PROSPECTIVE STUDY OF THE EFFECTS OF THE 1/29 ROBERTSONIAN TRANSLOCATION UPON THE FERTILITY OF MARCHIGIANA MALES

RITA DE CASSIA STOCCO DOS SANTOS
Pesquisador Científico III
Instituto Butantan

IOSHIHIRO OHTSUBO
Professor Assistente
Faculdade de Medicina Veterinária e Zootecnia da USP

JANETE WALTER MOURA
Pesquisador Científico I
Instituto Butantan

NELSON HENRIQUE CARVALHO DE CASTRO
Médico estagiário
Instituto Butantan

VALQUIRIA HYPPOLITO BARNABE
Professor Adjunto
Faculdade de Medicina Veterinária e Zootecnia da USP

JOSE ANTONIO VISINTIN
Professor Assistente Doutor
Faculdade de Medicina Veterinária e Zootecnia da USP

CLOVIS DE ARAUJO PERES
Professor Adjunto
Instituto de Matemática da USP

WILLY BEÇAK
Pesquisador Científico VI
Instituto Butantan

SANTOS, R.C.S.; OHTSUBO, I.; MOURA, J.W.; CASTRO, N.H.C.; BARNABE, V.H.; VISINTIN, J.A.; PERES, C.A.; BEÇAK, W. Prospective study of the effects of the 1/29 Robertsonian translocation upon the fertility of Marchigiana males. Rev. Fac. Med. Vet. Zootec. Univ. S. Paulo, 26(1): 111-120, 1989.

SUMMARY: The effects of the 1/29 translocation on fertility of Marchigiana males were investigated on a prospective study. We have compared normal Nelore females artificially inseminated by an heterozygote Marchigiana sire (experimental group) with those resulting from a normal male insemination (control group). We could observe: 1. higher mean number of A.I./conception in the experimental group; 2. significant reduction of pregnant females at first service, in the experimental group; 3. no difference in the period between the A.I.(s) or in obortion rates, in both groups; 4. F1 obtained from experimental group were analysed and the 1/29 distribution does nor differ from 1:1. The reduction of fertility may be due to zygotic loss and/or production of aneuploid gametes. We have performed a meiotic and spermatic study in two heterozygote 1/29 translocated males resulting from Marchigiana x Nelore crossbreed. We found alterations in the spermatic characteristics of the heterozygote, mainly reduction of sperm concentration/mm^3. Meiotic analysis of a translocated male shows no association between any autosome and the sex vesicle.

UNITERMS: Reproduction of cattle; Fertility; Artificial insemination; Chromosome translocation

INTRODUCTION

The 1/29 translocation in cattle is well known. The translocation was described by GUSTAVSSON & ROCKBORN, 14 and since then, it was intensively studied, but mainly by retrospective analysis performed in Artificial Insemination (A.I.) centers. Prospective studies involving translocated animals are not known in the crossbreed Nelore x Marchigiana.

The main effect of this chromosomal aberrations occurs at the reproductive level, reducing the fertility of translocated males (4,6,9) and of daughters of translocated sires that were detected carriers (2,12,17,22,25) or that has not been karyotipically analysed. DYRENDAHL & GUSTAVSSON, 6 developed a study using SRB bulls unselected for fertility, comparing 45 chromosomically normal bulls to 20 translocated ones. The authors reported that sexual functions such as libido and serving ability were found to be quite normal in 1/29 carrier bulls, and they also detected small reduction in the concentration of semen and in total number of spermatozoa with normal wave motion and mobility. FRANCK et alii, 9 analysed the fertility in five 1/29 carrier bulls from the Charoles breed showing decrease of annual collected semen, spermatic motility and non-return rate of the females.

Meiotic studies in heterozygous sires revealed few aneuploid cells in MII, as compared to normal karyotypes (10,19,21).

KING et alii, 15,16 reported aneuploid karyotypes (61 chromosomes probably trisomic for chromosome 1) in embryos recovered from superovulated females inseminated with heterozygote semen, between 1 and 7 days. POPESCU, 23 in a study of 13 days embryos reported two embryos with 59 chromosomes and probably lacking one chromosome 1.

The present report concerns a prospective study upon the effects of the 1/29 translocation on fertility of translocated Marchigiana. CARRARA et alii, 5 detected 1/29 carriers among the Marchigiana sires that have been imported from Italy by A.I. centers in Brazil, to cross with Nelore females. In this report we investigate the fertility of a Marchigiana sire throughout A.I. of normal Nelore females, and the characteristics of the spermatic aspects of the translocated F1.

MATERIAL AND METHODS

We inseminated 50 normal females (Bos taurus indicus) after induction of estrus cycle synchronization, with prostaglandin F_2 alfa (PGF_2). The females were inseminated with semen from a Marchigiana heterozygote (Bos taurus taurus), 0 - 8 hr after onset of estrus. The same method, described by BARNABE et alii, 1 was performed on the control group. The control group was composed of 1135 normal Nelore females, that were inseminated in similar conditions with semen from a normal Marchigiana sire.

All the females (experimental and control groups) were synchronizated at once, and a second PGF_2 aplication was performed 11 days later, to those that did not present estrus signs (1).

Both groups were constituted by females that had one calving. The age distributions were similar, respectively 3 ± 0,23 years for the control animals and 3 ± 0,35 years for the experimental females.

The data obtained was compared statistically: - mean number of A.I. for conception by use of t/student test; - rate of pregnant females at first service by comparison of two proportions; - periods between A.I.(s) by t/student test; distribution of the translocation among F1 Marchigiana x Nelore products by X^2 test.

The cytogenetic analysis was performed in preparations obtained from short-term peripheral lymphocyte cultures (7), using G (26) and C (28) banding techniques.

The spermatic evaluation was **performed on a translocated male product, between 14 and 25 months** of age, in 10 monthly collections. We recorded the volume of semen, sperm concentration and morphology, according to the methods described in BLOM, 3; LAGERLOF, 18 and MANN, 20. As control we used the values considered normal for this crossbreed by the A.I. centers which allowed the use of the sires as semen donors, in comparable ages. The collections were done under similar conditions and from donors of the same age of the B. taurus/B. indicus F1 and we have compared the data by analysis of variances. Meiosis was studied in a 18 month male, under conditions similar to those that the animal used for spermatic analysis was subject to. After castration under local anesthesia, the cytologic preparations were obtained according to the methods described by EVANS et alii, 8. The frequencies of the gamete types detected in MII were statistically compared by means of test of Pearson and a likehood statistical test.

RESULTS

Tab. 1 presents the comparison between the mean number of A.I.(s) for conception in the experimental group and in the control one, also with the rates of pregnant females at first service and mean periods between A.I.(s) The mean number of A.I.(s) for conception in the experimental group (2.86) was higher than that found in control group (1.29) **($P \leq 0.01$). Comparing the** rates of conception, 24% in the experimental group and 81% in the control group, the experimental group showed reduction of pregnant females at first service ($P \leq 0.01$). We found no differences in the period between A.I.(s) between the experimental and the control groups, i.e., 20.6 ± 1.5 days and 20.2 ± 1.1, respectively ($P \leq 0.01$).

Tab. 2 shows the distribution of the 1/29 translocation among the 50 products obtained from the experimental group. There was not significant difference in sex ratio (23 females: 27

males) ($P \leq 0.05$) and in the ratio translocated to normal animals (20 translocated: 30 normal, $P \leq 0.05$). We found a significant difference ($P \leq 0.05$) in the proportion translocated females: normal females (7:16).

Tab. 3 presents the data from spermatic evaluation of a 1/29 translocated Marchigiana x Nelore sire. Comparing these results with the normal control we found a significant reduction of sperm concentration/mm ($P \leq 0.05$).

The meiotic study allowed us to analyse the different meiotic phases (Fig. 1). No association was detected between any autosome and the sex vesicle (Fig. 1c and 1d). The analysis of 96 cells in the diplotene revealed the presence of a chiasma in the centromeric region of the trivalent.

Different configurations may be assumed by the 1/29 trivalent in diakinesis (Fig. 2).

Tab. 4 presents the chromosome counts of 21 cells analysed in MII. Through a statistical X^2 test of Pearson ($P \leq 0.05$) we could verify that the frequencies of the gamete types detected are equivalent. Considering the types that could be formed by meiotic segregation of the 1/29 trivalent, we verified absence of some types. Using a likehood statistical test, we confirmed a significant alteration ($P \leq 0.05$) when compared to the expected gamete proportion.

DISCUSSION

Artificial Insemination (A.I.) in cattle and the large dissemination of carriers of chromosomal abnormalities led to a rapid spread of the 1/29 translocation through the world.

In Brazil, this abnormality was introduced by importation of affected males, specially from the Marchigiana breed, by A.I. centers (5,21).

There are many reports on the fertility reduction in carriers of the 1/29 translocation. The main effect that has been reported is an increase in the non-return rate in females, daughters of translocated sires (2,10,11,12,17,21,25) that have been or not karyotyped. The studies developed about fertility in male carriers reported normal characteristics (14), except by small but significant decrease in the number of spermatozoa in the first ejaculate (6).

In this report, we detect a significant reduction of the fertility in a heterozygous translocated Marchigiana bull through a higher number of A.I.(s) for concepton in the Nelore females inseminated with its semen and consequent decrease of the rate of pregnant females at first service. Considering that there was no alteration in the period between A.I.(s), obtained from the groups of females inseminated with normal or translocated sires, two alternative hypothesis may explain these results:

1. early elimination of zygotes, or
2. inviability of aneuploid gametes.

The cytogenetic studies that have been performed on early embryos recovered from females inseminated with a translocated sire revealed aneuploid karyotypes (15,16,24). Regarding the absence of reports about abortion, stillbirths or aneuploid products in 1/29 carriers, the first hypothesis seems to be favored.

We performed a spermatic analysis in a male translocated product and showed a reduction in the sperm concentration/mm^3, that would normally prevent the acceptance of the animal in A.I. centers. It is interesting to compare this fact with the data reported by GUSTAVSSON, 10 in his study upon the spermatic characteristics of 1/29 translocated bulls from A.I. centers, in Sweden, and to those obtained from DYRENDAHL & GUSTAVSSON, 6 which come from translocated sires not selected for A.I. centers. While GUSTAVSSON, 10 did not refer any abnormality in the spermatic analysis he performed, the animals that he studied with DYRENDAHL & GUSTAVSSON, 6 presented spermatic alterations similar, to the ones we describe here. We may consider that the selection in the animals studied by GUSTAVSSON, 10 was very efficient, in the sense that the general high standard genetic characteristics of the sires neutralize the negative low fertility characteristics due to the translocation.

At the meiotic level, although our sample is small, we show that the chromosome count frequencies in MII are different from the expected ones. This fact may be due to mechanisms of meiotic drive through a preferencial orientation of the trivalent in the MI spindle. Although we have great difficulties to obtain a larger sample of MII cells, considering the reduced number of reports about meiotic studies in 1/29 carriers and the significant different frequencies of the gamete types that we formed, we judge actually useful the report.

Frequencies of 1/29 carriers lower than 50% were found among daughters of heterozygous bulls (10,17,27). GUSTAVSSON, 10 correlated this fact to elimination through selection due to sterility of the translocated females but his data here obtained from retrospective analysis of A.I. center reports. Yet, KOVACS & CSUKLY, 17 report

50% of 1/29 female carriers in a survey of 302 daughters of heterozygous sire. Our sample is very limited, but we want to emphasize the significant difference found, when compared to the expected ratios. In our small sample of MII cells, we could not detect X, t 1/29 gametes, which may indicate some kind of selection reducting the 59,XX,t 1/29 karyotype among F1.

Our results, about spermatic and meiotic analysis in 1/29 male carriers, are supporting evidences for the etiologic action of not viable aneuploid gametes reducing the fertility in 1/29 sires. However, these data are not enough to explain the great reduction of fertility we detected. Apparently anueploid embryos and not viable gametes may be concomitant, reducing the fertility in 1/29 animals.

The reduction of fertility shows the deleterious effect of the 1/29 translocation. This effect leads to consistent increase in the costs of the insemination process, such as amount of semen to be used and the time consumed to obtain the product.

ACKNOWLEDGEMENTS: The authors would like to regret O.P. Ferraz and L.A.T. Dias for technical colaboration and M.E. Barbosa Silva for editorial help.

SANTOS, R.C.S.; OHTSUBO, I.; MOURA, J.W.; CASTRO, N.H.C.; BARNABE, V.H.; VISINTIN, J.A.; PERES, C.A.; BEÇAK, W. Estudo prospectivo dos efeitos da translocação Robertsoniana 1/29 sobre a fertilidade de machos Marchigiana. Rev. Fac. Med. Vet. Zootec. Univ. S. Paulo, 26(1):111-120,1989.

RESUMO: O presente estudo refere-se aos efeitos da translocação 1/29 sobre a fertilidade de bovinos machos Marchigiana, de forma prospectiva. Comparamos fêmeas Nelore normais, inseminadas artificialmente com sêmen de touro Marchigiana heterozigoto (grupo experimental) com aqueles resultantes de inseminação com sêmen de touro normal (grupo controle). Os resultados revelaram: 1º nº médio significantemente aumentado de Inseminações Artificiais/concepção, no grupo experimental; 2. redução significante de fêmeas prenhes ao 1º serviço, no grupo experimental; 3. comparando-se os grupos experimental e controle, não se verificou diferença nas taxas de aborto ou no período entre inseminações; 4. a distribuição de translocados 1/29 não diferiu da proporção 1:1, dentro da F1 obtida do grupo experimental. A redução de fertilidade pode ser devida a perda zigótica e/ou produção de gametas aneuplóides. Desenvolvemos estudos meióticos e espermáticos em 2 touros heterozigotos 1/29, resultantes do cruzamento Nelore x Marchigiana. Pudemos verificar alterações nas características espermáticas do heterozigoto, principalmente redução da concentração de espermatozóides/mm^3. A análise meiótica de um macho F1 translocado mostrou não haver associação entre autossomos e a vesícula sexual.

UNITERMOS: Reprodução, bovinos; Fertilidade; Inseminação artificial; Cromossomos, translocação

TABLE 1 - Mean number of A.I. for conception in the control group (females inseminated with normal semen) and in the experimental group (females inseminated with heterozygous translocated sire semen)

	No. FEMALES	MEAN NUMBER OF A.I./CONCEPTION	CONCEPTION AT FIRST A.I.(%)	PERIOD BETWEEN A.I (DAYS)	ABORTION(%)
Control group ...	1135	1.29 ± 0.39	81	20.2 ± 1.1	0.85
Experimental group	30	2.86 ± 1.69	24	20.6 ± 1.5	-

TABLE 2 - F1 Marchigiana X Nelore products from A.I. with a heterozygous 1/29 translocated Marchigiana sire (experimental group).

KARYOTYPE	NUMBER OF PRODUCTS	%
60, XY	14	28
59, XY, t 1/29	13	26
TOTAL	27	54
60, XX	16	32
59, XX, t 1/29	7	14
TOTAL	23	46
TOTAL NORMAL	30	60
TOTAL 59, t 1/29	20	40
TOTAL	50	100

TABLE 3 - Spermatic evaluation of a 1/29 translocated Marchigiana X Nelore sire.

NUMBER OF COLLECTION	VOLUME	MOTILITY (%)	CONCENTRATION MM X 1000	MAJOR DEFECTS (%)	MINOR DEFECTS (%)	TOTAL OF DEFECTS (%)
1	12.0	50	120	21.0	11.5	32.5
2	12.0	30	80	21.5	11.5	33.0
3	10.0	10	160	15.0	10.5	25.5
4	5.0	50	320	18.0	10.0	28.0
5	8.0	60	200	18.0	10.0	18.0
6	13.0	30	160	22.0	11.5	33.5
7	10.0	60	72	12.5	12.0	24.5
8	8.0	40	72	18.5	10.5	29.0
9	5.0	60	560	9.0	13.5	27.5
10	6.0	60	320	12.0	11.0	28.0
X	8.9 ± 2.8	45 ± 16.3	260 ± 45.6	16.75 ± 4.2	11.20 ± 1.0	29.95 ± 2.88
CONTROL	5 - 4	60	300-2000	20	25	30

TABLE 4 - Karyotypes of cells analysed in MII from F1 male heterozygous 1/29 translocated product.

KARYOTYPE	NUMBER OF CELLS	(%)
Normal	8	38.1
30, Y	5	23.8
30, X	3	14.3
Balanced	8	38.1
29, X + t 1/29	5	23.8
29, Y + t 1/29	3	14.3
TOTAL (a)	16	76.2
Unbalanced		
30, Y + t 1/29 + 1	3	14.3
30, X + t 1/29 + 1	0	-
30, Y + t 1/29 + 29	0	-
30, X + t 1/29 + 29	0	-
29, Y + 1, - 29	0	-
29, X + 1, - 29	2	9.5
29, Y - 1, + 29	0	-
29, X - 1, + 29	0	-
TOTAL	5	23.8
TOTAL (a + b)	21	100

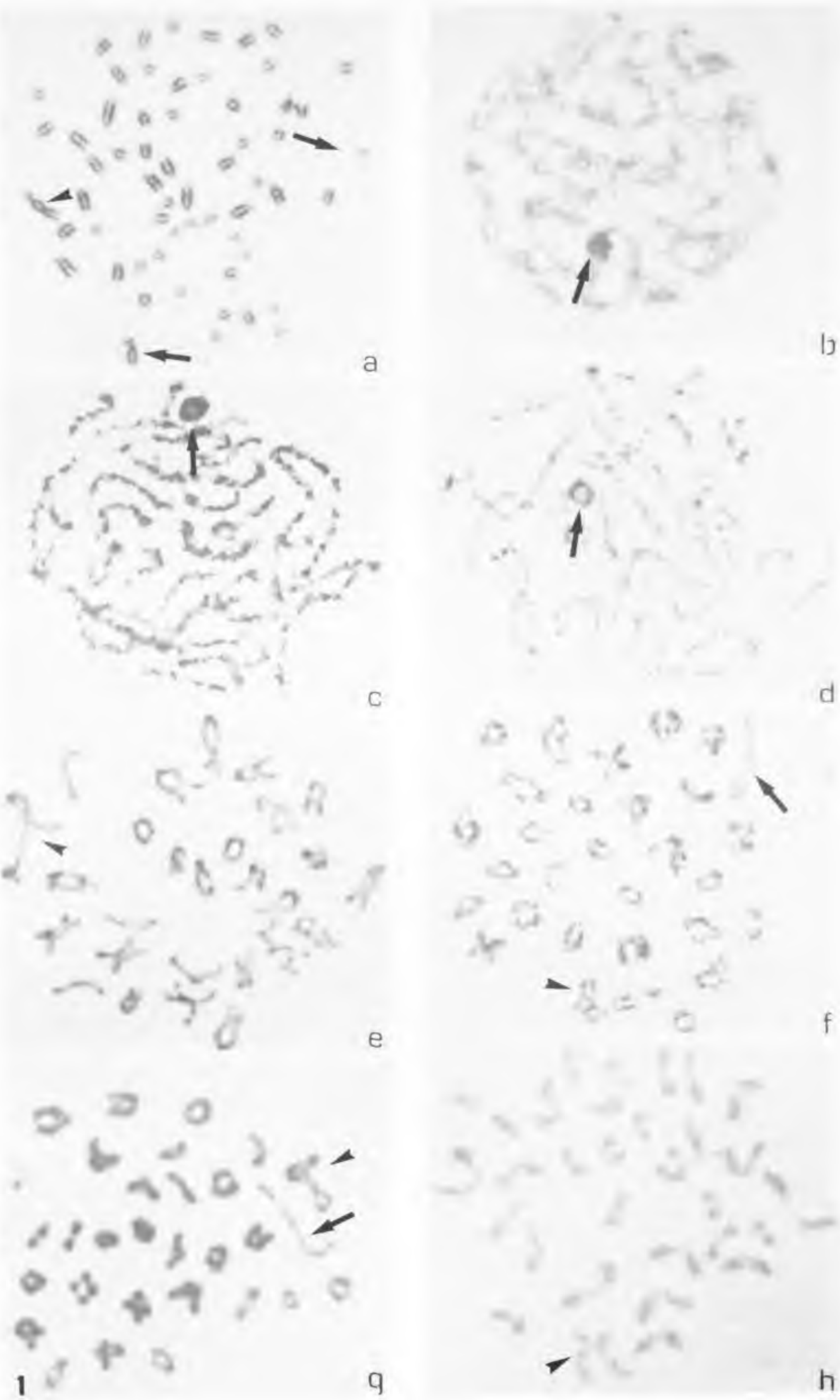
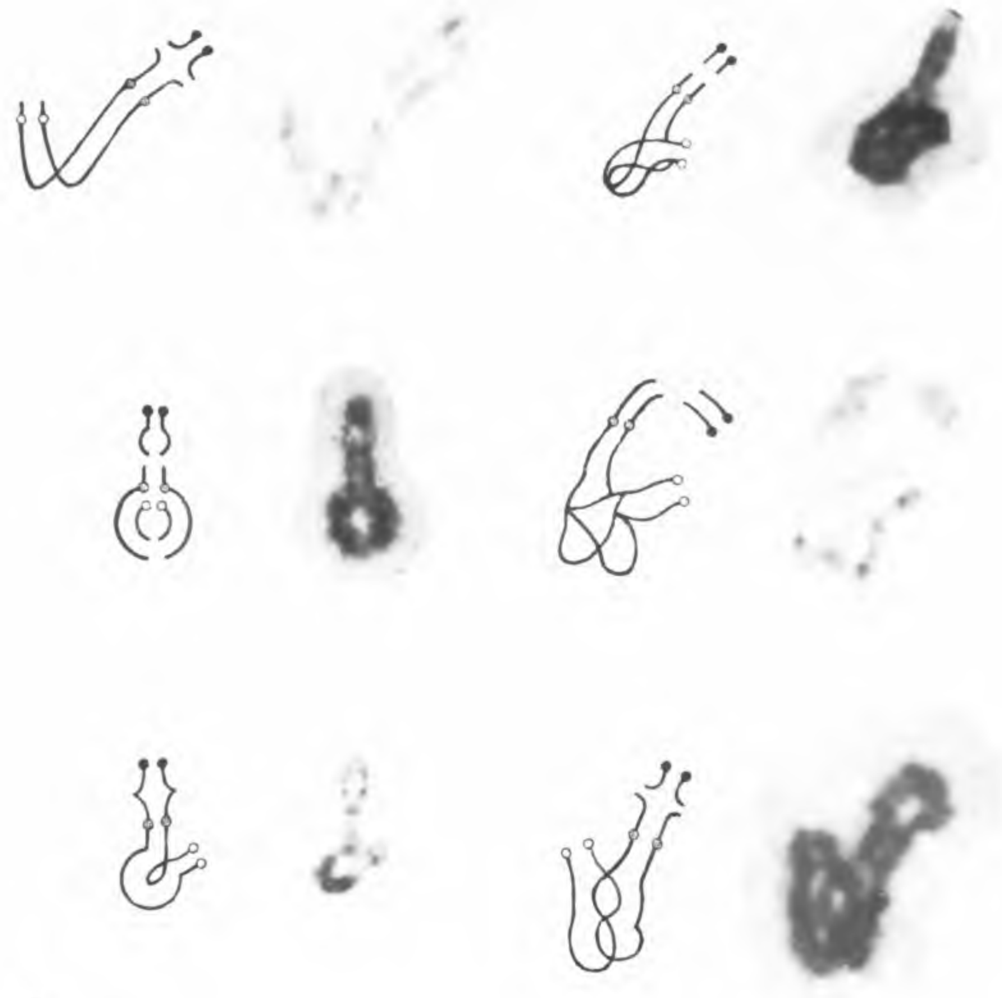


FIGURE 1 – Different phases in meiosis of 1/29 translocated heterozygous male carriers: a – spermatogonium metaphase; b – leptotene; c – zigotene; d – pachytene; e – diplotene; f – diakinesis; g – metaphase I; h – metaphase II; 1/29 translocated chromosome; → XY pair.



2

FIGURE 2 – Different configurations assumed by the 1/29 trivalent in diakinesis.

REFERENCES

1 - BARNABE, R.C.; BARNABE, V.H.; MUCCIOLO, R.G. Utilização da prostaglandina F2 alfa (PGF2 alfa) na sincronização do ciclo estral em bovinos. I. Efeitos segundo o local de deposição nos órgãos genitais. Rev. Fac. Med. Vet. Zootec. Univ. S. Paulo, 13:195-207, 1976.

2 - BETANCOURT, A. Fertility data from daughters of a Santa Gertrudes bull with chromosomal translocation 1/29. Beitr. trop. Landwirtsch. Vet.-Med., 23: 77-80, 1985.

3 - BLOM, E. Ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermiogram. Medd. St. vet. Serumlab., 541:125-139, 1972.

4 - BONGSO, A. & BASRUR, P.K. Chromosome anomalies in Canadian Guernsey bulls. Cornell Vet., 66:476-488, 1976.

5 - CARRARA, M.; BEÇAK, W.; CARRARA, F.E. Anomalia cromossômica em bovinos da raça Marchigiana. In: SIMPOSIO NACIONAL DE REPRODUÇAO ANIMAL, 3., São Paulo, 1978. Anais. p.85-86.

6 - DYRENDAHL, I. & GUSTAVSSON, I. Sexual functions, semen characteristics and fertility of bulls carrying the 1/29 chromosome translocation. Hereditas, 90:281-289, 1979.

7 - ELDRIDGE, F. Bovine lymphocyte methods. Mann Chrom., 23:176-179, 1982.

8 - EVANS, C.P.; BRICHON, G.; FORD, C.E. An air - drying method for meiotic preparation from mammalian tests. Cytogenetics, 3:289-294, 1964.

9 - FRANCK, M.; LAURENT, C.; FROGET, J.; SUREIN, J.M.; COURSAT, F. Chromosomal abnormalities and fertility of carriers. The 1/29 Robertsonian transolocation. In: EUROPEAN COLLOQUES ON CYTOGENETIC OF DOMESTIC ANIMALS, 4., Uppsala, 1980. p.55-61.

10 - GUSTAVSSON, I. Cytogenetics, distribution and phenotypic effects of a translocation in Swedish cattle. Hereditas, 63:68-169, 1969.

11 - GUSTAVSSON, I. Culling rates in daughters of sires with a translocation of a centric fusion type. Hereditas, 67:65-74, 1971.

12 - GUSTAVSSON, I. Chromosomes of repeat breeder heifers. Hereditas, 68: 331-332, 1971.

13 - GUSTAVSSON, I. Distribution and effects of the 1/29 Robertsonian translocation in cattle. J. Dairy Sci., 62:825-835, 1979.

14 - GUSTAVSSON, I. & ROCKBORN, G. Chromosome abnormality in three cases of lymphatic leukaemia in cattle. Nature, London, 203:990, 1964.

15 - KING, W.A.; LINARES, T.; GUSTAVSSON, I. Cytogenetic of preimplantation embryos sired by bulls heterozygous for the 1/29 translocation. Hereditas, 94:219-224, 1981.

16 - KING, W.A.; LINARES, T.; GUSTAVSSON, I.; BANE, A. Presumptive translocation type trisomy in embryos sired by bulls heterozygous for the 1/29 translocation. Hereditas, 92:167-169, 1980.

17 - KOVACS, A. & CSUKLY, S. Effect of the 1/29 translocation upon fertility in Hungarian Simmental cattle. In: EUROPEAN COLLOQUES ON CYTOGENETIC OF DOMESTIC ANIMALS, 4., Uppsala, 1980. p.35-

42.

18 - LAGERLOF, N. Morphologische Untersuchungen über Veranderungen im Spermabild und in den Hoden bei Bullen mit verminderter oder aufgehobener Fertilitat. Acta path. microbiol. scand., (suppl. 19):254, 1934.

19 - LOGUE, D.N. & HARVEY, M.J.A. Meiosis and spermatogesis in bulls heterozygous for a presumptive 1/29 Robertsonian translocation. J. Reprod. Fertil., 54:159-165, 1978.

20 - MANN, T. The biochemistry of semen and the male reproductive tract. London, Mathuen, 1946.

21 - PINHEIRO, L.E.L. Avaliação dos efeitos da translocação Robertsoniana 1/29 sobre a reprodução de bovinos Pitangueiras. Jaboticabal, 1983 (Tese de livre docência - Faculdade de Ciências Agrárias e Veterinárias - Campus de Jaboticabal, UNESP)

22 - PINHEIRO, L.E.L.; FERRARI, I.; LOBO, R.B. Robertsonian translocation in imported bulls utilized at artificial insemination centers in Brazil. Rev. bras. Genét., 2:135-143, 1979.

23 - POPESCU, C.P. A study of meiotic chromosomes in bulls carrying the 1/29 translocation. Ann. Biol. anim., 18:383-389, 1978.

24 - POPESCU, C.P. Cytogenetic study on embryos sired by a bull carrier of 1/29 translocation. In: EUROPEAN COLLOQUES ON CYTOGENETIC OF DOMESTIC ANIMALS, 4., Uppsala, 1980. p.182-186.

25 - REFSDAL, A.O. Low fertility in daughters of bulls with 1/29 translocation. Acta vet. scand., 17:190-195, 1976.

26 - SEABRIGHT, M. The use of proteolytic enzymes for the mapping of structural rearrangements in the chromosomes of man. Chromosoma, 36:204-210, 1972.

27 - STRANZINGER, G.F. & FORSTEN, N. Autosomal chromosomal translocation of Piebald cattle and Brown cattle. Experientia, 32:24-27, 1976.

28 - SUMMER, A.T. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res., 75:304-306, 1972.

Recebido para publicação em: 04/08/88
Aprovado para publicação em: 18/01/89