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RELATIONSHIP BETWEEN FERTILITY, FOLLICLE COUNT AND BLOOD BIOCHEMICAL COMPONENTS IN EGYPTIAN BUFFALOES

By

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ABSTRACT

The objective of present study was investigate the relationship between ovarian antral follicle count (AFC), serum alkaline phosphatase (ALP), alanine transferase (ALT), aspartate amino transferase (AST), progesterone and cortisol; and fertility to develop the selection tools for improve the reproductive performance of buffaloes. Antral follicle count (AFC) used as a basic indicator for selection. 21 non-pregnant, cyclic buffaloes were monitored AFC using ultrasonography. Scanning of the ovaries was performed on day 3 and day 5 of estrus and blood samples were collected from 3-5 day during early luteal phase (EL) and 13-15 day of estrus, during luteal phase (L) for measuring serum ALP, ALT, AST, progesterone and cortisol. Counting of AFC based on antral follicle with (≥3mm) in diameter, animals divided into high group G1 (≥ 9 AFC) and low group G2 (< 9 AFC). The results indicated that, the main values of days open were lower in G1 (70.30) than in G2 (103.38) and number of services per conception were lower in G1 (1.3) than G2 (2.57) respectively. Differences were significant (P≤ 0.001). Blood serum Levels of progesterone concentration were higher in EL and L with G1 (0.53 and 4.31 ng/ml) than G2 (0.19 and 1.69 ng/ml), while levels of cortisol concentration were lower in EL and L with G1 (1.32 and 1.52 µg/dl) than G2 (1.97 and 2.33 µg/dl) respectively. Differences were significant (P≤ 0.001). Blood serum Levels of ALP concentration were lower in EL and L with G1 (121.66 and 138.56 u/l) than G2 (165.79 and 185.5 u/l) respectively. Also, the levels of AST concentration were lower in EL and L with G1 (42.94 and 47.48 u/l) than G2 (53.41 and 59.36 u/l) respectively. While, the levels of ALT concentration were higher in EL and L with G1 (26.66 and 23.43 u/l) than G2 (22.44 and 20.43 u/l) respectively. Differences were significant (P≤ 0.001).

In conclusion, there was a positive relationship between high AFC and good fertility parameters or high fertility. Also high levels of blood serum concentration of (progesterone and ALT) were associated with a good fertility parameters or high fertility. On the other hand

high levels of blood serum concentration of (cortisol, ALP and AST) were associated with the weak fertility parameters or low fertility. So that AFC, progesterone, cortisol, ALP, ALT and AST could be an important indicators for selection female buffaloes with high reproductive performance.

Key wards:

Antral follicle count (AFC), fertility, progesterone, cortisol, ALP, ALT, AST, buffalo.

INTRODUCTION

Animal products especially milk and meat, have become limited and insufficient with increasing of the world population. Improving reproductive performance of animals is very important. Buffaloes are the first local source of milk production. Interest in buffalo breeding has tremendously increased worldwide due to the fundamental role played by the species in many climatically disadvantaged agricultural systems **(Gasparrini B., 2013).**

Infertile buffaloes mean a direct loss in milk production, whereas reduced calf crops hamper the selection efficiency in long term dairy herd improvement **(Baghel, 2006).**

Antral follicle count (AFC) reflects the ovarian follicle reserve and has been linked to fertility in cows **(Mossa *et al.*,2012;Martinez *et al.*,2015)**. Because AFC reflects the reserve of ovarian follicles, it is expected that age would impact AFC negatively **(Cushman *et al.*,2009)**. Reports in the literature have documented improved reproductive parameters and response to reproductive biotechnologies in cattle with increased antral follicle count **(Ireland *et al.*, 2011; Rico *et al.*, 2012 and Silva-Santos *et al.*, 2014a)**.

Biochemical parameters are commonly employed as useful indicators of health as well as nutritional status of many species, and thus help in diagnosis of metabolic diseases and management of infertility as well as low productivity in farm animals **(Amle *et al.*, 2014 and Kaminski *et al.*,2014)**. Cortisol is one of these biochemical parameters. The high cortisol production may effect on female cows reproductive system, leading to ovum and ovulation development **(Shugaba *et al.*, 2010)**. Progesterone is important for reproductive performance. Prostaglandin E2 (PGE2) production is stimulated by progesterone **(Wooding *et al.*,1996)**, concentrations of progesterone on the day of PGF2α-induced luteolysis had a positive linear correlation with subsequent fertility **(Diskin *et al.*, 2006)**. Besides that, alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) are also

important biochemical parameters. According to **Chaurasia *et al.,* (2016)** concentrations of blood serum ALP, and AST were higher in repeat breeder than normal cyclic buffaloes, while ALT concentration was lower. Therefor these biochemical parameters are useful for evaluate fertility of buffalo cows. The main objective of this investigation was study the relationship between antral follicle count, some biochemical components and fertility to develop the selection techniques to be more applicable, easier and not expensive for reproductive performance improvement in buffaloes.

MATERIAL AND METHODS

The present study was carried out at Mehallet MousaExperimental Station,Animal, Production Research Institute, Agricultural Research Center Ministry of Agriculture, and Egypt.

Animals:

21 non-pregnant, cyclic buffaloes with range of body weight between 450-600 kg. These animals were housed in semi shaded open pens and they were fed to meet both maintenance and milk production requirements according to **APRI (1997)**. The ration consisted of concentrate fed mixture berseem hay, corn silage and rice straw. Animals were fed twice daily. Animals were observed for estrus, twice a day using a teaser bull.

Ultrasonography:

Ultrasound scanning were done by ultrasound machine (digital ultrasound diagnostic imaging system, model Dp-30 vet. 50/60 HZ, shenzhen, mindray bio-medical. electronics, co. LTD, 7.5 MHz linear array transducer and depth 4.3) on day 3 and 5 of oestrus cycle, and ovarian AFC were estimated at the time of ultrasonography based on the number of follicles with ≥3mm in diameter and animals were classified into two groups: (1) high group (G1) with ≥ 9 AFC, and (2) low group (G2) with < 9 AFC **(Qaisar SHAHZAD *et al.,* 2015)**.

Blood sampling:

Blood samples were collected from 3-5 day of estrus during early luteal phase (EL) **(Mondal and Prakash, 2002b)** and from 13-15 day of estrus during Peripheral progesterone concentrations are rise to peak in luteal phase (L) **(Ahmed *et al.,* 1977 and Bachalaus *et al.,* 1979)**.Collecting blood samples were centrifuged at 3000 rpm for 10 minutes. Serum was stored at -20 until analysis.

Biochemical analysis:

Serum hormones

Determination of progesterone and cortisol hormones was performed by radioimmunoassay technique in representative serum samples. Kits of diagnostic products corporation, (DCP) Los Angles, USA with ready antibody coated tubes used according to the procedures outlined by manufacturer.

Serum enzymes:

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) were assayed by IFCC Enzymatic-UV method and alkaline phosphatase (ALP) by DGKC Kinetic- optimized method using commercial kits (Spinreact, GIRONA Spain).

Experimental design

In this study 3 experiments, the first one to investigate relationship between AFC and fertility parameters, experiment 2 to study relationship between AFC and serum hormones (progesterone and cortisol) and experiment 3 to access relationship between AFC and serum enzymes (AST, ALT and ALP).

Statistical analysis:

Data were analyzed according to **SAS (2002)**.

RESULTS AND DISCUTION

The results of this study were as follows: firstly, ovarian AFC were ≥ 9 in 13 animals which classified as a high group (G1) with ≥ 9 AFC, while 8 animals were classified as a low group (G2) with < 9 AFC. In experiment1: relationship between AFC and fertility parameters presented in (Table 1) and indicated that, the days open of animals of G1 were lower than G2 and its main values were 70.30 and 103.38 days in G1 and G2 respectively. Differences between these groups were significant (P≤ 0.01). In addition, the mains of the number of services per conception were lower in G1 (1.30) than G2 (2.57) respectively. Differences were significant (P≤ 0.01). These results referred to the AFC were associated with fertility parameters. AFC classification was highly associated with fertility parameters selected as indicators of reproductive performance **(Martinez *et al.,* 2015)**. AFC reflects the ovarian follicle reserve and has been linked to fertility in cows **(Mossa *et al.,* 2012 and Martinez *et al.,* 2015)**. In addition, improved reproductive parameters and response to reproductive

biotechnologies in cattle with increased antral follicle count **(Ireland *et al.,* 2011; Rico *et al.,* 2012; Silva-Santos *et al.,* 2014a)**. The size of reproductive structures has already been previously reported to be related to the AFC in Holstein cows, where greater ovarian volumes were associated with greater AFCs **(Ireland *et al.,* 2008)**. The association between antimullerian hormone (AMH) and AFC has been widely reported **(Ireland *et al.,* 2008; Rico *et al.,* 2011; Monniaux *et al.,*2012 and Batista *et al.,* 2014)**. Considering the low cost of AFC evaluation, and its obvious relationship with AMH, perhaps the AFC concentrations are more indicative of fertility in genetic selection processes **(Renata Maculana *et al.,* 2017)**.

Table (1): Relationship between antral follicle count (AFC) and fertility parameters.

groups	Fertility parameters	
	Days open (days)	Number of services per conception
G1	70.30±0.92[a]	1.30±0.13[a]
G2	103.38±1.65[b]	2.57±0.26[b]

G1=high group (≥ 9 AFC), G2= low group (< 9 AFC), Mean values of groups for each item with different superscripts in the same column are significantly different (P ≤ 0.001).

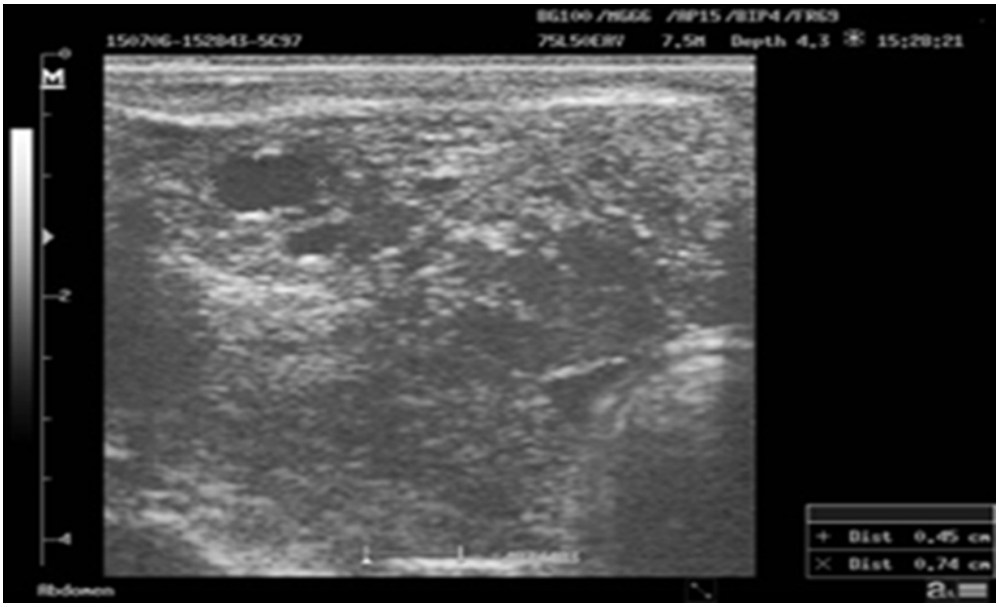

Fig. (1): Ovarian antral follicle count (AFC) ≥3mm in diameter.

In experiment2: The results of relationship between AFC and levels of progesterone and cortisol concentration presented in (Table 2). These results revealed that, the high levels of progesterone concentration were observed with G1 in each phases EL and L (0.53 and 4.31 ng/ml), while the low levels were in G2 (0.19 and 1.69 ng/ml) respectively. Differences were significant (P≤ 0.01). The reduced concentration of progesterone in plasma of low-AFC females was associated with decreased capacity of luteal and granulosal cells to produce progesterone, and reduced abundance of STAR and mRNA for STAR and LH receptor in CL **(Jimenez-Krassel *et al.,* 2009)**. Low progesterone concentration in cattle is associated with reproductive problems, such as embryonic mortality and slower development of the endometrium **(Inskeep, 2004; Diskin and Morris, 2008)**. The number of follicles in the ovary was associated with concentrations of progesterone, as described by **Martinez *et al.* (2016)**. On the other hand, the high levels of cortisol concentration in EL and L phases were related to G2 (1.97 and 2.33 µg/dl), and the low levels were with G1 (1.32 and 1.52 µg/dl) respectively, and the differences were significant (P≤ 0.01). The association between high levels of cortisol concentration and low AFC may be due to cortisol inhibit the secretion of GnRH and LH from a hypothalamic- pituitary system, resulting in the inhibited growth of follicles as well as estrogen and progesterone hormones due to lake Adenosine triphosphate (ATP) that serves to the activate cyclic Adenosine monophosphate (c AMP) as intracellular messenger **(Joana da Costa Freitas *et al.,* 2017)**. The decrease of LH affected the decreased growth of follicles **(Joana da Costa Freitas *et al.*2017)**. In addition, according to **Smith (2005)**, the increased level of cortisol hormones cause decreased production of progesterone hormones.

Table (2): Relationship between AFC and serum hormones (progesterone and cortisol)

Groups	progesterone (ng/ml)		Cortisol (µg/dl)	
	EL	L	EL	L
G1	0.53 ± 0.20 [a]	4.31 ± 0.08 [a]	1.32 ± 0.04 [a]	1.52± 0.007 [a]
G2	0.19 ± 0.19 [b]	1.69 ± 0.03 [b]	1.97 ± 0.07 b	2.33 ± 0.07 [b]

G1=high group (≥ 9 AFC), G2= low group (< 9 AFC), EL= early luteal phase, L= luteal phase, Mean values of groups for each item with different superscripts in the same column are significantly different (P ≤ 0.001).

In experiment 3: the results of relationship between AFC and concentrations of AST, ALT and ALP enzymes presented in (Table 3). Present results indicated that the high levels of ALP concentrations in EL and L phases were with G2 (165.79 and 185.5 u/l), and the low levels were with G1 (121.66 and 138.56 u/l) respectively, and the differences were significant (P≤ 0.001). **Gandotra *et al.* (1993)** reported higher level of ALP activity in repeat breeder cattle and buffaloes as compared to normal cyclic cattle and buffaloes. **Chandrakar (1999)** found serum activity concentration to be significantly higher in repeat breeder than normal fertile cows. **Chaurasia *et al.,* (2016)** recorded that, the serum alkaline phosphatase (ALP) activity was significantly increased in repeat breeder followed by that in normal cyclic buffaloes. Decreased level of ALP activity in normal cyclic as compared to Repeat breeders might be due to enhanced folliculogenesis resulting in increased pace of conception while reverse is true in repeat breeder **(Sharma *et al.,*1986 and Chaurasia *et al.,* 2016)**.Alkaline phosphatase (ALP) is associated with prominent atresia **(Wise,1987)**, and is inversely correlated to the follicle size **(Mimoune Nore e*t al.,* 2018)**. The positive correlation between the activity of ALP and progesterone levels may indicate that ALP is a useful metabolic indicator of follicular atresia **(Wise,1987)**.Also levels of AST concentration in EL and L phases were high with G2 (53.41 and 59.36 u/l), but these levels were low with G1 (42.94 and 47.48 u/l) respectively, differences were significant (P≤ 0.001). These results were agreement with findings of **(Chaurasia *et al.,*2016)**, they found that AST activity was higher in repeat breeder than normal cyclic buffaloes and differences between two groups were significantly different. AST enzyme activity was indicative of increased physiological activity and pathological condition of the tissue and the possible cause of increased AST activity level may be uterine tissue damage in repeat breeder cows and buffaloes **(Chaurasia *et al.,* 2016)**. The high levels of ALT concentrations in ELand L phases were with G1 (26.66 and 23.43 u/l), while the low levels of ALT concentration were with G2 (22.44 and 20.43 u/l) respectively and the differences were significant. Significantly lower activity of ALT in infertile group of heifers than normal group **(Sharma *et al.,*1986)**. The level of ALT was significantly lower in repeat breeder than normal cyclic buffaloes **(Chaurasia *et al.,* 2016)**. ALT activity level was higher in normal cyclic cattle and buffaloes than the repeat breeder cattle and buffaloes **(Gandotra *et al.,*1993).** Higher level of serum ALT activity might be due to increase in metabolic activity mediated by physiological activity during oestrus cycle condition **(Chaurasia *et al.,* 2016**).

Table (3): Relationship between AFC and serum enzymes (AST, ALT and ALP).

Groups	ALP (u/l)		ALT (u/l)		AST (u/l)	
	EL	L	EL	L	EL	L
G1	121.66± 0.38[a]	138.56± 0.35[a]	26.66± 0.44[a]	23.43± 0.043[a]	42.94± 0.059[a]	47.48± 0.041[a]
G2	165.79± 0.48[b]	185.5± 0.41[b]	22.44± 0.051[b]	20.43± 0.06[b]	53.41± 0.04[b]	59.36± 0.04[b]

G1=high group (≥ 9 AFC), G2= low group (< 9 AFC), EL= early luteal phase, L= luteal phase, Mean values of groups for each item with different superscripts in the same column are significantly different (P ≤ 0001).

This study indicates that AFC has a positive relationship with the fertility parameters and associated with the high fertile animals which they have a less open days and number of services per conception. In addition to, positive relationship between AFC and blood serum concentrations of progesterone and ALT, while it has a negative relationship with blood serum concentrations of cortisol, ALP and AST.

From these results it's clear that high AFC and a high levels of blood serum progesterone and ALT concentration were associated with the good fertility parameters or high fertility.

On the other hand low AFC and high levels of blood serum cortisol, ALP and AST concentration were associated with the weak fertility parameters or low fertility **(Diskin *et al.*, 2006, Ireland *et al.*, 2011and Chaurasia *et al.*, 2016)**.

CONCLISION

High AFC and high levels of blood serum progesterone and ALT concentration were related to high fertility,while, low AFC and high levels of blood serum cortisol, ALP and AST concentration were related to low fertility. AFC, progesterone, cortisol, ALP, ALT and AST could be an important indicator to improve the reproductive performance of female buffaloes.

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العلاقة بين عد الحويصلات المبيضية ومكونات الدم البيوكيميائية والخصوبة في الجاموس المصري.

عماد فوزى علي المغربى - سيد احمد محمود

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ان الهدف من الدراسة الحالية هو دراسة العلاقة بين عدد الحويصلات المبيضية (AFC) وانزيم الفوسفاتيز القاعدي (ALP) ،و ألالانين ترانسفيريز (ALT) ،و الأسبارتات امينو ترانسفيريز((AST)،و البروجسترون و الكورتيزول والخصوبة لتطوير أدوات الاختيار لتحسين الأداء التناسلي للجاموس. وتستخدم الحويصلات المبيضية كمؤشر اساسي للاختيار.

الملخص العربي

تم فحص مبايض عدد 21 جاموسة ليست عشار بالموجات فوق الصوتية في اليوم الثالث والخامس من دورة الشياع كما تم اخذ عينات دم من هذه الحيوانات من اليوم الثالث الى اليوم الخامس من دورة الشياع اثناء مرحلة الجسم الاصفر المبكر, هذا بالاضافة الى اخذ عينات دم في اليوم الثالث عشر حتى اليوم الخامس عشر في مرحلة الجسم الاصفر لقياس مستويات تركيز كل من انزيم الفوسفاتيز القاعدي (ALP) ،و ألانين ترانسفيريز (ALT) ،و الأسبارتات امينو ترانسفيريز (AST)،و البروجسترون و الكورتيزول في سيرم الدم .

تم عد الحويصلات المبيضية التي قطرها 3 ملليمتر او اكثر, كما تم تصنيف الحيوانات طبقا لعدد الحويصلات المبيضية الى مجموعة اولى (1) التي كان عدد الحويصلات على المبايض 9 فاكثر و مجموعة ثانية (2) حيث كان عدد الحويصلات على المبايض اقل من 9 .

اشارت النتائج الى ان متوسط عدد الايام من الولادة حتى الاخصاب كان اقل في المجموعة الاولى (70.3) من المجموعة الثانية (103.38) , هذا بالاضافة الى ان متوسط عدد التلقيحات اللازمة للاخصاب كانت اقل في المجموعة الاولى (1.3) من المجموعة الثانية (2.57) وكانت الفروق معنوية ($P\leq 0.001$).

مستويات تركيز هرمون البروجستيرون في سيرم الدم كان اعلى في المجموعة الاولى في كل من مرحلة الجسم الاصفر المبكر ومرحلة الجسم الاصفر (0.53 و 4.31 نجم/ملل) من المجموعة الثانية (0.19 و 1.69 نجم / ملل).

مستويات تركيز هرمون الكورتيزول في سيرم الدم كان اقل في المجموعة الاولى في كل من مرحلة الجسم الاصفر المبكر ومرحلة الجسم الاصفر (1.32 و 1.52 ميكروجرام / دلتر) من المجموعة الثانية (1.97 و 2.33 ميكروجرام / دلتر) وكانت الفروق معنوية ($P\leq 0.001$).

مستويات تركيز انزيم الفوسفاتيز القاعدي في سيرم الدم كان اقل في المجموعة الاولى في كل من مرحلة الجسم الاصفر المبكر ومرحلة الجسم الاصفر (121.66 و 138.56 وحدة / لتر) من المجموعة الثانية (165.79 و 185.85 وحدة / لتر).

وايضا مستويات تركيز و الأسبارتات امينو ترانسفيريز كان اقل في المجموعة الاولى في كل من مرحلة الجسم الاصفر المبكر ومرحلة الجسم الاصفر (42.94 و 47.48 وحدة / لتر) من المجموعة الثانية (53.41 و 59.36 وحدة / لتر).

بينما كانت مستويات تركيز الالانين ترانسفيريز في سيرم الدم اعلى في المجموعة الاولى في كل من مرحلة الجسم الاصفر المبكر ومرحلة الجسم الاصفر (26.66 و 23.43 وحدة / لتر) من المجموعة الثانية (22.44 و 20.43 وحدة / لتر).

وكانت الفروق معنوية ($P \leq 0.001$). ونستخلص من هذه الدراسة انه توجد علاقة ايجابية بين كل من الحويصلات المبيضية ذات العدد المرتفع (اكثر من 9 حويصلات) قياسات الخصوبة الجيدة او الخصوبة المرتفعة, وايضا المستويات المرتفعة من تركيز البروجستيرون و الالانين ترانسفيريز لها علاقة ايجابية مع قياسات الخصوبة الجيدة او الخصوبة المرتفعة.

ومن جهة اخرى فان المستويات المرتفعة من تركيز هرمون الكورتيزول و انزيم الفوسفاتيز القاعدي و الأسبارتات امينو ترانسفيريز لها علاقة سلبية مع قياسات الخصوبة الجيدة او الخصوبة المرتفعة حيث انها مرتبطة بالخصوبة المنخفة. ولهذا فان الحويصلات المبيضبة و البروجستيرون والكورتيزول وانزيم الفوسفاتيز القاعدي و ألالانين ترانسفيريز و الأسبارتات امينو ترانسفيريز من الممكن ان تكون مؤشرات مهمة للاختيار او الانتخاب لتحسين الاداء التناسلي لاناث الجاموس المصري.