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RELATIONSHIP OF BLOOD METABOLITES, MINERALS AND THYROID HORMONES DURING ESTROUS CYCLE WITH POST-PARTUM FERTILITY IN EGYPTIAN BUFFALOES

By

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ABSTRACT

This study aimed to determine the relationship of some blood biochemicals, thyroid hormones profiles and minerals during different stages of the estrous cycle and in relation to follicular size with early conception in Egyptian buffaloes during the postpartum period. According to pregnancy diagnosis, 30 out of 43 buffaloes became pregnant (69.7%), 20 animals being pregnant (46.6%) within 90 day-postpartum (1st group, G1) and 10 animals (23.25%) within 120 day postpartum (2nd group, G2). The estrous cycle was classified into four stages (I: 0-4d, II: 5-10d, III: 11-17d and IV: 18-21d). Follicles were grouped into three diameter classes: class 1 (3 to 5 mm), class 2 (6 to 9 mm), and class 3 (≥10 mm). Results showed that overall mean of serum total proteins (TP), albumin (AL), globulin (GL), glucose, cholesterol (CH) and triglycerides (TG) concentrations were higher ($P<0.001$) in G1 than in G2. Overall mean of TP and glucose levels increased ($P<0.05$) from I to II stage, then showed insignificant changes at the following stages, while GL and CH levels significantly ($P<0.05$) increased from I up to III stage, then showed insignificant change, but AL and TG levels were not affected significantly by estrous stage. Overall mean of TP, AL, GL and TG levels were not affected significantly by follicular size. However, glucose concentration increased ($P<0.05$) by increasing follicular size from I to III class, while CH concentration increased ($P<0.05$) only from I to II class. Concentrations of serum T3 and T4 as well as Zn and Se contents were higher ($P<0.001$) in G1 than in G2. As affected by follicular size, Se content increased ($P<0.05$) by increasing follicular diameter. However, concentration of T3and T4, and Zn contents were not affected by follicular size.

The blood composition regarding biochemical, mineral and thyroid hormones provides a useful indication of the requirements for lactating buffaloes during early stage of postpartum period. Thyroid hormones and biochemical metabolites concentration have relationships considerably with the stage of estrous cycle and follicular size. There were changes in the biochemical composition of blood happened during estrous stages, and follicular development, indicating a relationship of blood metabolites, mineral and thyroid hormone with the reproductive status and metabolic activity of the lactating buffaloes. Based on the obtained results, breeders should take in mind the change in energy balance and feeding requirements during the early stage of postpartum period to obtain higher pregnancy rate within 90 day-postpartum

Keywords:

Buffaloes, pregnancy rate, blood biochemical, estrous cycle, follicle size.

INTRODUCTION

Reproductive efficiency of females is assessed by its ability to produce a viable offspring at the expected intervals. The postpartum period is vulnerable period for diseases in the reproductive life of the female **(Swanson, 1989).** There are many factors affecting duration of postpartum intervals like age, body weight, body condition, nutritional status, blood biochemical, season and suckling. Earlier studies conducted abroad indicate that certain blood parameters can indicate the postpartum reproductive performance in cattle **(Heuer *et al.,* 1999; De Vries and Veerkamp, 2000).**

Blood biochemical parameters employed as useful indicators of health as well as nutritional status, help in diagnosis of metabolic diseases, infertility and low productivity in farm animals **(Piccione *et al*., 2010**; **Amle *et al*., 2014; Kaminski *et al*., 2014)**. Blood biochemical changes that occur during early postpartum period are closely related with physiological statues and reproductive performance of dairy animals. A decline in reproductive efficiency during the postpartum period might be caused by high milk production, low plane of nutrition, energy balance and heat stress (**Chapa *et al.,* 2001; Grummer, 2007**). Postpartum metabolic changes induced by mismatching of energy requirements and energy intake may lead to negative energy balance, which impacts the subsequent fertility, also assessing metabolic profile of dairy animals can reveal the reasons behind differential fertility **(Jorritsma *et al.,* 2003).**

Low reproductive efficiency in buffaloes is a major problem facing buffalo production

(Sharma, 2003; Ahmed, 2006). Blood profiles might be a potential aid in characterizing the problem. The blood components are mirror, which reflects the healthy condition of animals. So, the biochemical studies are very important for clinicians in the field during interpretation of their findings **(Nebel and Jobst, 1998).** The changes in blood constituents can reflect the physiological condition as well as nutritional and health status of cows **(Huszenicza *et al.,* 2002).**

Biochemical concentrations in the follicular fluid of the bovine ovary fluctuate considerably with the stage of estrous cycle, follicular size and status, and presence of large follicles **(Tabatabaei and Mamoei, 2010)**. In cattle, trace elements are among the antioxidants which can positively affected fertility **(Baldi *et al.,* 2000)**. Moreover, thyroid hormones were found to have a large and effective role in the reproductive process. In this line, **Suriyasathaporn (2000)** mentioned that thyroid hormones are part of the complex hormonal mechanism that regulates steroidogenesis in the ovary. The thyroid hormones maintain the homeostasis of energy and protein metabolism, thermo-regulation, growth and productivity parameters **(Huszenicza *et al.,* 2002).** The analysis of the functional activity of the thyroid gland is relevant for assessing breeding qualities of animals **(Ladanova *et al.,* 2018).**

Further studies are required to determine the relationship between concentration of blood biochemical, minerals and thyroid hormone at different stages of post-partum period.

Therefore, the goal of the present work was to determine the relationship of some blood biochemicals, thyroid hormones profile and minerals during different stages of the estrous cycle and in relation to follicular size with early conception of Egyptian buffaloes during the postpartum period.

MTERIAL AND METHODS

This study was carried out at Mehallet Mousa Experimental Station belonging to Animal Production Research Institute (APRI), Agricultural Research Center, located in the North Nile Delta, Kafr-El-Sheikh Governorate, Egypt, during the period extending from November 2017 to February 2018.

Animals feeding and management:

Total of 43 multiparous Egyptian lactating buffalo with 3 -5 parities, 6 -7 years old and 450-600 kg live body weight were used in the present study. All experimental animals had normal reproductive tract and normal cyclic activity and were kept indoors in semi-shaded pens.

They were fed on diet that met both maintenance and milk production requirements according to **APRI (1997),** consisting of clover (2nd and 3rd cuts), rice straw and concentrate feed mixture (33% un-decorticated cotton seed, 22% corn, 21% wheat bran, 14% rice bran, 3% molasses, 3% limestone, 1.2% common salt and 2.8% calcium). Drinking water was available all day times.Chemical composition of feedstuffs were determined according to**AOAC (1990)** and presented in (Table 1).The experimental buffaloes were milked twice daily**.**

During post-partum period of 120 days, all animals in heat were naturally served with fertile buffalo bulls. According to pregnancy diagnosis, 30 out of 43 buffaloes became pregnant (69.7%), 20 animals being pregnant (46.6%) within 90 day-postpartum (1st group, G1) and 10 animals (23.25%) within 120 day postpartum (2nd group, G2).

Table (1): Chemical composition of the feed ingredients (on DM basis).

Ingredient	DM %	Chemical composition on DM basis (%)					
		OM	CP	EE	CF	NFE	ASH
Concentrate feed mixture	**89.9**	**88.7**	**16.1**	**4.8**	**18.4**	**49.4**	**11.3**
Clover (2nd cut)	**15.8**	**85.4**	**15.2**	**1.8**	**20.8**	**47.6**	**14.6**
Clover (3rd cut)	**19.7**	**86.8**	**13.7**	**3.0**	**23.4**	**46.7**	**13.2**
Rice straw	**92.5**	**83.9**	**3.7**	**1.3**	**36.4**	**42.5**	**16.1**

DM: dry matter; OM: organic matter, CP: crude protein; CF: crude fiber; EE: ether extract; NFE: nitrogen free extract.

Reproductive management:

Heat detection, service and pregnancy.

Buffaloes were observed twice daily at an intervals of 12 h by experienced herd man for at least one hour for estrous signs. Buffaloes stand to be mounted by the teaser bull were considered in estrus.

On day ≥45 of postpartum period, all experimental animals in heat were inseminated with fertile bull naturally according to morning/evening system. On day 25 post-service, non-return animals were ultrasonography investigated to indicate pregnancy, while those returned to estrus were re-served. Thereafter, number of conceived animal during 90 or 120 days postpartum was recorded and pregnancy rate was calculated. Days open was calculated by subtracting day of last calving from day of fertile service.

Experimental design:

Before pregnancy, the estrous cycle of all pregnant buffaloes (30 animals) was classified into four stages (I: 0-4d, II: 5-10d, III: 11-17d and IV: 18-21d) according to **Ali *et al.* (2003).** Ovarian maps were drawn at each examination and sizes of ovarian follicles greater than 3.0 mm in diameter were recorded, then animals were grouped according to three follicular diameter classes: class 1 (3 to 5 mm), class 2 (6 to 9 mm), and class 3 (≥10 mm).

Ultrasound Examinations:

Ovaries were examined by trans rectal ultrasonography using ULTRSCAN Model 900, 5 MHz and "Falco, Easote/Piemedical, Maastricht, the Nether lands", 6-8 MH2 Linear array transducer (Alliance Medical Int) In midmorning on every 3 days during the second estrous cycle of the experiment.

Blood sampling:

Blood sample collection was initiated when the animals showing estrus symptoms within 0-4, 5-10, 11-17 and 18-21 d of estrous cycle. Also, blood samples were collected during follicular development of three diameter classes I: 3-5 mm, II: 6 - 9 mm), and class 3 (≥10 mm). About 10 ml of blood was drawn from the jugular vein of each animal in a clean vacutainer tube without anticoagulant, centrifuged at 3000 rpm for 10 min; clear serum free from hemolysis. Glucose concentration was assayed immediately in fresh samples of serum, while other serum parameters were determined in samples stored at - 20 ^{o}C.

Blood assays:

Blood serum samples were analyzed for determining concentration of various biochemical metabolites (total proteins, albumin, triglycerides, glucose and total cholesterol) using reagent test kits supplied commercially (Bio diagnostic, Egypt). The globulin concentration in serum was calculated by subtracting albumin concentration from total proteins concentration.

Trace elements (zinc and selenium) content and thyroid hormones profile (triiodothyronin and thyroxinprofiles) were determined. Trace elements including selenium (Se) and zinc (Zn) serum concentrations were determined using atomic absorption spectrophotometer as outlined by**Varley*et al.*(1980).**Direct radioimmunoassay techniquewas performed for the determination of thyroid hormones (triiodothyronine and thyroxine) in blood serum. Chemical kits (Diagnostic Products Corporation, DCP, and Los Angloes, USA) with ready antibody coated tubes were used according to the procedures outlined by the manufacturer.

Statistical analysis:

The Data were statistically analyzed using factorial design (2 groups x 4 estrous stages or 3 follicular classes) within **SPSS (2008)** program. The significant differences among means of estrous stage or follicular classes were done by Multiple Range Test of **Duncan (1955)** and set at $P<0.05$).

RESULTS AND DISCUSSION

Blood metabolites changes:

Effect of estrous cycle stages.

Overall mean of serum total proteins (TP), albumin (AL), globulin (GL), glucose, cholesterol (CH) and triglycerides (TG) concentrations were significantly ($P<0.001$) higher in G1 than in G2. Overall mean of TP and glucose levels significantly ($P<0.05$) increased from I to II stage, then showed insignificant changes at the following stages, while GL and CH levels significantly ($P<0.05$) increased from I up to III stage, then showed insignificant change, but AL and TG levels were not affected significantly by estrous stage (Table 2).

The effect of interaction between group and estrous cycle stage was significant on concentration of TP ($P<0.01$), GL ($P<0.001$), glucose ($P<0.01$) and CH ($P<0.05$), while AL and TG concentrations were not affected significantly by this interaction (Table 2).

The significant interaction on TP, GL, glucose and TG concentrations reflected increase of TP, glucose and TG levels in G1 from I to II stage with nearly constant in G2 during estrous cycle. However, GL Concentration showed an opposite trend of change in G1 and G2 at all estrous cycle stages. Generally, concentration of all metabolites studied were higher in G1 than in G2 at all estrous cycle stages Fig. (1).

Table (2): Concentration of serum metabolites in buffaloes of G1 and G2 at different estrous cycle stages.

Variable	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Effect of group (G):						
G1	7.30±0.072	3.59±0.046	3.70±0.050	70.09±0.508	82.44±0.758	22.49±0.280
G2	5.74±0.049	2.40±0.068	3.34±0.054	66.17 ±0.561	69.67 ±0.569	19.36±0.267
P-Value	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
Effect of estrous stage (S):						
I (0-4)	6.39±0.137^{b}	3.20±0.116	3.20±0.073^{c}	65.45±0.912^{b}	75.61±1.285^{c}	20.92±0.562
II (5-10)	6.88±0.177^{a}	3.32±0.139	3.56±0.061^{b}	69.54±0.794^{a}	77.30±1.153^{bc}	21.55±0.538
III (11-17)	7.00±0.186^{a}	3.22±0.128	3.78±0.087^{a}	69.95±0.728^{a}	78.79±1.613^{ab}	21.81±0.427
IV (18-21)	6.83±0.157^{a}	3.05±0.131	3.78±0.059^{a}	70.18±0.630^{a}	81.02±1.905^{a}	21.54±0.441
P-Value	0.0076**	0.0912^{NS}	0.0001***	0.0006***	0.05*	0.7163^{NS}
Effect of interaction (G x S):						
P-value	0.0030**	0.4475^{NS}	0.0006***	0.0097**	0.0250*	0.5473^{NS}

$^{a, b \text{ and } c}$:Means denoted within the same column with different superscripts are significant at P<0.05.
NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. * Significant at P<0.001.**

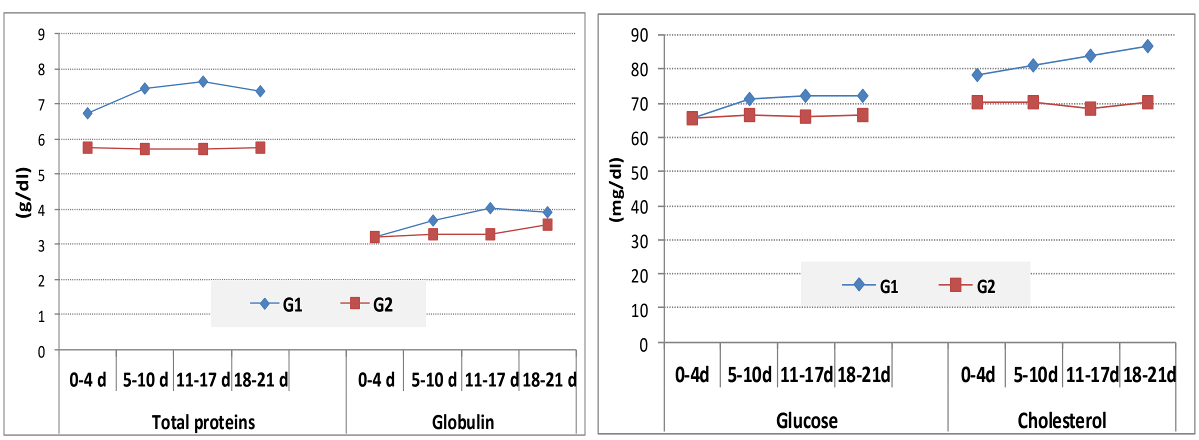

Fig. (1): Change in level of serum total proteins, globulin, glucose and cholesterol of buffaloes in G1 and G2 at different estrous cycle stages.

Generally, values of TP and their fractions are well within the range reported by several authors in buffaloes **(Agarwal *et al.,* 1985; Quayam *et al.,* 1990; Veena *et al.* (2015)**.

Many research studies on the relationship between blood biochemical parameters and reproductive performance of buffaloes are debatable and sometimes contradictory **(Kumar *et al.,* 2010; Ghuman *et al.,* 2011; Khan *et al.,* 2011)**. The changes in the biochemical constituents of the blood are important indicators of reproductive status of dairy animals **(Perveen and Usmani, 1993)**.Inthis respect,**Alavi-Shoushtari *et al.*(2006**) mentioned that blood protein concentration is directly affected by some conditions such as stage of estrous cycle, pregnancy, lactation period and stress. In accordance with the present trend of serum TP in both groups, **Veena *et al.* (2015)** found that blood TP concentration was higher in buffalo cows showing estrus within 2 months than in those showing estrus after 2 months (7.14 vs. 7.08 g /dl). However, blood TP level did not reveal any significant difference between group of animals which conceived within 3 months and other groups that conceived after 3 months (7.18 vs. 7.09 g/dl)**.**

In bovine, **Tabatabaei and Mamoei (2010)** reported that biochemical metabolites concentration in the follicular fluid of the ovary was found to be fluctuated considerably with the stage of estrous cycle. Although, **Veena *et al.* (2015)** reported insignificant difference in the blood total proteins concentration between cows which conceived within 3 months or more than 3 months, the present study indicated marked differences in serum total proteins and their fraction, being higher in G1 than in G2. Similarly, **Alavi-Shoushtari *et al.* (2006**) reported that serum TP in peripheral blood of cows was found to be lower during the estrous stage than other stages. This reduction in TP concentration at estrus was attributed to a reduction in serum $\alpha 1$, $\gamma 1$ and $\gamma 2$ globulin during estrous stage. In goats, **Yaqub *et al.* (2011)** found also low serum concentration of TP during estrous phase of estrous cycle. Generally, the lower level of serum proteins may cause deficiency of certain amino acids required for the synthesis proteins in the body.

Blood glucose appears to be one of the key nutrients affecting ovarian activity in farm animals which plays an important role in ovarian metabolism. So, the maintenance of physiological levels of glucose is a perquisite for optimum fertility and reproductive success in buffaloes. It increases luteinizing hormone, follicle stimulating hormone, insulin, growth hormone and insulin like growth factor **(Arshad *et al.,* 2005)**. Similar to the present results,

Veena ***et al.*** **(2015)** observed significant increase in blood glucose in buffalo cows which came to estrus within 2 months compared to those which came to estrus after 2 months postpartum. However, **Singh** ***et al.*** **(2004)** reported that, the level of glucose did not influence early or late occurrence of postpartum estrus in buffaloes. Plasma glucose may be involved in steroid synthesis in the ovary and luteal function and thus, might strongly influence the interval from calving to conception **(Reist** ***et al.,*** **2003)**. The increased serum glucose levels during different estrous cycle stages in G1 compared with G2 may suggest negative energy balance in G2 that may affect the subsequent fertility **(Bertoni** ***et al.,*** **1999)**.

Cholesterol plays a significant role in the ovarian physiology. Cholesterol is a precursor of steroid hormones and its higher blood concentrations in the pregnant buffaloes group suggested a better ovarian activity **(Ashmawy, 2015).** In accordance with the present results, **Veena** ***et al.*** **(2015)** found that cholesterol proportions were higher in animals which came in heat within 2 months than those came in heat after 2 months postpartum. The same authors add that there was significant correlation between blood cholesterol and first postpartum heat. Dairy animals with higher plasma cholesterol are more likely to express estrous as lipids are the precursors of gonadal steroid hormones **(Jorritsma** ***et al.*** **2003)**.

Regarding the observed increase in triglycerides level in G1 than in G2 in the present study, **Kor and Moradi (2013)** indicated that triglycerides might be the alternate sources of energy for cells in follicles. In disagreement with the present results, several authors found that serum triglyceride concentration is not related to resumption of postpartum ovarian cyclicity in cattle **(Jayachandran** ***et al.,*** **2013; Kumar** ***et al.,*** **2015).**

<u>Effect of follicular size:</u>

Overall mean of serum total proteins (TP), albumin (AL), globulin (GL), glucose, cholesterol (CH) and triglycerides (TG) concentrations were significantly ($P<0.001$) higher in G1 than in G2. Overall mean of TP, AL, GL and TG levels were not affected significantly by follicular size. However, glucose and CH levels were affected significantly ($P<0.05$) by follicular size. Glucose concentration showed continuous and significant ($P<0.05$) increase by increasing follicular size from I to III class, while CH concentration showed significant ($P<0.05$) increase only from I to II class (Table 3).

Table (3): Concentration of serum metabolites in buffaloes of G1 and G2 with different follicular sizes.

Variable	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Effect of group (G):						
G1	7.10±0.044	3.22±0.047	3.88±0.057	69.92±0.560	82.59±0.458	21.93±0.247
G2	5.42±0.084	2.20±0.060	3.22±0.109	64.73±0.506	69.15±0.777	19.44±0.267
P-Value	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
Effect of follicle size (F):						
Class I: 3-5 mm	6.42±0.132	2.90±0.086	3.53±0.103	66.05±0.723[c]	76.26±1.171[b]	20.64±0.346
Class II: 6-9 mm	6.59±0.183	2.86±0.120	3.73±0.121	68.31±0.711[b]	78.78±1.314[a]	21.29±0.383
Class III: ≥10 mm	6.61±0.167	2.89±0.121	3.72±0.096	70.21±0.909[a]	79.29±1.525[a]	21.38±0.429
P-Value	0.091 NS	0.908 NS	0.208 NS	0.0001***	0.0022**	0.201NS
Effect of interaction (G x F):						
P-value	0.0011**	0.05*	0.4626NS	0.0001***	0.0199*	0.1439NS

a, b and c:Means denoted within the same column with different superscripts are significant at P<0.05.
NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. * Significant at P<0.001.**

The effect of interaction between group and follicular size was significant on concentration of TP (P<0.01),AL(P<0.05),glucose (P<0.001)andCH (P<0.05),while GL and CH concentrations were not affected significantly by this interaction (Table 3). The significant interaction on TP, AL, glucose and TG concentrations reflected increase of TP, glucose and CH levels in G1 by increasing follicular diameter from 3-5 up to >10 mm with slight decrease in G2, but all previous concentrations were higher in G1 than in G2 with different follicular diameters Fig. (2).

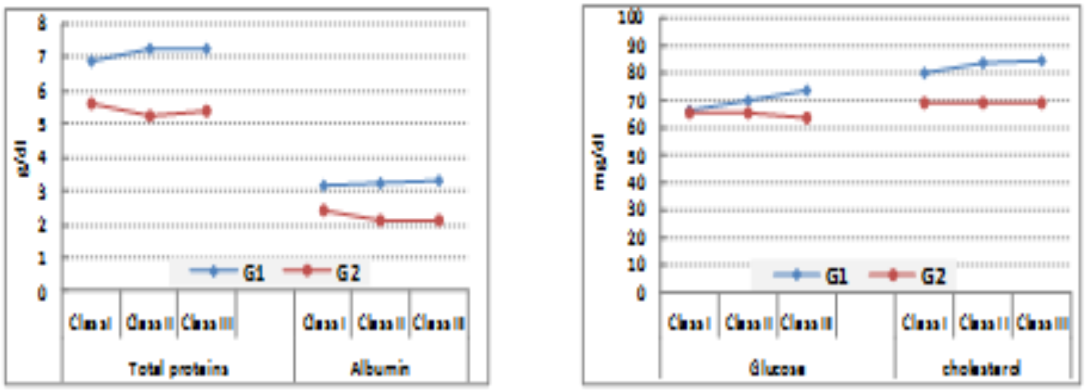

Fig. (2): Serum concentration of total proteins, albumin, glucose and cholesterol of buffaloes in G1 and G2 with different follicular sizes.

In buffaloes during estrus, **Kumar *et al.* (2015)** found that both of total protein and albumin concentrations in follicular fluid were significantly higher, may be due to increase in permeability of blood vessels across the basement membrane, which are in agreement with those found in buffaloes **(Arshad *et al.*, 2005).**

In bovine, **Tabatabaei and Mamoei (2010)** reported that biochemical metabolites concentrations in the follicular fluid of the ovary were found to be fluctuated considerably with follicle size and presence of large follicles.**Wise (1987)** reported that,the high correlation between total proteins contents in serum may be due to a substantial part of portion contents of follicular fluid originates from the serum. At estrus, during ovulation, some follicular fluid containing albumin is picked by fimbria which play a role in sperm capacitation by inducing cholesterol efflux. **Lucy *et al.*, (1992)** found direct relationship between positive energy status at early postpartum and diameter of largest follicle on day 10 postpartum. However, **Hassan *et al.* (2018)** found significant decreases in TP concentration by increasing the size of follicles in camels. Generally, TP and AL affect oocyte development and quality **(Kumar *et al.*, 2015).** The present results agreed with the results of **Veena *et al.* (2015)** on concentration of glucose in buffaloes**. Hassan *et al.*(2018)** found significant ($P<0.05$) increase in glucose concentration of the follicular fluid with increasing the follicular size in camels. **Al-Rubaeae (2015)** found that, the follicular glucose concentration was significantly increased ($P<0.05$) from small to large follicles. **Kor and Moradi (2013)** reported that follicular fluid concentration of glucose was significantly ($P<0.05$) differ with follicles sized categories. **Razzaque *et al.*,(2012)** observed that as the follicle size increases glucose levels in the fluid also increase in cows and buffalos. This suggests that glucose metabolism is less intense in large follicles of cyclic animals as compared to small follicles of acyclic one. An increase in the volume of follicular fluid and increased permeability of the blood follicle barrier during follicular growth **(Bagavandoss *et al*., 1983)** could be attributed to higher glucose levels in large size follicles **(Gosden *et al*., 1988).** Also, hypoglycaemia leads to depression in hypothalamic functions causing loss of ovarian activity which is due to failure of release of gonadotropin hormone. This may explain improving pregnancy rate of buffaloes in G1 than in G2. Follicle size got enlarged at estrus; glucose concentration was increased simultaneous with increase in follicle size **(Leroy *et al.,* 2004; Tabatabaei and Mamoei, 2010)**. Larger follicles in estrus have less glucose metabolism (per follicular fluid volume) than small ones in anestrus, in turn results in less uptake of follicular fluid glucose by granulosa cells **(Gosden *et al.,* 1988).** 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 hypoglycemic state in buffaloes reduced the hypothalamic-hypophyseal-ovarian axis signal transmission leading to anestrus condition **(Sharma *et al.,* 1998).**

In accordance with the present results, some authors reported increased cholesterol concentration with the increase in follicular size in cows **(Nandi *et al.,*2008)** and in goats **(Mishra *et al.,*2003)**. In camels, **Hassan *et al.,*(2018)** found also significant ($P<0.05$) increase in cholesterol concentration of the follicular fluid with increasing the follicular size.

Kor andMoradi (2013)reported that follicular fluid concentration of cholesterol significantly ($P<0.05$) differ with follicles sized categories. Concentration of total cholesterol significantly decreased ($P<0.05$) by increasing follicular size **(Al-Rubaeae (2015)**. Contrary, **Abd Ellah *et al.* (2010)** found no significant differences in the concentrations of cholesterol among follicles sizes in buffaloes; this could be attributed to the difference in age and/ or type of feed offered to the animal. In contrast to results of TG,**Al-Rubaeae(2015)** found that concentration of triglycerides significantly increased in large follicles as compared to small follicles.

Also, **Kor and Moradi (2013)** reported that follicular fluid concentration of cholesterol significantly ($P<0.05$) differ with follicles sized categories.

<u>Thyroid hormone profile and mineral contents:</u>

Effect of follicular size.

Concentrations of serum T3 and T4 concentrations as well as Zn and Se contents were significantly (P<0.001) higher in G1 than in G2. As affected by follicular diameter, Se content significantly (P<0.05) increased by increasing follicular size. However, concentration of T3 and T4 concentration, and Zn content were not affected significantly by follicular size (Table 4). The effect of interaction between group and follicular size was significant on Se content (P<0.001), while T3 and T4 concentrations, and Zn content were not affected significantly by this interaction (Table 4). The recorded significant interaction effect on Se content was reflected from marked increase in Se content by increasing follicular diameter in G1 as compared to unchanged Se content with increasing follicular size in G2 Fig. (3).

Table (4): Concentration of metabolic and thyroid hormones, and minerals in serum buffaloes of G1 and G2 with different follicular sizes.

Variable	Hormonal profile		Mineral content	
	T3 (ng/ml)	T4 (µg/dl)	Zn (µg/dl)	Se (µg/dl)
Effect of group (G):				
G1	**104.49±0.478**	**3.67±0.051**	**131.08±0.349**	**140.90±0.502**
G2	**92.93±0.828**	**2.90± 0.054**	**122.54±0.517**	**122.48±0.530**
P-Value	**0.0001*****	**0.0001*****	**0.0001*****	**0.0001*****
Effect of follicle size (F):				
Class I: 3-5 mm	**99.61±1.146**	**3.41±0.930**	**127.51±0.796**	**132.63±1.435[b]**
Class II: 6-9 mm	**101.10±1.142**	**3.36±0.095**	**128.22±1.038**	**135.58±1.857[a]**
Class III: ≥10 mm	**101.20±1.418**	**3.48±0.095**	**128.96±0.832**	**136.07±1.832[a]**
P-Value	**0.215 NS**	**0.464 NS**	**0.119 NS**	**0.0001*****
Effect of interaction (G x F):				
P-Value	**0.0645NS**	**0.5127NS**	**0.1381NS**	**0.0032****

a, b and c:Means denoted within the same column with different superscripts are significant at P<0.05. NS: Not significant. ** Significant at P<0.01. * Significant at P<0.001.**
T3 Ttriiodothyronine, T4: Thyroxine.

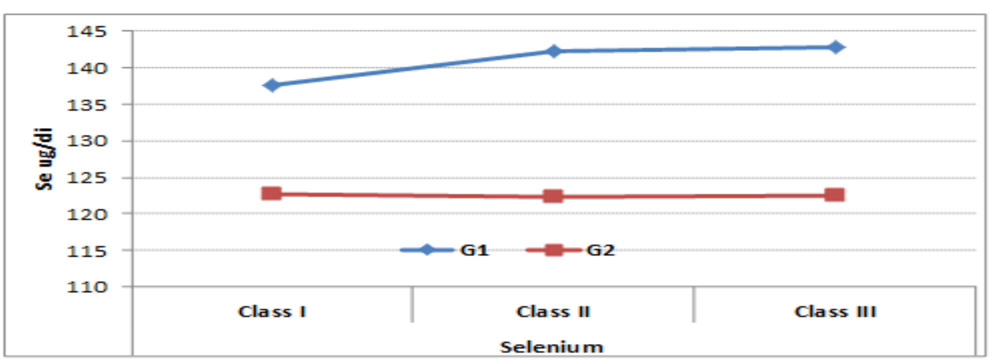

Fig. (3): Serum concentration of selenium of buffaloes in G1 and G2 with different follicular sizes.

In similarity with the obtained results, no significant differences were found in T3 or T4 concentration at different stage of the estrous cycle in mare **(Johnson, 1986)**. However, **Spicer *et al.* (2001)** mentioned that thyroid hormones in cattle may have stimulatory effects on ovarian function acting at the level of granulose cells. **Alkalby *et al.* (2012)** found that T_4 concentrations were significantly higher in blood plasma than in follicular fluid in small and large follicles, while no significant differences were found in concentrations of T_3 between blood plasma and follicles. In cows, **Ashkar *et al.* (2010)** reported that the concentrations of T_4 were significantly greater (P<005) in large follicles compared with small follicles, but T3 and T4 concentrations were greater (P<0.05) in serum than follicular from small and large follicles. **Barbara *et al.* (2006)** in cows described that concentrations of T_4 in small follicles, were significantly (P<0.05) higher than in large follicles and no differences were found in concentrations of T_3 in both groups of follicles.

In general, the positive correlation between circulating thyroid hormone concentrations and energy balance is well known in many species including cattle **(Nikolič *et al.,* 1997; Capuco *et al.,* 2001; Cassar-Malek *et al.,* 2001).** In this line, **Mityashova *et al.*(2018)** mentioned that cows with short open days period (<100 days) cows have a greater ability to implement the energy-saving adaptive mechanism associated with a reduced thyroid activity than animals with long open days period (>100days). During the postpartum period, a decrease in serum T4 and T3 levels is less pronounced and occurs later (or absent at all) in long open days period(>100days) than in animals with short open days period (<100days) cows.

CONCLUSION AND RECOMMENDATION

The blood composition regarding biochemical, mineral and thyroid hormones provides a useful indication of the requirements for lactating buffaloes during early stage of postpartum period. Thyroid hormones and biochemical metabolites concentration have relationships considerably with the stage of estrous cycle and follicular size.

There were changes in the biochemical composition of blood happened during estrous stages, and follicular development, indicating a relationship of blood metabolites, mineral and thyroid hormone with the reproductive status and metabolic activity of the lactating buffaloes. Based on the obtained results, breeders should take in mind the change in energy balance and feeding requirements during the early stage of postpartum period to obtain higher pregnancy rate within 90 day-postpartum.

REFERENCES

Abd Ellah, M. R.; Hussein, H. A. and Derar, D. R. (2010): Ovarian follicular fluid constituents in relation to stage of estrous cycle and size of the follicle in buffalo. Vet. World, Vol. 3, 263-267.

Agarwal, S.P.; Pandey, N.N. and Umashankar (1985): Serum protein, inorganic phosphorus and blood glucose in relation to different phases of reproduction in crossbred cattle. Indian J. Anim. Reprod. 6: 23-25.

Ahmed, W.M. (2006): Adverse conditions affecting ovarian activity in large farm animals. Proceeding of the 3 International Conference of Veterinary Research Division, National Research Centre, Cairo, Egypt, 251-253.

Alavi-Shoushtari, S.M.; Asri-Rezai, S. and Abshenas, J. (2006): A study of the uterine protein variations during the estrous cycle in the cow: a comparison with serum proteins. Anim. Rep. Sci. 96 (1 - 2): 10 - 20.

Ali, A.; Abdel Razek, A.; Abdel Ghaffar, S. and Glatzel, P.S. (2003): Ovarian follicular dynamics in buffalo cows (Bubalus bubalis). *Reprod. Domest. Anim*. 38, 214-218.

Alkalby, J.M.A.; Bushra, F.H. and Fahad, T.A. (2012): Study on some hormonal and biochemical constituents of follicular fluid and blood plasma in buffaloes.Bas.J.Vet.Res.Vol.11,No.1,90-102.

Al-Rubaeae, H.M. (2015): Studies on some metabolites, ionic and hormonal composition in ovarian follicular fluid and blood serum in relation to size of the follicle in Iraqi buffaloes. J. of Kerbala University, 13 (2) Scientific: 253-263.

Amle, M.; Patodkar, V.; Shelar, R. and Birade, H. (2014): Serum biochemical levels of repeat breeder cross bred cows under rural condition of Satara district of Maharashtra. International Journal of Advanced Veterinary Science and Technology, 3 (1): 109 -113.

AOAC methods (1990): Association of Official Analytical Chemists (AOAC) INTERNATIONAL.

APRI (1997): Monthly report on the herd production and reproduction status of APRI buffalo breeding experimental stations, tune 1997, (personal communication).

Arshad, H. M.; Ahmad, N.; Rahman, Z.; Samad, H. A.; Akhtar, N. and Ali, S. (2005): Studies on some biochemical constituents of ovarian follicular fluid and peripheral blood in buffaloes. Pakistan Vet J. 25(4): 189-193.

Ashkar, F.A.; Bartlewski, P.M.; Singh, J.; Malhi, P.S.; Yates, K.M.; Singh, T. and King, A.W. (2010): Thyroid hormone concentrations in systemic circulation and ovarian follicular fluid of cows. SK, Canada Exp. Biol. 235:215-221.

Ashmawy, Neama, A. (2015): Changes in Peripheral Plasma hormone Concentrations and metabolites during the last trimester of pregnancy and around Parturition in the Egyptian Buffalo and Baladi cows. International Journal of Advanced Research 3 (11): 1377 - 1390.

Bagavandoss, P.; Midgley, A. R. and Wicha, M. (1983): Developmental changes in the ovarian follicular basal lamina detected by immunofluorescence and electron microscopy. Journal of Histochemistry and Cytochemistry, 31: 633 - 640.

Baldi, A.; Savoini, L.; Pinoti, E.; Chei F. and Dello-Orto, V. (2000): Effect of vitamin E and different energy sources on vitamin E status, milk quality and reproduction in transition dairy cows. Journal of Veterinary Medicine, 47: 599 - 608.

Barbara,B.; Tomasz,S.; Jan,U.; Dariusz,G.; Bogdan,L.; Piotr,B.; Anna,S and Jolanta,S. (2006): Free thyroid hormones and cholesterol in follicular fluid of bovine ovaries. *Bull Vet Inst Pulawy* 50, 189-193.

Bertoni, G.; Lombardelli, R. and Orioli, F. (1999): Postpartum reproductive and metabolic behaviour of fertile and infertile dairy cows with different genetic merit. Associazione Scientifica di Produzione Animale (ASPA), Pia cenza, 21-24 June, 413 - 415.

Capuco A.V.; Wood D.L.; Elsasser T.H.; Kahl S.; Erdman R.A.; Van Tassell, C.P.; Lefcourt, A. and Piperova, L.S. (2001): Effect of somatotropin on thyroid hormones and cytokines in lactating dairy cows during ad libitum and restricted feed intake. J. Dairy Sci., 84, 2430 - 2439.

Cassar-Malek I.; Kahl S.; Jurie C. and Picard C. (2001): Influence of feeding level during postweaning growth on circulating concentrations of thyroid hormones and extrathyroidal 5'-deiodination in steers. J. Anim. Sci., 79, 2679 - 2687.

Chapa, A. M.; McCormick, M. E.; Fernandez, J. M.; French, D. D.; Ward, J. D. and Beatty, J. F. (2001): Supplemental dietary protein for grazing dairy cows: reproduction, condition loss, plasma metabolites and insulin. J. Dairy Sci. 84, 908-916.

De Vries, M.J. and Veerkamp, R.J. (2000): Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci., 2000; 83: 62-69.

Duncan, D.B., 1955. Multiple range and Multiple F test. Biometrics, 11:10.

Ghuman, S.P.S.; Singh, J.; Honparkhe, M.; Ahuja, C.S.; Dhami, D.S.; Nazir, G.; Gandotra, V.K. (2011): Differential fertility in dairy buffalo: Role of thyroid and blood plasma biochemical milieu. Iranian J. Appl. Anim. Sci., 1: 105-109.

Gosden, R. G.; Hunter, R. H. F.; Telfer, E.; Terrance, C. and Brown, N. (1988): Physiological factors underlying the formation of ovarian follicular fluid. Journal of Reproduction and Fertility, 82:813:825.

Grummer, R. R. (2007): Strategies to improve fertility of high yielding dairy farms: management of the dry period. Theriogenology 68, S281-S288.

Hassan, M.S.; Al-Nuaimi, A.J.; Al-Yasari, A.M. and Jameel, Y.J. (2018): Study the effects of follicular size on some biochemical follicular fluid composition in she camel (*Camelus dromedarius*). Adv. Anim. Vet. Sci. 6 (8): 341-346.

Heuer, R.; Schukken, Y.H. and Dobbelaar, P. (1999): Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield and culling in commercial dairy herds. J. Dairy Sci., 1999; 82: 295-304.

Huszenicza, G.; Kulcsar M. and P. Rudas (2002): Clinical endocrinology of thyroid gland function in ruminants. Vet. Med. Czech, 47: 199-210.

Jayachandran, S.; Nanjappan, K.; Muralidharan, J.; Selvaraj, P. and Manoharan, A. (2013): Blood biochemical and mineral status in cyclic and postpartum anestrus buffaloes. International Journal of Food, Agriculture and Veterinary Sciences, Vol. 3 (1), pp. 93 - 97.

Johnson AL. (1986): Serum concentrations of prolactin, thyroxine and triiodothyronine relative to season and estrous cycle in the mare. J Anim Sci 1986; 62 (4): 1012-1020.

Jorritsma, R.; Wensing, T.; Kruip, T.; Vosa, P. and Noordhuizen, J. (2003): Metabolic changes in early lactation and impaired reproductive performance in dairy cows. *Vet. Res.* 34, 11-26.

Kaminski, P.; Jerzak, L.; Sparks, T.H.; Johnston, A.; Bochenski, M.; Kasprzak, M.; Wisniewska, E.; Mroczkowski, S. and Tryjanowski, P. (2014): Sex and other sources of variation in the haematological parameters of White Stork Ciconia chicks. Journal of Ornithology, 155:307-314.

Khan H. M.; Mohanty, T. K.; Bhakat, M.; Raina V. S. and Gupta A. K. (2011): Relationship of Blood Metabolites with Reproductive Parameters during Various Seasons in Murrah Buffaloes. Asian-Aust. J. Anim. Sci.Vol. 24, No. 9: 1192 – 1198.

Kor, N. M. and Moradi, K. (2013): A Review of biochemical metabolites concentration and hormonal composition of ovarian follicular fluid in domestic animals. Annu Rev Res Biol. 3 (3): 246-255.

Kumar, S.; Saxena, A. and Ramsagar (2010): Comparative studies on metabolic profile of anestrous and normal cyclic murrah buffaloes. *Buffalo Bull*. 29, 7-11.

Kumar, S.; Balhara, AK.; Kumar, R.; Kumar, N.; Buragohain, L.; Baro, D.; Sharma, RK.; Phulia, SK. and Singh, I. (2015): Hemato-biochemical and hormonal profiles in post-partum water buffaloes (*Bubalus bubalis*), Veterinary World 8 (4); 512-517.

Ladanova, M.; Stekol'nikov, A.; Plemyashov, K.; Meboniya E. and Anipchenko P. (2018): Analysis of the level of thyroid hormones in cows indifferent periods of lactation Saint-Petersburg State Academy of Veterinary Medicine, St. Petersburg, Russia.

Leroy, J.L.M.R.; Vanholder, T.; Delanghe, J.R.; Opsomer, G.;Van Soom, A.; Bols, P.E.; Dewulf, J. and Kruif, A. (2004): Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post-partum. *Theriogenology,* 62 (6): 1131-1143.

Lucy,M.C.;Staples,L.M. and Thatcher,W. W.(1992): Energy balance and size of and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows.*J.Dairy Sci.*74: 473.

Mishra, O. P.; Pandey, J. N. and Gawande, P. G. (2003): Study on biochemical constituents of caprine ovarian follicular fluid after superovulation"Asian Aust. J.Anim. Sci.,Vol.16,1711-1715.

Mityashova O; Solomakhin, A.; Smekalova, A.; Montvila, E.; Lebedeva, I. and Ernst L.K. (2018): Alterations in blood thyroid levels during the postpartum period are related to the reproductive ability of primiparous dairy cows resumed cycling activity. Federal Science Center for Animal Husbandry, Podolsk, Russia.

Nandi, S.; Girish Kumar, V.; Manjunatha, B. M.; Ramesh, H. S. and Gupta, P. S. (2008): 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, 186-196.

Nebel, R.L.and Jobst, S.M. (1998): Evaluation of systematic breeding programs for lactating dairy cows: a review. J. Dairy Sci., 81(4):1169 -74.

Nikolic J.A.; Samanc H.; Begovic J.; Damjanovic Z.; Dokovic R.; Kostic G.; Kramanovic J., and Resanovic, V. (1997): Low peripheral serum thyroid hormone status independently affects the hormone profiles of healthy and ketotic cows during the first week postpartum. Acta Vet. (Belgr.), 47, 3-14.

Perveen, S. and Usmani, R.H. (1993): Peripartum profiles of certain haematological and biochemical parameters in normally calving buffaloes. J. Anim. Hith. Prod., 13:55 - 60.

Piccione, G.; Casella, S.; Lutri, L.; Vazzana, I.; Ferrantelli, V. and Caola, G. (2010): Reference values for some haematological, haemoato chemical and electrophoretic parameters in the Girgentanagoat. Turkish Journal of Veterinary and Animal Sciences, 34 (2):197-204.

Quayam, S.A.; Devanthan T.G. and Pattabiraman, J.R. (1990): Serum total protein and blood levels during pre-peri and postpartum Murrah buffaloes. Indian. J. Anim. Sci. 60: 140-142.

Razzaque, W. A. A.; Rao, M. M.; Mahapatra, P. S.; Pandey, A. K.; Sudhir Kumar.; Kafil Hussain and Singh, R. (2012): Biochemical composition of Follicular fluid in buffalo ovaries. Indian Veterinary journal, 89: 24.

Reist, M.; Erdin, D.K.; Von Euw, D.; Tschumperlin, K.M.; Leuenberger, H.; Hammon, H.M.; Morel, C.; Phillipona, C.; Zbinden, Y.; Kunzi, N. and Blum, J.W. (2003): Postpartum reproductive function: association with energy, metabolic and endocrine status in high yielding dairy cows. *Theriogenology,* 59: 1707-1723.

Sharma, M.; Bisnoi, P.C. and Mohanty, B.P. (1998): Serum constituents in indigenous and crossbred cattle. Indian Journal of Animal Science 68 474 - 475.

Sharma, R.K. (2003): Reproductive problems of buffaloes and their management. Compendium of lectures delivered to the Cent. Institute of Research on Buffalo, Hisar, India, 119-126.

Singh, A.; Saxena, M.S. and Prasad, J.K. (2004): Levels of trace minerals, glucose and haemoglobin in relation to occurrence of post-parturient oestrum in buffaloes. Indian J. Anim Reprod., 25: 58-60.

Spicer, L. J.; Alonso, J. and Chamberlain, C. S. (2001): Effects of thyroid hormones on bovine granulose and thecal cell function in vitro: dependence on insulin and gonadotropins. J Dairy Sci. 84:1069 -1076.

SPSS (2008): Statistical Package for the social sciences. Release 16, SPSS INC, Chicago, USA.

Suriyasathaporn, W. (2000): Negative energy balance in postpartum dairy cows: its effect on clinical mastitis and reproductive performance. Ph.D. thesis, Utrecht University, Department of Farm Animal Health.

Swanson, L.V. (1989): Interaction of nutrition and reproduction. J. Dairy Sci. 72:805.

Tabatabaei, S. and Mamoei, **M. (2010):** Biochemical composition of blood plasma and follicular fluid in relation to follicular size in buffalo. Springer, 20: 441.

Varley, H.; Genlock, A.H. and Bell, M. (1980): Practical Clinical Chemistry. Vol.1.General topics commen test. 5th ed. William Heinemann Medical books Ltd, London, UK.

Veena, M. P.; Maheswarappa Gowrakkal; Girish Kumar, V.; Gupta P.S.P. and Narayana Swamy M. (2015): Relation of various physiological blood parameters with the postpartum reproductive efficiency in cattle. International Journal of Biomedical Research 2015; 6 (10): 780-785.

Wise, T. (1987): Biochemical analysis of bovine follicular fluid: albumine, total protein, lysosomal enzymes, ions, steroids and ascorbic acid content in relation to follicular size, rank, atresia classification and day of estrous cycle. J. Anim. Sci., 64: 1153-1169.

Yaqub, L.S.; Ayo, J.O.; Rekwot, P.I.; Oyeanusi, B.I.; Kawu, M.U.; Ambali, S.F.; Shittu, M.; Abdullahi, A. (2011): Changes in serum proteins and urea during the oestrous cycle in Red Sokoto goats. Adv. Appl. Sci. Res. 2 (6): 197-205.

العلاقة بين بعض المواد التمثيلية، الأملاح المعدنية وهرمونات الغده الدرقيه اثناء دورة الشياع مع خصوبة الجاموس المصرى في فترة ما بعد الولادة

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الملخص

تهدف هذه الدراسة الى تحديد العلاقة بين بعض المواد الكيميائية الحيوية وهرمونات الغدة الدرقية والمعادن في الدم اثناء المراحل المختلفة لدورة الشياع وعلاقة ذلك بحجم الحويصلات المبيضية مع الحمل المبكر للجاموس المصري خلال فترة ما بعد الولادة.

ووفقا لتشخيص الحمل كان معدل الحمل الكلى 69.7% (43/30) ،20 من الحيوانات46.6% (43/20) اصبحوا عشار خلال ال 90 يوم بعد الولادة (المجموعة الاولى) و10 حيوانات 23.25% (43/10) اصبحوا عشار خلال ال 120 يوم بعد الولادة (المجموعة الثانية).

قسمت الحيوانات طبقا لدورة الشياع الى 4 مراحل (المرحلة الاولى من 0-4 يوم والمرحلة الثانية من 5-10 يوم والمرحلة الثالثة من 11-17 يوم والمرحلة الرابعة من 18-21 يوم). وقسمت الحيوانات طبقا لحجم الحويصلات المبيضية الى ثلاث اقسام على حسب قطرها القسم الاول (3-5مم) والقسم الثاني (6-9مم) والقسم الثالث (أكبر من او يساوى 10مم)

وقد اوضحت النتائج ما يلى:

كان المتوسط العام لتركيز كل من البروتين الكلى والألبيومين و الجلوبيولين والجلوكوز والكوليسترول والدهون الثلاثية في سيرم مرتفع معنويا (P<0.001) في المجموعة الاولى عن المجموعة الثانية. زاد المتوسط العام لمستوى البروتين الكلى والجلوكوز معنويا (P<0.05) بين المرحلة الاولى والثانية لدورة الشياع، وكان التغير غير ملحوظ معنويا في المراحل الاخرى، بينما زاد مستوى الجلوبيولين والكوليسترول معنويا (P<0.05) بين المرحلة الاولى وحتى الثالثة وكان غير معنوي فى المرحله الرابعه، ولم يتأثر مستوى الألبيومين والدهون الثلاثية معنويا بمراحل الشياع. لم يتأثر المتوسط العام لمستوى كل من البروتين الكلى والألبيومين والجلوبيولين و الدهون الثلاثية معنويا بحجم الحويصلات المبيضية. وزاد تركيز الجلوكوز معنويا (P<0.05) بزيادة حجم الحويصلات المبيضية من القسم الاول وحتى القسم الثالث، بينما زاد تركيز الكوليسترول معنويا (P<0.05) فقط من القسم الاول للثاني . كان تركيز كل من هرمونى الغده الدرقيه وايضا الزنك والسيلينيوم أعلى معنويا (P<0.001) فى المجموعة الاولى عن المجموعة الثانية. كان تأثير حجم الحويصلات المبيضية معنويا (P<0.05) لمحتوى السيلينيوم في سيرم الدم وزاد بزيادة قطر الحويصلات المبيضية. لم يتأثر هرمونى الغده الدرقيه والزنك بحجم الحويصلات المبيضية.

نستخلص من هذه الدراسة:

أن مكونات الدم البيوكيميائية والهرمونات الدرقية والمعادن مؤشرا مفيدا لمتطلبات الجاموس الحلاب اثناء فترة ما بعد الولادة وكانت لها علاقة بمراحل دورة الشياع وحجم الحويصلات المبيضية. وحدث تغير في التركيب الكيميائي الحيوي للدم خلال المراحل المختلفة لدورة الشياع وتطور الحويصلات المبيضية ، مما يشير إلى وجود علاقة بين مكونات الدم وهرمونات الغدة الدرقية والمعادن حسب الحالة التناسلية والحالة التمثيلية في الجاموس الحلاب.

بناءً على النتائج التي تم الحصول عليها ، يجب على المربين مراعاة التغيير في ميزان الطاقة ومتطلبات التغذية خلال المرحلة المبكرة من فترة ما بعد الولادة للحصول على معدل حمل أعلى خلال 90 يومًا بعد الولادة.