COMPARATIVE STUDY BETWEEN HORMONAL BLOOD SERUM AND OVARIAN FOLLICULAR FLUID DURING SEASON AND OUT SEASON IN BITCHES

Alaa Habeeb Abboud , Taher.A.Fahad
Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

(Received 2 October 2018 ,Accepted 9 October 2018)

Keywords: Follicular fluid, bitches, ovaries.

Corresponding author:taher1952@yahoo.com

ABSTRACT

The functions of the ovaries are controlled by many exogenous and endogenous factors, including changes in the biochemical and endocrine glands that occur in the follicular fluid during the breeding season in the bitches. The aim of this study was to determine and compare the concentrations some hormonal in the peripheral circulation and follicular fluid of bitches during the breeding season. For this purpose, ovaries collected from adult bitches immediately after ovariectomy, and blood samples were also collected from these bitches before and after season. The follicular fluid and blood serum samples were analyzed for hormonal concentrations using commercial kits.

The results showed that the concentrations of estrogen, progesterone and testosterone in blood serum at season (54.31±0.49, 20.75 ±0.12, 0.75±0.05) respectively. While the concentrations of estrogen, progesterone and testosterone in blood serum at out season (10.88±0.39, 0. 61±0.036, 0.063±0.042) respectively. The concentrations of estrogen, progesterone and testosterone in follicular fluid at season (69.9±0.44, 28.46±0.82, 0.331±0.65) respectively. While the concentrations of estrogen, progesterone and testosterone in follicular fluid out season (there are no found ovarian follicles).

The present study a significantly higher ($P< 0.05$) in blood serum and follicular fluid at season than that out season blood serum.

INTRODUCTION

Follicular fluid (FF)is 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). FF provides a very important micro-environment for the development of oocytes. FF is a product of both the transfer of blood plasma constituents that cross the blood follicular barrier and of the secretory activity of granulosa and thecal cells (2). FF is an avascular compartment within the mammalian ovary, separated from the perifollicular stroma by the follicular wall that constitutes "blood-follicle barrier"(3) 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 (4). FF originate from somatic cells, that produce factors related to it is metabolic activity (2). FF is derived plasma from blood flow through the capillaries as a result of the activity of granulosa cells, resulting in an osmotic gradient. As a result of the convergence of the granular cells with each other and the reconfiguration of cellular links from cell to the cell (5). FF composition has been under intensive studies, especially in recent times to increase knowledge and development of follicles, acolyte maturation and follicular artesian (6). FF consists of many nutrients, growth factors, hormones, electrolytes, and enzymes (7), it is reasonable to think that some biochemical characteristics of the FF surrounding the oocyte may play a critical role in determining oocyte quality and the subsequent potential to achieve fertilization and embryo development. The analysis of FF components may also provide information on metabolic changes in blood serum, as the circulating biochemical milieu may be reflected in the composition of FF (8). The biochemical method utilizes the ratio of estrogen and progesterone in FF as criteria to determine follicular health (9) (10). The synthesis of steroid hormones by the developing follicle is dependent upon the presence and activation of several key proteins (11) (12), although gonadotropins play a major role in the regulation of follicle function (13). The ovaries are the major source of estrogens, androgens and progesterone found in peripheral blood (14). Hormonal concentrations in the follicular fluid of ovary fluctuate considerably with the stage of the cycle follicular size and status (10). The aim of this study was to

determine and compare the concentrations of some hormonal composition in peripheral and follicular fluid of bitches in Basra province.

MATERIALS AND METHODS

Animal housing and management: Twelve bitches local Iraqi bitches were used with an average body weight range between 20 - 25 Kg, and their ages between 2–4 years ages. The bitches were housed in individual cages for bitches in the College of Veterinary Medicine / University of Basrah. All animals were exposed to the same conditions, including temperature, management fees and acclimatize, adaptive to the cage environment for one month before the experiment. The animals were treated for common gastrointestinal and antiparasites by giving a single dose of Ivermectin (200 microgram) /kg body weight/SC. And examined for any infection by giving a course of systemic antibiotic that they were healthy before the beginning of the study.

Collect blood samples: Blood samples were collected using disposable syringe 10 ml from the cephalic vein during the breeding season before operation collected from each animal and also collected blood in outer season, in anticoagulant tube. These tubes were then carried to the laboratory. Blood tube was placed in centrifuge at 5000 rpm for 5 minutes for the purpose of obtaining the serum, then serum was drawn by a micro- pipette and placed in the abendrove tube, and kept at (-20C) until analysis.

Collection of ovaries: Twelve pairs of ovaries were collected from mature female dogs. After ovarian resection, the ovaries were placed in a plastic container. The plastic container containing the mature ovaries was then transferred to the laboratory after the end of the operation. In the laboratory, the ovaries were washed with a normal saline solution. Each ovary was then examined for the presence of mature follicles.

Collect follicular fluid samples: follicular fluid was aspirated using disposable sterilized syringe. The follicular fluid samples were centrifuged at 5000rpm for 5 minutes, and stored at -20C until analysis.

Hormonal Analysis: The hormonal test performed using the serum which was

extracted from blood samples and follicular fluid, collected without anticoagulant and frozen until the time of the examination. The hormone test includes estrogen (E_2), progesterone and testosterone using a device called ELISA Micro wells machinehormones assay with the kit hormones of USA. Enzyme-Linked Immunosorbent Assay (ELISA) is an effective method used for estimating ng/ml to pg/ml materials in the solution, ELISA ng / ml to g / ml of solution. Based on these criteria an enzyme was used to detect the association of antibodies to the antigen (Ag) (Ab). Converting the enzyme from colorless to a colored enzyme, a reference to Ag: Ab binding (15).

RESULTS

Table 1: Comparative concentrations of hormone constituents in blood serum in season and out season.

Hormones	M± SE (In Season)	M± SE (Out Season)
Estradiol-17β(pg/ml)	54.31 ±0.49 a	10.88±0.39 a
Progesterone (ng/ml)	20.75±0.12 b	0. 61± 0.36 b
Testosterone (ng/ml)	0.75±0.05 c	0.063 ± 0.042 c

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

The results in table (1) showed significantly higher ($P< 0.05$) between hormones in season and out season in blood serum.

Table 2: Comparative concentrations of hormone constituents in follicular fluid in season and out season.

Hormones	M±SE (In Season)	M±SE (Out Season)
Estradiol-17β(pg/ml)	69.9 ±0.44 a	_
Progesterone (ng/ml)	28.46±0.82 c	_
Testosterone (ng/ml)	0.331±0.65 b	_

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

The results in table (2) showed significantly higher ($P< 0.05$) between hormones in season and out season in follicular fluid.

Table 3: Comparative concentrations of hormone constituents between season and out season in follicular fluid and blood serum.

Hormones	M± SE In follicular fluid		M±SE In blood serum	
	In season	Out season	In season	Out season
Estradiol-17β (pg/ml)	69.9 ±0.44 a	_	54.31 ±0.49 a	10.88±0.39 A
Progesterone (ng/ml)	28.46±0.82 c	_	20.75±0.12 b	0. 61± 0.36 B
Testosterone (ng/ml)	0.331±0.65 b	_	0.75±0.05 c	0.063 ± 0.042 C

Value with small and large letters within a row differ significantly ($p<0.05$)

The results in table (3) showed significantly higher (P< 0.05) between hormones in season and out season in follicular fluid, also there are found significantly higher (P< 0.05) between hormones in season and out season in blood serum.

DISCUSSION

The results obtained during the study showed an increase in the concentration of hormones (estrogen, progesterone and testosterone) in serum and follicular fluids during breeding season may be due to healthy environment the provision of an appropriate and in terms of cleanliness of place, temperature and humidity as well as the provision of meat food as well as the use of antibiotics and antiparasites. In our study there were concentrations of estrogen, progesterone and testosterone in follicular fluid and blood serum in season were significantly higher (P<0.05) than blood serum in out the season. There is an important role for both estrogen and progesterone in maintaining the pregnancy and prolong the period in anestrus (16). The higher estrogen concentrations and lowered progesterone concentrations in the breeding season have been recorded during this study. This finding is in agreement with previous studies the results (17, 18, 19). Estrogen concentrations during this study were higher in follicular fluid in season than that blood serum in out the season. This finding is in agreement with previous study (20).

The progesterone has been recognized as an essential for ovulation, establishment and maintenance of pregnancy, and mammary gland development as well as for the expression of sexual behavior in mammals (21, 22). The progesterone concentration in blood serum was less in out season than that follicular fluid in season. This finding in agreement with previous studies (23, 24). The progesterone concentration, low found in this observation agrees with previous study (25). It has been found that progesterone indicates a low level of luteolysis that occurs in the absence of pregnancy (26).

Testosterone is changed into estrogen, the female sex hormone. In this study, testosterone is increasing level in follicular fluid at season is higher than that in blood serum out the season . This finding is in agreement with previous study (27).

CONCLUSION

The present study concluded that

1-Estrogen level in follicular fluid were higher in season than that out season(there are no found ovarian follicles).

2- Progesterone level in out season lower than that season in blood serum.

3-Progesterone level in follicular fluid in a season was higher than that blood serum in out the season.

4- Testosterone level in follicular fluid in a season was higher than that blood serum in out the season.

دراسة مقارنة بين الهرموني مصل الدم وسوائل المبيض الحويصلي خلال الموسم وخارج الموسم في اناث الكلاب

الاء حبيب عبود , طاهر عبدالحسين فهد

فرع الجراحة والتوليد البيطري ،كلية الطب البيطري، جامعة البصرة، العراق

الخلاصة

يتم التحكم في وظائف المبيض من قبل العديد من العوامل الخارجية والداخلية ، بما في ذلك التغيرات في الغدد البيوكيميائية والغدد الصماء التي تحدث في السائل الجريبي خلال موسم التكاثر في الكلبات. كان الهدف من هذه الدراسة هو تحديد ومقارنة بعض تراكيز الهرموني في الدورة الدموية الطرفية وسوائل الجريبي من اناث الكلاب خلال موسم وخارج موسم التناسل. لهذا الغرض ، تم جمع المبايض من اناث الكلاب البالغة بعد استئصال المبيض مباشرة ، كما تم جمع عينات الدم من هذه الحيوانات قبل وبعد موسم التناسل. تم تحليل عينات مصل الدم وسوائل الدم لتراكيز الهرمونات باستخدام مجموعات تجارية.

وأظهرت النتائج أن تراكيز هرمون الاستروجين والبروجسترون والتستوستيرون في مصل الدم في الموسم (54.31±0.49 , 20.75 ±0.12 , 0.75±0.05)على التوالي. بينما تراكيز هرمون الاستروجين والبروجسترون والتستوستيرون في مصل الدم في خارج الموسم (0.61±0.36, 0.063±0.042, 10.88±0.39) على التوالي. وكانت تراكيز هرمون الاستروجين والبروجسترون والتستوستيرون في السائل الجريبي في الموسم (69.9 ±0.44, 28.46±0.82, 0.331±0.65) على التوالي. في حين أن تركيزات هرمون الاستروجين والبروجسترون والتستوستيرون في السائل الجريبي خارج الموسم (لا توجد الجريبات المبيض).

الدراسة الحالية أعلى بكثير (P <0.05) في مصل الدم وسوائل الجريبي في الموسم من مصل الدم خارج الموسم.

REFERENCES

1. **Gerard, N., Loiseau,S.,Duchamp,G.,andSeguin,F**.(2002). Analysis of the variations of follicular fluid composition during follicular growth and maturation in the mare using proton nuclear magnetic resonance (HNMR).Reprod.124:241-248.

2. **Fortune JE.** (1994). Ovarian follicular growth and development in mammals. Biol Reprod 50,225–232.
3. **Bagavandoss, P., Midgley, A.R.and Wicha, M**. (1983). Developmental changes in the ovarian follicular basal lamina detected by immunofloresence and electron microscopy.J.Hist.Cytchem.31, 633-640

4. **Peters, Hand McNatty, K.P**. (1980). The Ovary, 1st ed., Granad.Great Britain: Paul Elek.
5. **Rodgers RJ, Irving-Rodgers HF.** (2010). Formation of the ovarian follicular antrum and follicular fluid. Biology of Reproduction 82, 1021–1029.
6. **Mishra, O.P., Pandey, J.N. and Gawande, P.G**. (2003). Study on biochemical constituents of caprine ovarian follicular fluid after superovulation. Asian Australas. J. Anim Sci., 16: 1711-1715.
7. **Das GK, Khan FA.** (2010). Summer anoestrus in buffalo-a review. Reprod Dom Anim 45, 483–494.

8. **Leroy JL, Vanholder T, Delanghe JR, Opsomer G, Van Soom A, Bols PE, Dewulf J, de Kruif A.** (2004). Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. Theriogenology 62,1131–1143.

9. **relandJJ, Roche JF**.(1982). Development of antral follicles in cattle after prostaglandin-induced luteolysis: changes in serum hormones, steroids in follicular fluid, and gonadotropin receptors.Endocrinology.111:2077-2086.

10. **KruipTA, Dieleman SJ.** (1985).Steroid hormone concentrations in the fluid of bovine follicles relative to size, quality and stage of the estrus cycleTheriogenology; 24:395-408.

11. **Lala,DS.,Rice,DA.and Parker,KL**.(1992). Steroidogenic factor I,a Key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi tarazu-factor I. Mol Endocrinol;6:1249-1258.

12. **Boa.B and Maverick, HA.** (1998). Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves; 76:1903-1921.

13. **Soboleva T.K.,Peterson A.J.,Pleasants A.B., McNatty K.P.,and Rhodes F.M**.(2000).A model of follicular development and ovulation in sheep and cattle.Anim Reprod Sci; 58:45-57.

14. **Kanchev L.N., Dobson H., Ward W.R..And Fitzpatrick R.J.** (1976). Concentrations of steroid in bovine peripheral plasma during the estrus cycle and the effect of betamethasone treatment. Journal of Reproduction and Fertility; 48:341-5

15. **Ma, H.; Shieh, K. and Lee, S**.(2006). Study of ELISA technique. Nature and science, 4(2): 312-318.

16. **Thota, C.; Gangula, P.R.R.; Dong, Y.L. and Yallampalli, C.** (2003). Changes in the expression of calcitonin receptors-like receptor, receptor activity-modifying protein (RAMP) 1, RAMP2 and RAMP3 in rat .

17. **Ginther, O. J.; Bergfelt, D. R. ; Kulick, L. J. and Kot, K**. (2000). Selection of dominant follicles in cattle: role of estradiol.

18. **Beg, M. Bergfelt,A.; D. R.,; Kot, K. M. C. Wiltbank, O. J. and Ginther,** (2001). Follicular fluid factors granulose-cell gene expression associated with follicle deviation in cattle. Biol. Reprod. 64, 432-441.

19. **Komar, C. M.; Berndtson, A. K. ; Evans, A. C. O. and Fortune , J. E.** (2001). Decline in circulating estradiol during the periovulatory period is correlated with decreases in estradiol rogen, in messenger RNA for P450 aromatase P450 17α-hydroxylase, in bovine preovulatory follicles. Biol. Reprod., 64:1797-1805

20.S. **Fahiminiya; K. Reynaud; V.Labas ; S.Batard; S. Chastant-Maillard and N. Gérard** (2010), Steroid hormones content and proteomic analysis of canine follicular fluid during the preovulatory period, Reproductive Biology and Endocrinology 2010, 8:132.

21. **Lydon JP, Demayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA, Shyamala G, Conneely OM, Omalley BW** (1995). Mice lacking progesteronereceptor exhibit pleiotropic reproductive abnormalities. Genes; 9: 2266–2278.

22. **Kim J, Bagchi IC, Bagchi MK.(**2009). Control of ovulation in mice by progesterone receptor-regulated gene networks. Mol Hum Reprod 2009; 15:821–828.

23. **Arimbawa, I.W.P.; Trilaksana, I.G.N.B. and Pemayun, C.G.O.** (2012). Gambaranhormonprogesteronsapibaliselamasatusiklus estrus. Indonesia MedicusVeterinus 1(3):330336

24. **Mukasa-Mugerwa, E. and Tegegne, A.** (1989). Peripheral plasma progesterone concentration in Zebu (Bosindicus) cows during pregnancy. Reprod. Nutr. Dev,29, 303. find online

25. **Mann, G. E., & Lamming, G. E.** (2007). The influence of progesterone during early pregnancy in cattle. Reproduction in Domestic Animals, 34(3-4), 269-274

26. **Romano, J.E.** (2011). Pregnancy diagnosis in cattle. Veterinary population medicine. College of Veterinary Medicine, University of Minnesota.

27. **Abbott, D.H.; Barnett, D.K.; Bruns, C.M. and Dumesic, D.A**.(2005). Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome Human Reproduction Update; 11: 357–374.