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Original article

Synaptonemal complex analysis in goats carrying the 5/15 Robertsonian translocation

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Summary – Synaptonemal complexes were analysed by electron microscopy in 2 bucks heterozygous for the 5/15 Robertsonian translocation. The *cis* configuration (free homologous 5 and 15 chromosomes on the same side of the 5/15 translocated chromosome) was found in all 50 cells examined. This feature is considered a prerequisite for the development of balanced gametes. No association between the sex bivalent and trivalent was observed.

meiosis / synaptonemal complex / trivalent / Robertsonian translocation / goat

Résumé – L'analyse du complexe synaptonémique de 2 boucs porteurs de la translocation robertsonienne 5/15. *L'analyse du complexe synaptonémique a été effectuée, en microscopie électronique, chez 2 boucs hétérozygotes pour la translocation robertsonienne 5/15. La configuration cis (chromosomes homologues 5 et 15 situés du même côté du chromosome-transloqué 5/15) a été trouvée dans les 50 cellules examinées. Cette caractéristique est considérée comme une condition préalable au développement de gamètes équilibrés. Aucune association entre le bivalent sexuel et le trivalent n'a été détectée.*

méiose / complexe synaptonémique / trivalent / translocation robertsonienne / chèvre

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INTRODUCTION

Electron microscopic (em) analysis of synaptonemal complexes (SC) in the meiotic cells of domestic animals carrying chromosomal aberrations began with the works of Switonski *et al* (1987) and Gabriel-Robez *et al* (1986).

Karyotype analysis of 2 *Saanen* male goats with $2n = 59$ chromosomes indicated the presence of a submetacentric chromosome. The G-banding technique was used to identify the marker chromosome as a fusion of chromosomes 5 and 15. Little research has been documented concerning SC in goats possessing either normal or translocated chromosomes. To investigate the effect of the 5/15 translocation on the reproductive capacity of the heterozygous animals, SC formation and the behaviour of the trivalent 5/15; 5;15 was studied at the pachytene stage of meiosis.

MATERIALS AND METHODS

Two *Saanen* male goats (*Capra hircus*), both 59, XY, t(5;15) heterozygous for the translocation were studied. Hemi-castration was immediately followed by testicular dissection with a scalpel, in Hank's medium.

The preparations of microspread samples were made according to the technique presented by Solari (1980). A droplet of the testicular cell suspension was added to approximately 5 ml of a 0.5% NaCl hypotonic solution. The spread nuclei were then picked up by touching slides pre-coated with plastic film on the surface of the solution. The slides were immediately immersed in a Coplin jar containing SDS fixative (4% paraformaldehyde and 0.03% SDS, pH 8, adjusted with sodium tetraborate buffer) and incubated for 5 min at room temperature. The slides were then held on the surface of 0.4% Photoflo, pH 8 for 30 s and allowed to air-dry. Silver nitrate staining was performed as described by Howell and Black (1980). Nuclei were selected by light microscopy and covered with 50/75 mesh grids. The plastic film, with the attached nuclei and grids, was detached from the slide by floating on water and collected with resined paper. Under em, the magnification was 1 600 ×. Micrographs were enlarged 5 ×. The SC lengths (mm) were measured individually. Intact nuclei containing a complete SC set were used to construct karyotypes. The normal autosomal bivalents were arranged by decreasing size and aligned by the kinetochore. Each trivalent 5/15, 5 and 15 was put in the position of bivalent 5 (see fig 2 below). The XY bivalent occupied the last position. The trivalent 5/15, 5 and 15 was photographed individually and enlarged 4 200 × for detailed analysis. The position of the kinetochores of the free homologues 5 and 15 was considered *cis* when the free kinetochores 5 and 15 were located on the same side of the 5/15 chromosome, and *trans* when the free kinetochores 5 and 15 were located on opposite sides. In 50 cells containing the sex bivalent, the presence or absence of association of the trivalent 5/15, 5 and 15 and the sex bivalent was noted.

RESULTS

Twenty-seven autosomal bivalents, one autosomal trivalent and the sex bivalent (figs 1 and 2) were observed in spermatocyte preparations from the heterozygous 5/15 translocated buck ($2n = 59$). In all the analysed nuclei (50), the free homologous chromosomes 5 and 15 paired in the *cis* configuration (figs 3 and 4). In the trivalent, the pericentromeric region of the free homologous 5 and 15 was not paired at early pachytene (fig 3). At mid-pachytene, heterosynapsis was observed between the pericentromeric regions of the free homologues 5 and 15. At late pachytene, the synaptic adjustment was complete and the centromeric tips of the free homologues 5 and 15 were completely paired with the translocated corresponding arms (fig 4). The kinetochores, however, did not fuse and stayed in juxtaposition. The trivalent 5/15, 5 and 15 was not observed to be associated with the sex bivalent.

DISCUSSION

In this paper, the trivalent showed the pericentromeric region of the free homologues 5 and 15 not paired at early pachytene. Late synapsis seems to be a characteristic of the trivalents as is described in bovine (Switonski *et al*, 1987), and *Lemur* hybrids (Moses *et al*, 1979). In chinese hamster bivalents, the centromeric region is also the last to form SC (Moses, 1977).

The kinetochore of the free homologues 5 and 15 paired preferentially in the *cis* configuration with their homologue portions in the 5/15 translocated chromosome and were visible throughout the pachytene. Moses *et al* (1979) observed the same phenomenon while studying trivalents in *Lemur* hybrids under em. Data obtained with *Capra hircus* agree with the results of Moses *et al* (1979) who suggested that both acrocentric kinetochores maintain the *cis* configuration independently during synapsis. This excludes the probability of variable pairing faces and leads to the conclusion that a single pairing face on the translocated chromosome determines the plane of SC assembly.

The *cis* configuration was also found in the rodent, *Sigmodon fulviventer*, by light microscopy (Elder and Pathak, 1980) and no fertility reduction was observed.

When the heterozygous buck was crossed with normal homozygous or heterozygous, females, there was no fertility reduction according to Gonçalves (personal communication). These data may be interpreted as an indirect sign of a non-random segregation of the trivalent chromosomes. Moses *et al* (1979) noted that the normal fertility rate observed in *Lemur* hybrids in related to a mechanism which increases the frequency of balanced gametes. In addition, such a mechanism would specify the symmetric arrangement of free homologues of the trivalent at pachytene.

The hypothesis which suggests that the symmetry presented by the translocated chromosomal arms may influence the configuration, either *cis* or *trans*, should be considered. It may be well accepted that the more asymmetric the translocation arms, as in bovine translocation 1/29, the greater the probability of observing the *trans* configuration. Previous studies which present trivalents in the *cis* configuration only support this assumption since cattle with t(4;8) (Bouvet *et al*, 1989),

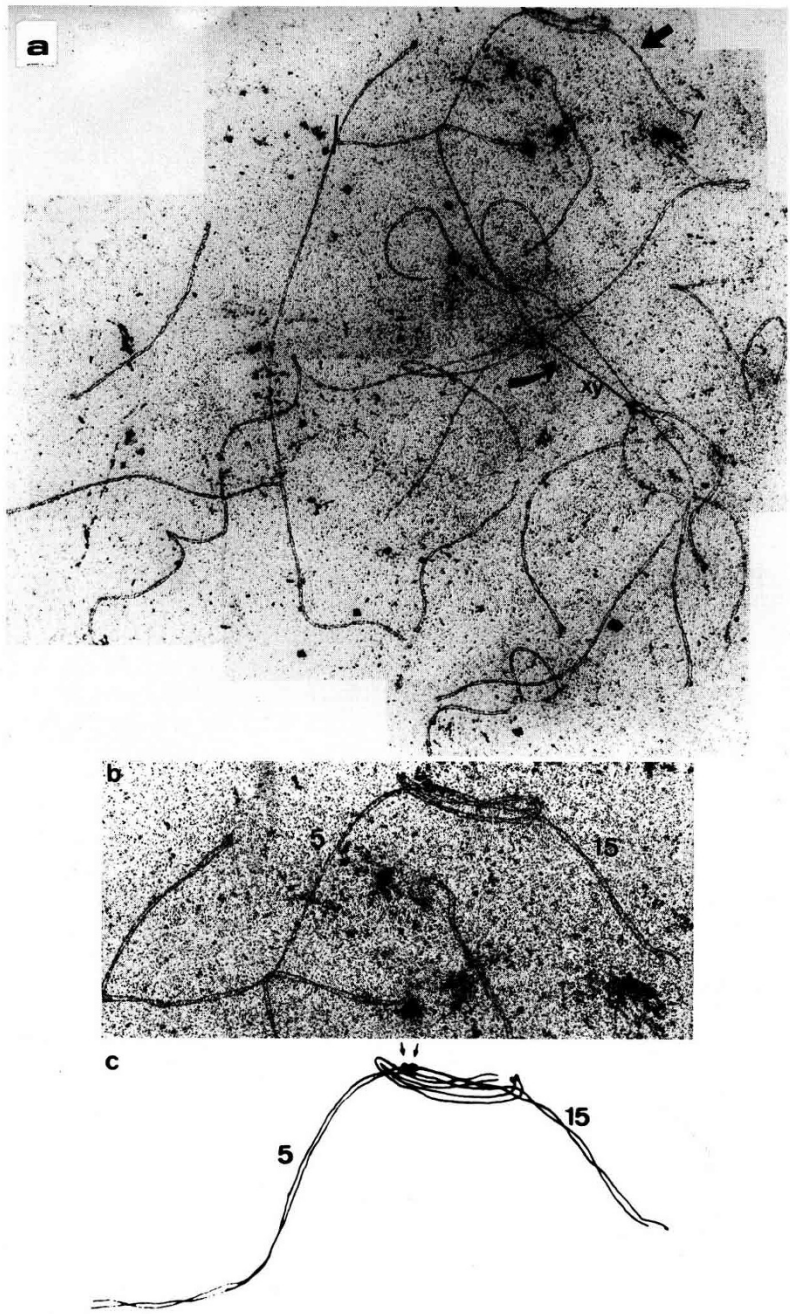


Fig 1. a) Surface-spread nucleus of a spermatocyte from the 5/15 Robertsonian translocation goat showing 29 elements, including one autosomal trivalent (straight arrow), 27 autosomal bivalents, and the XY bivalent (curved arrow). Note that the trivalent complex is the longest and that there is no association with the XY bivalent. **b)** The trivalent at higher magnification. Notice an autosome over the pericentromeric region of the trivalent. It is possible to identify the trivalent. **c)** Schematic drawing of the trivalent. Arrows indicate the position of the kinetochores. Magnification: **a)** 6 250 ×, **b)** 8 000 ×.

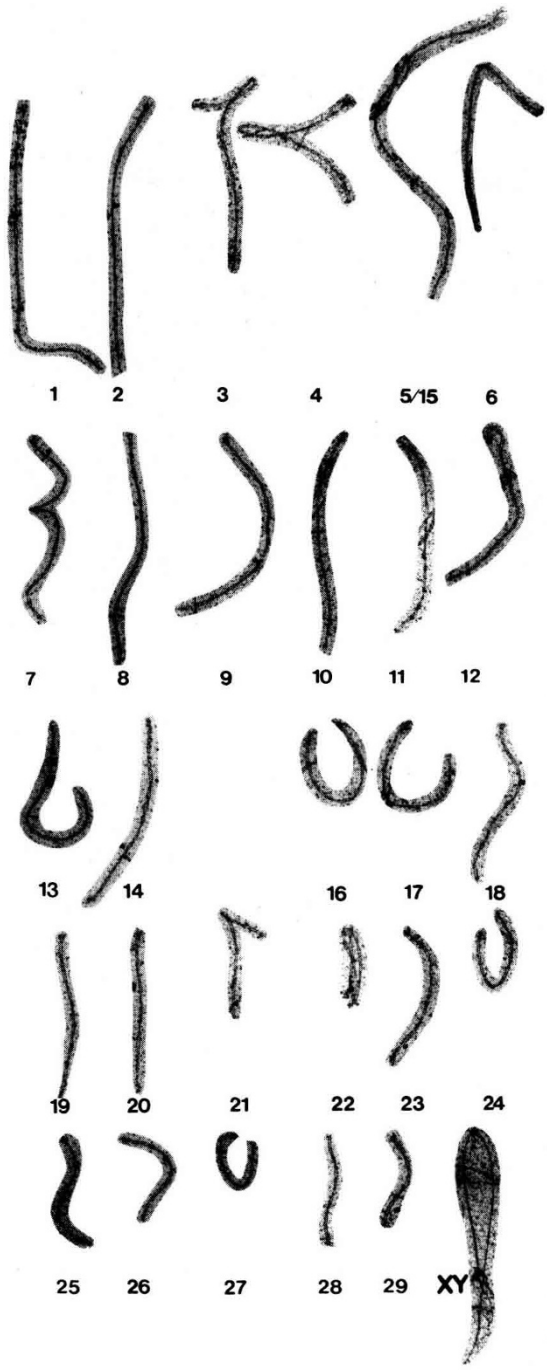
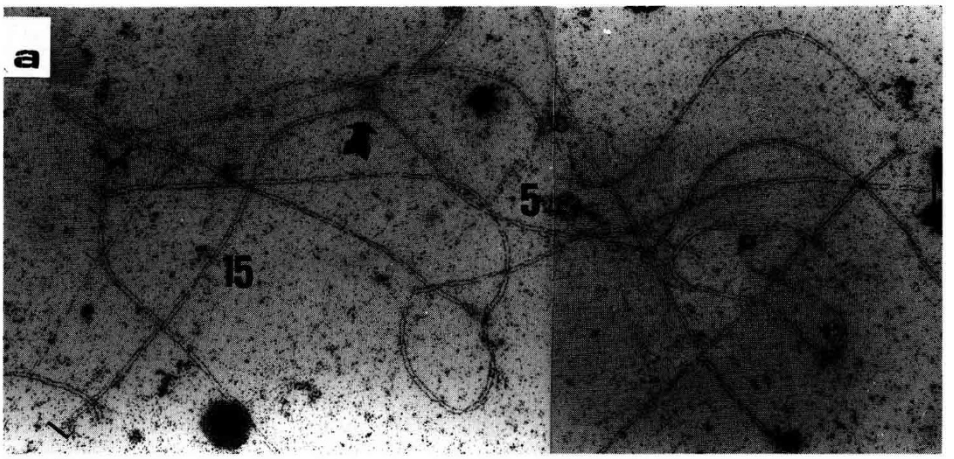


Fig 2. SC karyotype of the cell shown in figure 1. The autosomal bivalents are arranged in order of decreasing size and aligned by the kinetochore. Magnification: 6 600 ×.



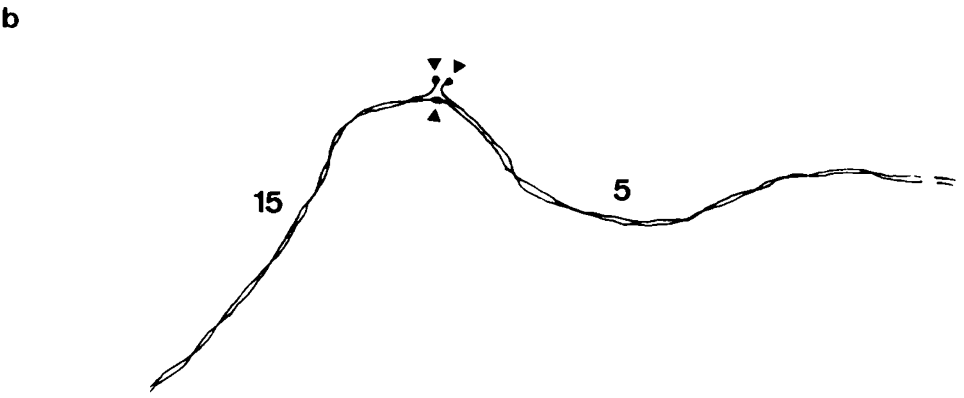


Fig 3. a) Trivalent configuration in a spermatocyte from the buck heterozygous for a 5/15 Robertsonian translocation. Chromosomes 5 and 15 and the 5/15 translocated chromosome are unpaired in the pericentromeric region at early pachytene stage. The kinetochores of the free homologues 5 and 15 are in the *cis* position. Magnification: 8 000 ×. **b)** Schematic drawing of the trivalent. Arrows indicate the position of the kinetochore.

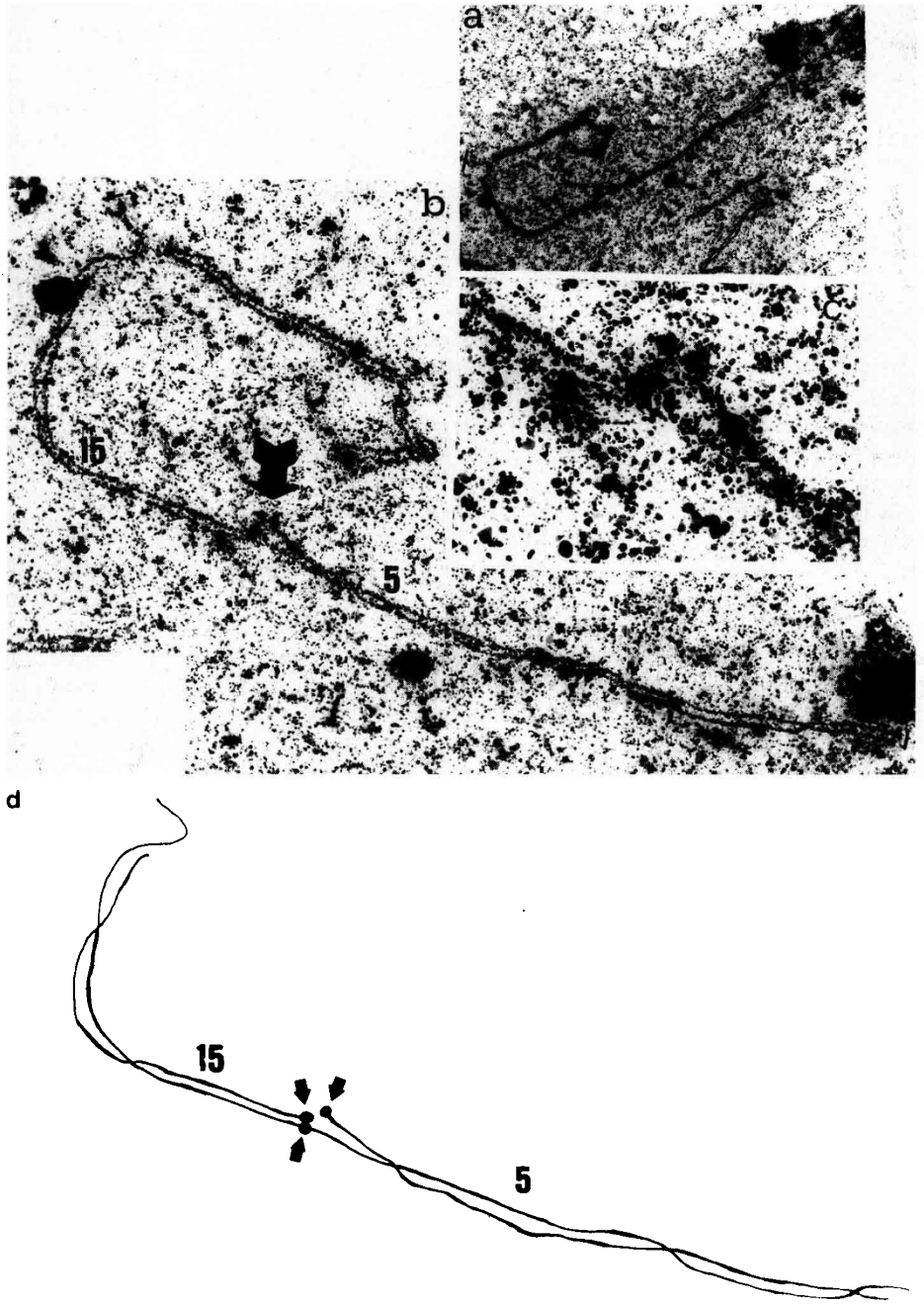


Fig 4. a) Chromosomes 15 is completely paired with its corresponding arm in the 5/15 translocation, but chromosome 5 is still unpaired proximally. The trivalent **(b)** and the kinetochore region **(c)** at higher magnification. **d)** Schematic drawing of the trivalent. Arrows indicate the position of the kinetochores. Magnification: **a)** 6 600 ×; **b)** 17 500 ×; **c)** 41 600 ×.

rats with t(10;11) (Elder and Pathak, 1980) and humans with t(13;14) (Luciani *et al*, 1984) present very symmetric translocated chromosomal arms. Perhaps the *trans* configuration becomes less probable as observed if the chromosomal arm ratio (long arm/short arm) approaches 1.00.

There are morphological differences among the trivalents that result from centric fusion in bovines, humans, rodents and caprines, and they may influence the association with the sex bivalent. The variation in the position of the acrocentric chromosomes neighbouring the sex vesicle produces free parts with a stronger tendency to pair with the sex chromosomes. This variable tendency could explain the variation which occurs in the phenotypic expression of fertility in men with centric fusion (Johannisson *et al*, 1987). Bouvet *et al* (1989) suggest that the absence of association between the sex bivalent and the autosomal trivalent could explain the normal spermatogenesis presented by cattle carrying chromosomal translocations, while mice and humans carrying similar translocations are infertile due to the presence of such associations.

In the present case, the trivalent was not observed in association with the sex bivalent. In studies on humans (Johannisson *et al*, 1987) and mice (Forejt *et al*, 1981) it was assumed that the reciprocal and Robertsonian translocations may bring about either sterility or infertility if the unpaired autosomes, or autosomal segments, pair with the X chrosomome. The authors have suggested that the proximity of the autosomal segment with the X chromosome may interfere with its inactivation process by harming the germinative cell. This kind of association has often been found in infertile men with balanced Robertsonian translocation between chromosomes 13 and 14 (Luciani *et al*, 1984) and 14 and 21 (Rosenmann *et al*, 1985). In all of the micrographs observed, the phase of unpairing in the pericentromeric region of the acrocentric 5 and 15 was restricted to the beginning of pachytene when the Y chromosome had also started pairing with the X chromosome.

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