

The cytogenetic screening of South African artificial insemination bulls

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Summary – Following the discovery of the 1/29 chromosome translocation in Swedish artificial insemination (AI) bulls, cytogenetic evaluation of South African AI bulls was initiated. The present paper reports on the 269 bulls screened over the past 11 yr. The 1/29 chromosome translocation was found in Brahman and Brown Swiss bulls while chimerism was present in the Friesian bulls. The overall incidence of chromosome abnormalities in these bulls is 1.49%. Although the incidence is low, discovery of the abnormalities serves to illustrate the importance of cytogenetic screening in the AI industry.

cattle / chromosome abnormality / translocation / chimerism

Résumé – Le contrôle cytogénétique des taureaux sud-africains d'insémination artificielle. A la suite de la découverte de la translocation chromosomique 1/29 chez des taureaux d'insémination artificielle (IA) suédois, l'examen cytogénétique des taureaux d'IA sud-africains a été entrepris. Cet article concerne les 269 taureaux examinés au cours des 11 dernières années. La translocation chromosomique 1/29 a été trouvée dans les races Brahman et Brune des Alpes, et par ailleurs le chimérisme existe chez les taureaux de race Frisonne. L'incidence globale des anomalies chromosomiques chez ces taureaux est de 1,49%. Bien que ce taux soit faible, la découverte de ces anomalies illustre l'importance d'un contrôle cytogénétique pour les centres d'IA.

bovin / anomalie chromosomique / translocation / chimérisme

INTRODUCTION

Artificial insemination has, since its inception, revolutionised the cattle breeding industry. There is, however, an ever-present danger in its widespread application; undiagnosed genetic defects and less severe chromosome abnormalities may be spread rapidly through the use of a carrier bull's semen. Examples can be found in the amputated calf syndrome present in Friesian cattle (Wriedt and Mohr, 1928; Meyer *et al*, 1980), the high incidence of the 1/29 chromosome abnormality in Swedish Red and White cattle (Gustavsson, 1969) and inherited immunodeficiency diseases such as agammaglobulinaemia (Perryman, 1979).

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These examples illustrate the importance of effective screening procedures for all bulls used in the artificial insemination industry. Present screening techniques are based on pedigree history, progeny testing, semen quality testing, libido, linear trait selection and chromosome analysis.

With the advent of recombinant technology, it will be possible in future to screen for a host of important genetic defects using a battery of defect-specific DNA probes.

Since the discovery of the 1/29 chromosome abnormality in various cattle breeds and its effect on fertility (Refsdal, 1976; Blazak and Eldridge, 1977; Gustavsson, 1979; Kovacs and Csukly, 1980; Popescu, 1977, 1982; Swartz and Vogt, 1983; Foulley and Frebling, 1985), chromosome screening has become an important component of bull evaluation.

Following the discovery of the 1/29 translocation in Swedish AI bulls (Gustavsson, 1969), cytogenetic evaluation of South African AI bulls was initiated in 1977 to ensure that chromosome abnormalities were not spread by the use of carrier bulls.

This paper reports on the cytogenetic study of bulls used in the South African AI industry over the past 13 yr.

MATERIALS AND METHODS

Peripheral blood samples taken from 269 bulls comprising 20 cattle breeds (table I) were submitted for cytogenetic analysis. These samples were taken from bulls stationed at AI centres belonging to the Artificial Insemination Co-operative.

Routine screening was performed on Giemsa-stained metaphase spreads obtained from whole blood cultures. The culture method was adapted from that of Moorhead *et al* (1960). In this method, concanavalin A (0.01 mg/ml) was used instead of phytohaemagglutinin. The chromosome spreads were routinely stained in 5% Giemsa and 10 well dispersed metaphase spreads were screened for structural or numerical aberrations.

The chromosomes of animals found to deviate from the standard karyotype were identified by means of an R-banding technique adapted from Popescu (1975), Popescu *et al* (1982) and Di Berardino and Ianuzzi (1982).

The lymphocyte cultures were exposed to 5'Brdu (50 µg/ml) for 5 h prior to harvesting. The air-dried, rinsed slides were passed through a series of alcohol grades to water, rinsed in phosphate buffer and stained in acridine orange for 15 min. After rinsing for 30 min, the cells were mounted in a drop of buffer and sealed with nail varnish. Banded metaphase spreads were photographed with Zeiss Epifluorescence equipment on Agfaortho 25 film.

In instances where a chromosome abnormality was found, follow-up studies ascertained the origin and extent of the abnormality in the population.

RESULTS

A study of the metaphase spreads confirmed the presence of 29 acrocentric autosomal pairs and one pair of sex chromosomes, a large submetacentric X chromosome and a small metacentric Y chromosome in the *Bos taurus* and *Bos indicus* (Sanga) bulls. In the *B indicus* (Zebu) bulls, the Y chromosome is represented by a small acrocentric chromosome.

Table I. AI bulls screened.

<i>Breed</i>	<i>Application</i>	<i>Type</i>	<i>No of bulls</i>
Ayrshire	Dairy	<i>Bos taurus</i>	16
Brown Swiss	Dairy/Beef	—	6
Charolais	Beef	—	1
Devon (North and South)	Beef	—	7
Friesian	Dairy	—	108
Guernsey	Dairy	—	20
Hereford	Beef	—	10
Jersey	Dairy	—	19
Pinzgauer	Beef	—	7
Red Poll	—	—	1
SA Angus	—	—	10
Short Horn	—	—	2
Simmentaler	—	—	12
Sussex	—	—	5
Brahman	—	<i>Bos indicus</i> (Zebu)	13
Santa Gertrudis	—	— (Zebu)	3
Afrikaner	—	— (Sanga)*	2
Bonsmara	—	— (Sanga)*	26
Drakensberger	—	— (Sanga)*	4
Nguni	—	— (Sanga)*	2
Total			269

* *Bos indicus* (Sanga) cattle are indigenous to Southern Africa.

Screening of the Giemsa stained and subsequent R-banded metaphase spreads revealed the presence of abnormalities in the Friesian, Brahman and Brown Swiss cattle (table II). Giemsa stained and R-banded karyotypes are shown in figures 1, 2 and 3.

Table II. Chromosome abnormalities in the bulls screened.

<i>Breed</i>	<i>Animals</i>		<i>Abnormality</i>	
	<i>Screened</i>		<i>Type</i>	<i>No</i>
Friesian	108		60, XX/XY	2
Brahman	13		59, XY, t (1; 29)	1
Brown Swiss	6		59, XY, t (1; 29)	1

Following the discovery of the chromosome abnormalities, subsequent studies revealed that the Friesian bulls were both co-twins to heifers and that the Brahman bull was descended from carrier cattle imported from the United States of America. In the case of the Brown Swiss bull, the most recent discovery, the origin has as

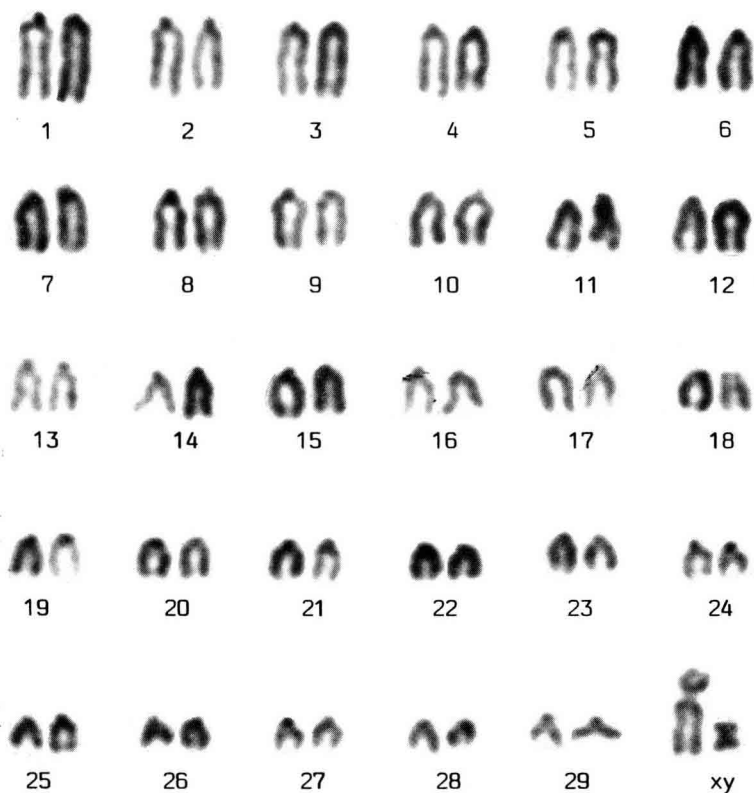


Fig 1. A Giemsa stained karyotype of the *B taurus* and *B indicus* (Sanga) bull.

yet not been ascertained but the normal familial pattern of inheritance has been established (results not shown).

DISCUSSION

The results in table II show that structural chromosome abnormalities appear in one beef and one dual purpose breed, while there is a total absence in the 4 dairy breeds screened. The chimerism that does appear in the Friesian breed cannot be considered a primary chromosome defect. The lack of chromosomal aberrations in the pure dairy breeds is in accordance with the findings of teams across the world (see reviews by Popescu, 1977 and 1982; Gustavsson, 1979; Foulley and Frebling, 1985). Possible reasons for this absence are the pure breeding practices and the constant selection for fertility in these breeds. Any animal which does not meet the required standard, irrespective of the reason, is automatically culled from the production herd.

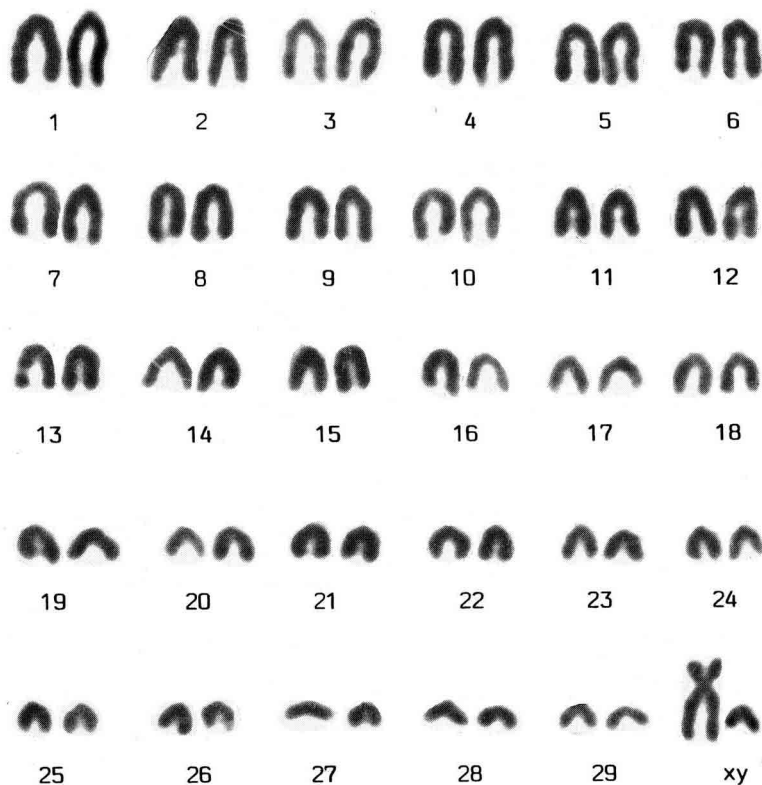


Fig 2. A Giemsa stained karyotype of a *B indicus* (Zebu) bull.

As the sample sizes are very small, these results do not give any indication of the incidence of the particular abnormality in the national herd. Each case should be investigated individually before such predictions are possible.

The chimerism found in the 2 Friesian bulls was traced to the presence of female co-twins during pregnancy. The observed incidence of 1.85% (2 of the 108 bulls) is slightly higher than the expected figure of 1% for twins of opposite sex. The average twinning rate in South African Friesian cattle is 2% (Friesian Cattle Breeder's Society, 1989, unpublished results). This discrepancy is possibly due to the small sample size.

The condition, known as freemartinism in the heifer co-twin, is caused by an allantochorionic anastomosis during embryo development. This condition was described as far back as 1911 (Tandler and Keller, 1911) and has since been studied extensively by Short *et al* (1969). In South Africa, the condition was first described by Gerneke in 1969. The condition affects the fertility of the heifers and, although some of the bulls may not be affected, infertility has been reported. It is, therefore, advisable to remove such bulls from the selection programme (Dunn *et al*, 1968; Gerneke, 1969; Short *et al*, 1969; Stafford, 1972; and unpublished results, Animal and Dairy Science Research Institute).

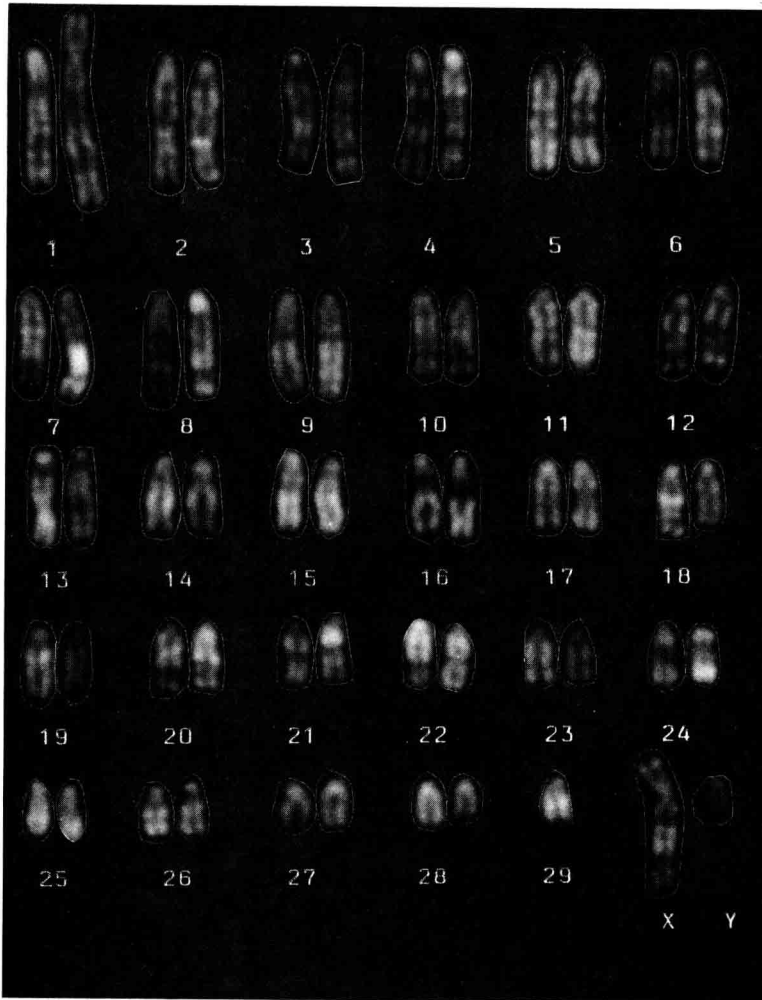


Fig 3. An R-banded karyotype of a 1/29 translocation heterozygote bull (*B indicus* - Zebu).

The occurrence of twinning in cattle should be carefully monitored during any long-term programme aimed at increasing the fertility of a particular breed. An increase in the twinning frequencies would immediately oppose any gains in fertility and this would necessitate a re-evaluation of the selection programme in use.

The 1/29 chromosome translocation found in the Brahman bull was introduced into the Southern African herd by Brahman cattle imported from the United States of America during the 1960's. The subsequent investigation showed that the anomaly was confined to a particular family and was not present in a random sample of unrelated Brahman cattle (Nel *et al*, 1988).

The most recent abnormality found was that in a Brown Swiss bull. The preliminary study shows a familial pattern of inheritance with the translocation present in the dam and maternal half-sister.

During the period 1974–1986, Brown Swiss semen was imported from the United States of America, Austria, West Germany and Switzerland. It is possible that some of this semen may have originated from a carrier bull. Translocation carrying Brown Swiss cattle have been reported in Switzerland and the United States of America. The incidence in these 2 herds is 0.4% and 2.4% respectively (Blazak and Eldridge, 1977; Tschudi, 1984). The investigation will continue and the detailed results will be published at a later stage.

It is interesting to note that the 1/29 Robertsonian translocation was not found in the 12 AI Simmentaler bulls tested, despite the fact that the anomaly is present in the South African herd. Tests carried out on 3 stud farms revealed an incidence of 20% (25 of the 125 cattle investigated) in the herds screened (unpublished observations).

As most of the sires used on these farms originated from a single stud herd, it cannot be construed as a national herd average but merely serves as an indication of a higher than average proportion of translocation carrying cattle. Based on the results of Gustavsson (1979) a figure of $\approx 2.7\%$ would appear to be a reasonable world average. This study has been suspended because of the reluctance of the Simmentaler Breed Society to allow the screening of their cattle.

A similar situation is present in the indigenous Nguni cattle where an incidence of 10.2% was established in the 305 cattle studied (Nel *et al*, 1985). After facing near-extinction, attempts are underway to increase the number of Nguni cattle: a culling programme was therefore initiated to ensure that stud animals are free of the 1/29 translocation. No abnormalities were found in the 2 AI bulls screened.

The overall incidence of chromosome abnormalities in South African AI bulls is low, 1.49% of which only 0.74% are heritable abnormalities. Nevertheless, the abnormalities found in the bulls screened illustrate the importance of these tests in situations where a specific animal is extensively used, in, for example, artificial insemination and, to a lesser extent, embryo transfer from an elite corps of females.

Cytogenetic laboratories worldwide perform an extremely valuable function and it is imperative that the tests be continued and expanded to include the testing and certification of bulls selected for import and export purposes. The introduction of the 1/29 chromosome translocation into the South African Brahman herd as well as the suspected introduction into the Brown Swiss herd through the use of imported material is indicative of the important role of cytogenetic screening.

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