

# Ultrastructure of the germinative epithelium in a goat with a 5/15 Robertsonian translocation

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## ABSTRACT

The spermatogenesis of two goats bearing a 5/15 Robertsonian translocation was investigated by electron microscopy. There was no dramatic change in the morphology of the cells of the spermatic line. All cells of the seminiferous epithelium seemed quite normal at the ultrastructural level. However a certain disturbance in the cell localization and some morphological abnormalities involving nuclear structure were seen. Spermatocytes and spermatids normal in appearance were observed, but a great number of cells presented two or more nuclei. These cells were frequently seen to become degenerated during spermatogenesis. We believe that unbalanced spermatocytes degenerate during the process and only some spermatocytes succeed in fertilizing gametes.

## INTRODUCTION

The Robertsonian translocation of centric fusion is a special type of chromosomal translocation which takes place between non-homologous acrocentric chromosomes. It is the most widespread type of translocation in mammals.

The normal karyotype of the caprine species has 60 acrocentric chromosomes. In some animals submetacentric or metacentric chromosomes may appear as a result of centric fusion. In this case the number of chromosomes is reduced to 59 in the heterozygotes, and 58 in the homozygotes. In the bovine species, a number of heterotriplets are formed, which leads to a certain degree of chromosomal nondisjunction, resulting in aneuploid gametes and consequently in reduction of fertility. In the caprine species heterozygote trivalents are also formed, but apparently chromosome segregation proceeds in a quite regular manner, resulting in the production of balanced fertile gametes.

Cytogenetic analysis of a male Saanen goat, with  $2n=59$  chromosomes, using the G band technique (Oriani, 1987), showed the presence of a submetacentric chromosome, product of a centric fusion between acrocentric chromosomes five and 15. Gonçalves (1988), observing the results of controlled mating of the translocation carriers and comparing the data obtained with those of nontranslocated animals, concluded that in the 5/15 heterozygous animals there was no reduction of fertility.

An ultrastructural study of the 5/15 translocation seminal epithelium of heterozygous goats was performed in order to study germ cell development.

## MATERIAL AND METHODS

Two three-year-old Saanen goats, both carrying a 5/15 translocation in heterozygosis, were studied.

The goats were castrated and small pieces of testicular tissue were fixed in 2.5% glutaraldehyde in

0.1M sodium cacodylate buffer, pH 7.2, and post-fixed in 1% osmium tetroxide in the same buffer. Inclusion was made in Epon-812, after dehydration in increasing concentration acetone series. This sections obtained with a diamond knife were double stained with uranyl acetate and lead citrate.

## RESULTS AND DISCUSSION

Ultrastructural studies of seminiferous epithelium of 5/15 translocated goats showed some alterations. All cellular types were found, however some disorganization and germ cell displacement in the epithelium was observed (Figures 1A and 1B). The basal lamina appeared irregular and was formed by several sheets of concentric layers (Figure 1A).

The Sertoli cells maintained their elongated form, with nuclei positioned near the basal lamina, and cytoplasmic prolongations, extending towards the seminiferous tubule lumen (Figure 1A).

The spermatogonia were also found beneath the basal lamina and their morphology was entirely normal. They appeared as spherical nucleated cells, containing disperse euchromatin and prominent nucleoli; abundant cytoplasm rich in smooth endoplasmic reticulum, with small mitochondria grouped around masses of an electron-dense material.

The spermatocytes I were characterized by ellipsoidal nuclei containing the synaptonemal complexes, indicative of chromosomal pairing (Figure 2B) (indications of trivalent formations were not observed). Their cytoplasm showed a well-developed Golgi complex and small mitochondria (Figure 2A). Some spermatocytes I appeared multinucleated (Figure 3A), and showed cytoplasmic and nuclear signs of degeneration.

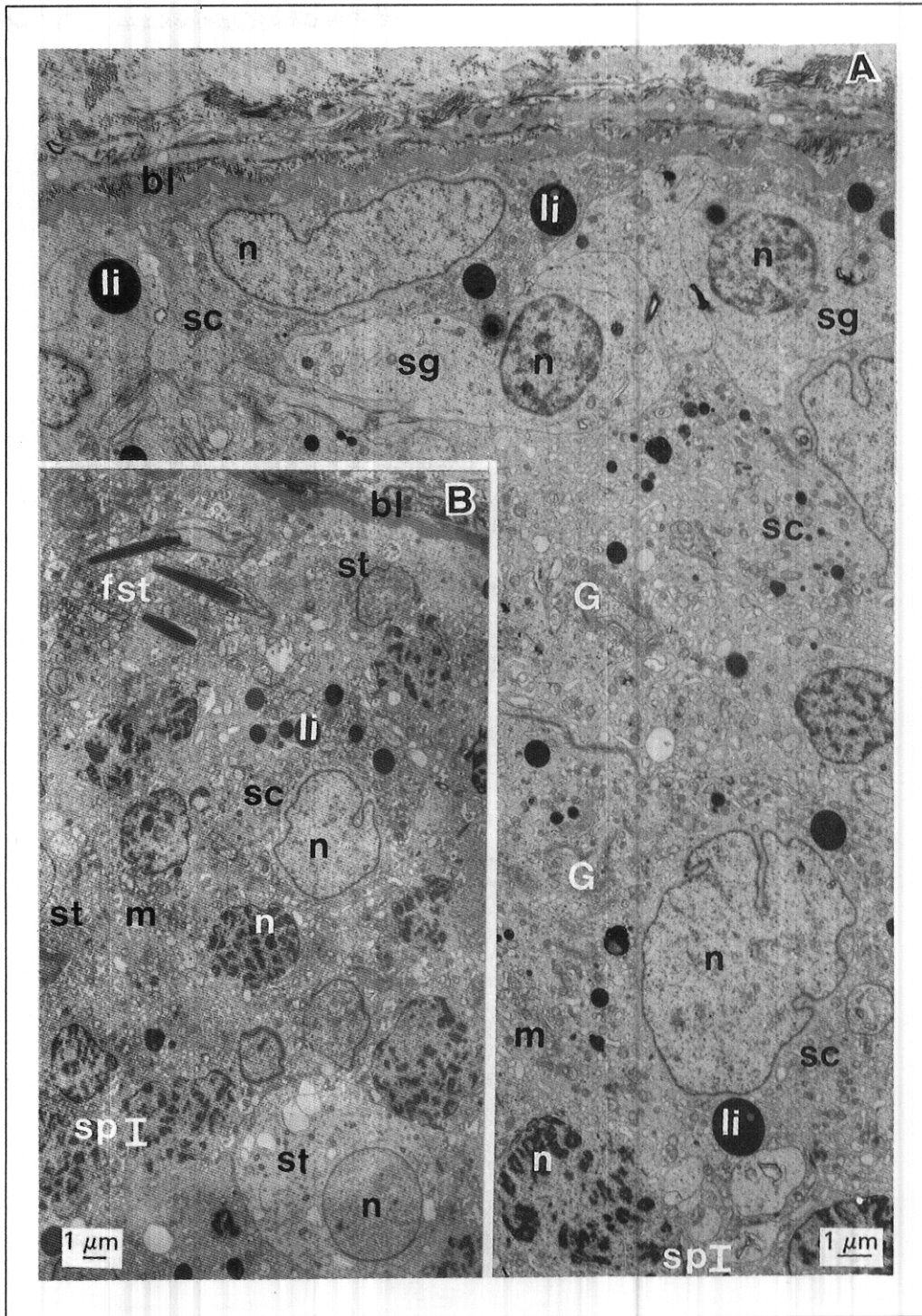
Degenerative changes in the nuclei were characterized by the enlargement of the perinuclear space. Carioteccal leaflets were pulled apart, forming vesicular structures (Figure 3B). Chromosome condensation was increased and sometimes the carioteca appeared broken down. The nuclei of these multinucleated cells were seen to be closely packed at this stage (Figure 3C).

Sometimes spermatids appeared normal but others were multinucleated (Figure 4). Nuclear elongation and flagellum formation were rarely seen in abnormal spermatids (Figure 4B). Nevertheless, a few mature spermatids with two nuclei were observed (Figure 4C). Most of the degenerated multinucleated spermatids showed similar degenerative alterations, i.e. nuclear deformation and cariotecal disruption.

Very few reports of chromosomal abnormalities in goats are found in the literature. Padeh *et al.* (1971) and Gonçalves (1988), through cytogenetic studies among the descendents of carriers of the translocation 5/15, observed that the mating of 59XY,T X 59XX,T gave a proportion of 1:2:1. When this proportion was compared with the expected proportion of 1:6:1, the occurrence of meiotic selection with elimination of the *aneuploid cells during gametogenesis was concluded*, resulting therefore in normal fertility. The same was observed by Bruère (1975) and Chapman and Bruère (1975) in the ovine species, where the presence of translocations did not result in decreased fertility. However, Padeh *et al.* (1971) observed that the proportion of multiple births in the progeny of heterozygotic animals is somewhat lower than normal. When single births were compared to multiple births, it was found that translocated chromosomes are much more frequent in offspring born as singles, whereas the normal chromosome complement appeared more frequently among offspring born in multiple births. This effect might be explained by the particularly high uterine mortality among twinships including at least one heterozygotic or homozygotic translocation. This effect was not observed by Gonçalves (1988). Chapman and Bruère (1975) demonstrated that as in mice, in the heterozygous ovines, the Robertsonian translocation resulted in a large percentage of aneuploid gametes, but the fertility rate of females mated with the carriers remained unchanged.

From the present study, we infer that the aneuploid cells casually formed during spermatogenesis, degenerate before gametic maturation. This idea is shared by Roosen-Runge (1973). The unbalanced karyotype is not transmitted to the descendents, and developmental anomalies of the germ cells occur before their maturation, and lead to death of the carrier cells. A common manifestation of heterozygous translocation carriers is an incomplete pairing of homologous chromosomes, and this had led to the hypothesis that "unsaturated pairing regions of meiosis lead to the death of the spermatocytes in which they occur" (Miklos, 1974). Apparently, only a few cells carrying the aberration can mature. Since a great number of spermatozoa are always produced, neither cellular degeneration during spermatogenesis nor the casual presence of some anomalous mature spermatozoa compromises fertility.

The analysis of synaptonemal complex behavior in a caprine carrier of the same translocation that the 5 and 15 telocentric chromosomes attain complete synapsis with their corresponding arms in the translocated 5/15 (Amaral, 1991). In all samples analysed, the

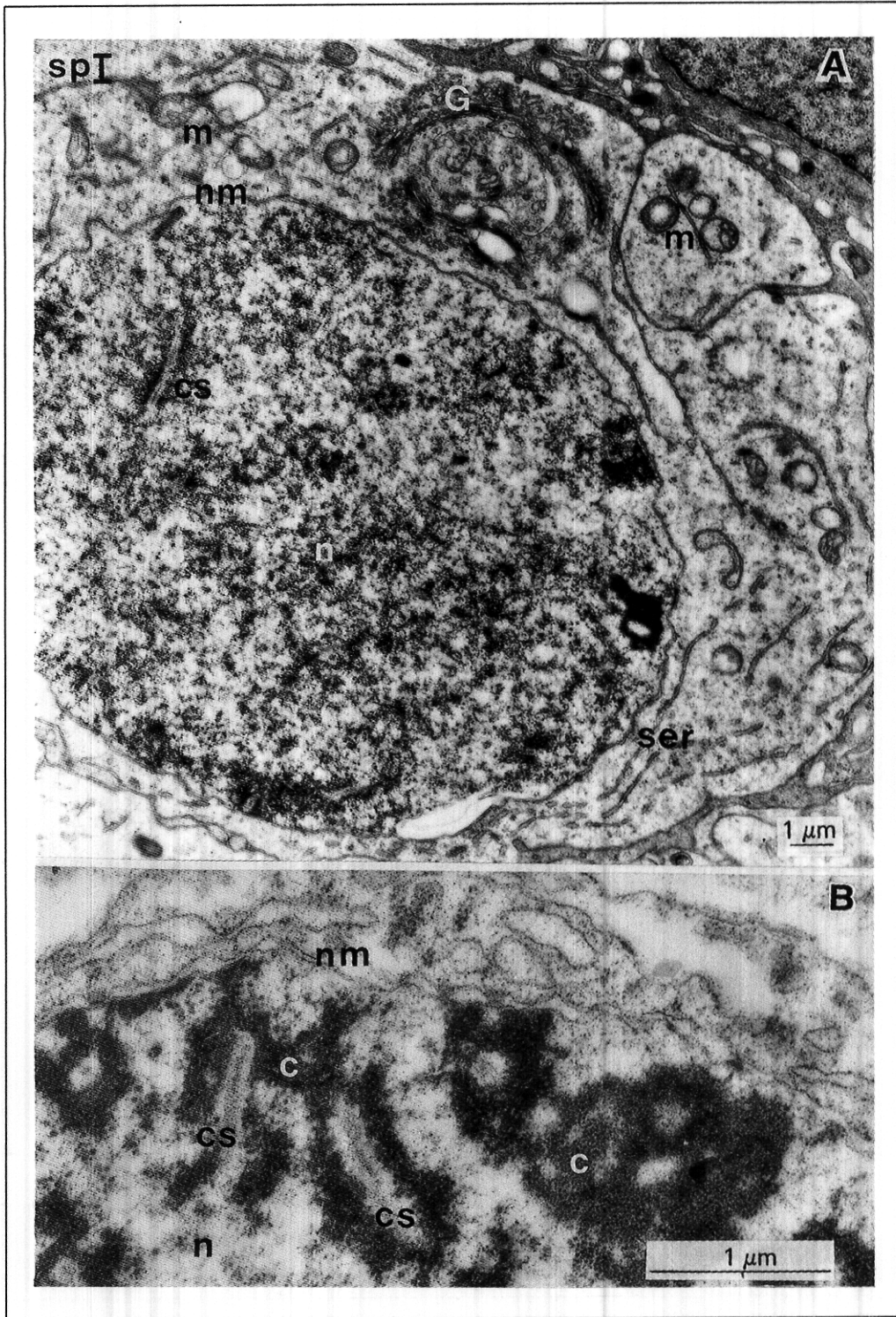


**Figure 1** - A) and B) General view of seminiferous epithelium of a boar carrier of the Robertsonian 5/15 translocation. sc = Sertoli cells; bl = basal lamina; fst = final spermatids, n = nuclei, sp I = spermatocyte I, st = spermatid, sg = spermatogonia; li = lipid; G = Golgi complex; m = mitochondria.

"cis" configuration was observed in the trivalent. According to Amaral (1991) this configuration may be considered as a requisite for normal disjunction, resulting in balanced gametes. Bovine carriers of the 1/29 Robertsonian translocation normally display the configuration "trans" in 32% of meiosis. This configuration

seems associated with the asymmetry of translocated chromosome arms, leading to a failure of synapsis, which would explain the decrease in fertility observed in these animals (Switonski *et al.*, 1987).

These results showed that although Amaral (1991) did not observe pairing abnormalities in the

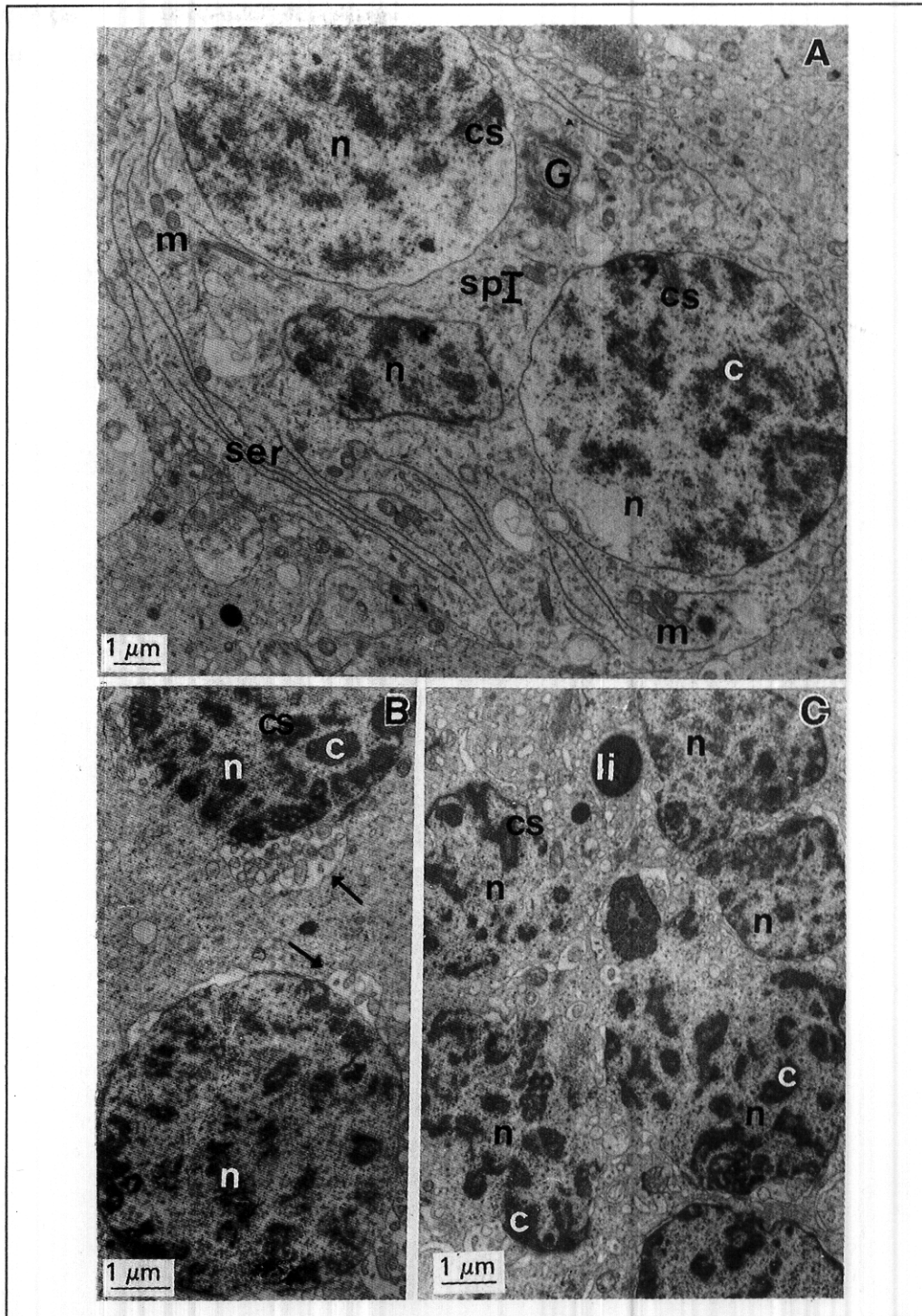


**Figure 2** - A) Normal spermatocyte I (sp I). B) Synaptonemal complexes (sc) from a spermatocyte I nucleus (n). ser = Smooth endoplasmic reticulum; ne = nuclear envelope; c = chromatin.

trivalents, they may occur in some cells, demonstrating the occurrence of cellular degeneration, as observed by Miklos (1974).

It was not possible to determine the mechanisms involved in the formation of a multinucleated cell. The nondisjunction caused by the translocation, gives

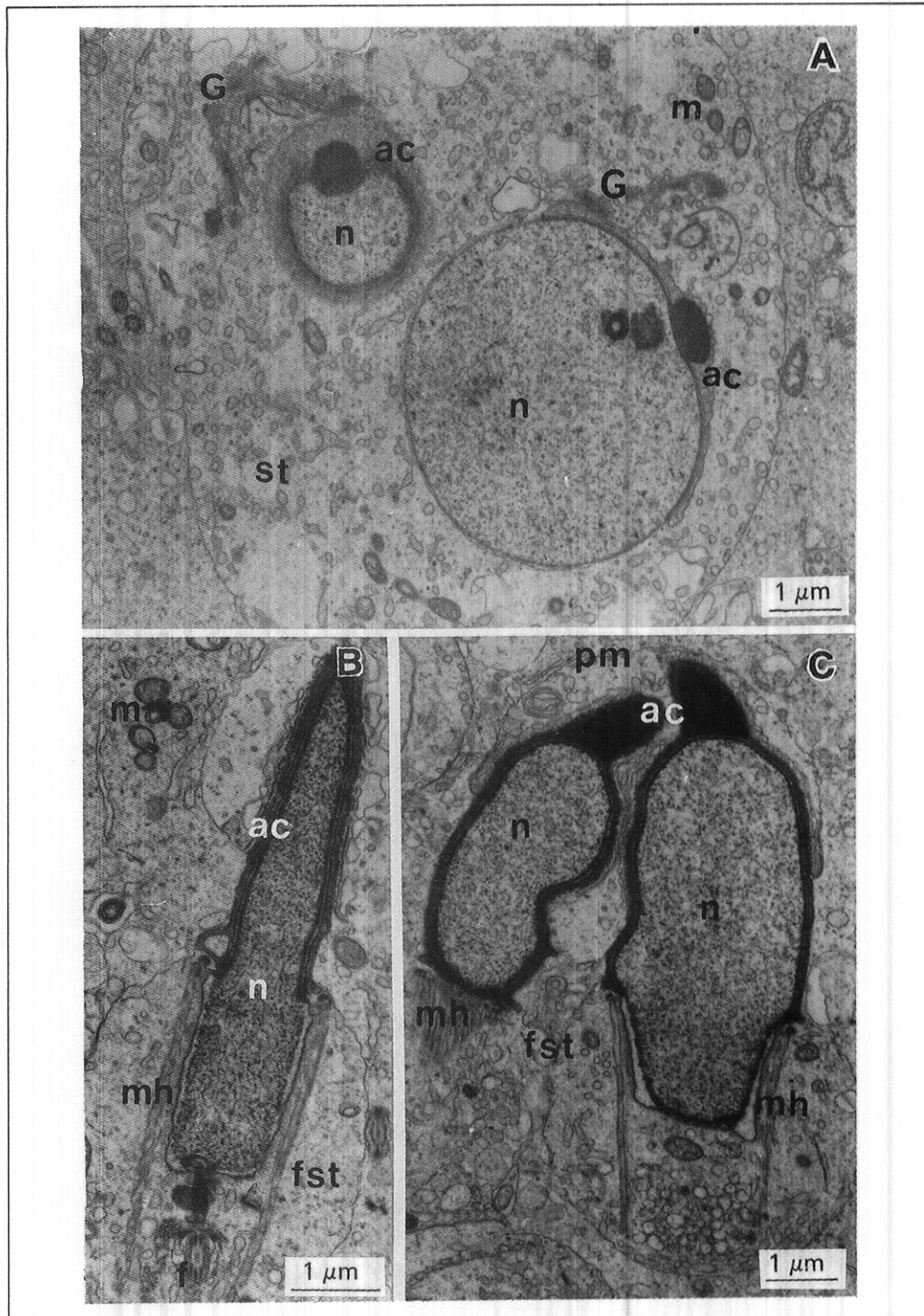
rise to an aneuploid nucleus, and it is not expected to result in two nuclei. The presence of two nuclei in the cells would be expected to result from a normal karyokinesis associated with an absence of cytokinesis (Matano, 1971). The cytoplasmic division depends upon the action of the actin microfilaments, as shown by the



**Figure 3** - Abnormal features of spermatocyte I. A) Spermatocyte I (sp I) with three nuclei (n). B) Perinuclear space dilatations (arrows). C) Nuclei (n) grouped in spermatocyte I, with nuclear envelope (ne) disruption. sc = Synaptonemal complex.

cytochalasin arrest of cytokinesis. As the cell always has a pool of G actin, it would be expected that the substance promoting the actin polymerization was lacking in the aberrant cells. Knudsen (1958) found that regardless of the cause, the alterations resulting from testicular degeneration occur in cellular division

mechanisms due to impairment in the microtubule and microfilament formation. These alterations would also be responsible for the erroneous location of germ cells in relation to the seminiferous tubule lumen. Another possibility is that the trivalent formed by the translocated chromosome is delayed during the anaphasic



**Figure 4** - Spermatids from 5/15 translocation carriers. A) Binucleated (n) spermatid (st). B) Normal final spermatid (fst). C) Binucleated (n) final spermatid (fst). ac = acrosome; f = flagellum; mh = manchette.

movements and in telophase a nuclear envelope would be formed around it. Evidence that this could be occurring is the different sizes of the cellular nuclei observed in Figures 3A and 4A. However, the nuclear position in the cell can also be responsible by these size differences in the plane of sections. The degeneration of these cellular types also suggests that in the aneuploid gam-

etes, the chromosomic anomalies are so intense that they would result in failure of spermatozoan development. In fact, Handel (1987) described genes with postmeiotic transcription that would be associated with spermatogenesis and fertilization promotion, whose anomalous transcripts could be the cell degeneration promoters.

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## RESUMO

O presente trabalho pretende estudar a espermatogênese de animais portadores de translocação Robertsoniana. A nível ultra-estrutural o epitélio seminífero apresentou um aspecto próximo ao normal. Todas as células da linhagem espermatogênica foram vistas, mas observou-se um certo desarranjo na localização dos tipos celulares. Apareceram espermátócitos primários e espermátides normais, mas um número considerável de células apresentaram dois ou mais núcleos. Tais células, via de regra, evoluíram para a degeneração. Com base nos achados observados acreditamos que os gametas desbalanceados sofrem degeneração durante o processo de espermiogênese.

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