Research paper

Klinefelter (XXY) syndrome in Sahiwal cattle: Distinguishable physical traits for early detection in field condition

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ABSTRACT

Karyological abnormalities of sex chromosomes, most often leads to reproductive dysfunction in livestock. Almost all of the dairy cattle are invariably prone to the chromosomal aberrations in varying,but rare percentages, across the globe.Thezebucattle (*Bos indicus*)breeds of Indian subcontinent are seems to have fewer occurrences of such abnormalities. As chromosomal aberrations are mostly heritable, particularity if present in germ line cells, the screening of young males for such aberrations has been made mandatorybefore selecting for breeding by many of the countries, including India. A young male of Sahiwal, a well-known dairy breed of zebu cattle has been tested for chromosomal abnormalityduring pre-requisite screeningand found to possessabnormal karyotype with 61,XXY condition. The condition,first time reported in Sahiwal cattle was equivalent to Klinefelter syndrome of human being. During initial growing stage, the growth of affected male calf was found to be insignificantly different from other normal male calves; however, at later young stage, it started showing slightly feminine body conformations. In paper, we reported some important physical traits of the young male with XXY condition,which are distinguishable from the animals of normal karyotypeof same age group. Distinguishing physical traits, as conformational markers may identify syndromic young malesbefore selection in field condition;although, the condition should always be validatedthrough karyological analysis.

Keywords: Klinefelter syndrome, Sahiwal, chromosomal abnormality, XXY

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INTRODUCTION

Chromosomal abnormalities may account for approximately one fifth of the total embryonic and fetal loss. Chromosomally abnormal embryos,ifsurvivedhave shown slow development consequently inborn metabolic errors, congenital defects, diseased and syndromic conditions, impaired growth and reproduction, after birth. The chromosomal aberrations are heritable, if those are present in germ line cells. Various studies reveal that about 25% of the abnormalities are attributed to the errors in recombination and separation of chromosome during meiosis at the time of gametogenesis, while rest of the abnormalities occur around the time of fertilization.

Aneuploidy – a condition with any deviation from normal diploid number of the chromosomes – of sex chromosome, with presence of variable number of Xand Y chromosomes is the main characteristics of Klinefelter syndrome (Polani et. al. 1958). Different sex chromosome related aneuploidic conditions - pure XXY to mosaics of XX/XXY or XY/XXY have been observed in cattle (Zhang et al., 1994). Earlier cases of XXY in *Bos taurus* cattle has also been found to be associated with hypogonadism and azoospermia leading to sterility. Along with many abnormalities, incomplete development of reproductive organs leading toinfertility to complete sterility is amongmajor consequences of the syndrome.In humans, the syndrome is found to be associated with male sterility, characterized by hypogonadism and azoospermia (Klinefelter et al., 1942). The condition has been observed in many of the species including livestock (Molteni et al., 1999).Karyological abnormalities of sex chromosomes, most often leads to reproductive dysfunction in livestock.

The zebu cattle (*Bos indicus*) breeds of Indian subcontinent are seems to have fewer occurrences of such abnormalities.Sahiwal, one of the most potential breed of *Bos indicus* cattle of Indian subcontinent is well known for its high milk production in tropical and sub-tropical conditions. The breed is also acknowledged for its resilience to heat stress, tick and disease tolerance, the breed is now distributed across the world. Similar to other zebu breeds, the Sahiwal is also seems to have low frequency of chromosomal and other genetic mutations compared to the taurine breeds,however, the presence of any mutation could never be denied in this breed. In this paper, we described the physical appearance of a XXY condition similar to Klinfelter syndrome in human in young Sahiwal male and confirmed the condition by the karyological analyses. Generally, such karyological analysis is a pre-requisite and mandatory for all bovine males to be used for semen production in government organization in India.Minimum Standards of Protocol and Standard Operating Procedures for Bovine Breeding published by Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, Government of India, in 2014, it is now necessary that all animals be karyotyped

to rule out any chromosomal defects. Young bulls are preliminary screened for various genetic defects including karyological before final selection under multi stage selection programme.

MATERIAL AND METHODS

Karyological study was conducted as routine screening of five young malesof Sahiwal cattle. All the males were of 2 years of age and healthy and were maintained in same management and nutritional conditions in a government farm in Northern India. Peripheral blood was collected from Jugular vein in a heparinized vacutainer tube in sterilized condition. The sample was transported to the lab within 24 hrs of collection in cool and dry conditions with maintaining the temperature of 4 °C. The sample was put for lymphocytes culture immediately after reaching the laboratory. A 0.5 ml blood was cultured in RPMI media at 37 °C for 72 hours. The 72-hour lymphocyte culture was performed from whole blood in standard medium (RPMI 1640-Sigma, St. Louis, USA) supplemented with 15% of fetal calf serum,penicillin and streptomycin (100 IU/ml and 0.1 mg/ml of culture medium, respectively), and pokeweed mitogen (2.5 µg/L of culture medium, SIGMA, St. Louis, USA). To arrest the somatic cells at metaphase stage, colchicine (Sigma, India) 2 µg/mL was added for one hour before harvest. The cells were harvested by centrifugation at 1000 rpm for 20 minutes followed by hypotonic treatment with 0.075 M KCl for 20 minutes at 37°C and fixed thrice in Carnoy's fixative (3:1 ratio of methanol and glacial acetic acid). Finally, cell suspension was dropped on slides and air dried. Slides were stained with 2% Giemsa stain and DPX mounted. At least 30 Metaphase spreads for each animal were analyzed under bright field microscopy and karyotyping was done by using automatic karyotyping software(Genus).

Physical characteristics of these young male of Sahiwal cattle were recorded and compared. The physical characteristics were recorded through visual appraisal, which included body appearance, coat colour, body musculature, gonadal physical attributes. Behavioural attributes were also recorded for these young males.

RESULTS AND DISCUSSION

Karyotypingof samples

Each species of domestic animals has specific chromosomal set, regarding the number as well as the form. Related species possess high chromosomal analogy. Although, differences in apparent chromosomal numbers the fundamental numbers as well structure remain same in two different but genetically close species. Cattle possess 29 pairs of autosomes acrocentric and X (submetacentric) and Y (metacentric in taurine and acrocentric in indicine cattle) sex chromosome.

During karyological study of the male Sahiwal, every metaphase revealed aneuploidy condition with total 61 chromosomes in count. Further observation confirmed the presence of two X chromosome along with Y chromosome in each of the chromosomal spread (Figure 1) and designated as 61, XXY karyotype. Since, the condition was observed in all of the chromosomal spread, the condition seems to be pure 61, XXY instead of any mosaic condition. This was the first observation of 61, XXY genetic condition in any male among about one thousand young cattle males of different breeds, which were screened during 2015-2019.

Klinefelter syndrome is hard to diagnose at early stage of life in human and other species. However during growth the secondary sex characteristics start pointing out towards the aneuploidy condition.The condition seems to be arisen most of the time due to meiotic non-disjunction of sex chromosome pair in either of male or female gamete and further fertilization with normal counterpart gamete. Some time, mitotic division during early embryogenesis may also lead to mosaic condition with mix of cells of normal and aneuplodic karyotype (Rieck, 1984). The frequency of the Klinefelter syndrome is reported from 1:500 to 1:1000 males in different livestock species. Aneuploidic conditions associated with sex chromosomes are mostly tolerated in nature because of dosage compensation - chromosomal inactivation mechanism, wherein other than X chromosome(s) are genetically inactivated during early embryo stage (Lyon, 1961).

In cattle XXY condition and their mosaics have been found to be associated with bilateral testicular hypoplasia, oligospermia or azoospermia, degenerated seminiferous tubules. Although the symptoms worsen after puberty and animals could be easily identifiable with complete sterility (Popescu, 1990). However, the animals with mosaic

Fig. 1. Karyological (chromosomal spread) showing 61, XXY condition of Sahiwal male Genetic testing (karyotyping) is the initial diagnostic for identifying the syndromic condition.

Fig. 2. Physical appearance of Klinefelter syndrome (61, XXY) young Sahiwal male

Fig. 3. Physical appearance of contemporary normal Sahiwal male

karyotype may show different stages of reproductive abnormality. In our study, we found pure XXY condition, the bilateral testis hypoplasia and hypogonadism was most evident.

Physical attributes

The affected Sahiwal male with 61, XXY condition was largely deviated frommaleness (masculine attributes) in its physical appearances and behaviour, and not in concordance with that of a normal male (60, XY) of same age group. Physical characteristics of the male were quite different to the contemporary males of the same breed kept in similar management conditions.

The physical attributes of affected male were as follows.Coat colour was light and evenly toned,without darkening coat colour, normally seen in growing males. Appearance of the animal was slim, taller with longer legs much more closely to female body (Figure 2). The calf showed poor muscle tone with thin, loose and less musculature all over the body, similar to feminine body conformation. Rear view of the calf was also indicated similar feminine appearance with longer hip region, elongated back with shorter base. Skin was thin and loose overall, but more in umbilical region. Head was proportionately smaller to the body. Face is elongated and tapered. Foreheadis elongated and less fleshy.Poleis prominent and more elevated. Lean musculature on occipital bones above the eyes was also evident in the animal. Shoulders were with less musculature, thin and narrow. Thin cannon bone region and less prominent dewclaw were also prominent characteristics. Less thick skin folds in dewlap. Rib cage was prominentlyappeared on body than the normal male. Humpwas comparative small with low curvature at wither. Flank was less deep due to more presence of musculature attached to the rear legs.Affected animal was showing elevated rump with more pointed hook bones. Limbs were thin, seemingly long with less musculature, specifically on thigh region in comparison to male calves of the same age group. Musculature was also found to be loose in perineum region at rear part of the affected male.

A deep furrow with folded musculature at lower part of the inguinal region was also observed different from normal cattle males which show tight musculature without any cleft in the region. Although during younger stage, the growth of animal was found to be insignificantly different from other male calves, however, at later stage it was not able to show the male related secondary characteristics, and showed slight feminine body conformation. Behaviourally, the aggression with low energy level was also found to be low comparatively. The testicles of the animal were also found underdeveloped inside the scrotum, reflecting testicular atrophy. Secondary sex characteristics were much less prominent. Skin on penile-prepuce was seen to be more folded, without any significant penile protrusion.

Fig. 4. Physical appearance of inguinal region, gonads and prepuce in affected male

Table 1: Comparison of physical characteristics in Klinefelter (XXY) syndrome and normal males

Attributes	Normal male	Klinefelter (XXY) syndrome male
Appearance	Strong, broader and stout, body cylindrical	Thin, slim and taller appearance; more closely to female body
Body coat colour	More dark, with differential tone	Less dark, evenly toned
Musculature	Well-developed musculature, Prominent on shoulder and rump	Less musculature on body
Skin	Thick and tight	thin and loose
Dewlap	Thick skin folds on dewlap	Less thick skin folds on dewlap
Head	Proportionatehead to the body, broader and fleshy forehead	Proportionately smaller head to the body,elongated and less fleshyforehead, prominent and more elevated pole
Face	Broader	Elongated and tapered.
Eye	Well-muscled eyebrows, ring like appearance around the eyes	Lean musculature on eyebrows, no discolouration around the eye
Hump and wither	Well elevated hump with high curvature at wither, musculature shoulders	Less elevated hump with low curvature at wither, narrow shoulders with less musculature
Rib cage	Broader rib cage without any visibility of ribs	Narrower rib cage with prominently visible ribs
Umbilical region	Tight fleshy skin	Loose skin flap
Rump	Muscular and sloppy rump	elevated rump with more pointed hook bones
Inguinal region	Tight skin on perineum	loose in perineum,deep furrow like appearance in inguinal region
Appendages	Cylindrical appearance, broader cannon, prominent dewclaw, deep flank,good musculature on thighs	longer and thinner appearance, thin cannon, less prominent dewclaw,less deep flank,less musculature on thighs
Body growth	Fully grown	Less grown
Penile region	Tight skin with clear penile protrusion	Skin on penile-prepuce is more folded, no penile protrusion
Gonadal growth	Normal grown testicles (Normogonadal)	No testicular development (hypogonadism)
Behavioural traits	Alert and aggressive	slightly dull,no aggression

In conclusion, 61, XXY condition may occur, although in rare case in cattle similar to other species. Most of the physical attributes were towards feminine type, despite of presence of male gonads, although underdeveloped in the young male with 61, XXY karyotype. Physical traits in XXY conditions were found to be distinguishable from the animals of normal genotypeat younger stage. Largely feminine physical attributes along with underdeveloped male gonads may be early identifiable physical traits forKlinefelter (XXY) syndrome in cattle; however, the condition should be confirmed by karyological analysis.

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