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CHROMOSOMAL ABNORMALITIES IN INFERTILE BUFFALO WITH SPECIAL FOCUS ON FRAGILE SITES

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SUMMRY

Thirty-eight breedable female buffalo were used to identify the relation between chromosomal abnormalities and infertility, with a special focus on the fragile sites located on the buffalo genome. Animals were classified into three groups according to rectal examination and fertility records. These groups consisted of 18 sterile heifers, 10 repeat breeder buffalo cows and 10 fertile buffalo cows as a control group. Blood samples were collected from the animal's jugular vein under aseptic conditions. Standard blood culture technique was performed to identify the different types of chromosomal abnormalities and their frequencies with special focus on the fragile sites in buffalo genome.

Incidence of numerical abnormalities was almost similar in all the studied groups, ranged from 0.8 to 1.2%. The appearance of polyploidy was the unique type among the numerical abnormality observed. However, incidence of structural abnormalities was high (20.4%) in the sterile heifer group followed by the repeat breeder group (17.7%), whereas the lowest percentage (10.2%) was in the control group. Incidence of autosomal aberrations was almost similar in the studied groups, 12.3 % and 14.6% in repeat breeder and sterile heifer groups, respectively compared to 9.2% in the control. Whereas, incidence of X-chromosome abnormalities was 5.4 % and 5.8% in repeat breeder and sterile heifer groups, respectively compared to 1% in the control. Different types of structural abnormalities were considerably observed in sterile heifers followed by repeat breeder group and the control, except that of autosomal chromosomal gaps and chromatid breaks in X-chromosome, which had no clear trend. The X-chromosomes were more prone to these aberrations than autosomal in infertile animals. The phenomenon of fragile sites was not observed between those animals in the studied groups.

***Keywords**: Buffalo, sterility, repeat breeder, chromosome aberrations, fragile sites.*

INTRODUCTION

Infertile buffalo are one obstacle that hinders the breeders to improve the buffalo reproductive performance and leads to economic losses in the production of meat and milk. The role of chromosome abnormalities in reproductive problems in man and domestic animals has been well documented during the past few decades. In cattle,

structural alterations of the Robertsonian translocation type are known to cause varying degrees of subfertility (Gustavsson, 1969; Dyrendahl and Gustavsson, 1979; Popescu, 1990). However, effects of other types of structural aberrations on the fertility of carrier bulls and cows are not well documented.

Fragile sites are defined as specific points liable to breaks, and located on different chromosomes or chromosome parts. It was firstly discovered in humans by Sutherland (1977). The biological significance of these fragile sites in human is reported to be related to many chromosomal rearrangements that may lead to cancer and tumor types (Sutherland and Baker, 2000; Richards, 2001). In farm animals such as pigs, few reports have been published on the occurrence of chromosomal fragile sites (Yang and Long, 1993; Riggs *et al.*, 1993; Ronne, 1995). These workers found that fragile sites were correlated with the same places which commonly known as chromosomal rearrangements. Fragile sites were reported also in cattle but with little occurrence and in the X chromosome only (Uchida *et al.*, 1986). In buffalo only two reports were published concerned with the localization of fragile sites on the buffalo chromosomes. Pires *et al.* (1998) Localized a fragile site on the X chromosome in the Brazilian buffalo. In contrast, Mahrous and Ahmed (2000), in the Egyptian buffalo chromosomes found that it is located on two biarmed chromosomes only (2p13, 2q21 and 5q21).

The aim of this study was to identify the types and frequencies of different chromosomal abnormalities in subfertile buffalo, its possible relation with the infertility in buffalo, with a special focus on the fragile sites in the buffalo genome.

MATERIALS AND METHODS

Animals and management:

Out of 313 breedable female buffalo at Mehallat Mousa Research Station, Animal Production Research Institute, 18 heifers and 10 buffalo cows were considered as problem buffalo culled for reproductive disorders. These animals were diagnosed as free of reproductive diseases and considered to have fertility problems where no pregnancy was recorded. Young heifers were considered to have fertility problem if they reached more than two years of age and / > 350 kg body weight and no pregnancy was recorded, although enter mating group more than three times. In addition, the rectal examination for these heifers revealed certain degree of atrophy in genitalia. Buffalo cows were considered to have fertility problem, if the female received more than three-non fertile service (repeat breeder). The buffalo were housed in shaded open yard. They were fed on concentrate mixtures, berseem (*Trifolium axlexndrium*) and rice straw according to live body weight and milk production. Berseem hay was occasionally offered whenever green berseem was not available.

Experimental design:

Animals were classified into three groups according to their rectal examination and fertility records. The first group included 18 infertile buffalo heifers; the second group included 10 repeat breeder buffalo cows while the third group included 10 fertile buffalo cows as a control group.

Cytogenetical analysis:

conditions in heparinized tubes. For obtaining the chromosomes, the standard blood culture technique was performed. Blood lymphocytes were cultured in 4 ml of tissue background media (RPMI 1640); this media was previously supplemented with 1 ml fetal calf serum and 0.1 ml Phytohamaegltinin (PHA) as a mutagenic agent. The tube was mixed well and incubated at 38 °C for 72 hours (at hour 70, 0.1 ml from colchicines (0.05%) was added to the culture). Hypnotic treatment was performed with 0.075 M KCL for 20 min. at 38 °C. The cells were washed and fixed in a solution consisted of glacial acetic acid: methanol (1:3). The chromosomal suspension was dropped on cold wet slides then flamed to dry. The slides were stained with 10% Giemsa. For each animal, 50 well metaphase spreads in the control group and 100 well metaphase spreads in the infertile groups were examined for total chromosomal count, and the types of chromosomal abnormalities. Metaphases affected with chromosomal abnormalities were de-stained and reexamined after G-banding as described by Seabright (1971) to allow the identification of fragile sites location. The fragile sites were identified as a site of non staining gaps or breaks at specific points on the chromosomes and their frequencies are more than three times per individual (Fundia and Larripam, 1989). The classification of fragile sites was done according to the nomenclature established in standard karyotypes of the river buffalo according to the report of the committee for the standardization of banded karyotypes of the river buffalo (ISCNDB, 2000 and SKRB, 1994) .

Statistical analysis:

Heterogenecity test was performed to observe the heterogenecity or the error among the animals within each group. Correlations were estimated according to SAS (2000).

RESULTS

Cytological analysis of chromosomal abnormalities indicated that percentage of incidence of numerical abnormalities was almost similar in all the studied groups ranged from 0.8 to 1.2% (Table 1). The appearance of polyploidy was the unique type of numerical abnormality observed. However, the percentage of incidence of structural abnormalities was higher (20.4%) in sterile heifer groups followed by repeat breeder group (17.7%), whereas the least percentage (10.2%) was in the control.

Table 1. Incidence of total structural and numerical abnormalities in Egyptian buffalo

Groups	Total examined metaphases	Normal chromosomes		Chromosomal abnormalities			
				Total structural abnormalities		Numerical abnormalities	
		No	%	No	%	No	%
Control	500	444	88.8	51	10.2	5	1.0
Repeat breeder	1000	815	81.5	177	17.7	8	0.8
Sterile heifers	1800	1412	78.4	367	20.4	21	1.2

Structural abnormalities were recorded separately for autosomal and X-chromosome abnormalities as shown in table (2). Incidence of autosomal aberrations was almost similar in the studied groups, 12.3 % and 14.6% in repeat breeder and sterile heifer groups, respectively compared to 9.2% in the control. Whereas, incidence of X-chromosome abnormalities was 5.4 % and 5.8% in repeat breeder and sterile heifer groups, respectively compared to 1% in the control. i.e., incidence of X-chromosome abnormalities in repeat breeder and sterile heifer groups being five times (5.4%-5.8 %) as compared to the control (1%).

Table 2. Incidence of autosomal and X-chromosome abnormalities in Egyptian buffalo

Groups	Total examined metaphases	Structural abnormalities			
		Autosomal abnormalities		X-chromosome abnormalities	
		No	%	No	%
Control	500	46	9.2	5	1.0
Repeat breeder	1000	123	12.3	54	5.4
Sterile heifers	1800	262	14.6	105	5.8

The data concerning all types of structural abnormalities (gaps and breaks) were recorded separately for autosomal and X-chromosome abnormalities as shown in table (3). Different types of structural abnormalities were higher in the sterile heifer group followed by repeat breeder group and the control. This trend was not clear in the autosomal chromosomal gaps and the chromatid breaks in the X-chromosome.

Table 3. Percentage of incidence of different types of structural chromosome abnormalities in Egyptian buffalo

Groups	Structural abnormalities											
	Chromatid gaps				Chromosomal gaps				Chromatid breaks			
	A		S		A		S		A		S	
	No	%	No	%	No	%	No	%	No	%	No	%
Control	14	2.8	2	0.4	11	2.2	2	0.4	15	3.0	1	0.2
Repeat breeder	39	3.9	18	1.8	19	1.9	5	0.5	50	5.0	27	2.7
Sterile heifers	81	4.5	29	1.6	34	1.9	19	1.1	114	6.3	40	2.2

Groups	Structural abnormalities			
	Chromosomal breaks			
	A		S	
	No	%	No	%
Control	6	1.6	0	0
Repeat breeder	15	1.5	4	0.4
Sterile heifers	33	1.8	17	0.9

A = Autosomal
S = X- chromosomal

Figure (1) is showing a normal buffalo metaphase spread as well as some metaphases having chromosomal abnormalities. Frequencies of occurrence of chromatid and chromosome gaps and breaks in the X-chromosome revealed that in infertile animals X-chromosomes were more prone to these aberrations than auto-chromosomes.

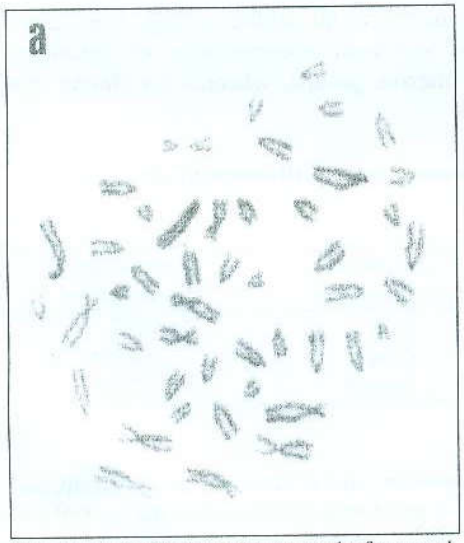

Figure (1-a): Metaphase spread of normal a Buffalo cows.

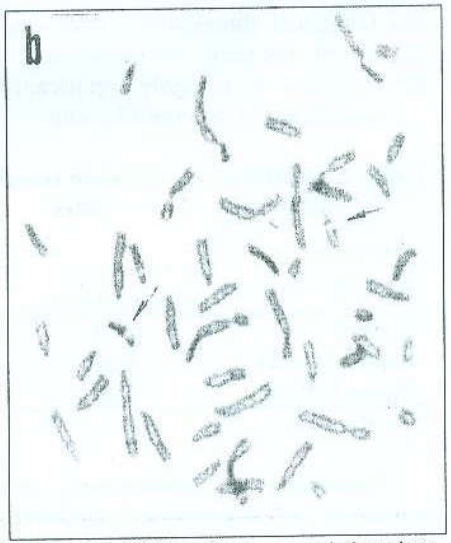

Figure (1-b): Metaphase spread showing chromatid break and chromosome gap.

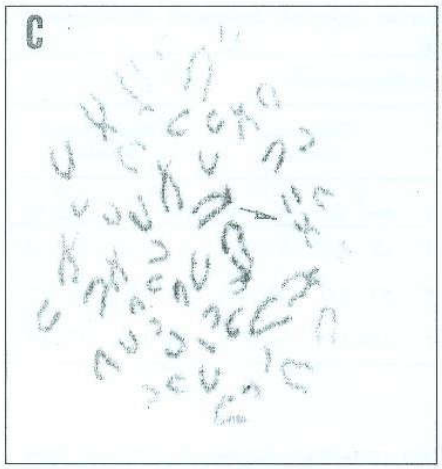

Figure (1-c): Metaphase spread showing a showing a chromosome break.

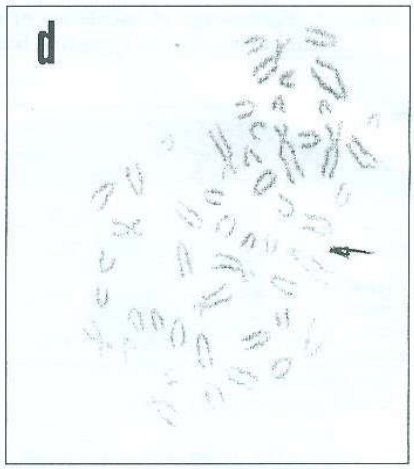

Figure (1-d): Metaphase spread break in the sex-chromosome.

Relationships between X-chromosome and numerical, structural and autosomal abnormalities are shown in table (4). Correlation coefficient between X-chromosome and numerical abnormalities was non-significant in all studied groups. Correlation coefficient between X-chromosome and structural abnormalities & autosomal abnormalities were highly significant in infertile groups, whereas correlation was non-significant in the control group.

Table 4. Relationships between sex-chromosome and numerical, structural and autosomal abnormalities

Groups	Numerical abnormalities	Structural abnormalities	Autosomal abnormalities
Control	-0.15	0.45	- 0.37
Repeat breeder	0.40	0.94**	0.83**
Sterile heifers	-0.30	0.91**	0.79**

** = P < 0.01

Concerning the heterogeneity test between individuals in each group, no significant differences were noticed for any type of aberrations within the control and repeat breeder groups. Meanwhile, the sterile heifers group showed significant differences in autosomal chromatid breaks and X-chromosomal chromatid breaks that were reflected on the total structural abnormalities of this group (Table 5).

Table 5. Heterogenecity values for different types of chromosomal abnormalities in the studied groups of Egyptian buffalo

Groups	Structural abnormalities									Numerical abnormalities
	Chromatid gaps		Chromosomal gaps		Chromatid breaks		Chromosomal Breaks		Total	Polyploidy
	A	S	A	S	A	S	A	S		
Control	01.7	08.0	04.5	08.0	04.3	09.0	10.7	00.0	01.8	05.0
Repeat breeder	08.4	03.1	03.7	09.0	03.3	03.2	03.2	09.3	18.8	07.0
Sterile heifers	18.0	11.6	13.7	18.7	37.9**	31.1	09.1	11.6	69.9**	16.5

A = Autosomal
S = Sex chromosomal

Phenomenon of fragile sites (break in the same place of chromosome) on the specific chromosomes was not observed between all animals in the studied groups.

DISCUSSION

From the results obtained in this study, incidence of numerical abnormalities was almost similar in all the studied groups, ranged from 0.8 to 1.2%. However, incidence of total structural abnormalities was high (20.4%) in the sterile heifer group followed by repeat breeder group (17.7%), whereas the least percentage (10.2%) was in the fertile group. Incidence of X-chromosome abnormalities ranged from 5.4 to 5.8% in repeat breeder and sterile heifer group (five times) as compared to fertile group (1%). Therefore, X-chromosomes were more prone to aberrations in the infertile animals than autosomal chromosomes. Even though these figures cannot be easily taken into consideration as a predisposing factor for infertility due to many reasons: 1) The abnormalities detected in this study are non-inherited type of abnormalities since it were detected in the lymphocyte, which is a somatic cell. 2) The gaps are a type of abnormality that appear to represent a single effect on the chromosome, therefore it is likely to indicate a damage in the mutational sense and may be easily repaired by the repair mechanism of the animal (Evans, 1962). 3) The cells that carry gaps and breaks usually die and are excluded from the cell population. The fate of the cells that carry similar abnormalities in human lymphocytes was studied by Iakovenko and Sapacheva (1984); Das and Sharma (1987); Kusakabe *et al.* (1999); Hoffmann *et al.* (1999). They agreed that these abnormalities may lead to the death of the carrying cell and subsequently it would be eliminated from cell population. They also indicated that some health problems may arise from the death of these cells but no effect on the fertility was reported.

Gustavsson (1971) studied the chromosome abnormalities in some repeat breeder cattle heifers and reported that this phenomenon is associated with the Robertsonian translocation and not gaps or breaks. From another side, Popescu (1990) reported that abnormalities in the chromosome structure from the gap and break types have no

phenotypic expression on fertility, while translocations have an effect. This is also a confirmation to our result that there is no real association between gaps, breaks and reduced fertility.

The reason for a chromosomal abnormality is an environmental mutagen and the different ratios of abnormalities exhibited by the animals are usually due to the impact of environmental conditions that may indirectly affect cell mutation. This agrees with the observation of Bongso and Basrur (1976) who failed to prove any relation between gaps and breaks from one side and fertility from the other side. The author attributed this abnormality to a variety of agents including bacterial, viral or mycoplasmal infection. The results of fragile site analysis and the non-clear trend for chromosomal abnormalities confirm the genetic stability of the buffalo genome. In general, all these abnormalities are considered from the non-inherited abnormality type that causes the death of the carrying cells as mentioned earlier in this discussion. Although a fragile site on the buffalo X chromosome was reported in Brazilian buffalo, no proved correlation between this fragility and fertility of carrier animals was reported (Pires *et al.*, 1998).

Concerning the gross chromosomal abnormalities such as translocations inversions, trisomics and monosomics, a very limited case of reported animals carrying such abnormalities, was observed in Egypt, India and Italy during the last 20 years. In Egypt, only two cases were reported, the first was a 10/13 Robertsonian translocation (De Hondt *et al.* 1986), with no available data on fertility. The second was a female carrying a mosaic mixoploid trisomic for chromosome 10 (Hassanane and El-Kholy, 1995), and this female was infertile. In India, Balackrishnan and Yadav (1984) reported a case of pericentric inversion in the fourth chromosome, a case of X-chromosome trisomy, and a case of secondary constriction in the fourth chromosome. Prakash *et al.* (1992 and 1994) reported two cases of X-chromosome monosomy and trisomy respectively. Freemartin in Indian buffalo was reported by Balakrishnan *et al.* (1981). In Italy the only European country that is interested in buffalo, Iannuzzi *et al.* (2000) reported a case of X chromosome monosomy in a sterile buffalo. Moreover Iannuzzi *et al.* (2001) reported also a case of 50, XY gonadal dysgenesis (Sawyer's syndrome) in a female river buffalo.

The results obtained from the present study showed and confirmed the previously reports of Hassanane (1991) who failed to find any relation between chromosome abnormality and subfertility in buffalo. A general conclusion could be addressed that the buffalo genome has a chromosomal stability against environmental mutagens. The absence of the fragile sites in the studied animals is a confirmation to our conclusion .This differs than the case of pig when fragile sites are 38 points linked with the translocation points (Yang and long, 1993).

The reason for fertility problems may be due to environmental or managerial factors rather than genetic factors, especially that the subfertile animals were fertile in previous seasons or may be fertile in a next season. The study demonstrated that cytogenetical analysis for somatic cells (lymphocytes) could be an indicator to a mutation or abnormalities happened simultaneously in the oocytes and subsequently affected the fertility, especially one oocytes is produced during each sexual cycle.

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الشـــذوذ الكروموسومي في الجاموس منخفض الخصوبة مع التركيز علي أماكن الضعف في الكروموسوم

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أستخدم في هذه الدراسة ثمانية وثلاثون من إناث الجاموس الناضجة بغرض التعرف علي طبيعة العلاقة بيـــن التغـــيرات الكروموســـومية والإضرابات التناسلية مع التركيز علي أماكن الضعف الكروموسومية في التركيـــب الكروموســـومى. قســـمت الحـــيوانات إلي ثلاثة مجاميع طبقا لنتيجة الفحص التناسلي وسجلات الخصوبة، اشتملت المجموعة الأولي علي ١٨ عجلة عقيمة و المجموعة الثانية علي ١٠ جاموسات متكررة التلقـــيح و المجموعـــة الثالثة علي ١٠ جاموسات خصبة (مجموعة مقارنة). جمعت عينات الدم من الوريد الوداجي للحيوانات تحت ظروف التعقيم و مضادات التجلط المطلوبة. أجريت تقنية مزرعة الدم للتعرف علي الأنمـــاط المخـــتلفة مـــن الشـــواذ الكروموســـومية و معدل تكرار حدوثها مع التركيز علي أماكن الضعف الكروموسومية في التركيب الكروموسومى للجاموس.

أظهـــرت نـــتائج الدراســـة أن نســـبة حدوث الشواذ الكروموسومية العددية متشابهة تقريبا في مجاميع الحـــيوانات تحـــت الدراســـة حيـــث تراوحـــت النســـبة بين ٠,٨ و ١,٢ %. و كان تضاعف المجموعة الكروموســـومية هو النوع الوحيد المشاهد بين أنماط الشواذ الكروموسومية العددية. في حين كانت أعلي نســـبة لحـــدوث التغيرات التركيبية في كروموسومات مجموعة العجلات العقيمة (٢٠,٤%) تليها مجموعة الجاموســـات مـــتعددة التلقيح (١٧,٧%) في حين أظهرت المجموعة المقارنة أقل نسبه حدوث (١٠,٢%). علـــــي الجانب الآخر كانت نسبة حدوث التغيرات الكروموسومية الجسدية متقاربة في المجموعات الثلاث (١٢,٣% ، ١٤,٦%، ٩,٢% في المجموعة المتكررة التلقيح و العجلات العقيمة و المقارنة علي التوالي)، بينما كانت نسبة حدوث حالات الشذوذ الكروموسومية في كروموسومات الجنس هى٥,٤%، ٥,٨%، ١,٠% فـــي المجموعـــة المتكررة التلقيح و العجلات العقيمة و المقارنة علي التوالي. و علي وجه العموم ظهرت الأنـــواع المخـــتلفة مـــن التغيرات الكروموسومية التركيبية بمعدل أعلي في مجموعة العجلات العقيمة تليها المجموعـــة المـــتكررة التلقيح ثم أخيرا المجموعة المقارنة علي الترتيب فيما عدا التغيرات الكروموسومية الجســـدية ذات الفجـــوة و الكروموســـومات الجنســـية المكسورة حيث لم تظهر توجها واضحا في أي من المجموعات. أظهرت كروموسومات الجنس ميلا اكبر للتعرض لحدوث مثل تلك الحالات من الشواذ مقارنة بالكرموســـومات الجسدية في مجموعة العجلات العقيمة. وقد كانت ظاهرة أماكن الضعف في الكروموسوم غير مشاهدة في كل المجموعات موضع الدراسة.