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Relationship Between Ovarian Activity and Blood Lead Concentration in Cows and Buffaloes with Emphases on Gene Frequencies of Hemoglobin

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Abstract: Genetic constitutions as well as environmental pollution are among the main factors which predispose to infertility in farm animals. The current investigation was carried out to monitor the impact of blood lead concentration on ovarian activity in local Egyptian cows and buffaloes with special emphasis to genetic constitution. The genital organs of 103 cows and 185 buffalo-cows were examined by rectal palpation as well as ultrasonography. Blood (cows-buffaloes) and milk (buffaloes) samples were collected for monitoring serum progesterone level and lead concentration in blood and milk samples. Results revealed that buffaloes had more incidence of ovarian inactivity than cows with undetectable progesterone level in all animals suffering from inactive ovaries. Lead concentration was high in both blood (> 10 μg/dl) and milk (> 0.4 μg/dl) samples of animals have inactive ovaries. Concerning hemoglobin genotyping, the result revealed that HbAB is distinguish as a good gene marker for active ovaries in cattle, while in buffaloes, Hb^A gene completely predominate in buffalo-cows having active ovaries, No clear relationship could b e obtained between gene frequencies of hemoglobin and blood lead concentration in both species. It was concluded a tight relationship is found between high blood and milk lead concentrations and ovarian dysfunction in farm animals.

Key words: Ovary · Lead · Blood · Milk · Hemoglobin · Gene frequencies

INTRODUCTION

In Egypt, cattle (4 500 000) and buffaloes (3 920 000) are the main farm animals, mainly raised indoors and in small herds, with seasonal fluctuation in availability and quality of ration and these animals have somewhat low reproductive performance [1, 2].

Ovarian inactivity is the main cause of infertility in cattle and buffaloes in Egypt [2]. This phenomenon was attributed to multiple factors including malnutrition [3], parasitism [4], bad hygiene and pollution [5]. Such ovarian dysfunction is associated with reproductive failure and great economic losses in Egyptian farm animals, especially in buffaloes [5, 6].

Heavy metals are among the most dangerous pollutants that have a tendency to accumulate in different organs and tissues of exposed animals and disrupt different physiological function [7].

Lead is a pervasive and widely distributed environmental pollutant with no beneficial biological roles, despite its poisoning is more common in farm ruminants, which are considered most susceptible to its toxic effects [8]. Animals get access to lead from soil, water, feed and varied degrees of lead poisoning have been reported in animals reared around different polluted areas [9].

It is well known that most of the metal is accumulated in the blood and have many deleterious effects related to its circulating concentrations [10]. The main target organs of lead are the hematopoietic, nervous, and renal systems. Also, it has impairment effects on the reproductive and immune systems [8]. Moreover, It was reported that lead accumulated in milk of animals constituting public health concern [11].

Impact of genetic predisposition on metal toxicokinetis in the body is limited, there is increasing evidence that certain genetic polymorphisms modify lead toxicokinetis [12].

Hemoglobin is a large complex protein molecule, consists of four polypeptide chains and one hem.

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The four chains usually occur in two identical pairs alpha and non-alpha chains, which have very different amino-acid sequences and are under the control of separate non-allelic genes. Hemoglobin genotyping are genetically determined by two co-dominant autosomal alleles which produce three observable phenotypes that can distinguished by electrophoresis[13].

The current investigation was carried out to detect the possible inter-relationship between lead concentration in both blood and milk and ovarian activity in local Egyptian cows and buffaloes. Also, the role of genetic constitution in relation to ovarian activity and lead toxicity was investigated.

MATERIALS AND METHODS

The present study was carried out as a part of the National Research Centre Project No. **E-8041207.**

Animals: A total number of 103 local Egyptian polyparous cows and 185 buffalo-cows kept in small holder farms at villages of Lower Egypt was included in this study.

Experimental Design: A full case history and owner complain of each animal were recorded. General health condition was examined. Gynecological examination was carried out by inspecting genital organs through rectal examination using an ultrasound apparatus (PiaMedical Falcs e`Saote, Netherlands) with an endorectal linear array transducer (6-8 MHz) for two successive weeks at least. Also, the condition was confirmed later on by monitoring serum progesterone level.

Samples Collection: Samples of blood (with and without EDTA) were collected from all animals. Uncoagulated blood samples were used for performing blood lead as well as hemoglobin electrophoresis. Serum was separated from coagulated blood samples by centrifugation (x 3000 g, 15 minutes at 4°C) and kept at-20°C for assaying progesterone level.

Analysis

- Serum progesterone level was assayed by ELISA microwell technique using kits from DIMA (Germany). The kit had a sensitivity of 2.0 pg/ml with inter-and intra-run precision coefficient of variations of 2.9 and 4.85, respectively [14].
- Blood [15] and milk [16] lead concentrations were determined using graphite furnace atomic absorption spectrophotometer at a wave length of 283.7 nm.
- Whole blood was subjected to electrophoresis using agrose 2% at pH 8.5 to 9.0 for genotyping of hemoglobin loci and determination of gene frequencies according to Hardi-Weinbrg law [17].
- Data were computed and statistically analyzed [18].

RESULTS

Ovarian Activity in Examined Local Egyptian Cows and Buffaloes: Table 1 shows the incidence of animals which did not show estrous signs for at least 6 months after calving, especially buffalo cows (during the breeding season; September- May). These animals have small non-functioning ovaries when scanned with ultrasound (Fig. 1) and serum progesterone level <0.02 ng/ml (Vs. 0.48±0.06 and 4.91±0.79 and 0.49±0.02 and 3.66±0.84 in cyclic cows and buffaloes, respectively). Buffalo-cows showed high incidence of ovarian inactivity if compared with cows.

Blood and Milk Lead Concentrations in Examined Local Egyptian Cows and Buffaloes in Relation to Ovarian Activity: Table 2 reveals the relation between ovarian activity and blood lead concentration. It is clear that animals suffer from ovarian inactivity have significantly (P<0.01) high blood lead concentration than those had active ovaries.

Table 1: Incidence of ovarian inactivity in examined local Egyptian cows and buffaloes (%)

Species	Total number of examined animals	Inactive ovaries	
		Number	%
Cows	103	38	36.89
Buffalo-cows	185	113	61.08

Table 2: Blood and milk lead concentrations in examined local Egyptian cows and buffaloes in relation to ovarian activity (µg/dl).

Species	Samples	Ovaries	
		Active	Inactive
Cows	Blood	7.29±0.19	13.36±0.39**
Buffaloes	Blood	6.32±0.37	11.60±0.31**
	Milk	0.47±0.14	0.80±0.10**

**P< 0.01

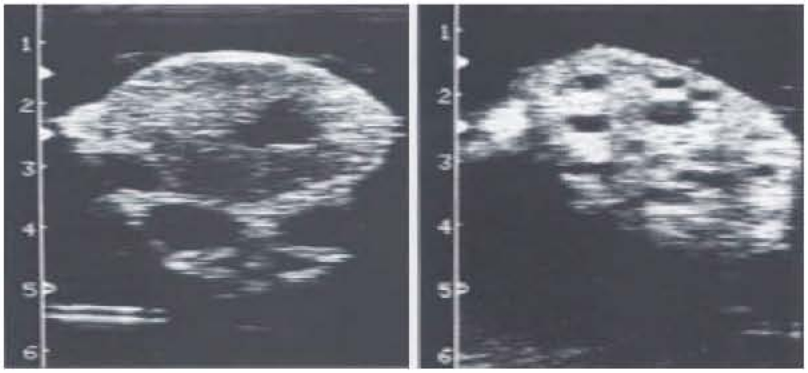

Fig. 1: Ovaries of local Egyptian cows examined by ultrasonography and show large follicular growth and regressed corpus luteum (Active ovary, Right) or small growing follicles (Inactive ovary, left).

Fig. 2: Electrophoretic pattern of hemoglobin in cows (N=16)

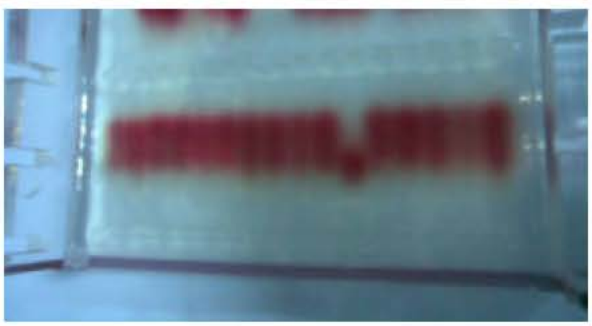

Fig. 3: Electrophoretic pattern of hemoglobin in Buffalo-cows (N=16)

Milk obtained from buffalo-cows suffering from ovarian inactivity was found to have significantly ($P<0.01$) higher lead concentration than those obtained from animals with active ovaries. It was 0.47±0.14 μg/dl in buffaloes with active ovaries vs. 0.80±0.10 μg/dl in animals suffer from ovarian inactivity.

Hemoglobin Genotypes in Relation to Ovarian Activity and Blood Lead Concentration in Examined Cows and Buffaloes: Figures2 and 3 show hemoglobin genotypes of cattle and buffaloes. It is clear that the hemoglobin loci in cattle are the four common bovine hemoglobin (A-B-C-D) which were found in different allelic frequencies (Hb^A=0.51, Hb^B=0.33, Hb^C=0.014 and Hb^D=0.01), while in buffaloes, it is consisted of two genotypes (HbAA and HbBB) with complete predominance of allele A (gene frequency 0.861 for Hb^A vs. 0.138 for Hb^B).

In cattle, HbAB was distinguished as a good gene marker for animals having active ovaries, while, in buffaloes, hemoglobin Hb^A is the completely predominated gene.

No clear relationship could be traced in gene frequencies of hemoglobin and blood lead concentration in the studied samples of both species.

DISCUSSION

The current investigations revealed that a considerable high percent of examined local Egyptian cows and buffaloes cows suffer from ovarian inactivity and have high blood lead concentration as compared to animals having active ovaries. Similar result was obtained by Avran *et al.* [19] who noticed that cattle farm at Romania suffered from high incidence of infertility with low pregnancy rate in animals had high blood lead concentration. Moreover, it was reported that blood lead concentration above 10 μg/dl is consider to be high with negative effects on many physiological functions including growth, reproduction and hematology [20].

Regarding the high incidence of ovarian inactivity in animals with high blood lead concentration, it is well known that lead induced reproductive toxicity and affect ovarian function and fertility of exposed animals, mainly due to both central and gonadal functional disturbance. [21, 22]. Centrally, exposure to lead resulted in reduction of hypothalamic GnRH levels [23], decreased LH and FSH concentrations [24] and interfere with pituitary hormone release via interaction with calcium-dependant secondary messengers system, which mediates hormone release from secretary granules storage [25]. At the gonads, lead has a direct effect, through affecting germinal epithelium [26], decreased gonadal weight or even act synergistically to reduce DNA gonadal content [27] and disturbed folliculogenesis due to its tissue accumulation [23].

In the present study, relatively high concentration of lead was found in collected milk samples from buffaloes, especially those animals suffering from ovarian inactivity. These results coincide with the result of Emam and El-Nabrawy [11] who found high level of lead in milk samples of cattle reared at Sharkia Governorate, Egypt.

Hemoglobin polymorphism of cattle and buffaloes was analyzed by means of electrophoresis. The current investigation showed the presence of two alleles of Hb (Hb^A and Hb^B) in buffaloes with complete predominance of Hb^A and these results confirm the finding of Bachmamn *et al.* [28] and Braemd [29]. Concerning the hemoglobin fraction of cattle, a difference was found in migration rates of different genotypes. The condition was found by many authors [30-32]. Schwellnns and Guerin [30] reported that the difference of mobility of the non-α chains of hemoglobin A, B, C. 1, F at different pH levels, while no difference was detected in the migration rate of their respective α chain. These results confirm the theory that genetic variation is restricted to the non-α chain of bovine hemoglobin. The gene frequencies of different hemoglobin alleles in the present study agree with the finding reported by Braemd [29] for East African Zebu except of HbG which did not found in Egyptian cattle breed. On the other hand, Shixin *et al.* [31] found that the Hemoglobin locus in yak is consisted of only one genotype HbAA, while in yellow cattle, it is consisted of 3 alleles (A, B,C). Through investigation on cattle and buffalo cows in which the Hb have became phenotyped and some reproductive indices were determined, the independent and simultaneous influence of the phenotypes of loci on the reproduction traits were analyzed for ovarian status (active or inactive). Results revealed that the favorable performance is found to be associated to HbAB and agree with the finding of Marcu *et al.* [32] and Boonprong *et al.* [33]. On the other hand, when hemoglobin polymorphism in Brahnan and seven southern African cattle breeds was investigated by means of starch gel electrophoresis, a difference was found between migration rates of HbC, showing that these are actually two separate variants. A comparison of the migration rate of α and β chains of all variants were encountered.

No clear relationship could be traced between gene frequency of hemoglobin and blood lead level in both cows and buffaloes under investigation and this may be attributed to the small number of samples. However such phenomena need further investigation.

It could be concluded that high blood and milk lead concentration in bovine is associated with endocrine disturbances with consequent ovarian dysfunction and great economic loss. Moreover, the milk from such animals may constitute public health hazards.

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