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CYTOGENETIC STUDIES IN RELATION TO FERTILITY IN CATTLE

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SUMMARY

Seventy-one Holstein-Friesian divided into five groups (Three groups of females and two groups of males) were investigated cytogenetically to identify and score any chromosomal aberration which may be encountered. The female groups were repeat breeder group (25 animals), and freemartin group (4 animals) in which the females are heifers aged from 3 to 4 years and the third group was pregnant heifers (25 animals control) aged 2 to 2.5 years. The male groups were young males aged from 12 to 18 month (8 animals with unilateral testicular orchidy) and breeding bulls (8 control animals) aged 3 years. Blood samples were collected from these groups for cytogenetical analysis. The highest percentage was detected for the total structural aberrations (25 68%) which are significantly higher than that observed for total numerical aberrations (11.84%) in the repeat breeders group. The freemartins had equal numbers of metaphases with xx and xy, and were found to have numbered (20 and 5%) of structural and numerical variations, the percentage of total chromosomal aberrations was 25%. The total structural aberrations in the affected male group (26.75%) were significantly higher than numerical variations (11.0%). The total numerical and structural aberrations, the percentage in the affected male group (37.75%) is significantly higher than that found in the normal control group (16.3%)

INTRODUCTION

Fertility is one of the most important perfomances of domestic animals, and it is very sensitive to the disturbance in genetic material (chromosomes and genes) Previous studies in cattle (Gustavsson 1971, 1977, Zahner et al., 1979, Swartz and Vogt 1983, De Hondt et al. 1988, and Mahrous 1991) sheep (Bruere 1969) and buffaloes (Hassanane 1991). Miyake and Kaneda (1990) recorded the chromosomal abnormalities in bulls with unilateral cryptorchidism. Kulekova (1990) examined cytogenetically some cases of calves with various congenital abnormalities. He found a case of trisomy of chromosome 19 and sex chromosome chimerism. Hazas et al. (1998) found some decrease of percentage of carriers of bovine hereditary chromosome abnormalities.

A freemartin has been defined as a sexual imperfect (Swett et al., 1940), usually sterile, female partner of a pair of heterosexual twins . The karyotype from leucocyte cultures in dizygotic twins showed sex chimerism (Fechheimer et al. 1963). The freemartin syndrome occurs in around 92% of bovine female born as a result of heterosexual twin pregnancies and exists although at much lower incidence in sheep and pigs (Marcum, 1974). Dunn et al., (1979) recorded thirteen chimeric xx/xy bulls born co-twin with freemartin while Zhang et al., (1992) found that six hundred calves (82.5%) from 727 female cows were determined to be freemartin. De Hondt et al (1988) and Mahrous et al (1993) found one case of freemartin in hybrid friesian cows. Slota et al. (1998) studied twenty-five bulls originated from dizygotic twins taken under cytogenetic control. In 17 of these animals, the xy cell line was increased and in 8 bulls, the xy cell line was decreased. The results obtained indicated that the tendency to eliminate the xx line is very individual and can be compared to individual immunological tolerance on skin grafts between dizygotic twins.

The aim of the present investigation is to study the relationship between infertility in cattle (heifers, bulls, and some cases of freemartin) and presence of some specific chromosomal aberrations.

MATERIALS AND METHODS

The material used in this work consists of five groups of cattle, collected from different farms in Egypt. The detailed clinico-cytogenetic studies have been carried out on cattle with reproductive problem. Holstin Friesian cattle raised in Egypt. The symptoms of reproductive disorders encountered in heifers were repeat breeding and anoestrum, while in case of bulls the reproductive anomalies were unilateral testicular orchidy and two others normal groups, the first was pregnant heifers and second breeding bulls used as control male group (8 animals, age 3 years). The number physical conditions, reproductive stage, and findings of rectal examination of control animals and those with reproductive problems are presented in table (1 a, b, c). Ten ml of blood were collected under aseptic conditions using heparinized vacutainer tubes, from all animals, these samples were used for cytogenetical analysis. Blood cells were cultured for 72 hrs in 5 ml RPMI media, 1 ml of fetal calf serum and 0.1

ml of phytohaemogglutinine (PHA). After incubation, the cells were treated with colchicine for 2 hrs, then with hypotonic solution (0.075M) KCL for 20 min at 38°C. After flxatin (3 methanol: 1 acetic acid), the cell suspension was dropped on wet slides, then flamed to dry. The slides were stained and examined. Data were statistically analysed using statistical analysis system after (S. A. S. 1987).

RESULTS

Cytogenetical analysis of the present study is presented in Tabel (2,3,4 dand 5).. Moreover Fig. 1 and Fig. 2 show some structural chromosome aberrations in all groups of animals. It clearly evident from the present data that the percentage of abnormal metaphases were significantly higher (P<0.05) in repeat breeder group than in control. Structural aberrations demonstrated that the chromatid gaps (11.76%) and total structural

Table (1a): The number, clinical signs, rectal findings and general health condition of heifers with fertility problems:

Clinical signs	No. of heifers	Findings of rectal examination	General Appearance
Repeat breeder	25	The ovaries had normal size and uterus to some extent was considered normal	Phenotypically normal, healthy animals
Anoestrum	4	In three of them, the ovaries and uterus were small sized, while in the fourth one the uterus was not found	Stear like, normal healthy animals

Table (1b): The clinical finding number and age of Friesian bulls

Clinical signs	No. of bulls	Findings of gross examination	Age
Unilateral testicular orchidy	8	Absence of the left testis and slight entargement of the other one	12-18 month

Table (1c): The reproductive stage of the control groups.

Sex	No. of animal	Reproductive stage	Age
Heifers	25	Pregnant animals	2-2.5
Breeding bulls	8	Phenotypically normal used either for AI or natural service	3 years (average)

Table (2) The numbers. percentages of different groups of chromosomal aberrations and statistical significance in female cattle groups.

Female groups	Number of animals	Number of metaphases examined	Number of cells with aberrations	Number of cells without aberrations	Chromosomal aberrations									Total aberrations
					Numerical aberrations			Structural aberrations						
					Poly	Peri	Total	Gaps	Breaks	Chrs gaps	Chrs breaks	Centric fusion	Total	
					No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %	
Control female	25	1250	125	1125	ef 33 (2.64)	c 63 (5.04)	C 96 (7.68)	fg 21 (1.68)	gh 13 (1.04)	i 0 (0)	h 5 (0.4)	h 9 (0.72)	D 48 (3.84)	2 *144 (11.52)
Repeat breeder	25	1250	462	788	de 46 (3.68)	b 102 (8.16)	B 148 (11.84)	a 147 (11.76)	cd 62 (4.96)	de 42 (3.36)	e 39 (3.12)	ef 31 (2.48)	A 321 (25.68)	1 *469 (37.52)

* Many metaphases speared have more than one aberration
- Chromatid breaks include deletions and fragments.
Values with the different small alphabetica, capital alphabetica and numbers are significantly each other at level (P<0.05).
- Poly: Polploidy Peri: Peridiploid Chrs: Chromosome

Table (3) The numbers, percentages of different groups of chromosomal aberrations and statistical significance in male groups.

Male groups	Number of animals	Number of metaphases examined	Number of cells with aberrations	Number of cells without aberrations	Chromosomal aberrations									Total Chromosomal aberrations
					Numerical aberrations			Structural aberrations						
					Poly	Peri	Total	Gaps	Breaks	Chrs gaps	Chrs breaks	Centric fusion	Total	
					No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %	
Control male	8	400	57	343	bcd 19 (4.8)	bc 25 (6.3)	B 44 (11)	def 10 (2.5)	ef 8 (2)	g 0 (0)	g 0 (0)	fg 3 (0.8)	C 21 (5.3)	2 *65 (16.3)
Affected male group	8	400	148	252	cde 14 (3.5)	ab 30 (7.5)	B 44 (11.3)	a 45 (11.3)	bc 21 (5.3)	cde 14 (3.5)	def 10 (2.5)	bed 17 (4.3)	A 107 (26.75)	1 *151 (37.75)

* Many metaphases speared have more than one aberration
- Chromatid breaks include deletions and fragments.
Values with the different small alphabetica. capital alphabetica and numbers are significantly each other at level (P<0.05).
- Poly: Polploidy Peri: Peridiploid Chrs: Chromosome

aberrations (25.68%) were significantly higher (P<0.05) in repeat breeder than in control animals.

Table (3) indicated that the highest ratio of chromosomal aberrations in bulls was detected in the affected groups for the chromatid gap (11.3%) and for the peridiploid aberrations (7.5%) followed by the chromatid break (5.3%) and

Table (4) The numbers, percentages of sex chromosome compement in freemartin group.

Serial number	Number of metaphases examined	XX	XX	XXX	XXY	XYY	XO	OY
		No. %	No. %	No. %	No. %	No. %	No. %	No. %
*1	50	12 24	25 50	0 0	13 26	0 0	0 0	0 0
2	50	28 56	22 44	0 0	0 0	0 0	0 0	0 0
3	50	21 42	26 52	0 0	0 0	0 0	2 1	1 2
4	50	30 60	20 40	0 0	0 0	0 0	0 0	0 0
Total	200	91 45.5	93 46.5	0 0	13 6.5	0 0	2 1	1 0.5

*Freemartin heifer without uterus and ovaries.

centric fusion (4.3%). The total structural aberrations (26.75%) was significantly higher (P<0.05) in male group than in control.

Table (4) indicated that the highest ratio of chromosome complement was 46.5% for xy sex chromosome for all the freemartin heifers followed by 45.5% for xx sex chromosome, while the ratio of xxy sex chromosome was 6.5%. Comparing the four animals, the third case had the highest ratio for xy sex followed by the case number one (50%).

Table (5) revealed that the percentage of chromatid gap and chromatid, break were the highest ratio (8.5%) and 8%, respectively) compared with the chromosomal gaps 2%, the chromosome break was 1% and the percentage of centric fusion was 0.5%. The total structural

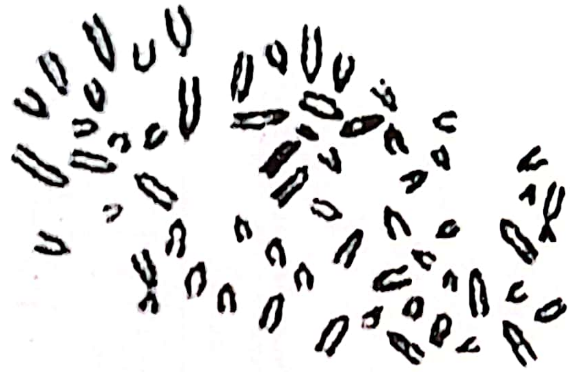

Fig. (1): A metaphase spread of a repeat breeder heifer showing chromatid gaps.

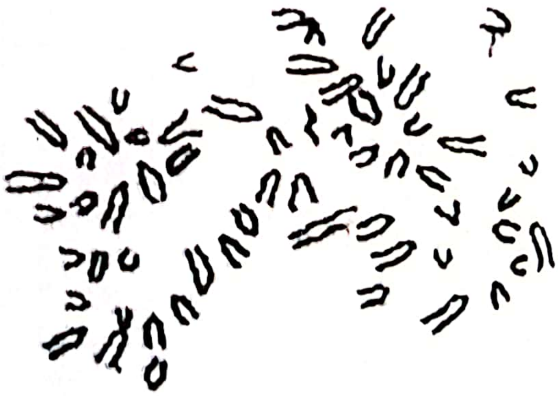

Fig. (2): Ametaphase spread of a repeat breeder heifer showing chromatid break.

Table (5) : Chromosomal aberrations in freemartin group.

Animal group	Number of animals	Number of metaphases examined	Number of cells with aberrations	Number of cells without aberrations	Chromosomal aberrations									Total aberrations
					Numerical aberrations			Structural aberrations						
					Poly	Peri	Total	Gaps	Breaks	Chrs gaps	Chrs breaks	Centric fusion	Total	
					No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %	
Freemartin group	4	200	50	150	0 (0)	10 (5)	10 (5)	17 (8.5)	16 (8)	4 (2)	2 (1)	1 (0.5)	40 (20)	50 (25)

- Chromatid breaks include deletions and fragments aberration (20%) was higher than total numerical variation (5%).

DISCUSSION

To study relation between fertility in cattle and chromosomal aberration, three groups of females and two groups of males were cytogenetically investigated. Polyploidy, which was found in both repeat breeder and control groups was not significant (3.68% and 2.64% respectively). These results were considered lower in comparing with the finding of Herschler et al 1962 (5.2-10.8) and of Darre et al 1970 who recorded the same findings. Although several correlations were suggested for abnormally high level of polyploid cells in case of inbreeding problems including repeat breeder (Zartman and Fechheimer, 1967), the nonsignificant lower percentages of polyploid could be attributed to the ealier suggestion that the high percentage of polyploid cells were considered as artifact of culture (Halnan, 1976). There is a significant difference between peridiploid in repeat breeder and control groups (8.16%) and 5.04%, respectively).

Regarding the findings recorded in table (2), it is clear that the chromatid aberrations were more closely related to infertility than chromosomal aberrations (Galloway and Norman, 1974). In a previous study, El-Nahass et al (1974) reported break and gaps in a heifer. Also El-Nahas et al (1976) observed a high percentage (42%) of gaps and breaks in young cows. In that study, there was a significant difference between repeat breeders and control groups (2.48% and 0.72%, respectively). In our study, the percentage repeat breeder was 11.76 which seemed higher than that of DeHondt et al 1988 (1.4%) and of Mahrous 1991 (1.5%). Moreover, the presence of centric fusion with significant difference agreed with those of Refsdal (1976) and Gustavsson (1979, 1980 and 1984). The high percentage of total chromosomal aberrations (49.07%) in the repeat breeder group was due to the high percentage of structural aberrations (37.52%) and not due to numerical aberrations (11.52%).

Table (3) shows that there was a significant difference between affected male groups and control group in chromatid gaps, breaks and chromosome gaps, while it was statistically higher in chromosome break. The increased rate of gaps and breaks observed in this study agreed with those recorded by Halnan (1976).

The freemartin syndrome occurs in 92% of bovine born as a result of heterosexual twin pregnancies. In the present study, the freemartins have a xx constitution in 45.5% of the metaphases examined, while the other metaphases were 46.5% xy constitution. The xy sex chromosome complement study was nearly similar to the findings of Marcum 1974 (47.6%), chromatid gaps and breaks were high as well, resulting in a very high number of structurally aberrant metaphases. Although xx /xy chimerism has been reported in cattle from a long time ago, there is still some new reports about some cases of freemartinism in cattle (Moreno et al. 1992). It seems also found that the xx/xy chimerism is in buffalo (Balakrishnan et al., 1981), sheep (Rynkiewicz-Szatkowska 1992), goat (El-Nahass et al. 1993) and in red deer (Stewert-Scott et al., 1990).

It can be concluded that, gaps and breaks either in chromatid or chromosomes play an important role in fertility problems. These losses of parts of chromatid or chromosomes mean the missing of genes carried. Increasing the frequencies of gaps and breaks accompanied mostly by losses of genes responsible for fertility performance. Repeat breeder, freemartinism and males with unilateral orchidy should be culled from breeding program.

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