International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:10, No:2, 2016

Seasonal Heat Stress Effect on Cholesterol, Estradiol and Progesterone during Follicular Development in Egyptian Buffalo

Heba F. Hozyen, Hodallah H. Ahmed, S. I. A. Shalaby, G. E. S. Essawy

***Abstract*—**Biochemical and hormonal changes that occur in both follicular fluid and blood are involved in the control of ovarian physiology. The present study was conducted on follicular fluid and serum samples obtained from 708 buffaloes. Samples were examined for estradiol, progesterone, and cholesterol concentrations in relation to seasonal changes, ovarian follicular size, and stage of estrous cycle. The obtained results revealed that follicular fluid and serum levels of estradiol, progesterone, and cholesterol were significantly lower during summer and autumn when compared to winter and spring seasons. With the increase in follicular size, the follicular fluid levels of progesterone and cholesterol were significantly decreased, while estradiol levels were significantly increased. Estradiol and progesterone levels were significantly higher in follicular fluid than blood, while cholesterol was significantly lower in follicular fluid than serum. In conclusion, the current study threw a light on the hormonal changes in the follicular fluid and blood under the effect of heat stress which could be related to the low fertility of buffalo in the summer.

***Keywords*—**Buffalo, follicular fluid, follicular development, seasonal changes, steroids.

I. Introduction

EXPOSURE to high ambient temperature is a major limitation on buffalo productivity leading to impairment of both production and reproduction performance and this effect is aggravated when heat stress is accompanied by high ambient humidity [1]. Buffalo is one of the most important animals among livestock that can play a vital role in solving the world-wide problem of deficiency of animal proteins particularly in Egypt as well as other developing countries [2]. However, summer temperatures in Egypt are extremely high, reaching 38◦C to 43◦C. Based on the historical records over a period of twelve years (1999–2010), the subtropical climate in Cairo is characterized by hot summer season (June–August) with averages 23◦C–35◦C of minimum and maximum temperatures and 74% mean temperature humidity index [3].

Ovarian function is central to all reproductive problems in buffalo including seasonal reproductive patterns [4]. Within the ovarian follicle, follicular fluid microenvironment was found to provide optimal conditions for the maturation and function of both granulosa cells and gametes by virtue of the essential biomolecules present in it [5]. Follicular fluid is mainly derived from blood besides the locally produced substances [6]. Steroid hormones including estradiol and progesterone that found in serum and follicular fluid are one of the major factors controlling follicular development [7]. Cholesterol is the precursor for steroid synthesis and high value of cholesterol in cyclic animals leads to more secretion of steroids during estrus due to increased ovarian activity [8]. Follicular fluid may be regarded as a biological window reflecting hormonal processes occurring in the microenvironment of the maturing oocyte before ovulation [9]. Consequently, studying follicular fluid microenvironment in buffalo provides a valuable insight into the process of normal follicular development as well as the pathogenesis of some reproductive problems [10]. The current study aimed to investigate the effect of environmental heat stress on concentrations of cholesterol, estradiol and progesterone in blood and follicular fluid harvested from different-sized ovarian follicles in buffalo.

Heba F. Hozyen and S. I. A. Shalaby are with the Dept. Reproduction and A. I., Veterinary Division, National Research Centre, Dokki, Giza, Egypt (Corresponding author: Heba, F. Hozyen, postal address: Tahrir street, Dokki, Giza, Postal code: 12622; e-mail: Drheba23@yahoo.com).

Hodallah H. Ahmed and G. E. S. Essawy are with the Dept. Physiology, Cairo University, Egypt.

II. Materials and Methods

A. Ovaries

Ovaries were collected from local slaughterhouse from 708 non-pregnant female buffaloes in good health and with clinically normal reproductive tracts. Immediately after slaughtering, both ovaries from each animal were collected in plastic bags containing 0.9% NaCl and transported in ice tank to be inspected at the laboratory.

B. Experimental Design

Follicles were collected over one year during different seasons. The stage of estrous cycle (follicular or luteal) was identified according to the presence or absence of the corpus luteum on the ovary according to [11], [12]. Follicular diameter was measured using a caliper and follicles were divided into three categories: small (≥3 mm), medium (4-9 mm) and large (≥10 mm) according to [13]. Ovaries with cystic follicles were excluded from the study.

C. Sampling

1. Follicular Fluid

The contents of the ovarian follicles of different size (small, medium and large) were aspirated using a 10 ml syringe attached to an 18 gauge needle and centrifuged at 3000 rpm for 10 min for separation of the fluid from the cell fraction.

Follicular fluids obtained from small and medium size follicles in each individual buffalo were pooled in one sample. Collected follicular fluid samples were kept at -20 C° until analysis.

2. Blood

Samples were collected during slaughtering for serum separation after centrifugation at 3000 rpm for 10 minutes. Collected serum samples were kept at -20 C° until analysis.

D. Measured Parameters

1. Estradiol

Estradiol levels were assayed using kits purchased from DRG, Germany according to [14].

2. Progesterone

Progesterone levels were assayed using kits from DRG, Germany as described by [15].

3. Cholesterol

Cholesterol concentrations were measured according to the method of [16] using kits purchased from Stanbio, USA.

E. Statistical Analysis

Data were analyzed statistically by one-way ANOVA except the difference between follicular and luteal phases in different size follicles which was analyzed by independent samples *t*-test using SPSS 16.0 for windows. Treatment means were compared by the least significance difference (LSD) at 5% level of probability.

III. Results

A. Effect of Follicular Size, Season of the Year and Phase of Estrous Cycle on Estradiol Levels (pg/ml) in the Follicular Fluid of Buffaloes

It is evident from values shown in Table I that the overall means of estradiol in follicular fluid from large follicles were significantly ($P < 0.01$) higher than medium ones. Also, the overall means of estradiol levels in follicular fluid of medium follicles were significantly ($P < 0.01$) higher than small follicles. Meanwhile, the overall means of follicular fluid estradiol decreased significantly ($P < 0.01$) in summer and autumn than winter and spring. Moreover, the overall means of estradiol during follicular phase were significantly higher than luteal phase in large and medium as well as small follicles.

B. Effect of Follicular Size, Season of the Year and Phase of Estrous Cycle on Progesterone Levels (ng/ml) in the Follicular Fluid of Buffaloes

As shown in Table II, the overall means of follicular fluid progesterone levels decreased significantly ($P < 0.01$) with the increase in follicle size. Furthermore, progesterone overall means of progesterone in the follicular fluid decreased significantly ($P < 0.01$) during summer and autumn than winter and spring. In addition, the overall means of progesterone during luteal phase were significantly ($P < 0.01$) higher than follicular phase in small, medium and large follicles.

C. Effect of Follicular Size, Season of the Year and Phase of Estrous Cycle on Cholesterol Concentrations (mg/dl) in the Follicular Fluid of Buffaloes

It is evident from values shown in Table III that the overall means of follicular fluid cholesterol concentration decreased significantly ($P < 0.01$) with the increase in follicular size. Also, cholesterol overall means were significantly ($P < 0.01$) lower during summer and autumn than winter and spring. No significant changes were detected in the overall mean of cholesterol concentrations between follicular and luteal phases in different follicle classes.

D. Effect of Season of the Year and Phase of Estrous Cycle on Levels of Estradiol (pg/ml), Progesterone (ng/ml) and Cholesterol (mg/dl) in the Serum of Buffaloes

It is clear from data presented in Table IV that the overall means of both serum estradiol and cholesterol levels in buffalo were significantly ($P < 0.05$) lower in summer and autumn than winter and spring. The overall means of serum progesterone levels in buffalo were significantly ($P < 0.05$) lower during summer season than both spring and winter seasons. Overall means of estradiol levels in serum were significantly ($P < 0.01$) higher during follicular than luteal phases. On the other hand, the overall mean of serum progesterone levels was significantly ($P < 0.01$) higher in luteal phase than follicular phase. However, no significant changes were reported in the overall means of serum cholesterol concentration between follicular and luteal phases.

E. Average Levels of Estradiol (pg/ml), Progesterone (ng/ml) and Cholesterol (mg/dl) in Follicular Fluid and Serum of Buffaloes

Table V clarifies that the overall means of follicular fluid estradiol and progesterone levels increased significantly ($P < 0.05$) than their levels in serum. Quite the opposite, the overall means of follicular fluid cholesterol concentrations decreased significantly ($P < 0.05$) in comparison with serum.

IV. Discussion

In the present study, the overall means of follicular fluid estradiol levels increased significantly during follicular growth from small to medium and large follicles. In contrast, small follicles had higher progesterone levels than medium follicles which in turn, had higher progesterone than large ones. It is known that both granulosa and theca cells of bovine follicles produce large amounts of progesterone which serves as a precursor for androgen and subsequently estrogen production [17]. Steroid content in follicular fluid reflects the synthetic capabilities of the granulosa and theca layers [18]. Therefore, the increase in estradiol concentration with the increase in follicle size may be due to an increase in the number of granulosa cells and aromatase activity with the growth and development of follicles [5]. The inverse relationship between follicular estradiol 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. Also, the lower level of progesterone in the follicular fluid of preovulatory follicles may be attributed to an increase in prostaglandin production [5]. On the other hand, [19] claimed that higher follicular fluid progesterone concentrations in small follicles may be indicative on ongoing follicular atresia.

Selection and dominance are two key events determining the fate of follicles and the increase in the estradiol production is an important feature of these processes [20]. For ovulation to occur under physiological conditions, a follicle must attain a minimum particular size (usually equal to or greater than the dominant size for the species) and produce sufficient estradiol to stimulate the LH surge [5]. The increased follicular fluid concentration of estradiol throughout folliculogenesis observed in the present study is in agreement with [21] and [5] in buffalo and [22] in cattle who all found more estradiol in the follicular fluid of large follicles. However, [23] reported that the medium sized follicles contained significantly higher estradiol compared to small and large follicles in buffaloes.

TABLE I
EFFECT OF FOLLICULAR SIZE, SEASON OF THE YEAR AND PHASE OF ESTROUS CYCLE ON ESTRADIOL LEVELS (PG/ML) IN THE FOLLICULAR FLUID OF BUFFALOES

Follicle size	Estrous phase	Summer	Autumn	Winter	Spring	Overall mean at different phases	Overall mean at different follicle size
Large	Follicular	$564.72^{aA}\pm4.99$	$604.1^{bA}\pm9.31$	$616.2^{bA}\pm0.68$	$607.10^{bA}\pm14.77$	$595.97^{A}\pm6.02$	$589.89^{A}\pm4.71$
	Luteal	$524.45^{aB}\pm16.29$	$564.70^{bB}\pm6.89$	$587.16^{bB}\pm7.01$	$587.93^{bB}\pm7.73$	$566.74^{B}\pm6.19$	
Medium	Follicular	$531.41^{aA}\pm5.04$	$585.53^{bA}\pm7.99$	$601.2^{bA}\pm0.68$	$591.46^{bA}\pm7.37$	$571.54^{A}\pm6.19$	$573.20^{B}\pm5.05$
	Luteal	$508.56^{aB}\pm28.95$	$547.69^{bB}\pm4.32$	$587.74^{cB}\pm4.35$	$569.98b^{cB}\pm4.59$	$556.21^{B}\pm7.55$	
Small	Follicular	$515.05^{aA}\pm9.49$	$581.21^{bA}\pm9.09$	$590.17^{bA}\pm0.68$	$584.04^{bA}\pm10.37$	$561.85^{A}\pm7.75$	$536.56^{C}\pm6.19$
	Luteal	$496.29^{aB}\pm20.17$	$523.43^{abB}\pm19.58$	$567.35^{bB}\pm4.95$	$558.01^{bB}\pm6.39$	$533.53^{B}\pm8.74$	
Overall mean at different seasons		$523.63^{a}\pm6.77$	$565.54^{b}\pm6.179$	$590.71^{c}\pm2.87$	$582.98^{c}\pm4.50$		

-Data are presented as means ± SE.
-Means having different superscripts (a, b) within the same raw are significantly different.
-Means having different superscripts in the same column (A, B, C), within each follicular size, differ significantly.
-N = 8 per group.

TABLE II
EFFECT OF FOLLICULAR SIZE, SEASON OF THE YEAR AND PHASE OF ESTROUS CYCLE ON PROGESTERONE LEVELS (NG/ML) IN THE FOLLICULAR FLUID OF BUFFALOES

Follicle size	Estrous phase	Summer	Autumn	Winter	Spring	Overall mean at different phases	Overall mean at different follicle size
Large	Follicular	22.19aA±2.29	21.88aA±1.45	26.97bA±0.94	25.45abA±0.92	24.58A±0.76	25.77A±0.49
	Luteal	24.67aB±1.18	25.32aB±0.47	29.23bB±0.79	27.60abB±1.28	26.99B±0.57	
Medium	Follicular	23.02aA±2.11	24.24abA±1.50	28.78cA±0.97	27.68bcA±1.05	26.12A±0.78	27.58B±0.54
	Luteal	27.27aB±1.43	27.11aB±1.29	31.52bB±1.05	30.32abB±0.73	29.04B±0.65	
Small	Follicular	25.65aA±1.24	26.79aA±1.25	30.74bA±0.63	28.52abA±0.70	27.99A±0.59	29.22C±0.42
	Luteal	29.33aB±0.59	28.76aB±0.81	32.28bB±1.17	31.37abB±0.77	30.41B±0.49	
Overall mean at different seasons		25.33a±0.73	25.65a±0.57	29.48b±0.45	28.47b±0.46		

-Data are presented as means ± SE.
-Means having different superscripts (a, b, c) within the same raw are significantly different.
-Means having different superscripts in the same column (A, B, C), within each follicular size, differ significantly.
-N = 8 per group.

The decreased follicular fluid progesterone with increased follicular diameter observed in the present study is comparable with earlier reports in buffalo [24], [25], cattle [19]. On the other side, a positive correlation between follicular size and progesterone concentrations was reported in buffalo [21]. Moreover, [26] reported that progesterone concentrations were not related to follicular size and were not different among small, medium, and large follicles in buffaloes.

Regarding the effect of seasons of the year on estradiol and progesterone in buffalo, the results of the current work revealed that the overall means of follicular fluid estradiol and progesterone levels were found to be lower in summer and autumn compared to winter and spring. These results are in accordance with [5] in buffalo who observed decreased follicular fluid estradiol levels during summer as compared with those in winter. Lower progesterone levels during hot months may be related to the adversely affected luteal function during the summer season [27]. In summer, the dominant follicle develops in a low LH environment and this results from reduced estradiol secretion from the dominant follicle leading to poor expression of estrus and hence, reduced fertility [28].

Hyperthermia has been shown to decrease ovarian blood flow [29] and to inhibit angiogenesis [30]. Blood flow and vascular density determine the follicular perfusion rate, which directly influences the rates of nutrient uptake and hormonal release by the follicle. In addition, estradiol content in the follicular fluid reflects the balance between production of the hormone by the cells and its clearance from the follicle to the circulation. Thus, decreased steroid hormonal follicular fluid content during hot seasons could be related to heat-stress-induced alteration in vascular responses [31]. During autumn, when air temperatures have decreased and cows are no longer exposed to thermal stress, conception rates remained lower than in the winter [32]. At the same time, chronic summer heat stress was found to cause an eight times decrease in androgen production by thecal cells in autumn which was accompanied by a significant decrease in estradiol concentration in the follicular fluid [33]. In this respect [31] stated that exposure to summer heat stress during the early stages of follicular development may impair later follicular function and decrease fertility in the autumn through a delayed effect of heat stress on follicular steroidogenesis.

TABLE III
EFFECT OF FOLLICULAR SIZE, SEASON OF THE YEAR AND PHASE OF ESTROUS CYCLE ON CHOLESTEROL CONCENTRATIONS (MG/DL) IN THE FOLLICULAR FLUID OF BUFFALOES

Follicle size	Estrous phase	Summer	Autumn	Winter	Spring	Overall mean at different phases	Overall mean at different follicle size
Large	Follicular	32.47a±0.89	32.63a±0.74	35.73b±0.84	33.87ab±0.53	33.73±0.44	33.64A±0.34
	Luteal	33.17a±0.89	32.44a±0.70	34.73b±0.82	34.02ab±0.90	33.55±0.54	
Medium	Follicular	34.90ab±1.02	33.54a±0.89	36.81b±0.90	36.52b±0.69	35.46±0.48	35.60B±0.34
	Luteal	34.78ab±0.63	33.54a±1.13	37.58c±0.71	36.54bc±0.82	35.75±0.48	
Small	Follicular	36.33ab±0.95	35.69a±0.60	37.87b±0.65	36.85b±0.93	36.69±0.44	36.79C±0.29
	Luteal	35.34a±0.64	36.74ab±0.81	37.82b±0. 70	37.57ab±0.81	36.89±0.40	
Overall mean at different seasons		34.46a±0.38	33.72a±0.42	36.83b±0.34	36.37b±0.36		

-Data are presented as means ± SE.
-Means having different superscripts (a, b) within the same raw are significantly different.
-Means having different superscripts in the same column (A, B, C) differ significantly.
-N = 8 per group

TABLE IV
EFFECT OF SEASON OF THE YEAR AND PHASE OF ESTROUS CYCLE ON LEVELS OF ESTRADIOL (PG/ML), PROGESTERONE (NG/ML) AND CHOLESTEROL (MG/DL) IN THE SERUM OF BUFFALOES

Parameters	Estrous phase	Summer	Autumn	Winter	Spring
Estradiol	Follicular	38.63A±1.55	46.45A±1.35	54.21A±1.76	52.01A±2.86
	Luteal	31.22B±1.81	37.35B±1.51	44.35B±1.27	42.28B±1.37
	Overall mean	34.93a±1.50	41.90b±1.53	49.28c±1.65	46.89c±1.40
Progesterone	Follicular	0.50A±0.027	0.50A±0.029	0.64A±0.017	0.56A±0.032
	Luteal	3.69B±0.22	4.59B±0.138	5.71B±0.15	5.55B±0.44
	Overall mean	2.09a±0.43	2.43ab±0.52	3.17b±0.66	3.34b±0.65
Cholesterol	Follicular	59.15±1.14	66.85±1.43	73.35±3.36	74.24±2.02
	Luteal	60.45±2.66	66.74±2.25	73.20±1.58	72.19±2.67
	Overall mean	59.47a±1.44	66.58b±1.32	73.70c±1.63	73.22c±1.64

-Data are presented as means ± SE. N = 8 per group.
-Means having different superscripts in the same column (A, B) within different parameters differ significantly.
-Means having different superscripts (a, b) within the overall mean of row are significantly different.

TABLE V
AVERAGE LEVELS OF ESTRADIOL (PG/ML), PROGESTERONE (NG/ML) AND CHOLESTEROL (MG/DL) IN FOLLICULAR FLUID AND SERUM OF BUFFALOES

	Estradiol (pg/ml)	Progesterone (ng/ml)	Cholesterol (mg/dl)
Follicular fluid (n= 192)	566.61^{A}±3.26	27.37^{A}±0.30	35.35^{A}±0.20
Serum (n=64)	43.41^{B}±1.00	1.10^{B}±0.08	68.35^{B}±1.00

-Data are presented as means ± SE.
-Means having different superscripts in the same column (A, B) differ significantly between follicular fluid and serum.

In the current study, the levels of estradiol and progesterone were decreased in serum of buffaloes during summer and autumn as compared with those in winter and spring. Megahed et al. [34] in buffalo and Ronchi et al. [27] in cows observed a decreased serum estradiol levels during summer as compared with those in winter. Moreover, plasma estradiol concentrations were found to be reduced by heat stress in bovine; an effect that is consistent with decreased concentrations of LH and reduced dominance of the selected follicle [35]. Reference [27] found lower peripheral progesterone levels in cows in hotter than in cooler months which are believed to be responsible for the poor expression of estrus and low conception rate during summer season. On the contrary, serum progesterone concentrations were observed to be significantly higher during summer compared to those in winter season in buffalo [11] and cow [36].

In the present work, ovarian follicular fluid estradiol and progesterone concentrations were higher than serum concentrations. Similar results were reported in buffalo [21], cattle [22]. It is well established that the main source of estradiol in the blood is the follicular estradiol, especially from the growing preovulatory follicle [5].

Cholesterol is known as a precursor of all steroid hormones including estrogen and progesterone in females [37]. In the present study, the overall means of follicular fluid cholesterol concentrations decreased significantly with the increase in follicle size. This could be in agreement with the pattern of follicular fluid steroids reported in the present work; the decrease in both cholesterol and progesterone levels could account for the rise of estradiol concentrations associated with increasing follicular size. In the same respect, [38] stated that the decreased cholesterol concentration with increased follicular size might be attributed to the conversion of cholesterol to steroid hormones, estrogen and progesterone during steroidogenesis.

These results are in agreement with those reported in buffalo [21]. However, the result of the present study differs from that reported by and [6] in cow and [39] in goat who all reported increased follicular fluid cholesterol concentration with the increase in follicular size. These contradictory results may be attributed to the difference in species. Reference [40] found no significant differences in the concentrations of follicular fluid cholesterol among follicles classified according to size in buffalo. This could be attributed to the difference in age and/ or type of feed offered to the animal.

In the current study, follicular fluid cholesterol overall means were significantly lower during summer and autumn than winter and spring. The marked decrease in follicular fluid cholesterol concentration in summer and autumn may be due to a decrease in acetate concentration, which is the primary precursor for the synthesis of cholesterol [41]. In the same respect, IT might be a result of negative energy balance and reduced dry matter intake due to heat stress [42].

The obtained overall means of follicular fluid total cholesterol didn't significantly differ between follicular and luteal phases in different seasons and within different follicle classes. In addition, [40] reported that follicular cholesterol levels did not differ during the stage of the estrus cycle in buffalo. On the other hand, [43] found higher cholesterol concentrations in follicular fluid during follicular phase in comparison with luteal phase in buffalo. The present study highlighted an association between serum cholesterol concentrations and summer heat stress as the overall means of cholesterol concentration in buffalo serum was found to be significantly lower in autumn and summer than winter and spring. Furthermore, no significant changes were reported in the overall means of serum cholesterol concentration between follicular phase and luteal phase. These results are in accordance with [44] in buffalo and [45], [46] in cow who found significant decrease in serum cholesterol with the increase in environmental temperature during summer.

The observed decrease in serum cholesterol concentration in summer and autumn may be a consequence of negative energy balance [41] and reduced dry matter intake due to heat stress [42]. In addition, the marked increase in glucocorticoid hormone level (in heat stressed animals) may be another factor causing the decline in blood cholesterol [47].

The obtained results indicated that the concentrations of cholesterol in serum were higher than in different sized follicles and this could be associated with the fact that a substantial part of cholesterol content in follicular fluid originates from serum [48]. Similar findings were observed by [47] in buffaloes and [41] and [19] in cows. In the present study, the reduction of blood cholesterol under heat stress may be responsible for insufficient steroid synthesis in the ovary, where cholesterol is a precursor for steroid synthesis [49].

References

[1] I. F. Marai, A. A. Habeeb, "Buffalo's biological functions as affected by heat stress". Livest. Sci., vol. 127, 2010, pp. 89-109.

[2] S. H. Abdel-Aziem, L. M.Salem, M. S. Hassanane, K. F. Mahrous, "Genetic analysis between and within three Egyptian water buffalo populations using RAPD-PCR", J. Am. Sci., vol. 6. 2010, pp. 217-226.

[3] M. M. Nour El-Din, "Proposed climate change adaptation strategy for the ministry of water resources & irrigation in Egypt", Joint programme for climate change risk management in Egypt, 2013.

[4] R. S. Manik, P. Palta, S. K. Singla, V. Sharma, "Folliculogenesis in buffalo (*Bubalus bubalis*)", Reprod. Fertil. Dev., vol. 14, 2002, pp. 315-325.

[5] F. A. Khan, G. K. Das, M. Pande, M. Sarkar, R. K. Mahapatra, U. Shankar, "Alterations in follicular fluid estradiol, progesterone and insulin concentrations during ovarian acyclicity in water buffalo (*Bubalus bubalis*)", Anim. Reprod. Sci., vol. 130, 2012, pp. 27- 32.

[6] S. Nandi, V. Girish Kumar, B. M. Manjunatha, H. S. Ramesh, P. S. Gupta, "Follicular fluid concentrations of glucose, lactate and pyruvate in buffalo and sheep, and their effects on cultured oocytes, granulosa and cumulus cells", Theriogenology, vol. 69, 2008, pp. 186-196.

[7] Y. Ys, M. J. Luo, Z. B. Han, W. Li, H. S. Sui, J. H. Tan, "Serum and follicular fluid steroid levels as related to follicular development and granulosa cell apoptosis during the estrous cycle of goats" Small Rumin. Res., vol. 57, 2005, pp. 57-65.

[8] S. C. Vohra, Dindorkar, Kaikini, "Studies on blood serum levels of certain biochemical constituents in normal cyclic and anestrus crossbred cows", Indian J. Anim. Reprod., vol. 16, 1995, pp. 85-87.

[9] G., Basini, B., Simona, S. E. Santini, F., Grasselli, "Reactive oxygen species and anti-oxidant defences in swine follicular fluids", Reprod. Fertil. Develop., vol. 20, 2008, pp. 269-274.

[10] K. H. El-Shahat, M. Kandil, "Antioxidant capacity of follicular fluid in relation to follicular size and stage of estrous cycle in buffaloes", Theriogenology, vol. 77, 2012, pp. 1513–1518.

[11] S. Mondal, V. Kumar, I. J. Reddy, S. Khub, "Progesterone and nucleic acid content of buffalo corpus luteum in relation to stages of estrus cycle", Indian J. Anim. Sci., vol. 74, 2004, pp. 710-712.

[12] P. Jaglan, G. K. Das, B. V. Kumar, R. Kumar, F. A. Khan, S. K. Meur, "Cyclical changes in collagen concentration in relation to growth and development of buffalo corpus luteum", Vet. Res. Commun., vol. 34, 2010, pp. 511-518.

[13] M. M. Dominguez, "Effect of body condition, reproductive status and breed on follicular population and oocyte quality in cows" Theriogenology, vol. 25, 1995, pp. 800-805.

[14] P. F. Hall, "Testicular steroid synthesis" in *The Physiology of Reproduction*. E. Knobil, J. Neill, Ed. Reven Press, New York, 1988, pp. 975-998.

[15] J. A. Katt, J. A. Duncan, L. Herbon, A. Barkan, J. C. Marshall, "The frequency of gonadotropin-releasing hormone stimulation determines the number of pituitary gonadotropin-releasing hormone receptors", Endocrinology, vol. 116, 1985, pp. 2113-2115.

[16] P. R. Finley, "Quantitative - enzymatic colorimetric determination of total cholesterol in serum or plasma", Clin. Chem., vol. 24, 1987, pp. 391-394.

[17] K. P. McNatty, D. A. Heath, K. M. Henderson, S. Lun, P. R. Hurst, L. M. Ellis, G. W. Montgomery, L. Morrison, D. C. Thurley, "Some aspects of thecal and granulosa cell function during follicular development in the bovine ovary", J. Reprod. Fertil., vol. 72, 1984, pp. 39-53.

[18] M. D. Reyes, M. L. Villagran, R. Cepeda, M. Duchens, V. Parraguez, B. Urquieta, "Histological characteristics and steroid concentration of ovarian follicles at different stages of development in pregnant and non-pregnant dairy cows", Vet. Res. Commun., vol. 30, 2006, pp. 161-173.

[19] J. F. Aller, S.S. Callejas, R. H. Alberio, "Biochemical and steroid concentrations in follicular fluid and blood plasma in different follicular waves of the estrous cycle from normal and superovulated beef cows", Anim. Reprod. Sci., vol. 142, 2013, pp. 113-120.

[20] M. A. Beg, O. J. Ginther, "Follicle selection in cattle and horses: role of intrafollicular factors", Reproduction, vol. 132, 2006, pp. 365-377.

[21] J. M. Alkalby, F. H. Bushra, T. A. Fahad, "Study on some hormonal and biochemical constituents of follicular fluid and blood plasma in buffaloes", Bas. J. Vet. Res., vol. 11, 2012, pp. 90-102.

[22] N. Hiromi, H. Seizo, A. Glen, M. Akio, T. Masafumi, "Classification of bovine follicles based on the concentration of steroids` glucose and lactate in follicular fluid and the status of accompanying follicles", J. Reprod. Dev., vol. 55, 2009, pp. 218-224.

[23] A. P. Parmar, V. M. Mehta, "Seasonal endocrine changes in steroid hormones of developing ovarian follicles in Surti buffaloes", Ind. J. Anim. Sci., vol. 64, 1994, pp. 111-113.

[24] M. Y. Mekkawy, H. A. Salem, M. Younis, R. Youssef, A. Azous, A. A. Farahat, "Estradiol, progesterone, thyrotrophic (TSH) and thyroid hormone concentrations in buffalo follicular fluid in relation to follicle size", Alexandria J. Vet. Sci., vol. 4, 1988, pp. 391-396.

[25] K. S. Khera, "Studies on the preovulatory and atretic follicles of buffalo ovary", Ph.D. Thesis, Indian Veterinary Research Institute, Izatnagar, India, 1989.

[26] P. Palta, N. Bansal, R. S. Manik, B. S. Prakash, M. L. Madan, "Interrelationships between follicular size, estradiol-17*ß*, progesterone and testosterone concentrations in individual buffalo ovarian follicles", Asian-Aus. J. Anim. Sci., vol. 13 (Supplement), 2000, pp. 272

[27] B. Ronchi, G. Stradaioli, A. VeriniSupplizi, U. Bernabuci, N. Lacetera, P. A; Accorsi, A. Nardone, E. Seren, "Influence of heat stress or feed restriction on plasma progesterone, oestradiol-17β, LH, FSH, prolactin and cortisol in Holstein heifers", Livestock Prod. Sci., vol. 68, 2001, pp. 231-241.

[28] Z. Roth, R. Meidan, R. Braw-Tal, D. Wolfenson, "Immediate and delayed effect of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows", J. Reprod. Fertil., vol. 120, 2000, pp. 83-90.

[29] A. Lublin, D. Wolfenson, "Effect on blood flow to mammary and reproductive systems in heat-stressed rabbits", Comp. Biochem. Physiol., vol. 115, 1996, pp. 277-285.

[30] L. F. Fajardo, S. D. Prionas, J. Kowalski, H. H. Kwan, "Hyperthermia inhibits angiogenesis", Radiat. Res., vol. 114, 1988, pp. 297-306
[31] Z. Roth, R. Meidan, A. Shaham-Albalancy, R. Braw-Tal, D. Wolfenson, "Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles", Reproduction, vol. 121, 2001, pp. 745-751.
[32] P. J. Hansen, "Effects of environment on bovine reproduction. in *Current Therapy in Large Animal Theriogenology*, R. S. Youngquist, W. B. Saunders, Ed. Philadelphia, 1997, pp. 403-415.
[33] D. Wolfenson, B. J. Lew, W. W. Thatcher, Y. Graber, R. Meidan, "Seasonal and acute heat stress effects on steroid production by dominant follicles in cows", Anim. Reprod. Sci., vol. 47, 1997, pp. 9-17.
[34] G. A. Megahed, M. M. Anwar, S. I. Wasfi, M. F. Hammadeh, "Influence of heat stress on the cortisol and oxidant-antioxidants balance during oestrous phase in buffalo-cows (*Bubalus Bubalis*): themo-protective role of antioxidant treatment", Repod. Domest. Anim., vol. 43, 2008, pp. 672-677.
[35] F. D. Rensis, R. J. Scaramuzzi, "Heat stress and seasonal effects on reproduction in the dairy cow", Theriogenology, vol. 60, 2003, pp. 1139-1151.
[36] A. Guzeloglu, J. D. Ambrose, T. Kassa, T. Diaz, M. J. Thatcher, W. W. Thatcher, "Long term follicular dynamics and biochemical characteristics of dominant follicles in dairy cows subjected to acute heat stress", Anim. Reprod. Sci., vol. 66, 2001, pp.15-34.
[37] Z. U. Rahman, S. A. Bukhari, N. Ahmad, N. Akhtar, A. Ijaz, M. S. Yousaf, I. U. Haq, "Dynamics of follicular fluid in One-humped Camel (*Camelus dromedaries*)", Reprod. Domest. Anim., vol. 43, 2008, pp. 664-671.
[38] N. M. Kor, K. Moradi, "A review of biochemical metabolites concentration and hormonal composition of ovarian follicular fluid in domestic animals", Annu. Rev. Res. Biol., vol. 3, 2013, pp. 246-255.
[39] O. P. Mishra, J. N. Pandey, P. G. Gawande, "Study on biochemical constituents of caprine ovarian follicular fluid after superovulation", Asian Aust. J. Anim. Sci., vol. 16, 2003, pp. 1711-1715.
[40] M. R Abd Ellah, H. A. Hussein, D. R. Derar, "Ovarian follicular fluid constituents in relation to stage of estrus cycle and size of the follicle in buffalo", Vet. World, vol. 3, 2010, pp. 263-267.
[41] J. L. Leroy, T. Vanholder, J. R. Delanghe, G. Opsomera, A. Van Sooma, P. E. Bols, A. Kruif, "Metabolite and ionic composition of follicular fluid from different-sized follicles and their relationship to serum concentrations in dairy cows", Anim. Reprod. Sci., vol. 80, 2004, pp. 201-211.
[42] C. E. Moore, J. K. Kay, R. J. Collier, M. J. VanBaale, L. H. Baumgard, "Effect of supplemental conjugated linoleic acids on heat-stressed Brown Swiss and Holstein cows", J. Dairy Sci., vol. 88, 2005, pp. 1732-1740.
[43] D. B. Acar, M. K. Birdane, N. Dogan, H. Gurler, "Effect of the stage of estrous cycle on follicular population, oocyte yield and quality and biochemical composition of serum and follicular fluid in Anatolian water buffalo", Anim. Reprod. Sci., vol. 137, 2013, pp. 8-14.
[44] D. N. Verma, S. N. Lal, S. P. Singh, O. M. Parkash, O. Parkash, "Effect of season on biological responses and productivity of buffalo", Int. J. Anim. Sci., vol. 15, 2000, pp. 237-244.
[45] I. F. Marai, A. A. Habeeb, A. H. Daader, H. M. Yousef, "Effect of Egyptian subtropical conditions and the heat stress alleviation techniques of water spray and diaphoretics on the growth and physiological functions of Friesian calves", J. Arid Environ., vol. 30, 1995, pp. 219-225.
[46] A. A. Habeeb, K. M. EL-Masry, A. I. Aboulnaga, T. H. Kamal, "The effect of hot summer climate and level of milk yield on blood biochemistry and circulating thyroid and progesterone hormones in Friesian cows", Arab. J. Nuclear Sci. Applic., vol. 29, 1996, pp. 161-173.
[47] H. M. Arshad, N. Ahmad, Z. U. Rahman, H. A. Samad, N. Akhtar, S. Ali, "Studies on some biochemical constituents of ovarian follicular fluid and peripheral blood in buffaloes", Pak. Vet. J., vol. 25, 2005, pp.189-193.
[48] H. K. Shabankareh, N. M. Kor, H. Hajarian, "The influence of the corpus luteum on metabolites composition of follicular fluid from different sized follicles and their relationship to serum concentrations in dairy cows", Anim. Reprod. Sci., vol. 140, 2013, pp. 109-114.
[49] M. A. Shehab-El-Deen, J. M. Leroy, M. S. Fadel, S. Y. Saleh, D. Maes, A. Van Soom, "Biochemical changes in the follicular fluid of the dominant follicle of high producing dairy cows exposed to heat stress early post-partum", Anim. Reprod. Sci., vol. 117, 2010, pp. 189-200.