Relationship Between Some Serum Microelements And The Ovarian Status In Buffaloes-A Clinical Study

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ABSTRACT

Fifty mature non pregnant female buffaloes were divided into 4 groups according to the ovarian findings. Group I consists of 12 animals exhibiting growing graffian follicles (follicular phase of the oestrous cycle) . Group II consists of 15 animals exhibiting corpora lutea (luteal phase of the oestrous cycle). Animals of gp.I and gpII representing the normal ovarian function (regular breeder gp). Group III consists of 10 animals with smooth inactive ovaries .Group IV consists of 13 animals with ovarian cystic follicles. Serum cupper (Cu), zinc (Zn), manganese (Mn) and iron (Fe), were estimated in these animals.

A significant lower serum levels of copper, zinc and manganese were recorded in animals having follicular phase than those in the luteal phase **(gp.I and gp.II).**On the other hand, serum iron level did not show any significant variation between animals of the two phases. Regarding buffaloes with ovarian disorders **(gp.III and gp.IV)** the studied microelements showed highly significant decreased levels than in regular breeder **(gp.I and gp.II).** It can be concluded that reduced levels of the afore-mentioned elements in association with ovarian dysfunctions my declare the beneficial effects of these elements for treatment of ovarian dysfunction in buffaloes.

INTRODUCTION

Buffaloes constitute a major source of meat, milk and dairy products in Egypt.Infertility problems among buffaloes are quite high and my constitute a challenging threat to our animal wealth. The reproduction in animals is controlled by many factors, of these are the microelements *(1-3).* It is well demonstrated that the deficiency of trace elements resulted in reduced fertility of the animal *(4-7).* Therefore, estimation of these elements may reflect the nutritional status and hense the reproductive performance of an animal.

The aim of this work was to study the serum levels of some trace elements (cupper, zinc,. manganese and iron) in relation to the ovarian status during follicular and luteal phases of the estrous cycle, as well as during ovarian dysfunctions in buffaloes.

MATERIAL AND METHODS

Animals

The current work was conducted on 50-non pregnant mature female buffaloes (aged from 5-12 years).These animals were allocated in several private farms at Fayoum province. Animals were fed mainly on darawa, wheat straw, rice straw and small amount of cotton seed cake. The general health condition of each animal and its reproductive history were recorded in a-sheet, beside foecal examination was done to ensure that animals were free from parasitic infestation. Rectal palpation of the genital organs was conducted six weeks to six months postpartum to detect the ovarian status. The animals were then classified into four groups according to the ovarian findings using the previous reported *(1,8)* criteria. The 1st group **(gp.I)** consists of 12 buffaloes exhibiting growing follicles on either ovaries (follicular phase of the estrous cycle). The 2nd group **(gp.II)** consists of 15 buffaloes exhibiting luteal structures on either ovaries (luteal phase of the estrous cycle).Animals of **gp.I and gp.II** has the normal ovarian functions, and called collectively, the regular breeder group. The 3rd group **(gp. III)** consists of 10 buffaloes, exhibiting complete smooth structure-less ovaries (about 1.5 cm thickness), without any detectable pathological changes in their genital tracts(smooth inactive ovaries) *(9).* The 4th group **(gp.IV)**, consists of 13 buffaloes, which had follicular theca cyst, that can be palpated as a fluid-filled smooth rounded structures raised above the surface with greater diameter (>3) cm on one or both ovaries (cystic ovaries),and the animals showing oestrous signs at short intervals (every 5 –7 days)and sometimes stayed for long period (2-3 days).

Sampling

Blood samples were collected individually by jugular vein puncture into a

McCartney vial of 20 ml capacity for serum separation. The blood samples were allowed to clot at room temperature, kept over night at 4 °C in a refrigerator. The clot was then gently separated. The tubes were centrifuged at 3000 rpm for 15 minutes to obtain clear non haemolysed serum which kept frozen at -20 °C until analyzed.

The serum levels of cupper, zinc, manganese and iron, were then determined *(10),* using atomic absorption spectrophotometer (Perkin-Elmer, 2380).

Statistical analysis:

The results were statistically analyzedusing **t. test** *(11).*

RESULTS

The estimated values of copper, zinc, manganese and iron were presented in table 1 and Fig 1,2. The mean values of copper, zinc, and manganese recorded in group.I (follicular phase) were significantly decreased than that recorded in group II (luteal phase) among the regular breeder group. On the other hand, serum iron level did not show any significant variation in both groups**(gp.I and gp.II),** Whears whereas, serum levels of the micro-elements were significantly reduced in gp.III and gp.IV, when compared with the regular breeder group**(gp.I and gp.II).**

Table 1: Serum levels of microelements (mean ± S.E) in the examined buffaloes.

Parameters	Normal cyclic			Abnormal cycling	
	Follicular phase	Luteal phase	Regular breeder	Smooth inactive ovaries	Follicular cyst
	(gp.I) (n=12)	(gp.II) (n=15)	(gp.III) (n=27)	(gp.IV) (n=13)	
Copper (µg/100ml)	135.46* ± 10.75	170.73 ± 12.45	160.35 ± 8.45	125.77** ± 7.6	98.71*** ± 6.36
Zinc (µg/100ml)	130.81*± 11.23	168.74 ± 10.76	138.98 ± 12.51	95.66** ± 9.58	80.31*** ± 7.11
Manganese (µg/100ml)	38.18* ± 3.35	56.40 ± 6.76	36.46 ± 4.23	23.38** ± 2.25	18.83*** ±1.96
Iron (µg/100ml)	200.76 ± 11.65	220.55 ± 12.60	218.06 ± 11.23	171.16** ± 9.84	130.38***±10.24

*Significant at $P < 0.05$, **Significant at $P < 0.01$, *** Significant at $P < 0.001$

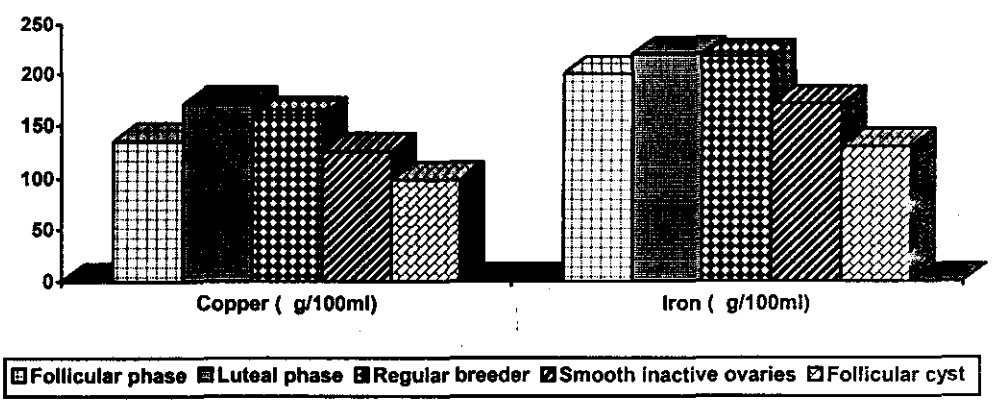

Fig. (1): Serum copper and iron levels in normal and abnormal cycling buffaloes.

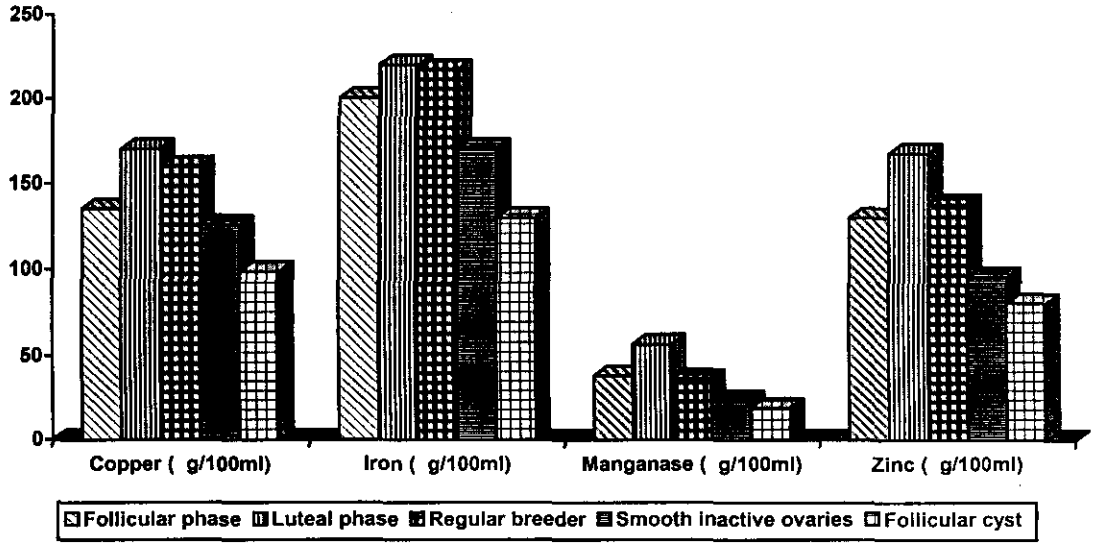

Fig. (2): Serum manganese and zinc levels in normal and abnormal cycling buffaloes.

DISCUSSION

The resumption of ovarian cyclic activity and oestrous behaviour may be the most important events to the animals ability to resume its breeding efficacy following parturition *(12).* Buffaloes are frequently subjected to severe dietary deficiency of trace elements, because of the biochemical composition of blood is under considerable haemostatic control, which is mediated by hormonal secretions and feed-back mechanisms which adjust the rates of absorption from the diet, excretion, deposition, and mobilization from body stores. But several factors operate against this equilibrium and this include; the imbalances in the availability of these trace elements , body stores to meet the requirements of reproduction and hence the hormonal insufficiency and enzymatic dysfunctions which leads to deviation from normality *(1,13, 14).*

Recently, the relationship between serum concentrations of some trace elements(cupper, iron, zinc,. manganese, selenium , molybdenum, cobalt, iodine, cadmium, aluminum, silicon and fluorine) and of several hormones including pituitary hormones and steroid hormones have been studied in animals *(14,15,16).* In the present investigation (table.1), the normal serum level of the microelements studied in regular cycling buffaloes **(gp.I and gp.II)** were in the normal ranges determined in buffaloes *(1,12,17).* However, the serum copper, zinc and manganese levels undergo cyclic variations in different phases of the oestrous cycle *(1,12).* In the present study, their levels were significantly decreased in the follicular phase **(gp.I)** than in the luteal phase (g**p.II**) of the oestrous cycle. These data suggests that serum levels of these trace elements is directly correlated with hormonal control of the oestrous cycle.

Findings regarding the effect of copper deficiency on the fertility of ruminants are conflicted. A relationship between the fertility of dairy cattle and their blood copper level *(13&18).* From the present work, it was evident that, the serum copper in the follicular phase**(gp.I)** was significantly lowered than the luteal phase **(gp.II)** among buffaloes with normal ovarian function (the regular breeder group). This may be due to that oestrogen stimulated in ceruloplasmin synthesis (cupper containing globulin of blood plasma). These findings was consistent with the previous records *(17,19,20).* The authers concluded that the serum cupper level was directly correlated with the blood oestrogen level. From the previous studies it was established that follicular growth is accompanied with gradual

increase in estrogen level in the blood until reaching its highest physiological level *(21)* .Hense,the decreased serum copper level in follicular phase than in the luteal phase (among the regular breeder group) is accepted

Furthermore, it was recorded from the previous studies that, zinc is necessary for nucleic acid metabolism, protein synthesis and cell growth, while its deficiency causes growth retardation *(22-24).* Moreover, it was evident from the previous studies that, the levels of oestrogens, corticosteroids and growth hormones were elevated during the follicular phase of the oestrous cycle *(15,21).* Therefore, decreased level of serum zinc in follicular phase **(gp.I)** than in luteal phase of the oestrous cycle **(gp.II)** in regular breeder animals may be due to the increased utilization of zinc under the effect of the aforementioned hormones.

Previous studies confirmed that manganese is necessary for normal fertility in ruminants and its deficiency lead to anoestrum, irregular return to oestrous, poor follicular development, low conception rate and silent heat *(13,22,25).* Also, some evidences suggest that manganese plays a role in the synthesis and the activity of gonadal hormones *(13).* In the present investigation (Table1), the level of manganese was significantly decreased in the follicular phase **(gp.I)** than in the luteal phase of the oestrous cycle **(gp.II)** with normal ovarian functions. Similar results were previously obtained *(19).* The highest level of manganese during the luteal phase of the oestrous cycle may be related to the involvement of manganese in the luteal activity as it reached to its maximal uptake during this phase *(19).*

Iron is an important element in the body and it is essential for life, because it is involved in the formation of haemoglobin inside RBCes which acts as oxygen carrier to supply the tissues with oxygen for oxidation process and release of energy for maintenance of life *(22,24).* Moreover, it was recorded that iron absorption plays an important role for hoemeostasis of iron metabolism *(12,22).* It is observed from the present study (Table I) that, there is no significant change in serum iron level between the follicular and luteal phases **(gps.I, II)** in the regular breeder buffaloes. These results can be explained by the fact that any decrease in iron absorption from the intestine under the effect of high oestrogen level, could be compensated by its withdrawal from stores in the liver or bone marrow and consequently iron level did not change during the normal ovarian function.

Regarding the levels of these trace elements in the serum of animals with ovarian disorders, they were decreased significantly in those buffaloes with smooth inactive ovaries and follicular cysts(**gp.III,** gp.IV) than that in the regular breeder group **(gp.I and gp.II).** These results are in consistent with the previous reported studies *(12,19).* Which showed that the regularity of ovarian cyclic activity and oestrous behaviour are in positive correlation with the higher levels of serum microelements. Also, it was reported that anaemia and deprived appetite associated with hypocuppraemia may adversely affect the general health condition including the endocrine system and consequently the ovarian activity *(26).* Hence animals with ovarian dysfunction tended to have highly significant decreased levels of these microelements in their serum. Moreover, animals showing anoestrum due to ovarian inactivity were treated by giving mineral mixture containing cupper, manganese, magnesium, zinc, iron, iodide and selenium *(27,28).* Therefore, the high significant reduced levels of copper, zinc ,manganese and iron in buffaloes with smooth inactive ovaries **(gp. III)** may be considered the immediate causes of infertilily in this group. While, buffaloes with ovarian follicular cysts **(gp.IV)** are accompanied by highly elevated serum oestrogen, which in turn reported to cause highly significant decreased levels of these microelements in their serum, when compared with the regular breeder group *(19, 21).*

It can be concluded that, buffaloes with ovarian dysfunctions exhibited highly significant lower levels of copper, zinc, manganese and iron in their serum, when compared with those exhibited normal ovarian activity. This may also reflect the beneficial effect of adding these elements to the ration of buffaloes to improve their fertility.

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الملخص العربي

دراسات باثولوجية إكلينيكية على العلاقة مابين بعض مكونات الدم والحالة الوظيفية للمبيض في الجاموس

محمد نجيب سعد سكران ، محمد زايد عبدالعزيز ، أحمد شعبان البخمى
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تم إجراء هذا البحث على عدد ٥٠ جاموسه ناضجة غير عشار يتراوح عمرها ما بين ٥- ١٢ سنة من مزارع خاصة فى محافظة الفيوم • تم تجميع بيانات كامله عن كل حاله وتشمل :

حاله التغذية لكل حيوان - الحالة التناسلية للحيوان - الفحص الظاهرى - الفحص المستقيمى للاعضاء التناسلية الداخلية وخصوصا

المبايض اعتبارا من ٦ أسابيع بعد الولادة وحتى ٦ شهور لمعرفة الحالة الوظيفية التى عليها المبيض - وتبعا لهذه الحاله تم تقسيم مجموعة الحيوانات تحت التجربة الى ٤ مجموعات وهم : المجموعة الاولى وعددها ١٢ وهى مجموعة الحالات التى جاءت فى فترة الشياع حيث كان على المبيض حويصلات جراف ناضجة - المجموعة الثانية وعددها ١٥ وهى مجموعة الحالات التى جاءت فى فترة ما بعد الشياع مباشرة حيث كان على المبيض الجسم الاصفر الناتج عن انفجار حويصلة جراف - واعتبرت حالات المجموعتين الأولى والثانية المجموعة الضابطة (وعددها ٢٧ حيوان) ، المجموعة الثالثة وعددها ١٠ حيوانات وكانت تعانى من عدم ظهور الشياع لفترة تزيد عن ٦ شهور وبفحص المبايض لها كانت ملساء بدون ظهور أى أعراض مرضية على الاعضاء التناسلية لها واعتبرت هذه المجموعة (مجموعة المبيض الخامل) وأخيرا المجموعة الرابعة وعددها ١٣ وكانت مجموعة الحيوانات التى تعانى من ظهور أعراض الشبق بصفة مستمرة وعلى فترات قصيرة وأعتبرت هذه المجموعة (مجموعة المبيض المتحوصل) حيث كان على المبيض حويصلة جراف مليئة بسائل وقطرها أكبر من ٣سم وتبرز على سطح المبيض

تم أخذ عينات دم من كل هذه المجموعات لفصل المصل منه واستخدم هذا المصل فى تقدير نسب بعض العناصر الكيميائية وخصوصا المعادن النادرة وهى (النحاس- الزنك - المنجنيز- والحديد) باستخدام جهاز الإمتصاص الذرى الطيفى وكانت النتائج المتحصل عليها كما يلى :

كان مستويات عناصر النحاس ،الزنك ، والمنجنيز فى مجموعة الحالات ذات حويصلة جراف أقل منها فىالحيوانات ذات الجسم الأصفر بينا لم يظهر عنصر الحديد أى تغيرات تذكر مابين المجموعتين •

بينما كان مستويات هذه العناصر فى الحالات المرضية المصابة بخمول المبايض والمبيض لمتحويصل أقل معنويا عما كانت علية فى مجموعة الحيوانات الضابطة •

استخلص من هذا البحث الى ضرورة إضافة مخلوط المعادن المحتوى على نسبة عالية من العناصر النادرة الى العليقة المقدمة للجاموس لتفادى حدوث مثل هذه الظواهر المرضية •