3.62 a
TSH (µlU/ml)	0.31±0.02 b	0.17±0.04 b	1.88± 0.57 a
PROGESTERONE (*gn*/ml)	1.30± 0.41 b	6.67±1.31 a	1.02± 0.20 b
ESTROGEN (pg/ml)	373.28± 92.49 b	863 194.16 a	57.42± 5.25 b
PROTEIN (g/dl)	2.82± 0.19 c	5.67±0.44 b	7.39±0.45 a
GLUCOSE (mg/ml)	75.19±1.90 b	56.94 ±2.83 c	121.71± 5.98 a
CHOLESTEROL (mg/dl)	78.08±1.62 b	59.97± 2.09 c	126.77±3.29 a

Value with different letters within a row differ significantly ($p<0.05$)

Table (1) shows the concentrations of T3, T4, TSH, progesterone, estrogen, total protein, glucose, and cholesterol in small and large follicular fluid and blood plasma.

These data demonstrated that the concentrations of T3, glucose, and cholesterol in small follicles were significantly higher ($p<0.05$) than the large follicles, while no differences were found in concentrations of TSH between small and large follicles.

The concentrations of T4, progesterone, estrogen, and total protein in large follicles were significantly higher (P< 0.05) than small follicles. The results of the present study also indicated that the, T4, TSH, glucose, cholesterol and total protein concentrations in the blood plasma were significantly higher (P<0.05) than the follicular fluid in both groups. While no differences observed between blood plasma and small follicles, in the concentrations of T3, progesterone and estrogen.

DISCUSSION

In the present study, follicular and plasma T3, T4, and TSH concentrations were in agreement with the findings of (18), who found in camel that concentrations of T3 were significantly greater (p<0.05) lower in large follicles than in small follicles and (19) in cows who reported that the concentrations of T4,were significantly greater (P<005) in large follicles compared with small follicles and T3 , T4 concentrations were greater (P<0.05) in serum than follicular from small and large follicles.

These results are not in agreement to those reported by (13), (20) in human and (21) in buffaloes , who observed that both T3 and T4 were present in follicular fluid fell within the normal range for serum and there was a positive correlation between serum T4 values and follicular fluid values . Our results also differed from those of (22) in cows who described that concentrations of T4 in small follicles, were significantly (P<0.05) higher than in large follicles and no differences were found in concentrations of T3 in both groups of follicles, while the concentrations of intra-follicular T3 were at least several times higher than the normal range for serum.

(23), (24) in cows also noted that the concentrations of T3 in the circulation is substantially lower than that of T4 , similar findings were observed in the present study.

The thyroid hormones may have stimulatory effects on ovarian function in cattle, acting at the level of granulose cells (25) .The differences in concentrations of T4 between groups of follicles may be related to growth and maturation of follicles in cattle (22).

In our study no differences were found in TSH concentrations between small and large follicles, while the concentrations of TSH were significantly higher (P<0.05) in

blood plasma than both follicular groups. These results differed from those of (26) in human who reported a positive correlation between serum and follicular fluid in concentrations of TSH. The differences might be due to species differences.

In our study , estrogen and progesterone concentrations in large follicles were significantly higher($P<0.05$) than the small follicles and plasma concentrations .Similar observations were made in buffaloes by (27) who found that estrogen and progesterone concentrations were significantly higher($P<0.05$) before and during estrus (large follicles) compared with metoestrus and and dioestrus (small follicles).

The estrogen concentrations reported in the present study are in agreement with those described by (28), (8), (29) and (30),(31) in cattle , (32)in buffaloes , (33) in mares who all found more estradiol in the follicular fluid of large follicles. The presences of large quantities of progesterone during proestrus and metoestrus has well established in bovine (30). Similar findings were observed in the present study.

The significant positive correlation between follicular estrogen and progesterone found in the present work is consistent with possibility that follicular progesterone serves as a precursor to androgen and subsequently estrogen production by follicles of buffaloes.

These results are not in agreement to those reported for bovine by (28) and (29) and (33) in mare who found a negative correlation between follicular size and progesterone concentrations.

The total protein concentrations of the follicular fluid in the present study was agreed with the findings of (34) in bovine, (27) in buffaloes, who found that the follicular fluid concentrations of total protein were significantly ($P<0.05$) higher in large follicles than small and medium follicles, while (21) in buffaloes, (35) in cows, found that the difference in total protein contents between the two follicular classes was nonsignificant.

In the present study, it was also observed that the total protein concentrations of blood plasma were significantly higher than those of small as well as large ,follicles which are in agreement with those found by (21) in buffaloes , similarly (35) observed that serum contents of total protein were significantly higher ($P<0.05$) than small , medium and large follicles in cows. The high correlation between total protein contents

in follicular fluid and serum suggest that substantial part of protein contents in follicular fluid originate from the serum (28).

Our results differed from those of (36) in goat and sheep, (37) in caprine, (38) in buffaloes, (4) in ewes and (18) in camel, who all observed that the total protein concentrations were lowered as the follicle size increase.

The present findings demonstrate that follicular concentrations of glucose were significantly higher ($P<0.05$) in small follicles than large follicles. Similarly, (18) in camel who observed that the concentrations of glucose were significantly lower in large follicles when compared with the small follicles. These results are also not in agreement to those reported of (27) and (21) in buffaloes, (31) and (39) in cows, (4) in ewes who all found that glucose concentrations were significantly increased as follicle diameter increased.

The present study was also observed that the glucose concentrations of blood plasma were significantly ($P<0.05$) than those of small as well as large follicles. These results are in agreement to those reported by (35) in dairy cows, (21) in buffaloes, who found that the glucose concentrations in blood plasma were significantly higher ($P<0.05$) than follicular fluid in both groups. This implies that the principal source of follicular fluid glucose is blood and very little glucose is synthesized locally by the granulosa cells of follicles.

The result of the present study also indicated that the cholesterol concentrations in small follicles were significantly higher ($P<0.05$) than large follicle, while the concentrations of cholesterol in blood plasma were significantly higher ($P<0.05$) than the follicular fluid in both groups.

These results are in agreement with That reported in Pig (40) and in buffaloes (38) and in camel, (18) who all found that the concentrations of cholesterol were significantly lower ($P<0.05$) in large follicles when compared with small follicles .

Cholesterol in follicular fluid derived from two sources, cellular synthesis from acetate and uptake from plasma lipoprotein. The decreased cholesterol concentrations in large follicles might be attributed to the conversion of cholesterol to steroid hormones, estrogen and progesterone during steroidogenesis.

However the results of the present study differed from that reported in goat (41) and (42) and in cows and (35) and in ewes (4), who all observed that the cholesterol

concentrations were significantly higher (P<0.05) in large follicles than in small follicles .

The results also differ from that noted by (21) in buffaloes and (22) in cows who found that no significant differences were detected in concentrations among follicles classified according to size. The result of present study also indicated that cholesterol concentration in blood plasma were signification higher (P<0.05) than the follicular fluid in both the group .these result are in agreement to these observed by (35) in dairy cow , (21) in buffaloes who found that plasma cholesterol concentration were signification higher (P<0.05) than that found in small and large follicles.

Cholesterol was the precursor for steroid synthesis and the follicular fluid contained only high-density lipoprotein , therefore , the vascular granulose cells of the follicles totally depended on the cholesterol from high-density lipoprotein , which was derives from the blood plasma by crossing the basement membrane of granulose cells(37).

دراسة بعض المكونات الهرمونية والكيموحيوية للسائل الجريبي وبلازما الدم في الجاموس

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الخلاصة

فسلجة المبايض يتم السيطرة عليها بواسطة العديد من العوامل الخارجية والداخلية وتشمل التغيرات الهرمونية والكيموحيوية التي تحدث في السائل الجريبي خلال دورة الشبق لذا هدفت الدراسة الحالية الى تحديد ومقارنة تراكيز بعض المكونات الهرمونية والكيموحيوية في الدورة الدموية المحيطية والسائل الجريبي . لهذا الغرض جمعت المايض من اناث الجاموس البالغة مباشرة بعد ذبحها وجمعت عينات الدم من هذا الحيوانات مباشرة قبل ذبحها في مجزرة البصرة.صنفت النماذج الاى مجموعتين حسب قطر الجريب المبيضي ،الصغيرة يتراوح قطرها من (4-8 mm) والكبيرة (>8 mm)ويتم تحليل السائل الجريبي وبلازما الدم بأستخدام العدة التجارية.

اظهرت النتائج ارتفاع معنوي ($P< 0.05$) في مستوى تركيز T_3 ،الكلكوز والكولسيترول مقارنة بالجريبات الكبيرة . بينما لم تلاحظ ظروف فروق معنوية في تركيز TSH بين المجموعتين ، أما تراكيز كل من T_4 ،البروجيسترون ،الاستروجين والبروتين الكلي كانت أعلى معنوياً ($P< 0.05$) في الجريبات الكبيرة مقارنة بالجريبات الصغيرة.

وأشارت نتائج الدراسة الحالية إلى ارتفاع معنوي ($P< 0.05$) في تركيز كل من TSH,T_4 ،الكلوكوز ،البروتين الكلي والكوليسترول في البلازما الدم مقارنة مع السائل الجريبي لكلا المجموعتين . ولم يلاحظ فروق معنوية في تركيز T_3 والبروجيسترون بين البلازما والجريبات..

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