

**A study on the birth of heterosexual river buffalo quadruplets
- a case report**

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ABSTRACT

The birth of live heterosexual quadruplets (three females and one male) in a river buffalo is a very rare event and hence it is documented. The cytogenetic study revealed the female calves and the dam exhibited $2n = 50$, XX chromosome complement and the male calf exhibited $2n = 50$, XY chromosome complement and this proves that the female calves are not freemartins, that in buffaloes the heifer calves born with bull calves need not always be freemartins.

Key words: quadruplets, freemartin, river buffalo

Introduction

River buffalo (*Bubalus bubalis*) are important domestic livestock for the Indian economy, with a population of 84 millions, producing about 60 per cent of country's total milk production. Buffaloes are uniparous species and twinning occurs relatively rarely.

Multiple births are undesirable in both cattle and buffaloes, though they have the potential to improve the efficiency of beef production. Twinning in beef cattle generally does not exceed 1 per cent (RUTLEDGE, 1975). Twinning in cattle ranges from about 1 per cent for beef breeds to about 4 per cent for dairy breeds.

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In dairy herds, the incidence of double births is higher (on average 4 to 5 per cent), and is strongly affected by the age and parity of the dam, ranging from about 1 per cent for heifers to nearly 10 per cent for older cows. Twinning rate may increase in cows over ten years of age, and the largest increase was observed between the first and second parity (BERRY et al., 1994; KINSEL et al., 1998; NIELEN et al., 1989). Twinning rate is also slightly influenced by seasonal effects, with a trend toward more multiple births during the spring (KINSEL et al., 1998; CADY and VAN VLECK, 1978) or autumn months (GREGORY et al., 1990). The nature of seasonal effects, although uncertain, is thought to be connected with changes in temperature, the duration of daylight or in feeding at conception.

Even in cattle, twinning (about 1 per cent) and triplets (less than 0.5 per cent) are common whereas quadruplets and quintuplets are uncommon. One such quadruplet birth was reported in a cow from Nanjing, China in 2003. Afterwards, in 2007, a Simmental cattle gave birth to five calves by artificial insemination but without application of hormone preparations of any kind (SABOLIĆ et al., 2007). But in buffaloes it was possible to trace a case (BALAKRISHNAN et al., 1981) of river buffalo triplets. Absolutely, no report could be traced with a buffalo giving birth to four live calves. However, twinning in buffaloes is reported to range from 0.015 to 0.63 per cent (TANTAWY and AHMED, 1957).

Materials and methods

Birth of quadruplets. A pleuriparous, graded Murrah buffalo gave birth to four live calves (three females and one male) in December, 2007 in a village in the Namakkal district of the Tamil Nadu state in India (Fig.1). The history of the animal was collected from the owner and the veterinarian who attended the case. The buffalo was a recently purchased animal and for the third calving it was served by a bull in the same location. No hormonal treatment had been given to the animal. Hence, the chance of hormonal induction of super-ovulation was eliminated. The details of the animal, and the blood samples collected both from the dam and the calves were used for this study.

Cytogenetic studies. The cytogenetic investigation was carried out to identify the chromosome complement as per the standard method (HALNAN, 1977).

Results and discussion

Details of the dam and calves. The birth weights of calves were recorded as 10.2, 10.1, 9.3 kg for three female calves and 9.2 kg for male calf, the lowest among the four calves. The actual weight of the dam after calving could not be recorded as there is no facility in the village. But the tentative weight was worked out using a formula method (PATIL and ULMEK, 2004) and the weight was 285.2 kg.



Fig.1 Heterosexual quadruplets on the day of birth

The unique heterosexual birth with three females and one male seemed to be a very rare event. In cattle, breed-wise multiple birth events have been recorded. There was a difference in twinning rates between dairy cattle and beef cattle breeds, with dairy cattle experiencing a higher frequency. The incidence in dairy cattle ranged from 1.3 per cent in Jerseys, 3.4 per cent in Holsteins and an 8.9 per cent in Brown Swiss. Small differences were reported in beef breeds, with Hereford cattle having the lowest incidence (0.4 per cent or one out of every 250 births) of twinning while Angus had 1.1 per cent incidence. The *Bos indicus* breeds experienced 0.2 per cent and 0.4 per cent twinning rates in Brahman and Santa Gertrudis, respectively (KIRKPATRICK, 2004).

Twins are classified as either fraternal or identical twins based on their origin. Fraternal twins originate from two separate fertilized ova due to multiple ovulations by the cow, while identical twins are a result of the single fertilized egg (embryo) splitting during early development. Therefore identical twins, like cloned animals, are genetically identical. Fraternal twins are more common than identical twins. It has been reported that only about 10 per cent of the naturally occurring twins in the cattle populations are identical. One phenomenon of fraternal twins is that when they are of different sexes, the female is very likely to be freemartin. It is estimated that 95 per cent of the heifers born co-twin to a bull are freemartins. These heifers should not be saved as replacement females for the herd.

Cytogenetic observation. The river buffalo (*Bubalus bubalis*) normally possesses $2n = 50$ chromosomes. The first five pairs of autosomes are submetacentric whereas all other chromosomes, including sex chromosomes, are acrocentric. The X chromosome is the largest acrocentric which could be identified even without GTG-banding. The Y chromosome is among the smaller acrocentric chromosomes (KUMAR and YADAV, 1991) and can be identified by conventional C-banding, as this is the best and simplest technique to distinguish sex chromosomes (KUMAR and YADAV, 1991; DI MEO et al., 1995). In the present study, all 100 metaphases screened for each animal exhibited 50 chromosomes.

The female calf and the dam exhibited $2n = 50$, XX chromosome complement and the male calf exhibited $2n = 50$, XY (Fig. 2). The Y chromosome was identified by conventional C banding procedure. This excludes any vascular anastomosis between the calves during fetal development. No sex chromosome chimaerism was identified in both male and female calves, excluding the possibility of female calves being freemartins.

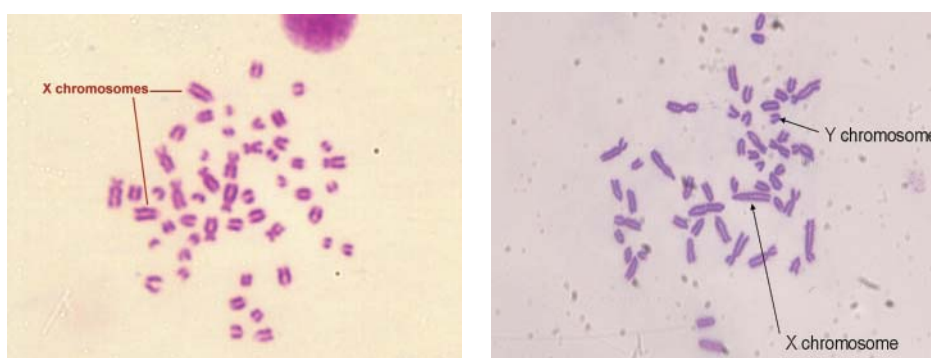


Fig. 2. Metaphase spreads from male and female calves showing normal chromosome complement (1000 X)

Freemartinism occurs more frequently in cattle than in any other species. Between 82.5 per cent (ZHANG et al., 1994) and 92 per cent (BUOEN et al., 1992) of heifers from mixed sex twin pregnancies are freemartins. The remaining 8 to 17.5 per cent of females develop correctly, presumably because the placental vessels fail to fuse or because the fusion occurs after the critical period of reproductive organ differentiation. The background of the placental anastomosis development is not clear. There are indications that breed type may influence the incidence of chimaerism in cattle (SUMMERS et al., 1984). The distinct differences observed between breeds indicate the hereditary tendency of placental anastomosis development. In a cytogenetic investigation (IANNUZZI et al., 2005) on 42 river buffaloes with reproductive problems in southern Italy, 10 freemartins (8 females and 2 males) were found with variable percentages of male and female blood

cells, the majority however showing similar percentages in both. Of the eight females, six showed normal body conformation, vagina and clitoris, while two showed some male traits (tight pelvis). The two males were apparently normal with reduced size of one testicle in one animal. In the present case the phenotypic conformation is normal except for the lower body mass of all four calves. In general, various chromosomal anomalies associated with infertility and reduced fertility have been documented in both humans and cattle, while few cases have been studied in river buffaloes.

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V. R. S. Kumar et al.: A study on the birth of heterosexual river buffalo quadruplets

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SAŽETAK

Rođenje živih heteroseksualnih četvorki (tri ženska i jedan muški mladunac) u azijskoga vodenoga bivola događa se vrlo rijetko. Citogenetska istraživanja pokazala su da su ženska telad i majka imali $2n = 50, XX$ kromosoma, a muško tele $2n = 50, XY$ kromosoma. To pokazuje da u ženske teladi nije bio izražen frimartinizam te da bivolske junice oteljene zajedno s bivolima nisu uvijek nužno frimartine.

Ključne riječi: četvorke, frimartinizam, azijski vodeni bivol
