



## Reproductive deficiency in bulls from synthetic breeds according to the type of crossbreed and the morphology of the Y chromosome

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### Abstract

We used 550 Braford and 214 Brangus-Ibagé bulls to study the association between Y chromosome morphology and the evaluation of breeding-soundness, karyotyping and breeding records were used to assess Y chromosome morphology. In general, no direct association was detected between the individual type of Y chromosome and reproductive fitness as previously estimated through breeding soundness examination. The type of breeding strategy used to form the synthetic breeds was also analyzed. The Braford breed is about 3/8 Zebu (*Bos taurus indicus*) and 5/8 Hereford (*Bos taurus taurus*) but when the breeding strategy used 1/4 Zebu animals to produce Braford bulls which were 3/8 Zebu a greater percentage of the bulls had to be culled due to reproductive problems. Our study shows that certain interactions between genotypes can be detrimental to the reproductive efficiency of 3/8 Zebu animals and that breeding strategies avoiding or favoring certain genotypes can be used to reduce the percentage of bulls from synthetic breeds that are culled due to problems with semen quality.

*Key words:* bulls, semen quality, Y chromosome, synthetic breeds of cattle.

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### Introduction

In spite of their common ancestral base Asiatic Zebu cattle (*Bos taurus indicus*) and European taurine cattle (*Bos taurus taurus*) exhibit several morphological and physiological differences (Wurster and Benirschke, 1968). The karyotypes of these two bovine sub-species are similar, consisting of 58 acrocentric autosomes and two sexual chromosomes, a submetacentric X chromosome and a Y chromosome whose morphology varies depending on the sub-species (Kieffer and Cartwright, 1968); the Y-chromosome found in *B. t. taurus* being submetacentric while that in *B. t. indicus* is acrocentric. These two types of Y chromosome also occur in bulls of the synthetic Brangus-Ibagé breed (Pinheiro *et al.*, 1979) and in 'Pé Duro' bulls (Britto and Mello, 1999) (an ancient European breed raised in Brazil) indicating miscegenation. According to Kieffer and Cartwright (1968) the difference in the Y chromosome between the *taurus* and *indicus* sub-species is a consequence of pericentric inversion and this was later con-

firmed by Pinheiro *et al.* (1980) based on G and C-band patterns. Halnan (1989) reviewed Y chromosome variants and fertility in more than 30 breeds and suggested that the Y chromosome could be used as a racial marker and to produce a genetic model capable of explaining the low reproductive rate of bulls derived from Zebu crosses in Australia. The lower fertility in Zebu crosses with European cattle could be due to small deletions or position changes between the synapse region of the X and Y chromosomes, or to alterations in genes participating in the regulation of reproduction.

It is known that the Y-chromosome possesses genes related to spermatogenesis (Foresta *et al.*, 1997), one example being the Sxra region of the rat Y-chromosome which contains genes related to the determination of the male gender and which participate during the last stages of spermatogenesis (Cooke, 1999). There is evidence from various species of irregularities occurring during the meiosis of F1 products and backcrosses, with fertility problems having been reported in the heterogametic sex in bovines (Basur and Moon, 1967), *Drosophila* (Lammissou *et al.*, 1996; Joly *et al.*, 1997; Snook, 1998) and rodents (Hale *et al.* 1993; Kaku *et al.*, 1995), the example in rodents being

particularly interesting because of the occurrence of fertile and infertile males depending on the type of backcross from which they arose (Hale *et al.* 1993).

A greater percentage of bulls with indicators of reduced potential fertility have been found in synthetic breeds in comparison with their parental pure breeds (Chenoweth *et al.*, 1996; Chacón *et al.*, 1999). In a population of 751 bulls raised in southern Brazil the culling rate due to alterations in semen quality was 47% for Braford bulls but only 18% for Hereford bulls raised under the same conditions (Moraes *et al.*, 1998). According to various authors between 80 and 90% of purebred animals have no reproductive problems (Johnson *et al.*, 1995; Bruner *et al.*, 1995; Gottschall and Mattos, 1997; Ribeiro Filho *et al.*, 1997; Kennedy *et al.*, 2002) but in their study of 48 crossbred bulls Rao and Rao (1991) found that only 47% could be considered reproductively sound.

The breeding strategies used to obtain synthetic breeds offers an ideal model to study a possible association between Y chromosome morphology and fertility because both types of Y chromosome coexist in the same racial group. The hypothesis which we tested was that there would be a greater incidence of alterations in the spermatogenesis of crossbred males due to gene modifications resulting from the rearrangement caused by variation in Y chromosome morphology which could lead to alterations in enzymes or other proteins. The objective of our study was to identify the relationships between Y chromosome morphological type, crossbreeding type and reproductive fitness of bulls from synthetic breeds of cattle.

## Material and Methods

We prepared karyotypes from lymphocyte cultures of 76 Braford and 21 Brangus bulls and identified the Y chromosome morphology for each bull. In addition we used genealogical analysis to investigate the type of Y chromosome present in 474 Braford and 193 Brangus-Ibagé bulls with known paternal genotypes.

The bulls used in this study had previously been evaluated for reproductive soundness according to the specifications of the Brazilian Society for Animal Reproduction (Government edict n. 26 from 05/09/1996, [www.agricultura.gov.br](http://www.agricultura.gov.br)) which states that to be considered as being fit for breeding a bull must present no more than 30% of abnormal sperm and > 50% sperm-motility with a vigor of > 3 on a 0-5 scale, bulls not conforming to these criteria were considered reproductively unfit for our study. The bulls were raised on two private properties and at South Embrapa Cattle & Sheep (Centre Embrapa, the Brazilian Agricultural Research Corporation) near the town of Bagé, Rio Grande do Sul state, southern Brazil. In summer the bulls grazed on native pastures, composed principally of *Paspalum sp.* and *Axonopus sp.*, and in winter on cultivated pastures consisting of

*Trifolium repens*, *Lotus corniculatus* and *Lolium multiflorum*, when the bulls were two years old at which point they were subjected to pre-sale breeding soundness evaluation.

For the 97 karyotyped bulls, the area around the tail-vein was cleaned and disinfected with 70% (v/v) alcohol and blood samples collected from the tail-vein using heparinized syringes, the samples being kept at 5-10 °C and transported to the laboratory within 24 h. Lymphocyte cultures were produced for each bull by adding four drops of blood to 10 mL of karyotyping medium containing phytohemagglutinin (Cultilab) and incubating the cultures for 72 h with gentle shaking for a few seconds every 12 h, 50 µL of a 0.025% (w/v) colchicine solution (Sigma) being added after 70 h incubation. After 72 h incubation cultures were centrifuged at 1000 revs min<sup>-1</sup> for 5 min and the supernatants separated, 4 mL of 0.075 molar potassium chloride was added to the pelleted cells to produce hypotonicity and the mixture allowed to stand for 15 min before adding 4 mL of Carnoy's fluid (3:1 (v/v) methanol/acetic acid) fixative and allowing the cells to fix for 24 h after which they were centrifuged and washed three times with the same volume of Carnoy fixative. Slides were made by placing a few drops of the fixed material onto cold microscope slides which were then flamed and stained with Giemsa and examined by clear-field microscopy at 1000x magnification, Y chromosome morphology being determined by examining five metaphases from each bull.

We also used genealogical data to investigate 667 bulls (474 Braford and 193 Brangus-Ibagé) with known paternal genotypes and compare the results with those from the karyotyped bulls. Genealogical data covering three years (1998-2000) were used for the Braford bulls and eight years (1992-2000) for the Brangus-Ibagé bulls. Table 1 presents the different matings used to obtain the animals with different degrees of crossbreeding established in the formation of the synthetic breeds. Both male and female 1/4 Hereford or Aberdeen Angus x 3/4 Nellore were obtained by mating Nellore bulls with 1/2 blood females but bulls with this genotype combination were not evaluated with respect their breeding soundness and data for this mating type is not included in Table 1.

The analysis of the association between the reproductive condition (fit or unfit) of the bulls and the morphological type of the Y chromosome (acrocentric or submetacentric) or the different matings was carried out by using the chi-square ( $\chi^2$ ) likelihood-ratio method using the PEPI package, Statistical Programs for Epidemiologists, version 3.0 ([www.usd-inc.com/pepi.html](http://www.usd-inc.com/pepi.html)) with the Yates correction being applied whenever the comparisons involved less than 100 animals.

**Table 1** - Matings used to obtain Braford (5/8 Hereford x 3/8 Nellore) and Brangus-Ibagé (5/8 Aberdeen Angus x 3/8 Nellore) synthetic bulls.

Type of mating	Offspring	Parents
1	1/2	Male Nellore x Female Hereford or Aberdeen Angus
2	1/4	Male Hereford or Aberdeen Angus x Female 1/2 Nellore
3	1/4	Male 1/2 x Female Hereford or Aberdeen Angus
4	3/8	Male Hereford or Aberdeen Angus x Female 3/4 Nellore
5	3/8	Male 3/4 Nellore x Female Hereford or Aberdeen Angus
6	3/8	Male 1/2 x Female 1/4 Nellore
7	3/8	Male 1/4 Nellore x Female 1/2 Nellore
8	3/8	3/8 x 3/8 Nellore

## Results

Of the sample of 97 bulls (76 Braford and 21 Brangus-Ibagé) used in the karyotype study, 55 were diagnosed as fit and 42 considered unfit for reproduction, Table 2 showing the frequencies of each type of Y chromosome as evaluated using karyotype analysis.

For the Braford group the percentage of fit and unfit bulls with the two Y chromosome types were similar

( $\chi^2 = 1.053$ ; DF = 1;  $p = 0.305$ ). In the smaller of Brangus-Ibagé sample although there was an apparent excess of fit bulls with an acrocentric Y chromosome there was also no significant difference between fit and unfit bulls ( $\chi^2 = 0.032$ ; DF = 1;  $p = 0.859$ ). In both breeds, no association was detected between Y chromosome morphology and reproductive fitness ( $\chi^2 = 0.681$ ; DF = 1;  $p = 0.409$ ). Excluding Y chromosome morphology from the analysis, there was a higher frequency of fit Brangus-Ibagé bulls than Braford bulls ( $\chi^2 = 5.222$ ; DF = 1;  $p = 0.022$ ).

Chromosome analysis revealed that genealogical data was imprecise only for the production of the 1/4 offspring (mating types 2 and 3), necessary for obtaining the 5/8 European and 3/8 Zebu crossbreds (Table 1), because a multiple sire mating system was used to obtain this genotype.

Table 3 shows the frequency of reproductively fit and unfit Braford bulls according to their genealogically determined Y chromosome morphology. In 471 Braford bulls (excluding bulls from mating type 4) the frequency of fit and unfit bulls was similar between mating types ( $\chi^2 = 9.949$ ; DF = 6;  $p = 0.127$ ). For bulls carrying an acrocentric Y chromosome there was a significant deviation between mating types ( $\chi^2 = 9.735$ ; DF = 4;  $p = 0.045$ ), unfit bulls being more prevalent in mating types 1 and 6 and less prevalent in types 5 and 8. In contrast, this interaction was not significant for bulls carrying a submetacentric Y chromosome ( $\chi^2 = 1.773$ ; DF = 3;  $p = 0.621$ ). In the genea-

**Table 2** - Cytogenetically determined frequency of the different types of Y chromosomes in Braford and Brangus-Ibagé bulls which had been classified by breeding soundness evaluation.

Breed	Y chromosome morphology						Total unfit + fit
	Unfit bulls			Fit bulls			
	Acrocentric number (%)	Submetacentric number (%)	Total unfit	Acrocentric number (%)	Submetacentric number (%)	Total fit	
Braford	25 (33)	13 (17)	38	30 (39)	8 (11)	38	76
Brangus-Ibagé	3 (14)	1 (5)	4	12 (57)	5 (24)	17	21
Total	28 (29)	14 (14)	42	42 (43)	13 (14)	55	97

**Table 3** - Genealogically determined frequency of the different Y chromosome morphological types found in reproductively fit and unfit Braford bulls.

Mating type	Y chromosome morphology						Total unfit + fit
	Unfit bulls			Fit bulls			
	Acrocentric number (%)	Submetacentric number (%)	Total unfit	Acrocentric number (%)	Submetacentric number (%)	Total fit	
1	19 (38)	-	19	31 (62)	-	31	50
2	-	33 (33)	33	-	66 (66)	66	99
3	12 (23)	-	12	40 (77)	-	40	52
4	-	2 (67)	2	-	1 (33)	1	3
5	4 (15)	-	4	23 (85)	-	23	27
6	27 (42)	-	27	38 (58)	-	38	65
7	-	37 (31)	37	-	83 (69)	83	120
8	3 (5)	14 (24)	17	8 (14)	33 (57)	41	58
Total	65 (14)	86 (18)	151	140 (30)	183 (38)	323	474

logical analysis, 44 bulls of different mating types sired the probands. For mating types 1 and 6 we found that 8 out of 17 half-sibs presented a frequency of unfit bulls outside the overall mean frequency (32%), contrasting with mating types 5 and 8 in which just 3 out of 11 half-sibs produced unfit sons at a frequency higher than the mean (data not shown).

Table 4 shows that for the 193 Brangus-Ibagé bulls investigated genealogically the frequency of fit and unfit bulls was similar for the different mating types ( $\chi^2 = 0.046$ ; DF = 1;  $p = 0.831$ ). The same was observed for the carriers of the submetacentric Y chromosome ( $\chi^2 = 0.114$ ; DF = 1;  $p = 0.736$ ). However, in bulls originating from mating type 8 there was a higher frequency of fit bulls carrying the submetacentric Y chromosome and a lower frequency of unfit bulls carrying the acrocentric Y chromosome ( $\chi^2 = 15.327$ ; DF = 1;  $p < 0.001$ ). A half-sib appreciation is difficult in the Brangus-Ibagé breed because there were 43 bulls from mating types 4 and 8 that sired the probands.

## Discussion

The crossbreeding strategies used to obtain synthetic breed bulls (Table 1) provides an ideal model to study the two types of Y chromosome morphology in the same population. Our cytogenetic and genealogical analysis shows that there was no significant direct association between the frequency of reproductively sound bulls and Y chromosome morphology for either of the racial groups. The genealogical data agrees with the cytogenetic analysis, a discrepancy being found for only 7.8% of animals from mating types 6 and 7 which had been produced by a multiple-sire mating system (Table 2), there being no other association between fertility indicators and Y chromosome type.

The data presented in Table 3 shows that Braford bulls suffered a culling rate (32%) higher than that described in the literature for purebred breeds (Johnson *et al.*, 1995; Bruner *et al.*, 1995; Gottschall and Mattos, 1997; Ribeiro Filho *et al.*, 1997), although similar breeding soundness culling rates have been reported for synthetic bulls (Chenoweth *et al.*, 1996; Moraes *et al.*, 1998; Chacon *et al.*, 1999). In contrast to what we found for the Braford

bulls, the reproductive fitness culling rate for the synthetic Brangus-Ibagé bulls was only 17% (Table 4).

The lack of association between the Y chromosome morphology and reproductive soundness suggests that the hypothesis that chromosomal rearrangement directly effects fertility must be rejected. A possible explanation for the high reproductive fitness culling rates for Braford bulls is that this could be related to mating types, as a significant difference in the frequency of unfit bulls carrying an acrocentric Y chromosome was observed. This conclusion is supported by the fact that for the Brangus-Ibagé bulls mating types 6 and 7 were not used to produce the population of these synthetic animals. This is one possible explanation as to why the culling rate for reproductive soundness in Brangus-Ibagé bulls is within the limits of what is considered normal for purebreds.

Table 3 shows that the highest frequency of Braford bulls with semen problems occur with 1/4 Nellore bulls. In mating type 6 the probands (all with acrocentric Y chromosomes) are sired by 1/2 Nellore bulls, contrasting with mating type 7 in which 1/4 Nellore bulls have both types of Y chromosomes because they could be sired by either of the original breeds. The bad semen quality found in some mating types could be due to the presence of an acrocentric Y chromosome because of the absence of the European genotype. The biochemical interactions that result in these negative fertility phenotypes could be due to genetic interactions that underlie complex epistasis (Johnson, 2000). This hypothesis is also supported by the familiar analysis in which only the male parents in mating types 1 and 6 presented higher frequencies of unfit sons.

Most probably a fraction of unfit bulls in the Brangus-Ibagé breed was due to reversible classical testicular degeneration caused by poor adaptation of the animals. Alternatively, in the Braford breed the increased rate of unfit bulls (~15%) could have been due to a permanent inherent condition derived from the interaction of genotypes and the different Y chromosome package in both subspecies. Further evidence that this peculiar testicular degeneration is a permanent condition comes from the maintenance of the semen pictures in unfit bulls for at least six months, the different capacity that unfit Braford bulls may have to select abnormal sperm along the epididymis, and that abnormal

**Table 4** - Genealogically determined frequency of the different Y chromosome morphological types found in reproductively fit and unfit Brangus-Ibagé bulls.

Mating type	Y chromosome morphology						Total unfit + fit
	Unfit bulls			Fit bulls			
	Acrocentric number (%)	Submetacentric number (%)	Total unfit	Acrocentric number (%)	Submetacentric number (%)	Total fit	
4	-	4 (22)	4	-	14 (78)	14	18
8	9 (5)	21 (12)	30	34 (19)	111 (64)	145	175
Total	9 (4)	25 (13)	34	34 (18)	125 (65)	159	193

spermatogenesis in these animals appears after the meiotic divisions and during sperm maturation (Horn *et al.*, 2002a, b; Horn *et al.*, 2003).

Further research is needed to confirm the results obtained in this study because different factors can cause bulls to have low quality semen, with synthetic breeds being specifically prone to additive and heterotic gene effects which can cause disparity in distinct genotype combinations. The economic losses associated with the high reproductive soundness culling rates of bulls of some synthetic breeds means that it is well worthwhile recommending that 1/4 Zebu females, or 1/4 Zebu males that were not originated from European parents, should not be used to produce 3/8 Zebu bulls because if producers could sell 10-15% more fit bulls their net income would increase by about 5%.

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