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Reproductive problems and their association with chromosomal anomalies in Dairy cattle and buffaloes

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Abstract

Reproductive disorders in farm animals hinder running profitable farm activities as they affect reproduction efficiency and consequently herd production. These are found to be commonly associated with chromosomal anomalies, which may be either numerical or structural ones. In present study, the incidence of reproductive problems in Karan Fries (KF) and Sahiwal (SW) cattle and Murrah (MU) buffalo herd was studied in cows and heifers for five years (2009-2013) followed by cytogenetic screening of physically available 180 reproductively abnormal and control females. The results indicated that maximum reproductive problems in cows were prevalent in the MU buffalo breed (49.57%), followed by KF (46.01%) and SW (35.20%) breeds of cattle. Based on incidence record of last five-year, cytogenetic screening of reproductively problematic abnormal animals through conventional cytogenetics technique was performed. Study revealed three cases of numerical anomalies i.e., two cases of autosomal mosaicism (KF 7263 and MU 5321) and one case of monosomy i.e. XO condition. Structural anomaly such as autosomal translocation was recorded in animal # SW 1985 in 100% of metaphase plates. Significantly higher percentage of gaps and breaks were identified in abnormal animals compared to control. The statistical analysis showed that the F values were highly significant between abnormal and control group for X chromosomal fragility, chromatid break, chromosome break and gaps.

Keywords: Anoestrus, repeat breeding, abortion, mosaicism, translocation

Introduction

Reproduction is a complex physiological process, regardless the species, breed or sex of the individual. Reproduction in all the species and breeds of animals is affected by genetics or environment as such and can lead to interruption in reproductive processes at any stage in gametogenesis, during transportation of the gametes to the site of fertilization or during the fetal, perinatal or neonatal growth phases. Animal geneticists and veterinarians have advanced in animal cytogenetic studies to predict the potential role of genes in reproductive defects in domestic animals. This development stems from advances and recognition in human genetics over the last century, highlighting that many reproductive problems may have a genetic basis.

Hereditary factors play an important role in infertility or sub-fertility caused in animals. It has been well-recognized that numerical and structural chromosomal aberrations can lead to reproductive disturbances not only in mammals but also in higher plants (Ford, 1982)^[1]. An area where the potential of veterinary cytogenetics can be fully exploited is reproduction since an inherited aberration that affects reproductive efficiency can hinder the success achieved over the years in animal breeding.

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, freemartins, early embryonic loss and retained placenta. These are diverse disorders that result in impaired reproductive function Fricke *et al* (2007)^[2]. The role of chromosomal abnormality as a cause of impaired reproductive function is crucial and is often associated with infertility in the carrier, early mortality of embryos and newborns, underdevelopment and degeneration of reproductive organs, poor semen quality, and decreased weight gain in young, as well as functional and, more rarely, phenotypic disturbances Ioana (2007)^[3]. Dairy heifers rearing and management

requires substantial financial investment. However, many times heifers turn out infertile or sub-fertile, which lead to hindrance to profit in cattle and buffalo oriented dairy industry. Possible losses due to reproductive problems are in terms of cost of rearing heifer till puberty, their lifetime production and future output from calves.

The productivity of cattle largely depends on their reproductive performance. For this reason, keeping in mind that it is necessary to keep breeding animals under cytogenetic control, particularly in cattle and buffalo population, the study was designed and incidence of reproductive problems in five years on an organized farm was calculated and its association with chromosomal anomalies was drawn.

Material and Methods

A. Study of Incidence of reproductive problems

All the experimental procedures involving animals were approved by the Institutional Animal Ethics Committee (IAEC), National Dairy Research Institute, Karnal, India. Incidence of reproductive problems in Sahiwal (SW) and Karan Fries

A. Criteria for selection

Animals calved in a particular year were studied for incidence of reproductive problems in the same year. Animal record was searched and information gathered from the date of calving.

The incidence of post-partum anoestrus in cows which failed to return to oestrus more than 50 days postpartum in cattle and 90 days in buffalo was recorded.

Incidence of ovarian cyst either follicular or luteal cyst were recorded as reported in the daily AI register and year wise auction list of animals at Livestock Research Station (LRS) in cows.

Incidence of delayed puberty was recorded in heifers that did not exhibit oestrus behaviour after 30 months in cattle and 35 months in buffaloes.

Incidence recording for repeat breeder syndrome was based on the number of services after calving. Those cows and heifers failed to conceive after 4 AI were recorded as repeat breeders in the herd.

In order to compare the problems a control group was selected by different criteria. It comprised of each breed with normal calving in the same year, without the history of anoestrus, abortion and irregular oestrus cycle. Control females for postpartum anoestrus were contemporaries that conceived in 50 and 90 days of the postpartum period in cattle and buffalo, respectively. In case of repeat breeder incidence study, control animals were contemporaries who conceived in less ≤ 4 AI. A control group for delayed puberty in heifers were animals that attend puberty before 30 months in the case of cattle and before 35 days in case of buffaloes

B. Cytogenetic studies

The animals for the proposed investigation were selected as abnormal (experimental) and normal (control) groups belonging to two species of dairy animals, viz., cattle and buffaloes. In total about 50 problematic animals were identified from each breed of cattle and buffalo i.e. Sahiwal (SW), Karan Fries (KF) and Murrah (MU), respectively. Similarly from each

breed of cattle and buffalo normal (control) animals (10 per breed) were chosen at random from the existing herd. The criterion for selection was the females with a record of three successive normal calving and completion of lactations each of 305 days or more. Thus, a total of 180 animals were investigated cytogenetically for different numerical and chromosomal anomalies.

In general, well-spread 50 Giemsa-stained metaphase plates were screened for each animal; however, in the case of some anomaly, 100 or more metaphases were examined. Slides were further subjected to C- and R- Banding methods for the identification of abnormal chromosomes.

Chromosome preparations: Blood sample of about 10 ml under strict aseptic care was collected in vacutainer tubes. Blood was brought to the laboratory and was stored in the refrigerator at 2-8 °C till used (1-3 days). Whole blood was used for setting up cultures according to the method described [Yadav and Balakrishnan \(1985\)](#) ^[4]. An aliquot of 0.5 ml blood was added to each culture glass bottle of 30 ml capacity containing 6 ml medium consisting of RPMI 1640, bovine foetal calf serum (10%), phytohaemagglutinin (10 µg/ml) and penicillin streptomycin (20 IU/ml). The cultures were incubated for 96 hours at 37.5±0.5 °C. Seven hours before harvesting of cultured cells, Bromo-deoxy-uridine (BrdU, 20 mg/ml) was added to each culture bottle. Subsequently, 45 minutes before harvesting of cultured lymphocytes drops (10 µg/ml) of colchicine solution was added to each culture bottle. Cultures were harvested with the routine method of hypotonic solution treatment and fixation with methanol : glacial acetic acid (3:1). Chromosome preparations were made on glass slides using the air-dried method.

Slides were stained with Giemsa (2% at pH 6.8) dye for conventional chromosome examination. Evaluation of a total of 50 metaphase spreads in each animal was done. To identify the anomaly slides were subjected to obtain R-banding with RB-FPG technique [Mukherjee and Yadav \(2004\)](#) ^[5] and C-banding with BSG method [Sumner \(1972\)](#) ^[6]. Details of band patterns of autosomes and X chromosome were compared and matched with standard karyotype to identify chromosome involved in mosaicism.

C. Statistical analysis

Statistical analysis using SAS 9.2 software was done for analysis of data obtained through reproduction records. Frequency of gaps, chromatid break, chromosomal break and X chromosome fragility was identified in 50 metaphase plates per animal in 50 experimental and 10 control animals, of the SW and KF cattle breed and MU buffalo breed. Among 2500 metaphase plates examined in 50 animals of each breed overall frequency of chromatid and chromosome break was determined. Per cent distribution of fragile sites in SW, HF and MU breed abnormal and normal groups was calculated. Mean values and standard deviations of chromosome abnormalities in cells of abnormal and control groups of each breed were estimated and the F test between abnormal and control was applied to determine significance using F values of the observed difference in the mean rate of X chromosome fragility, chromatid break/cell, chromosome break/cell and gap/cell within abnormal and control group comparison in each breed.

Results

Table 1: Incidence of anoestrus, repeat breeding and abortions during 2009-2013 in KF cows

Year	TBC	AN	AN%	RB	RB%	Both	Both%	AB	AB%	TI	TI%
2009	183	25	13.66	26	14.21	5	2.73	23	12.57	79	43.17
2010	159	26	16.35	17	10.69	2	1.26	24	15.09	69	43.40
2011	136	25	18.38	13	9.56	3	2.21	18	13.24	59	43.38
2012	150	39	26.00	25	16.67	1	0.67	15	10.00	80	53.33
2013	87	36	41.38	2	2.30	0	0.00	4	4.60	42	48.28
Overall	715	151	21.12	83	11.61	11	1.54	84	11.75	329	46.01

Table 2: Incidence of delayed maturity, repeat breeding and abortions during 2009-2013 in KF heifers

Year	TBH	DM	DM%	RB	RB%	AB	AB%	TI	TI%
2009	60	4	6.67	10	16.67	5	8.33	19	31.67
2010	54	6	11.11	3	5.56	8	14.81	17	31.48
2011	50	5	10.00	1	2.00	10	20.00	16	32.00
2012	57	2	3.51	6	10.53	3	5.26	11	19.30
2013	26	2	7.69	0	0.00	1	3.85	3	11.54
Overall	247	19	7.69	20	8.10	27	10.93	66	26.72

Table 3: Incidence of anoestrus, repeat breeding and abortions from 2009-2013 in Murrah cows

Year	TBC	AN	AN %	RB	RB %	Both	Both %	AB	AB %	TI	TI %
2009	103	31	30.10	12	11.65	1	0.97	10	9.71	54	52.43
2010	100	25	25.00	24	24.00	3	3.00	14	14.0	66	66.00
2011	73	18	24.66	5	6.85	0	0.00	5	6.85	28	38.36
2012	103	20	19.42	6	5.83	0	0.00	4	3.88	30	29.13
2013	85	48	56.47	1	1.18	0	0.00	3	3.53	52	61.18
Overall	464	142	30.60	48	10.34	4	0.86	36	7.76	230	49.57

Table 4: Incidence of anoestrus, repeat breeding and abortions from 2009-2013 in Murrah heifers

Year	TBH	DM	DM%	RB	RB%	AB	%AB	Total case	Total inc%
2009	21	6	42.86	4	28.57	4	28.57	14	66.67
2010	17	6	50.00	4	33.33	2	16.67	12	70.59
2011	40	8	57.14	1	7.14	5	35.71	14	35.00
2012	48	5	71.43	1	14.29	1	14.29	7	14.58
2013	33	4	50.00	3	37.50	1	12.50	8	24.24
Overall	159	29	18.24	13	8.18	13	8.18	55	34.59

Table 5: Mean values and standard deviations of chromosome abnormalities in abnormal and control groups cells of KF breed

S.No.	Group	X chromosome fragilityMean ± SD	Chromatid breaksMean ± SD	Chromosome breakMean ± SD	GapsMean ± SD
1	Abnormal	0.62 ± 0.85	7.12 ± 4.03	3.86 ± 3.42	1.86 ± 2.33
2.	Control	0.2 ± 0.13	0.9 ± 0.27	0.5 ± 0.16	0.4 ± 0.16

Table 6: Mean values and standard deviations of chromosome abnormalities in abnormal and control groups cells of Murrah breed

S.No.	Group	X chromosome fragilityMean ± SD	Chromatid breaksMean ± SD	Chromosome breakMean ± SD	GapsMean ± SD
1	Abnormal	10.54 ± 4.78	7.6 ± 3.14	3.6 ± 1.95	2.04 ± 1.80
2.	Control	2.5 ± 0.84	1.4 ± 0.51	0.6 ± 0.51	0.4 ± 0.51

Table 7: F values of the observed difference in the mean rate of X chromosome fragility, chromatid break/cell, chromosome break/cell and gap/cell within abnormal and control group comparison in KF breed

S. No.	Parameter	F
1	X chromosome fragility/cell	4.10*
2	Chromatid break/cell	21.28*
3	Chromosome break/cell	42.17*
4	Gaps/cell	20.51*

P<0.001

Table 8: F values of the observed difference in the mean rate of X chromosome fragility, chromatid break/cell, chromosome break/cell and gap/cell within abnormal and control group comparison in Murrah breed

S. No.	Parameter	F
1	X chromosome fragility/cell	31.72*
2	Chromatid break/cell	37.19*
3	Chromosome break/cell	14.23*
4	Gaps/cell	12.23*

$P < 0.001$

A. Incidence of reproductive problems in sahiwal and kf breed of cattle and MURRAH buffalo

The scrutiny of records of adult cows for 5 years (2009-2013) showed major reproductive problems such as anoestrus, repeat breeding and abortions in cows and heifers; however, in heifers delayed puberty was recorded instead of anoestrus.

The overall incidence of reproductive problems in SW and KF cows and MU buffalo cows during the last five years was 35.20%, 46.01% and 49.57% respectively (Table 1, 3 and 5). The overall incidence of anoestrus, repeat breeding and abortions, in Sahiwal cows were

Table 9: Incidence of anoestrus, repeat breeding and abortions during 2009-2013 in Sahiwal cows

Year	TBC	AN	AN %	RB	RB %	Both	Both %	AB	AB %	TI	TI %
2009	113	13	11.50	15	13.27	5	4.42	9	7.96	32	28.32
2010	112	21	18.75	22	19.64	5	4.46	10	8.93	38	33.93
2011	114	17	14.91	19	16.67	2	1.75	9	7.89	40	35.09
2012	72	8	11.11	6	8.33	0	0.00	11	15.28	22	30.56
2013	89	39	43.82	1	1.12	0	0.00	4	4.49	44	49.44
Overall	500	98	19.60	63	12.60	12	2.40	43	8.60	176	35.20

19.60%, 12.60% and 8.60% respectively, in KF were 21.12, 11.61 and 11.75%, respectively and in Murrah were 30.60%, 10.34% and 7.76%, respectively as shown in table 1, and 5. In case of heifers overall incidence in SW cattle, KF cattle and MU buffalo was 21.96%, 26.72% and 34.59%. The incidence of

delayed maturity (DM), repeat breeding and abortions in SW cattle heifers was 10.75%, 6.07% and 5.14%, respectively, in KF cattle heifers was 7.69%, 8.10% and 10.93%, respectively, in MU buffalo heifers was 18.24%, 8.18% and 8.18%, respectively (Table 2, and 6).

Table 10: Incidence of delayed maturity, repeat breeding and abortions during 2009-2013 in Sahiwal heifers

Year	TBH	DM	DM%	RB	RB%	AB	AB%	TI	TI%
2009	44	7	15.91	3	6.82	2	4.55	12	27.27
2010	31	3	9.68	4	12.90	2	6.45	9	29.03
2011	38	7	18.42	4	10.53	2	5.26	13	34.21
2012	50	4	8.00	1	2.00	1	2.00	6	12.00
2013	51	2	3.92	1	1.96	4	7.84	7	13.73
Overall	214	23	10.75	13	6.07	11	5.14	47	21.96

B. Cytogenetic analysis

B.1 Numerical chromosome anomalies in SW and KF cattle breeds and mu buffalo breed

Among the 50 KF problematic female cattle examined, all animals except KF 7263 revealed constitution of 29 pairs of acrocentric autosomes and a pair of submetacentric sex chromosomes (XX). Cytogenetic screening of heifer # KF 7263 revealed case of autosomal mosaicism as shown in fig. 1-4. Heifer had two cell lines, with cells carrying $2n=60$ and $2n=61$ chromosomes which was identified during routine screening of Giemsa stained slides (Fig 1-2). The cells with $2n=61$ has additional autosome 16 responsible for trisomy and leading to mosaicism condition which was identified using R banding technique (Fig 3-4). The cell percentage with 60, XX and 61, XX+16A cells was 83.5% cells and 16.5% cells, respectively. Cytogenetic screening of problematic 50 Murrah females showed that 48 females had chromosome complement similar to control (normal) with 50, XX. The other two animals showed deviation from the standards karyotype. Among this one female carried autosomal mosaicism (Fig 5-7), while other had 49, XO condition (Fig 8-9).

Among initial 50 metaphase plate screened in # MU 5321 during routine giemsa staining technique the overall occurrence

of different types of complements with 49, 50 and 51 chromosomes was 16.7, 76.7 and 6.6 per cent, respectively. Further, cytogenetic screening using R banding technique showed that the mosaicism was due to with or absence of autosome 11 in different cells consisting of 49, 50 and 51 chromosome number and thus had a chromosome configuration as 49, XX (-11) / 50, XX / 51, XX (+11) in cells with 49, 50 and 51 chromosome number, respectively.

Animal # Mu 6087, 3.5 years old heifer when screened cytogenetically with no observed oestrus behaviour showed XO condition with presence of 10 pairs of submetacentric autosomes, 38 acrocentric autosomes and one large acrocentric X chromosome in all the metaphase plates examined as depicted in figure 8. The heterochromatin specific C- banding technique used to identify autosomes and sex chromosomes showed one X chromosomes with prominent typical triangular centromeric band along with a thin intercalary band specific to buffalo (Figure 9) confirming the XO condition in buffalo heifer.

In the control group of animals no numerical anomaly was observed. Karyotype prepared for normal animals consisted of 29 pairs of acrocentric autosomes and one pair of submetacentric sex chromosomes in cattle. In buffalo karyotype comprised of pairs [1-5] of submetacentric autosomes, rest 20

pairs were acrocentric with sex chromosomes being longest one among them.

B.2 Structural chromosome anomalies in SW and KF cattle breeds and MU buffalo breed

Among 2500 metaphase plates examined each in 50 SW and KF cattle breed and 50 MU buffalo breeds, frequency of chromatid

and chromosome break was 355 and 217, 356 and 193 and 380 and 180, respectively in SW, KF and MU. While X chromosome fragility and gaps were 18 and 78, 31 and 93 and 527 and 102, respectively in SW, KF cattle and MU buffalo cows (Table 7 and Fig. 13-14). The percentage of structural anomalies in abnormal and normal group ranged from 27-50% and 4-10%, respectively.

Table 11: Per cent distribution of fragile sites in Sahiwal, HF and Murrah breed abnormal group

Breed	Structural anomalies										
	TCE	XF	XF(%)	CB	CB(%)	CHB	CHB(%)	Gap	Gap%	TAC	TAC(%)
SW	2500	18	0.72	355	14.2	217	8.68	78	3.12	668	26.72
KF	2500	31	1.24	356	14.24	193	7.72	93	3.72	669	26.76
MU	2500	527	21.08	380	15.2	180	7.2	102	4.08	1189	47.56

TCE= Total cells examined; CB = Chromatid break; CHB = Chromosome break TAC = Total abnormal cells

The percent of total structural aberration in SW, KF and MU breeds was 26.72, 26.76 and 47.56, respectively as shown in table 7. The percent of structural anomalies in SW, KF and MU control group was 4.6, 4.0 and 9.8 as shown in table 8. In SW breed abnormal animals out of the total 26.72 % abnormal cells percent of X chromosome fragility, Chromatid break, chromosome break and gaps were 0.72%, 14.2%, 8.68% and 3.12%, respectively. KF breed showed similar per cent of

abnormal cells, i.e., 26.76 consisting of 1.24% X chromosome fragility, 14.24% chromatid break, 7.72% chromosome breaks and 3.72% gaps. While, in MU breed out of total 47.56% distribution was as 21.08% X chromosome fragility, 15.2% chromatid break, 7.2% chromosome break and 4.08% gaps. Chromosome break percentage was higher in SW and KF while in case of MU buffalo X chromosome fragility was highest.

Table 12: Per cent distribution of fragile sites in Sahiwal, HF and Murrah breed normal group

Breed	Structural anomalies										
	TCE	XF	XF(%)	CB	CB(%)	CHB	CHB(%)	Gap	Gap%	TAC	TAC(%)
SW	500	2	0.4	15	3	6	1.2	1	0.2	24	4.6
KF	500	2	0.4	9	1.8	5	1.00	4	0.8	20	4.0
MU	500	25	5.00	14	2.8	6	1.2	4	0.8	49	9.8

TCE= Total cells examined; CB = Chromatid break; CHB = Chromosome break TAC = Total abnormal cells

Mean values and standard deviations of chromosome abnormalities in control and abnormal group for SW and KF cattle breed and MU buffalo is shown in table 9-11 which were

higher in abnormal compared to control in all the 3 breeds of cattle and buffalo.

Table 13: Mean values and standard deviations of chromosome abnormalities in abnormal and control groups cells of Sahiwal breed

S. No.	Group	X chromosome fragilityMean $\pm$ SD	Chromatid breaksMean $\pm$ SD	Chromosome breakMean $\pm$ SD	GapsMean $\pm$ SD
1	Abnormal	0.36 $\pm$ 0.98	7.1 $\pm$ 3.18	4.34 $\pm$ 2.73	1.56 $\pm$ 2.49
2.	Control	0.2 $\pm$ 0.42	1.5 $\pm$ 0.7	0.6 $\pm$ 0.7	0.1 $\pm$ 0.32

During incidence study of reproductive problems in the herd one animal (# SW 1985) came to attention because of its inefficient reproductive performance and was subjected to cytogenetic study. Study concluded presence of translocation which was inherited from parents as revealed on study of pedigree record. This was a case of delayed maturity since animal attained maturity after 1485 days (>4 yrs). Animal was morphologically normal, gynaecological examination also revealed normal, well developed genital tract. After first AI animal was inseminated 7 times consecutively and got pregnant. Animal calved a male calf on 26.11.2013, however, it was disposed young and was not available for karyotyping. Cytogenetic screening of giemsa stained 100 metaphase plates was done and karyotypes were prepared which confirmed presence of translocation in 100% plates. The heterochromatin specific CBG banding technique used to identify autosomes and sex chromosomes showed two submetacentric chromosomes of equal length in which centromeric band was absent. This observation debarred the possibility of occurrence of balanced reciprocal X-autosomal translocations. Further, R banding was

done and chromosomes were matched and arranged according to standard cattle karyotype (Fig. 10- 12).

Discussion

The incidence of reproductive problems in cows and heifers of each breed cattle and buffalo were recorded for five years. SW cows, showed the highest incidence of anoestrus (19.6%), followed by repeat breeding (12.6%) and abortions (8.40%). KF breed showed a trend as the highest incidence of anoestrus (21.12%), followed by abortions (11.75%) and repeat breeding (11.61%). MU buffalo cows reported highest incidence of anoestrus (31.46%), followed by repeat breeding. Sarder *et al* (2010) [7] reported 20.4% incidence of anestrus in cattle followed by repeat breeding (12.8%); Serur *et al* (1982) [8] reported the higher prevalence of anestrus (49.9%) followed by repeat breeding (15.1%). However, the highest incidence of repeat breeding was shown in several studies. Khair *et al* (2013) [9] in cattle showed highest cumulative incidence of repeat breeding and lowest incidence of abortions. Rabbani *et al*.

(2010)^[10] reported 15.69% repeat breeding followed by 9.74 % anoestrus and 5.99% abortion prevalence in buffaloes.

El-Bayomi *et al.* (2011)^[11] showed that the percent of total numerical aberrations for repeat breeder group was 19.95%, for vaginal prolapse group was 13.6% and for cows suffering from anoestrus was recorded as 21%. These results were significantly higher than that observed in the control group.

Sex chromosome mosaicism such as 60, XY / 61, XYY in cattle was reported by Krumrych *et al.* (2002)^[12], 59, XO/60, XX/61, XXX mixoploid in Pinzgauer x Angus heifer and 59, XO/ 60, XX/ 61, XO (with iso-X) mixoploid in Charolais was reported by Swartz and Vogt (1983)^[13]. Another animal MU 6087 had 49, XO condition known as Turner's syndrome. Yadav and Balakrishnan (1982)^[14] reported aneuploidies such as X chromosome trisomy in a ten year old buffalo and X chromosome monosomy (Yadav *et al.*, 1990; Prakash *et al.*, 1992; Iannuzzi *et al.*, 2000)^[15, 16, 17] have been reported in farm animals.

Structural chromosome anomalies in SW and KF cattle breeds and MU buffalo breed

Structural anomalies with an increased percent were reported. El-Bayomi *et al.* (2011)^[11] showed that the percent of total structural aberrations for repeat breeder group were 62% and vaginal prolapse it was 40% which were higher in comparison to control group as shown in present study.

Fragile sites are specific chromosome regions, which appear as nonstaining gaps or breaks in one or both chromatids, not in all metaphase spreads. The chromosome fragility on the autosomes and X chromosome (BTAX) has been associated with reproductive abnormalities, bald calf syndrome, and dwarfism (Lambi and Postiglioni, 1996)^[18]. The present study identified the higher frequency of gaps and breaks in the abnormal group (consisting of anoestrus, repeat breeding, and abortion cases) compared to control animals (Table 7 and 8). Farag *et al.* (1999)^[19] studied chromosomal fragility in different groups of reduced fertility, such as anoestrus and repeat breeding, other groups comprising uterine prolapse, heterosexual and isosexual twins which showed that the structural and numerical aberrations of the chromosomes were more frequent in abnormal females of cattle and buffaloes than the control groups. In cattle, the females with cystic ovary had the highest percentages of the chromosomal gaps and deletions (in autosomes and sex chromosomes), whereas the repeat breeder cases had the highest rates of chromatid breaks and centric fusions (in autosomal chromosomes). In buffaloes, the repeat breeder females had the highest proportion of the chromosomal gaps (especially in autosomal chromosomes).

The F values for X chromosome fragility, chromatid break, chromosome breaks and gaps were statistically significant ($P < 0.001$) between control and abnormal group as shown in table 12, 13 and 14. These findings are in accordance with El Bayomi *et al.* (2011)^[11] who reported percent of total structural aberrations were predominant and reached 62%, these results were significantly higher than observed for control group of repeat breeding animals. Saleh (2008)^[20] reported a highly

significant percent of gaps, breaks and fragments for anoestrus group. Farag *et al.* (1999)^[19] showed that the structural and numerical aberrations of the chromosomes were more frequent in abnormal females of cattle and buffaloes than the control groups. They reported that in cattle, the females with cystic ovary(ies) had the highest percentages of the chromosomal gaps and deletions (in autosomes and sex chromosomes), whereas the repeat breeder cases had the highest rates of chromatid breaks and centric fusions (in autosomal chromosomes). In buffaloes, the repeat breeder females had the highest proportion of the chromosomal gaps (especially in autosomes). Maity *et al.* (1996)^[21] recorded a highly significant percent for breaks 15% in cows suffering from repeat breeding and explained that gaps and breaks either in chromatid or chromosomes play an important role in fertility problems. The reproductively abnormal animals with higher percentage of chromosomal anomalies may be due to losses of part of chromosomes carrying genes responsible for reproduction, increasing their frequencies accompanied mostly by losses of genes responsible for fertility performance. Hassanane *et al.* (1995)^[22] reported that chromatid gaps and breaks are related to anoestrus with reduced fertility in animals. De Hondt *et al.* (1988)^[23] and El Nahass *et al.* (1974)^[24] found high percentages of cells with gaps or secondary constriction in infertile cattle.

Table 14: F values of the observed difference in the mean rate of X chromosome fragility, chromatid break/cell, chromosome break/cell and gap/cell within abnormal and control group comparison in Sahiwal breed

S. No.	Parameter	F
1	X chromosome fragility/cell	5.45*
2	Chromatid break/cell	20.26*
3	Chromosome break/cell	15.24*
4	Gaps/cell	62.11*

Apart from chromatids gaps and breaks other structural anomalies were also reported in reproductively abnormal animals. Sahiwal cattle (#1985) showed presence of autosomal translocation as shown in fig.10-12. Gustavsson *et al.* (1968)^[25] reported case of X autosome translocation *i.e.*, reciprocal translocation to be completely sterile while almost uninterrupted fertility was noted, initially, in an X-autosome translocation carrier cow identified in the United States (Eldridge, 1985)^[26]. Basrur *et al.* (2001b)^[27] reported X-AT carriers resulted in high rates of return to oestrus between days 28 and 60, abortions between days 121 and 235 after insemination; similar findings were witnessed in the present case. SW 1985 was a repeat breeder with return to oestrus in 30-45 days after insemination, which indicated resorption of post-fertilization zygotes or early embryos (Gustavsson, 1980; Popescu, 1990)^[28, 29]. The problematic female in the present study was morphologically and gynaecologically normal as revealed on per rectum examination, similar findings were reported by Di meo *et al.* (2011)^[30] in a five year old buffalo having centric fusion translocation. The female carrier had normal body conformation and internal sex adducts.



Fig 1: Giemsa Stained metaphase spread (left) and karyotype (right) of KF 7263 cells carrying 2n=60 chromosome

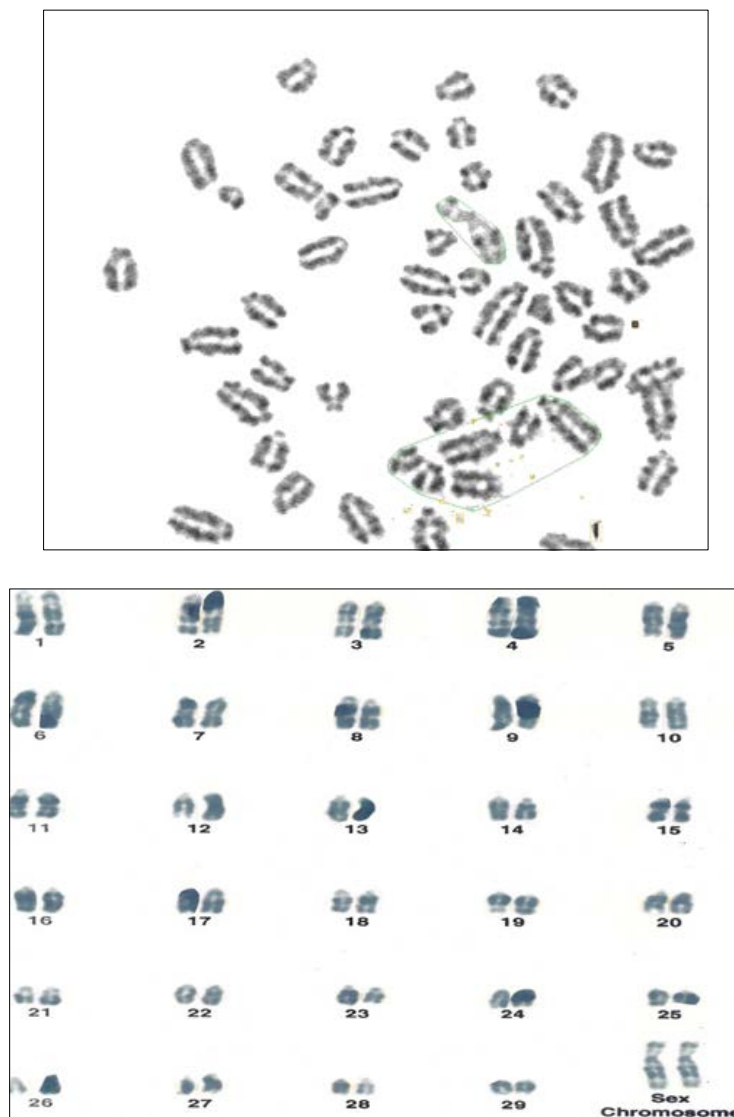


Fig 3 A: R- banded metaphase spread with 60, XX chromosomes

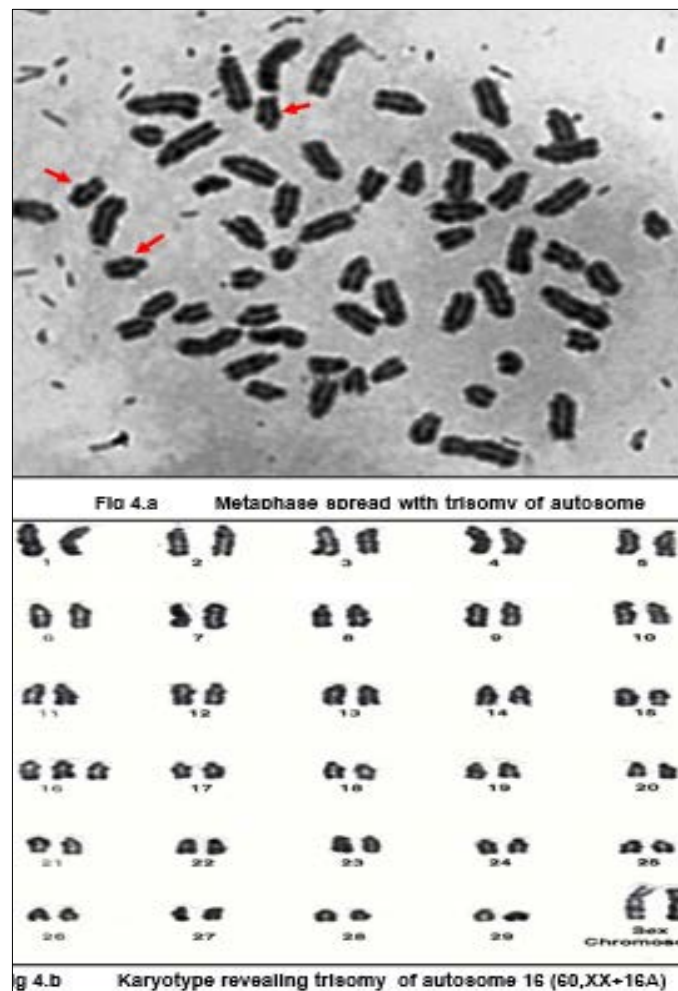


Fig 4: R- Banded metaphase spread and karyotype of KF 7263 carrying supernumerary chromosome

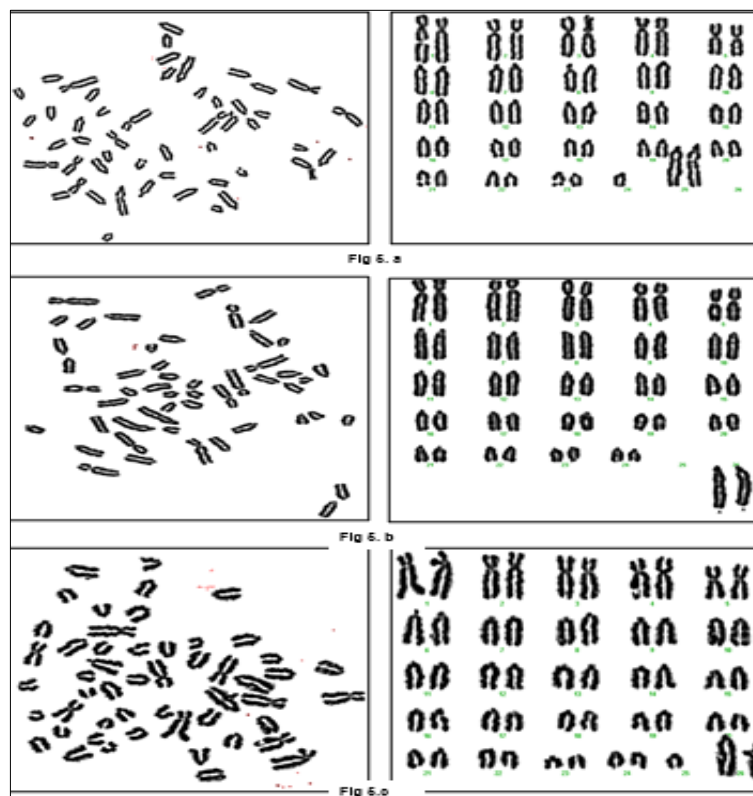


Fig 5: Giemsa stained metaphase spread and karyotype of buffalo cells carrying (a) 49, XX (b) 50, XX (c) 51, XX

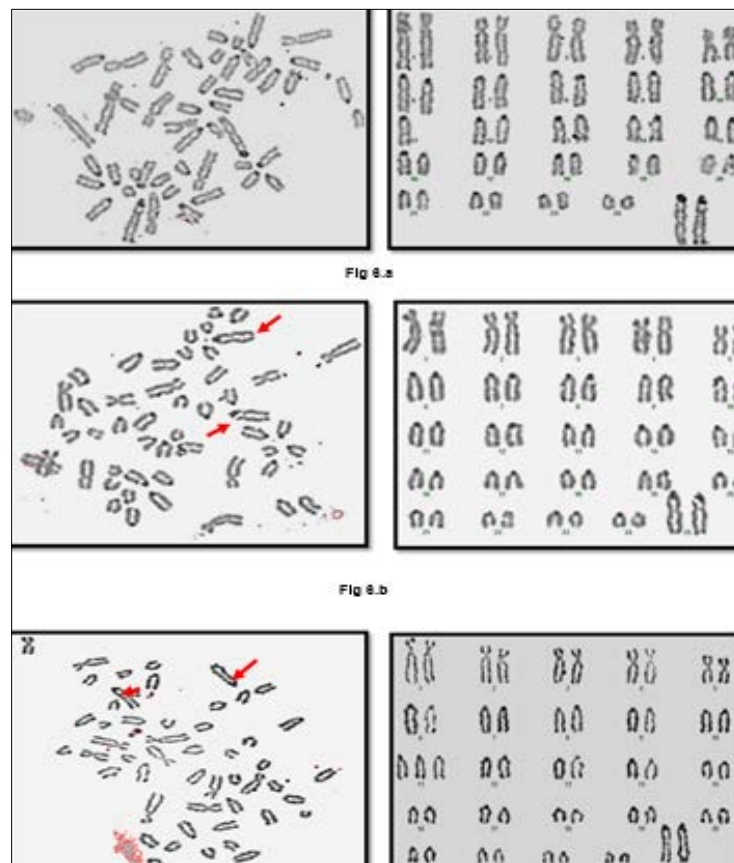


Fig 6: C- banded metaphase spread and karyotype of buffalo carrying (a) 49, XX (b) 50, XX (c) 51, XX+11. Note that arrows point out normal sex chromosomes

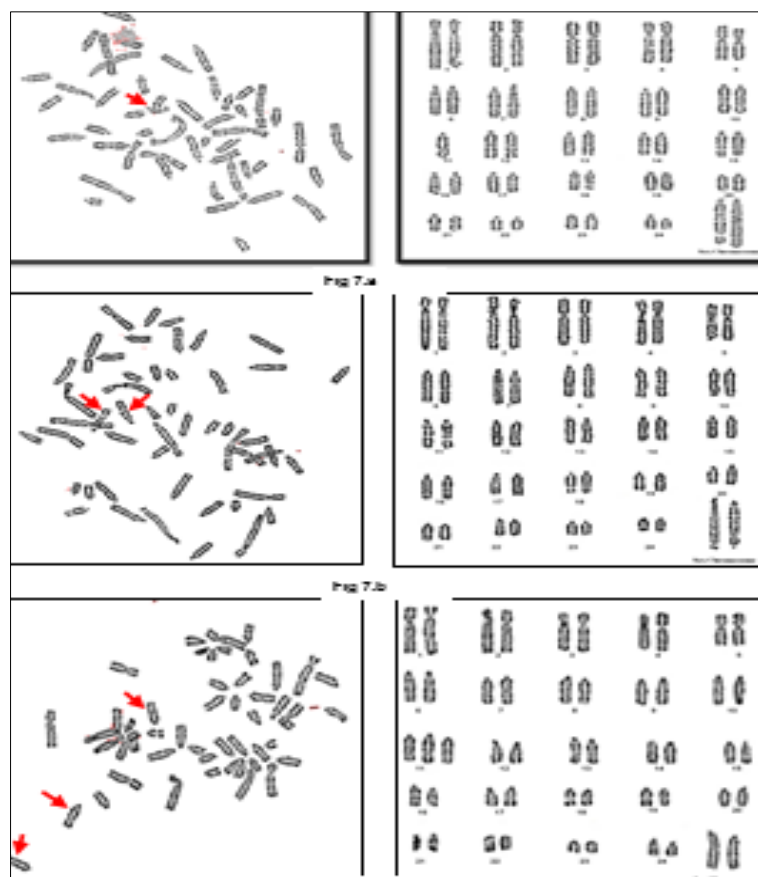


Fig 7: R- banded karyotype and metaphase spread of buffalo carrying (a) 49, XX (b) 50, XX (c) 51, XX+11. Note that arrows point out extra supernumerary chromosome 11

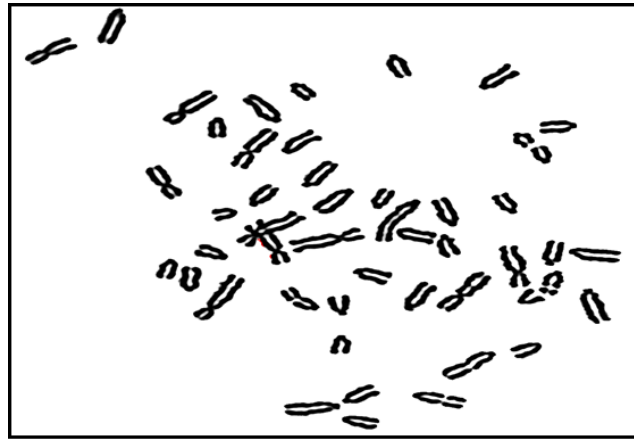


Fig 8: A Metaphase spread of female with 49, XO condition Xcondition

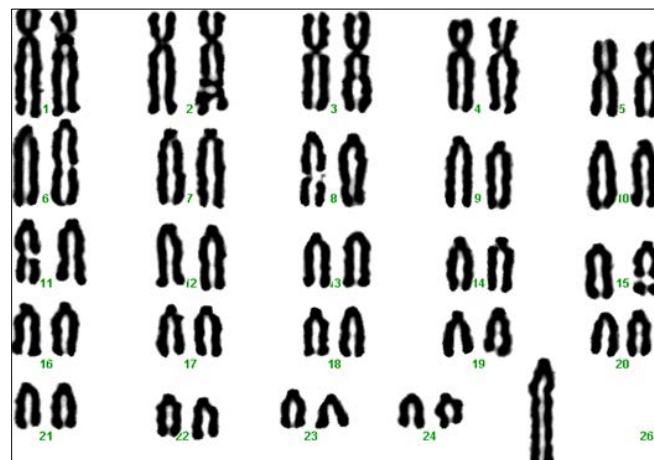


Fig 8: B Karyotype of female with 49, XO condition

Fig 8: Giemsa stained metaphase spread and karyotype of MU 6087 with 49, XO configuration



Fig 9: A Metaphase spread of female with 49, XO condition

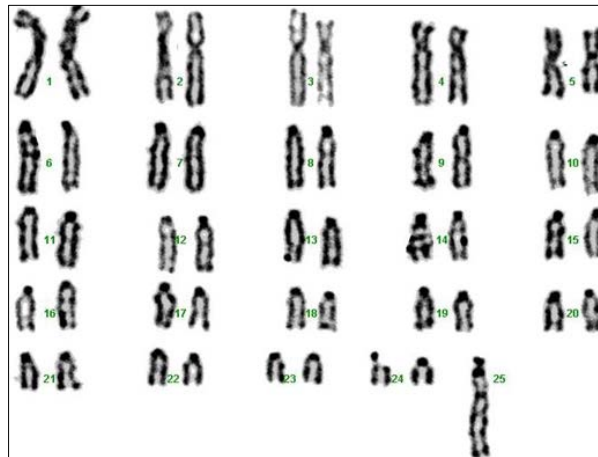


Fig 9: B Karyotype of female with 49, XO condition

Fig 9: C- banded metaphase spread and karyotype of MU 6087 with 49, XO configuration

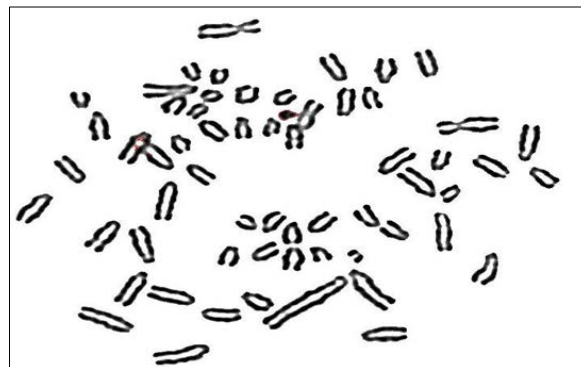


Fig 10: A Metaphase spread revealing translocation in SW 1985 cattle

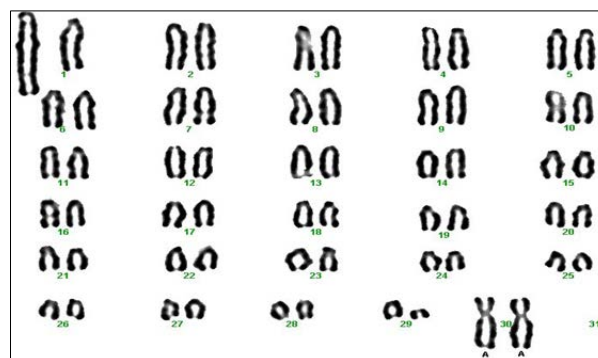


Fig 10: B Karyotype revealing translocation in SW 1985 cattle

Fig 10: Giemsa stained metaphase spread and karyotype of SW 1985 cattle showing translocation of autosome

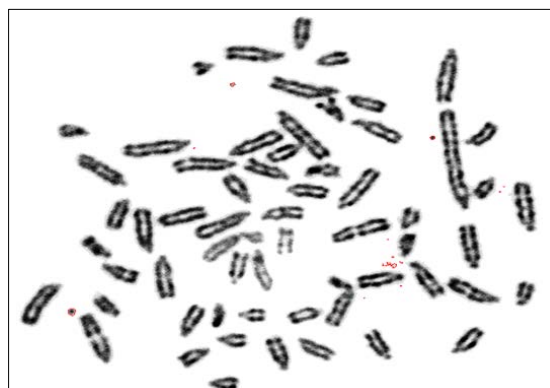


Fig 11: A Metaphase spread revealing translocation in SW 1985 cattle

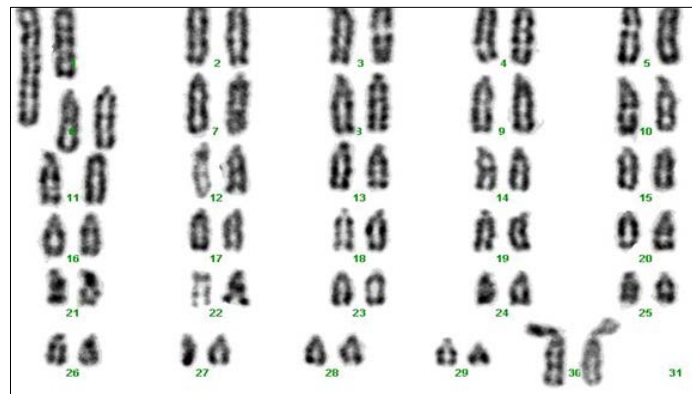


Fig 11: B Karyotype revealing translocation in SW 1985 cattle

Fig 11: R- banded metaphase spread and karyotype of SW 1985 cattle showing translocation of autosome

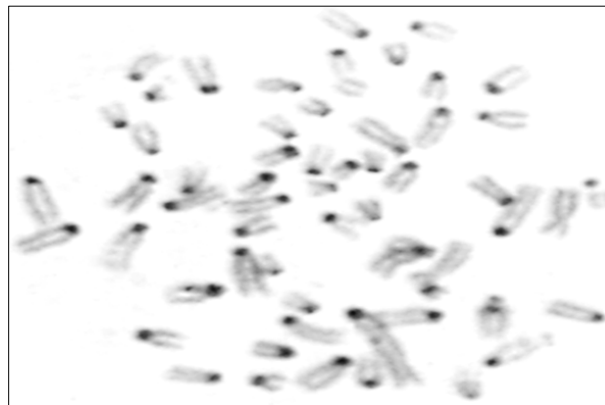


Fig 12: C- banded metaphase spread of SW 1985 cattle showing translocation of autosome

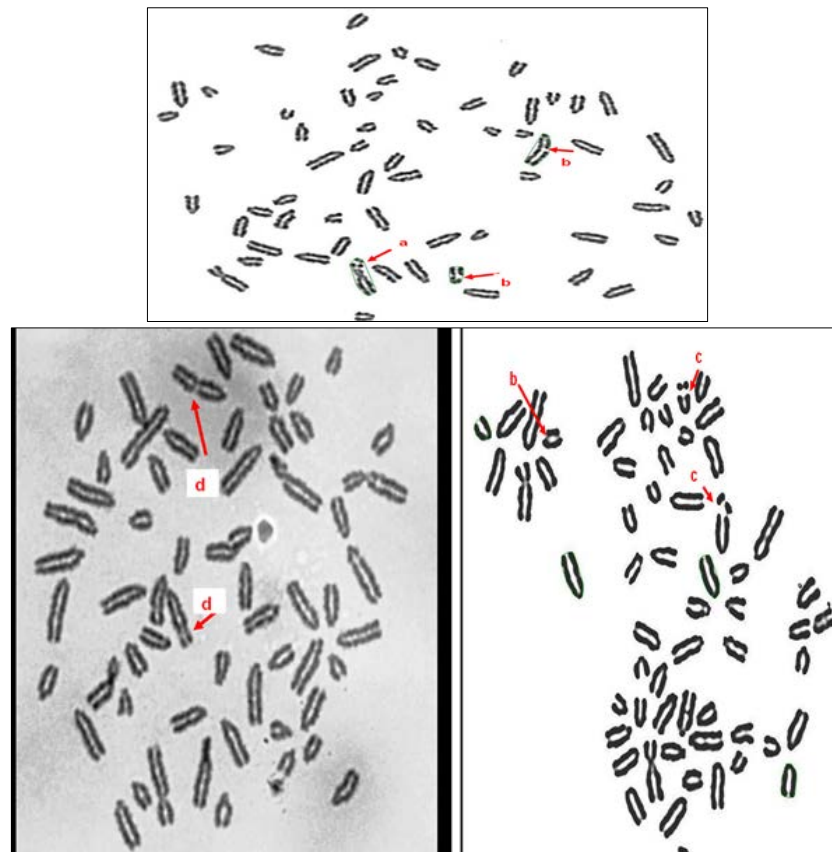


Fig 13: Structural chromosome aberration in cattle (a) X chromosome fragility (b) Chromatid break (c) chromosome break (d) gaps

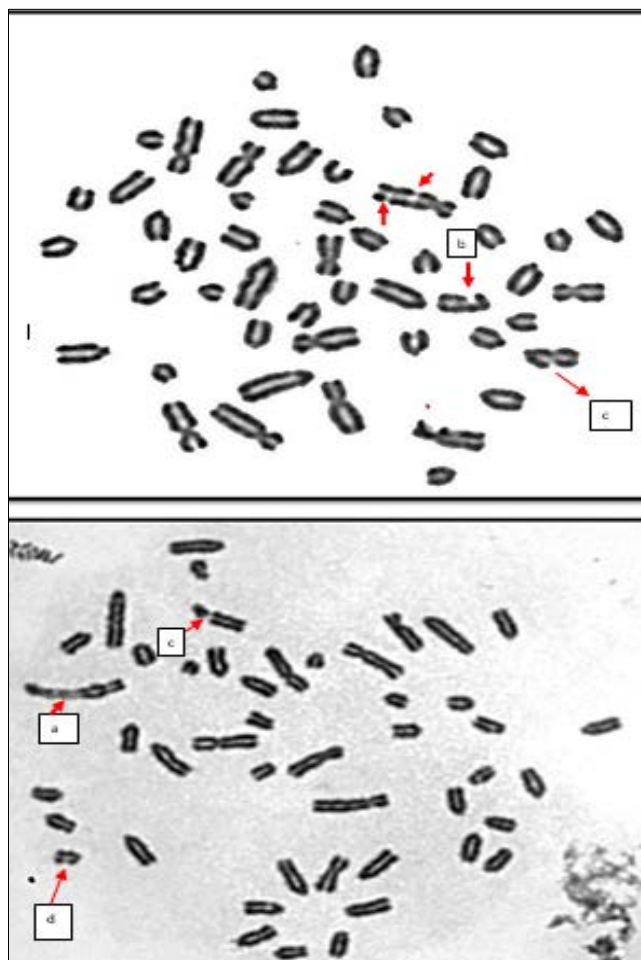


Fig 14: Structural chromosome aberrations in buffalo (a) X chromosome fragility (b) Chromatid break (c) chromosome break (d) gaps

Conclusion

The present study in reproductively problematic animals showed a wide range of anomalies including numerical and structural ones. These were identified only after recording problematic cases with prolonged history of reproductive problems such as anoestrus, repeat breeding, abortions and irregular cycles. It has been found that animals were treated many times by veterinarians, even then the occurrence remained the same, which emphasizes that the over expenses in terms of veterinary aid, management can be avoided by cytogenetic screening at an early age.

Competing interests

The authors declare that they have no competing interests

Authors' contributions

Dr. B R Yadav, has helped in planning the study and design of experiment, analysis and interpretation of data. Dr Rakesh Tonk and Dr Vaishali Sah had been involved in drafting the manuscript or revising it critically for important intellectual content.

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