Global Veterinaria 7 (4): 323-329, 2011
ISSN 1992-6197

Cytogenetic Analysis Related to Some Infertility Problems in Cattle

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Abstract: Cytogenetic studies were carried out on fertile and infertile Holstein Friesian cows in an effort to elucidate the role of chromosomal abnormalities in reducing fertility of this important producing animal. This study was carried out on thirty-one Holstein-Friesian cows representing by twelve sexually clinically normal and non pregnant cows as control group, eight cows suffering from repeat breeder, six cows suffering from anestrum and five cows suffering from frequently prolapsed vagina. Chromosomal preparations were made using blood culture from animals under study. About 50 metaphase spreads were screened to detect the chromosomal aberrations and prepare the karyotype. The results showed that the percent of total numerical aberrations for repeat breeder group was 19.95%, while the percent of total structural aberrations were predominant and reached 62%. The percent of total numerical aberrations for cows suffering from anestrum was recorded as 21%. These results were significantly higher than observed for control group. The percent of total numerical aberrations for vaginal prolapse group was 13.6%, while the percent of total structural aberrations was 40%. These results concluded that cytogenetic studies should be used as a diagnostic tool to determine the causes of low reproductive efficiency.

Key words: Chromosomes · Aberrations · Reproductive · Disorder

INTRODUCTION

Cattle have been known as the most important farm animal in Egypt, which show a peculiar type of reproductive performance.

Reproductive efficiency is a critical component of a successful dairy operation, whereas reproductive inefficiency is one of the most costly problems facing the dairy industry today. Reproductive disorders occur frequently in lactating dairy cows and can dramatically affect reproductive efficiency in a dairy herd. Some of the most common disorders include ovarian cysts, twinning, early embryonic loss and retained placenta. These are diverse disorders that are result in impaired reproductive function [1].

Cytogenetics applied to domestic animals was born with the discovery of (1/ 29) robertsonian translocation in Swedish cattle in 1964 especially when the deleterious effects on the fertility of carriers was demonstrated. It has expanded noticeably, covering not only the clinical aspects, but extending its interests also to evoluntionary, molecular, environmental and reproductive domains [2].

The role of chromosomal abnormalities as a cause of reproductive failure is very important and very often associated with infertility of carriers, early mortality of embryos and newborns, under development or degeneration of reproductive organs, poor semen quality and lower body mass increase in the offspring, as well as functional and more seldom phenotypic disturbances [3]. For this reason, it is necessary to keep breeding animals under cytogenetic control, particularly in cattle populations applying artificial inseminations, because the inherited aberrations can quickly become distributed in the next generations.

Alterations in chromosomes number and structure are the best known genetic based variations, which have direct effects on fertility and reproductive outcome in cattle [4]. Increased rates of chromatid breaks and gaps have been related to lowered fertility [5, 6]. High percentage of chromosomal aberrations were recorded in repeat breeder and anestrum animals [7].

A systematic survey of repeat breeder heifers has shown a high frequency of 1/29 translocation [8], mosaicism for various structural abnormalities of the

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chromosomes such as gaps and breaks [9, 10] and the highest rate of chromatid breaks, centric fusion of autosomal chromosomes and numerical aberrations were more frequent in repeat breeder females than fertile one [11].

The objectives of this study were establishing the karyotype of normal and abnormal Holstein Friesian cows suffering from some reproductive disorders (repeat breeder, anestrum, vaginal prolapse) and detecting the relationship between them and presence of some specific numerical and/or structural chromosomal aberrations.

MATERIALS AND METHODS

This work was conducted at the Genetics and Genetic Engineering Lab, Animal Wealth Development Department, Faculty of Veterinary Medicine, Zagazig University to investigate the effect of chromosomal abnormalities on the reproductive disturbance of cattle.

Animals Under Investigations: This investigation was carried out on 31 Holstein-Friesian cows from different private farms located at EI-Sharkia province. The age of cows ranged from 1-8 years old. Cows were maintained on standard ration correlated with their requirements. The cows were proved to be free from any detectable external, internal or blood parasites, brucellosis and tuberculosis.

The animals used were divided into twelve sexually clinically normal and non pregnant Holstein-Friesian cows as control group, eight cows suffering from repeat breeder, six cows suffering from anestrum and five cows suffering from frequently prolapsed vagina.

Chromosomal Preparation and Analysis: Under hygienic measures, 3 ml blood sample was collected from jugular vein of each animal into sterilized sodium heparinized vacutainer tube with special care in handling. The cultures were set up using RPMI media 1640 with L-glutamine, fetal calf serum, Penicillin Streptomycin and phytohaemagglutinin was added to stimulate mitotic activity of the lymphocytes and to facilitate the identification of chromosomes. The cultures were incubated for 72 hours at 38^{o}c the culture were harvested with 0.2 ml of 0.05% colchicine and hypotonic treatment (0.075 M KCl) and fixed in methanol: acetic acid (3:1). Air dried slide were prepared and stained in 10% Giemsa. About 50 metaphase spreads were screened to detect the chromosomal aberrations and prepare the karyotype [12].

Data Handling and Statistical Analysis: Data were analyzed using Chi- square procedure of the Statistical Analysis System package [13].

RESULTS AND DISCUSSION

Karyotypic Profile of Normal Fertile Cows (Control Group): The karyotypic profile of normal fertile Holstein Friesian cows under investigation showed that the diploid number of chromosomes is 2n = 60 chromosomes (29 pairs of acrocentric autosomes and one pair of submetacentric X chromosomes (Figure 1a and b).

Similar profile was observed by Sahaoo *et al.* [14]; Ibrahim [15] and Amaral [16].

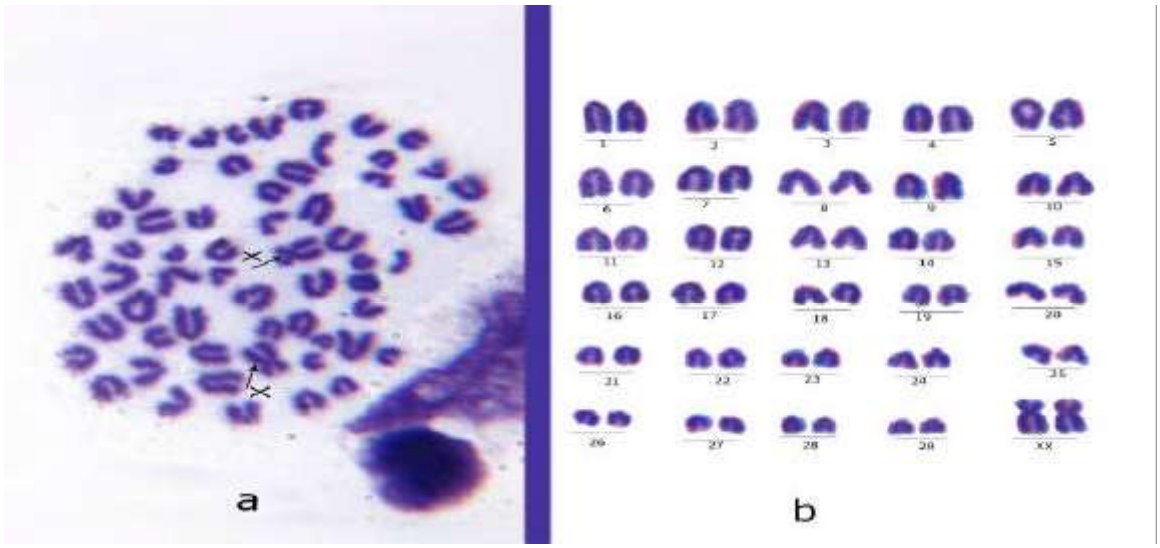

Fig. 1: Metaphases spread (a) and Karyotypic profile (b) of normal fertile Holstein-Friesian cow (2n= 60, XX).

Table 1: Total numerical and structural aberrations detected in different reproductive disorders:

Groups	No. of animal	M. No.	Numerical aberrations			Structural aberrations							Sex chromosomes aberrations		
			Poly	Aneu	Total	G	B	D	F	R	C.A.	Total	59, XO	60, XY	61, XXX
Control	12	600	6	16	22	8	14	21	4	2	9	58	3	0	1
% of aberrations			1	2.67	3.67	1.33	2.33	3.5	0.67	0.33	1.5	9.67	0.5	0	0.16
Repeat breeder	8	400	38	41	79	22	50	55	47	16	58	248	3	0	2
of aberrations%			9.5	10.45	19.95	5.5	12.5	13.75	11.75	4	14.5	62	0.75	0	0.5
X^2 cal			39.22**	24.28**	66.61**	12.92**	39.73**	34.46**	58.64**	16.2**	62.82**	307.05**	0.007ns	0ns	0ns
Anestrum	6	300	16	47	63	6	9	33	3	5	7	63	37	0	2
of aberrations%			5.33	15.67	21	2	3	11	1	1.6	2.33	21	12.33	0	0.06
X^2 cal			13.98**	49.94**	68.24**	0.22ns	0.13 ns	18.63**	0.01ns	3.04ns	0.39ns	21.11**	45.6**	0ns	.37ns0
Vaginal prolapse	5	250	14	20	34	17	20	26	6	1	30	100	1	0	0
% of aberrations			5.6	8	13.6	6.8	8	10.4	2.4	0.4	12	40	0.4	0	0
X^2 cal			14.31**	11.19**	26.71**	16.61**	13.32**	14.79**	3.19ns	0ns	42.17**	105.3**	0ns	0ns	0ns

M. No. = Metaphase number. Poly = Polyploidy. Aneu = Aneuploidy G = Gap. .

B = Break. D = Deletion. F = Fragment. C.A. = Centromeric Attenuation.

R = Ring chromosome X^2_{cal} Chi – square Value n.s. =Non-significant. ** =Highly-significant at ($p < 0.01$).

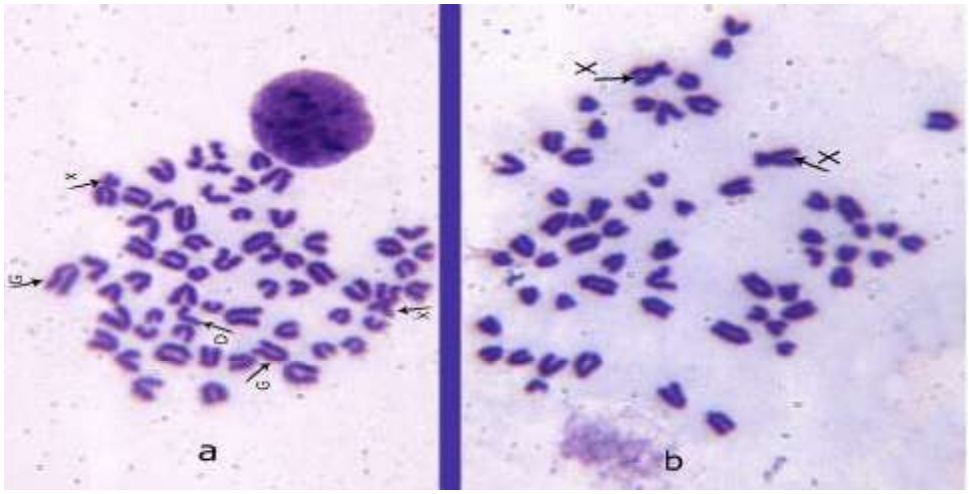

Fig. 2: Metaphases spread of normal fertile Holstein-Friesian cow showing (a): gap and deletion. (b): aneuploidy (2n -1 = 59, XX).

Chromosomal Aberrations Recorded in Control Group: The results of cytogenetic analysis of normal fertile cows were represented in table 1 which revealed that, the percent of total numerical aberrations was 3.67% including 1% polyploidy and 2.67% aneuploidy, while the percent of total structural aberrations was 9.67% including 1.33% gaps, 2.33% breaks, 3.5% deletions, 0.67% fragments, 0.33% ring chromosome and 1.5% for centromeric attenuations (Figure; 2). Regarding to the sex chromosomes aberrations, were manifested by 0.5% for monosomy –X (59, XO) and 0.16% for triosomy-X (61, XXX).

Structural changes of chromosomes resulted from the "breaking" of a part of the chromosome. When structural chromosome aberrations do not result in loss or gain of genetic material they are considered "genetically balanced" and not expressed in the phenotype of the carrier but genetically unbalanced structural chromosome aberrations resulted in serious problems [17].

Chromosomal Aberrations Recorded in Repeat Breeder Group: The percent of total numerical aberrations for repeat breeder group was 19.95% including 9.5% polyploidy and 10.45% aneuploidy, while the percent of total structural aberrations were predominant and recorded 62% including 5.5% gaps, 12.5% breaks, 13.75% deletions, 11.75% fragments, 4% ring chromosome and 14.5% centromeric attenuations (Table, 1) (Figures; 3 a, b, 4 a, b).

Both types of numerical aberrations recorded highly significant differences compared to normal group, moreover the total structural chromosomal aberrations showed highly significant differences than normal group. The sex chromosomes aberrations recorded no significant difference comparing to normal groups.

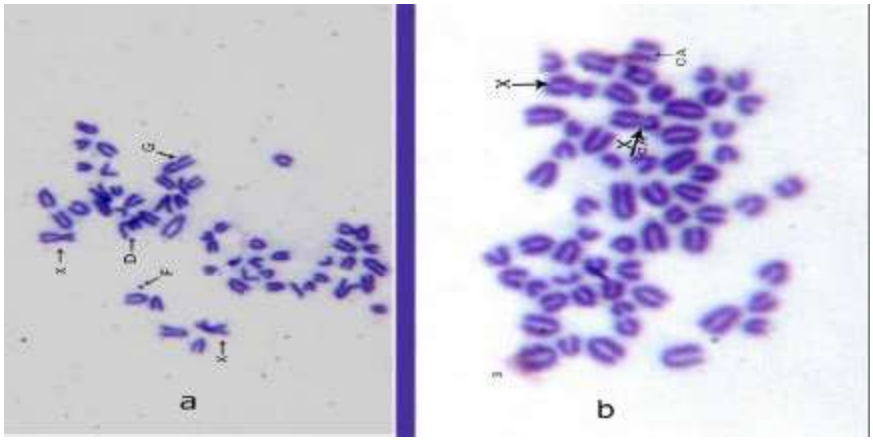

Fig. 3: Metaphases spread of Holstein-Friesian cow suffering from repeat breeder showing (a): deletion, fragment and gap (b): centromeric attenuation

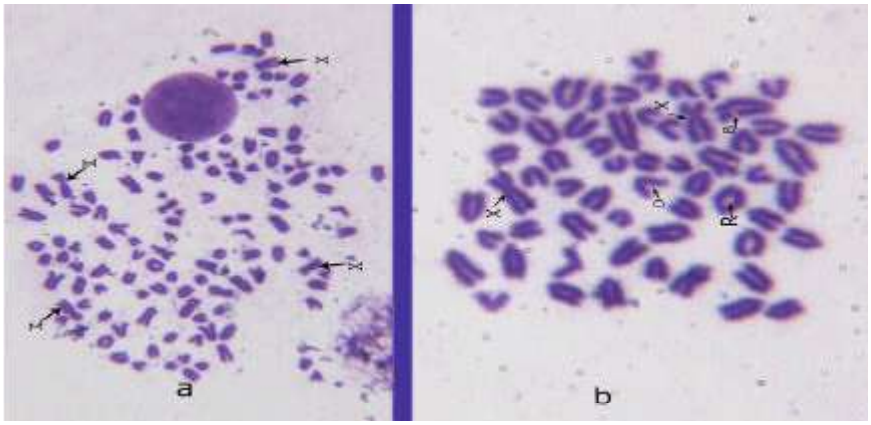

Fig. 4: Metaphase spread of Holstein-Friesian cow suffering from repeat breeder showing (a): polyploidy (4n=120, XXXX). (b): break, deletion and ring chromosome.

These results were agreed with those reported by *Maria and King* [4] as a highly significant difference for total numerical aberrations including polyploidy and aneuploidy between normal fertile 4.66% and repeat breeder group 18.9% and concluded that the numerical chromosomal aberrations associated with early embryonic death and return to service. Also, Maity *et al*. [18] recorded a highly significant percent for break 15% in cows suffering from repeat breeder and explained that gaps and breaks either in chromatid or chromosomes play an important role in fertility problems. These losses (gaps and breaks) of part of chromosome mean the missing of genes carried. Increasing their frequencies accompanied mostly by losses of genes responsible for fertility performance.

On the other hand these results disagree with Barik *et al.* [19] they stated that there were no chromosomal abnormalities were detected and chromosome length did not differ significantly between normal and repeat breeder cows.

Chromosomal Aberrations Recorded in Anestrum Group: Table 1 shows the results of cytogenetic analysis of cows suffering from anestrum (luteal cyst). The percent of numerical aberrations was recorded as 5.33% polyploidy and 15.67% aneuploidy (Figure; 5a) which results in 21% for total numerical aberrations. These results were significantly higher than observed for control group. The structural aberrations include 2% gaps, 3% breaks, 11% deletions, 1% fragments, 1.6% ring chromosome, 2.33% centromeric attenuations and 21% for total structural aberrations.

Deletions and total structural aberrations (Figure; 5 b) showed highly significant differences compared to normal group but there were no significant

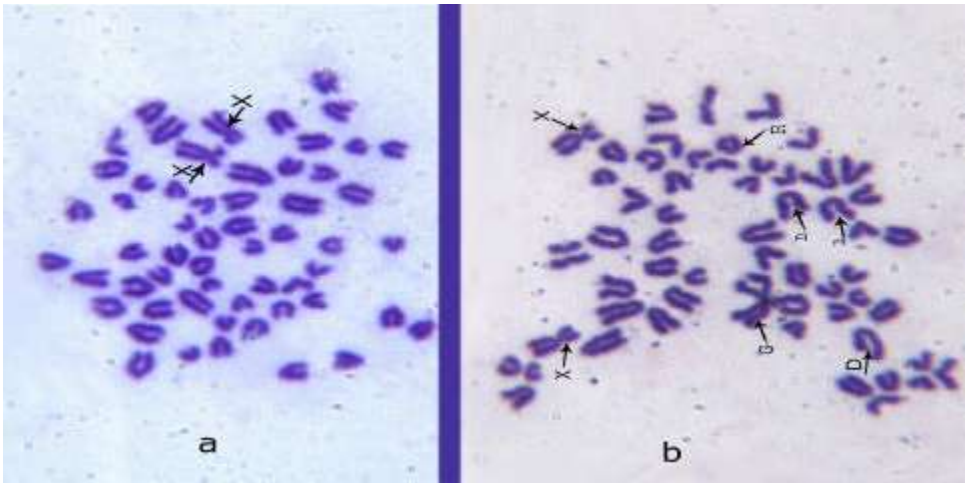

Fig. 5: Metaphases spread of Holstein-Friesian cow suffering from anestrum showing (a): aneuploidy (2n-3 = 57, XX) (b): deletions, gaps and ring chromosome.

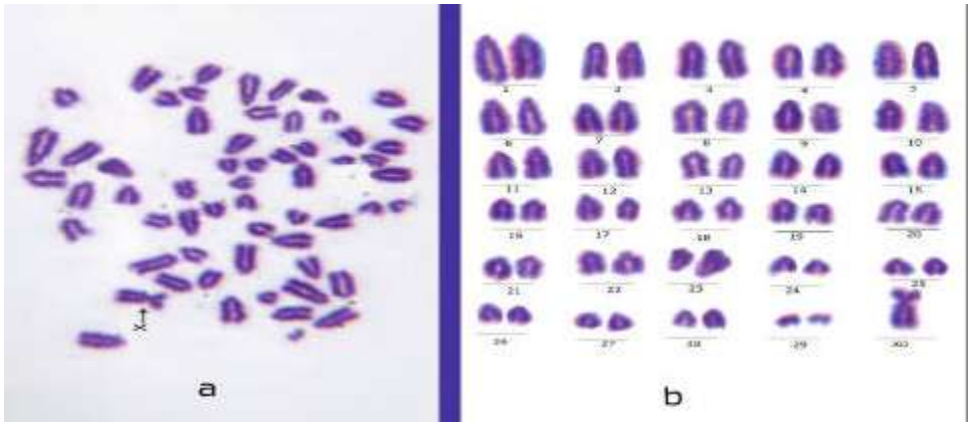

Fig. 6: (a) Metaphase spread of Holstein-Friesian cow suffering from anestrum showing aneuploidy (2n-1 =59, X0) (b): Karyotypic profile of Holstein-Friesian cow showing aneuploidy (2n-1 =59, X0)

differences between anestrum group and control group for centromeric attenuations, gaps, breaks, fragments and ring chromosome.

With respect to the sex chromosomes aberrations, there was a highly significant difference between monosomy-X (59, XO) for anestrum group 12.33% (Figure; 6a and b) and for control group 0.5%, while the rest of sex chromosomes aberrations recorded no significant difference comparing to normal groups.

The results of this study were nearly agreed with that obtained by *Ibrahim* [15]. He recorded a highly significant difference for total numerical aberrations between control 6.6% and anestrum group 25.71% which include 4.86% for polyploidy and 20.86 for aneuploidy, also recorded a highly significant difference between the control group 0.4% and anestrum group 6.57% for monosomy X. Aneuploidy arises as a result of non-disjunction of single chromosomes at cell division during the mitotic or meiotic events, the resulting primary products are trisomy (2n+1) and monosomy (2n -1). It has a controllable effect on fertility. Chromosomal abnormalities including sex chromosomes (monosomies or trisomies) or balanced structural rearrangements cause reproductive and health related problems of varying degree.

Also Hassanane *et al.* [20] illustrated that increased rates of chromatid breaks and gaps had been related to lowered fertility and anestrum in animals. Gaps and breaks mean the missing of genes carried by the deleted portion of chromosome. Increasing their frequencies accompanied mostly by losses of genes responsible for fertility performance.

On the other hand, these results disagree with results obtained by *Saleh* [21] who reported a highly significant percent for centromeric attenuations, gaps, breaks and fragments for anestrum group.

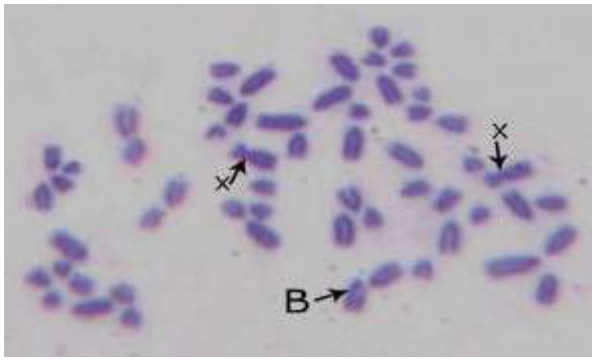

Fig. 7: Metaphases spread of Holstein-Friesian cow suffering from vaginal prolapse showing aneuploidy (2n-2 = 58, XX) and break.

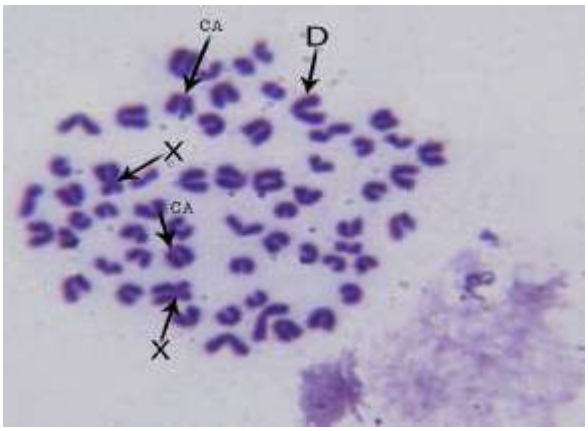

Fig. 8: Metaphases spread of Holstein-Friesian cow suffering from vaginal prolapse showing deletion and centromeric attenuation.

Chromosomal Aberrations Recorded in Vaginal Prolapse Group: Table 1 shows that the percent of total numerical aberrations for vaginal prolapse group was 13.6% consisting of 5.6% polyploidy and 8% aneuploidy, while the percent of total structural aberrations were 40% including 6.8% gaps, 8% breaks (Figure; 7), 10.4% deletions (Figure; 8), 2.4% fragments, 0.4% ring chromosome and 12% centromeric attenuations.

Numerical and structural changes on animal karyotype influenced on the reproductive performance and development of the animals, while numerical abnormalities usually have a visible effect; the structural abnormalities don't have visible effect and the carriers are phenotypically normal [22].

There were highly significant differences for all types of aberrations recorded except fragments and ring chromosome which showed no significant effect. The sex chromosomes aberrations recorded no significant difference in comparing to normal groups.

These results were nearly agreed with Saleh [21] as he obtained 10.75% for total numerical aberrations and 40.5% for total structural aberrations.

CONCLUSION

- Cytogenetic studies should be used as a diagnostic tool to determine the causes of low reproductive efficiency.
- The animals showing high levels of chromosomal aberrations should be culled from breeding programs.

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