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A cytogenetic study of heterosexual quadruplets of cattle (*Bos indicus*) - a case report

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ABSTRACT

The birth of live heterosexual quadruplets (three females and one male) in cattle (*Bos indicus*) is a very rare event and hence this study was conducted to observe the chromosome constitution of the dam and the heterosexual quadruplets. The cytogenetic study revealed sex chromosome chimerism (2n = 60, XX/XY) and preponderance of female cells (XX) in the heterosexual quadruplets, and the dam exhibited 2n = 60, XX chromosome complement. A high similarity of chimeric ratios was observed in all the calves and a relationship was also apparent between the chimeric ratio and the distribution of sex of fetuses.

Key words: quadruplet, *Bos indicus*, cytogenetics, chimerism, freemartin

Introduction

The incidence of twins (about 1 to 4 percent) and triplets (less than 0.5 percent) in cattle is common but quadruplets and quintuplets are uncommon (KUMAR et al., 2009). Sex chromosome chimerism (60, XX/XY) is a chromosome abnormality and a more common phenomenon occurring in multiple pregnancies in cattle than in any other species in which a female calf is born co-twin with a male calf (KANAGAWA et al., 1965; BASRUR and KANAGAWA, 1969; SMITH et al., 1977; PERETTI et al., 2008; KOZUBSKA-SOBOCINSKA

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et al., 2011; BISWAS et al., 2014). Chimerism is characterized by the presence of two cell lines with different genetic composition (60, XX/XY) and it may occur either artificially or naturally (SUDIK et al., 2001). A freemartin condition with serious reproductive system defects is thought to be the possible outcome of sex chromosome chimerism in multiple pregnancies in cattle. Freemartin arises due to the fusion of the placental membrane, which leads to reciprocal exchange of haematopoietic stem cells, hormones and other factors early in embryonic life (KOZUBSKA-SOBOCINSKA et al., 2011). Generally, the frequency of freemartins in cattle ranges from 82 to 95 percent of heterosexual twins (KOZUBSKA-SOBOCINSKA et al., 2011). However, fertile female twins in cattle (SMITH et al., 1977), in humans (SUDIK et al., 2001) with sex chromosome chimerism and in quadruplets of river buffalo (*Bubalus bubalis*) without chimerism (KUMAR et al., 2009) have also been reported. The cytogenetic technique (Karyotyping) is an important tool for detection of chromosome abnormalities after birth, thus it may be used for early diagnosis of XX/XY chimerism in animals (KANAGAWA et al., 1965). Early detection of chimerism could minimize the cost of rearing animals and it also prevents the multiplication of abnormal genetic materials through progenies. There are many published reports available on the cytogenetic aspects of twins and triplets, and we were able to find a case of quadruplets in cattle (*Bos taurus*) (BASRUR and KANAGAWA, 1969) and river buffalo (KUMAR et al., 2009). But so far no cytogenetic reports of quadruplets have been available in *Bos indicus* cattle. Therefore, the objective of the present study was to investigate the chromosome constitution of the dam and the quadruplet calves.

Materials and methods

Birth of quadruplets. Live quadruplet calves (three females and one male) were born from an indigenous Deshi cow (*Bos indicus*) on 1st March, 2013 in the village of Kanjialshi in Balurghat Block in Dakshin Dinajpur district of West Bengal, India. The history of the cow was collected from the owner and the veterinarian who attended the case. The animal was served by a crossbred bull (*Bos indicus* × *Bos taurus*) in the same location for her fourth calving. No hormonal treatment had been given to the cow. Hence, the chance of hormonal induction of super-ovulation was eliminated. The animal had a history of producing single calf in her previous three calvings.

Cytogenetic studies. The present study was conducted in the karyotyping laboratory, Frozen Semen Bull Station, Haringhata Farm, West Bengal. Cytogenetic analysis of the somatic chromosomes was carried out using the improved technique of in-vitro whole blood lymphocyte culture following the protocol of HALNAN (1977), with some modifications. All the chemicals and reagents, except those otherwise mentioned were purchased from Sigma-Aldrich, Co. (St. Louis, MO 63103, USA). The lymphocyte working culture media (100 mL) was composed of RPMI 1640, 15 percent fetal calf

serum, 0.25 mg lectin from phytolacca americana (pokeweed), 10 mg streptomycin and 6 mg penicillin, used after being filtered through a 0.2 µm filter (Millipore). About 8 mL working culture media, containing 0.5 mL blood in a 15 mL culture tube (Tarson, Kolkata, India) was placed in an incubator at 37 °C for 72 hrs. After 72 hrs incubation, each culture was harvested in four slides and the slides with good metaphase spreads were subjected to conventional Giemsa staining (GUSTASHAW, 1991) and C-banding (SUMNER, 1972). The stained slides were examined under a differential interference contrast microscope (Olympus) using a ×100 lens and photographed. A total of 200 metaphase chromosome spreads from each sample were analyzed.

Results and discussion

Cytogenetic analysis. The cattle (*Bos indicus/Bos taurus*) normally possess 2n = 60 chromosomes. All the autosomes (29 pairs) are acrocentric, whereas the X chromosomes are large sub-metacentric in both and Y chromosome is small acrocentric and metacentric or sub-metacentric in *Bos indicus* and *Bos taurus* respectively. The results of the present investigation showed that the diploid number (2n) of chromosomes of both the quadruplets and their dam were 60 with 58 autosomes and 2 sex chrosomes (Table 1).

Table 1. Distribution of sex chromosomes of the quadruplets and their dam

Type of animal	Chromosome Nº (2n)	Nº of cells counted	Nº of XX cells	(Percent)	Nº of XY cells	(Percent)
Female calf (F1)	60	200	192	96	8	4
Female calf (F2)	60	200	188	94	12	6
Female calf (F3)	60	200	176	88	24	12
Male calf (M1)	60	200	178	89	22	11
Dam	60	200	200	100		

The morphology of all the autosomes were found to be acrocentric, whereas the X and Y chromosomes were large sub-metacentric and small sub-metacentric respectively in quadruplets. The chromosome constitution of the dam was normal (60, XX) whereas the male calf, as well as female calves showed a mixture of two cell lines of either XX or XY, i.e. sex chromosome chimerism consisting of 60, XX/XY (Fig.1a and 1b). All the metaphase spreads in the present study showed the presence of two cell lines characterized by different sets of sex chromosomes (89 % XX: 11 % XY in the male calf; 96 % XX: 4 % XY in female calf no.1; 94 % XX: 6 % XY in female calf no. 2 and 88 % XX: 12 % XY in female calf no.3). The quadruplets examined in this study showed a preponderance of XX cells. A high similarity in the chimeric ratio was evident in the four calves in this study. The predominance of female (XX) cells and similarity in chimeric ratio in our study resembled the report by BASRUR and KANAGAWA (1969) for quadruplets in cattle.

The unequal proportion of male and female cells reported in this study was in accordance with other findings in multiple pregnancies (BASRUR and STOLTZ, 1966; HERSCHLER and FECHHEIMER, 1967; BISWAS et al., 2014). From the preponderance of XX cells in this study, it is evident that the distribution of the sexes of the fetuses may have influenced the chimeric ratio in multiple pregnancies.

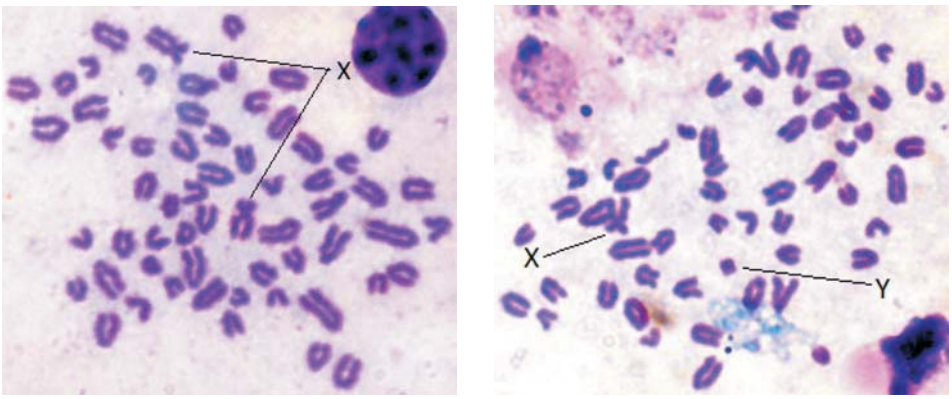

Fig. 1a and 1b. Metaphase chromosome spreads of heterosexual quadruplets showing sex chromosome chimerism (60, XX/XY)

The presence of two cell lines with different chromosome constitution in chimerism may arise artificially or naturally. Artificial chimerism can arise from transfusion of blood stem cells, either by intrauterine transfusion, by allogenic bone marrow transplantation or by organ transplantation. Natural chimerism is induced by fusion of two or more separate fertilized ova (fraternal twins) and splitting of a fertilized egg (monozygotic or identical twins) during early development. The exact reasons for the sex chromosome chimerism and the chimeric ratio in the blood cells of the quadruplets in the present study could not be explained. However, the reciprocal exchange of hematopoietic stem cells as a result of anastomoses of the placental vessels of the fetuses before or after the critical period of reproductive organ differentiation may be responsible for sex chromosome chimerism (KANAGAWA et al., 1965). A large amount of published literature shows that sex chromosome chimerism plays a significant role in the development of the freemartin condition in heifers (KOZUBSKA-SOBOCINSKA et al., 2011; BISWAS et al., 2014) and low semen production, with poor motility and high abnormal sperm and reduced androgen secretion in chimeric bulls from multiple pregnancies. There is also a possibility of development of a freemartin condition in all the female calves showing sex chromosome chimerism in the present study.

Conclusion

The cytogenetic study of live quadruplet calves with their dam was carried out in the present study. The chromosome constitution of the dam (60, XX) was normal but chimerism (60, XX/XY) was detected in all the quadruplets. A preponderance of female (XX) cells in all four calves was observed. A high similarity of chimeric sex ratios between male (XY) and female (XX) cells was found. A relationship was found between the distribution of the sex of the fetuses and chimeric ratios. The possible effect of sex chromosome chimerism is a freemartin condition in female calves and therefore heifer calves born with bull calves should not be used for breeding purposes. However, further study is required to know the effect of sex chromosome chimerism on the reproductive system of these quadruplet calves after puberty.

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SAŽETAK

Četiri živooteljena teleta različitog spola rijetka su pojava kod goveda (*Bos indicus*). U ovom radu obrađen je jedan takav slučaj (tri ženke i jedan mužjak) s istraživanjem kromosomske konstitucije majke i potomaka. Citogenetska analiza pokazala je kod raznospolnih četveraka kimerizam spolnih kromosoma (2n = 60, XX/XY) i pretežitu zastupljenost ženskih stanica (XX), a kod majke 2n = 60, XX kromosomski komplement. U sve teladi opažena je sličnost za stupanj kimerizma te njegova povezanost sa spolom plodova.

Ključne riječi: četverci, *Bos indicus*, citogenetika, kimerizam, frimartinizam