A STUDY OF MEIOSIS IN RAMS CARRYING MULTIPLE ROBERTSONIAN TRANSLOCATIONS

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN VETERINARY SCIENCE AT MASSEY UNIVERSITY

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ABSTRACT

A study was made of meiotic chromosomes in air-dried preparations from 30 genitally sound rams (*Ovis aries*). Three of the rams had a normal mitotic karyotype (2n = 54) and the remainder were carriers of the t_1, t_2 and t_3 Robertsonian translocations in various heterozygous and homozygous combinations.

The studies on the primary spermatocytes showed that the normal rams had a modal number of 27 chromosomal elements and the translocation-carrying rams were recorded with a modal number of either 24 or 25. The translocation in the heterozygous state was characterized by the presence of a trivalent in cells at the diplotene, diakinesis and metaphase I stages, while in the homozygous state it was characterized by the presence of a bivalent. The modal number of chromosomal elements was recorded in 90.5 to 97.3 percent of the cells at diakinesis and metaphase I in the translocation-carrying rams. The modal number of chromosomal elements was recorded in 88.3 percent of the cells in the normal rams. An association between the sex bivalent and a small autosomal bivalent was recorded in 3.5 to 5.3 percent of the cells at diakinesis and metaphase I in the translocation-carrying rams. Eight percent of the cells in the normal rams were seen with a sex bivalent association. The sex chromosomes were separated in 0.0 to 1.1 percent of the cells at diakinesis and metaphase I in the translocation-carrying rams, while 0.7 percent of the cells in the normal rams had separated sex chromosomes. However, aneuploidy involving the sex chromosomes was not observed in the secondary spermatocytes from the normal and translocation-carrying rams.

A total of 1,757 secondary spermatocytes were counted from 27 translocation-carrying rams and 103 secondary spermatocytes from 3 normal rams. The studies on the secondary spermatocytes showed that the modal number of 30 chromosome arms was recorded in 87.3 to 96.0 percent of the non-polyploid

cells in the translocation-carrying rams and 95.2 percent of the cells in the normal rams. No hypermodal cells were found in the normal rams. Hypermodal cells were recorded in 0.0 to 3.6 per cent of the non-polyploid cells in the translocation-carrying rams. There were individual variations in the percentage of hypermodal cells recorded in rams with identical karyotypes. There were also significantly greater numbers of cells with 29 chromosome arms than with 31 chromosome arms in the translocation-carrying rams. This suggested that chromosome loss due to lagging at anaphase I or technical manipulation, must have occurred in addition to non-disjunction. A significant surplus of secondary spermatocytes of normal karyotype and a deficit of 25,t_1t_3 were found in the metaphase II figures from the triple heterozygous rams. Significantly uneven distributions of segregation products were also found in the multiple translocation-carrying rams with karyotypes of 50,xy,$t_1t_2t_2t_3$ and 49,xy,$t_1t_2t_2t_3t_x$.

The translocation-carrying sheep have been shown to have good conception rates and an average breeding performance. This would suggest that only balanced (euploid) spermatozoa are involved in fertilization and the unbalanced (aneuploid) spermatocytes fail to mature into spermatozoa and are selected against or degenerate during spermatogenesis.

The evidence indicates that the fertility of the ram is unaffected by the presence of the translocation chromosomes and consequently these sheep should not be eliminated arbitrarily from our animal populations.

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CHAPTER 1

INTRODUCTION TO THE STUDY

Meiosis is the term given to the production from a single parent cell, of four cells, each with half the original number of chromosomes. The gamete end-products, of the two successive cell divisions, may be genetically different from the chromosomes of the parent cell due to crossing-over and assortment of genes by the exchange of chromatid segments between maternally derived and paternally derived homologues (Rhoades, 1961).

Although meiosis has been extensively investigated in plants and insects (Darlington, 1931; Rhoades, 1961; John and Lewis, 1965), it has only been in recent years that the process has been studied in detail in higher mammals. The early chromosome studies using testicular material were undertaken to confirm the diploid chromosome number.

A rapid expansion in our knowledge of mammalian chromosomes has resulted from the use of improved cytological techniques. The methods include the use of tissue culture techniques (Moorhead, Nowell, Mellman, Battips and Hungerford, 1960), autoradiography (Miller, 1970) and the techniques of fluorescent staining (Caspersson, Zech, Johansson and Modest, 1970; Hultén, 1974), C-banding (Hultén, 1971; 1974; Chandley and Fletcher, 1973; Chapman and Bruère, 1975; Logue, 1977; Scott and Long, 1980) and Giemsa banding (Sumner, Evans and Buckland, 1971; Stock, Burnham and Hsu, 1972; Deaven and Petersen, 1973) have been applied to both mitotic and meiotic material. The use of these techniques has helped to identify a number of small chromosomal abnormalities in man and the individual chromosomes in other species.

Most of the early studies in domestic animals were carried out on mitotic chromosomes and it has only been in recent

years that the meiotic chromosomes have been widely studied. However, these meiotic studies have, as in human studies, resulted in a greater understanding of the chromosomal aberrations found in these animals. The studies have been very important in determining the level of aneuploidy in the secondary spermatocyte cells. A high level of aneuploidy could be related to a reduction in the fertility of the animal due to the production of unbalanced zygotes and subsequent cause of embryonic loss.

A high frequency of centric fusions (Robertsonian translocations) has been described in man (Court Brown, 1967) and in several species of domestic animals (Bruère, 1974). The Robertsonian translocation (1/29) in cattle has been extensively investigated with particular reference to the effect of this translocation on the fertility of the animal (Gustavsson, 1969; 1971; Refsdal, 1976; King, Linares, Gustavsson and Bane, 1980; Popescu, 1980; King, Linares and Gustavsson, 1981). Robertsonian translocations have also been reported in other domestic animals including the pig (Hageltorn, Gustavsson and Zech, 1973), the goat (Popescu, 1972; Ricordeau, 1972) and the dog (Ma and Gilmore, 1971; Larsen, Dias and Cervenka, 1978). The effect of the Robertsonian translocations in the tobacco mouse and tobacco mouse hybrids has been of considerable interest and speculation to a number of workers (Gropp, Tettenborn and Von Lehmann, 1970; Cattanach and Moseley, 1973; Baranov and Dyban, 1976; White, Crandall, Raveché and Tjio, 1978).

The extensive occurrence of Robertsonian translocations in domestic sheep (Bruère, 1969; Bruère and Mills, 1971; Bruère, Chapman and Wyllie, 1972; Bruère, 1973; 1974) has led to considerable studies at Massey University into the effects of these translocations on fertility and their role, if any, on the development of the species (Chapman, 1974; Bruère and Ellis, 1979). A large flock of sheep with between one (2n = 53) and six translocations (2n =

48) has been bred. This thesis describes the studies carried out on the meiotic chromosomes of normal rams and rams with between two and five translocations.

The thesis has three main aims: -

1. To describe and compare the behaviour in the primary spermatocytes from normal rams and rams carrying translocations.

2. To compare the levels of aneuploid secondary spermatocyte frequency in normal rams and rams carrying translocations.

3. To describe the distribution of the segregation products in the secondary spermatocytes from translocation-carrying rams.

CHAPTER 2

LITERATURE REVIEW

2.1 CYTOGENETIC STUDIES IN SHEEP

2.1.1 The Establishment of the Diploid Chromosome Number of Sheep.

In earlier studies of the chromosomes of sheep, Wodsedalek (1922; 1929) reported the chromosome number of the male and female to be 33 and 34 respectively. Krallinger (1931), Bruce (1935), Butarin (1935) and Novikov (1935) reported that the diploid chromosome number was 60. Shivago (1930) and Pchakadze (1936) also made estimates of the diploid chromosome number of sheep.

Berry (1938) found a diploid chromosome number of 54 which was similar to the earlier report of Shivago (1930). Berry (1941) confirmed his earlier finding by applying the aceto-carmine method for mammalian chromosomes to the spermatogonial cells of domestic sheep. The diploid number of 54 for the sheep was later confirmed by Ahmed (1940), Makino (1943,a), Melander (1959), Nakanishi and Mizutani (1959), Bomsel-Helmreich (1959), Gimenez-Martin and Lopez-Saez (1962; 1966), Lopez-Saez and Gimenez-Martin (1963), Borland (1964) and Makino, Shimba, Sofuni and Ikeuchi (1967).

The wide variation in the estimates of diploid chromosome number of sheep reported by the early workers was expected since the techniques used in their studies yielded preparations greatly inferior to those produced by leucocyte culture techniques. For this reason it is likely that Krallinger (1931), Bruce (1935), Butarin (1935) and Novikov (1935) could have counted the two arms of one large metacentric chromosome as two chromosomes (Berry, 1941).

Nowell (1960) made the discovery that phytohaemagglutinin, a natural extract of the red bean, acted as a strong mito-

genic agent in stimulating the division of the leucocytes in peripheral blood. The hypotonic sodium citrate treatment, previously described by Hsu (1952) acted to spread the chromosomes; the basic technique for blood cultures was established (Moorhead *et al.*, 1960). Basrur and Gilman (1964) reported the first description of a whole blood culture method applicable to sheep and cattle. Their technique produced good quality well spread chromosomes and because of its simplicity the technique could be used for the chromosome examination of large numbers of animals.

Attempts have been made to classify the chromosomes of sheep according to their size (Ahmed, 1940; Berry, 1941; Makino, 1943a; Melander, 1959; Borland, 1964; Bruère, 1966). However, using conventional staining methods the identification of the individual chromosomes in the karyotype was difficult as many are morphologically similar. The use of Quinacrine fluorescence techniques (Evans, Buckland and Sumner, 1973; Hansen, 1973) and Giemsa banding techniques (Evans *et al.*, 1973; Zartman and Bruère, 1974; Buckland and Evans, 1978) have made it possible to identify every chromosome in the sheep's complement.

2.1.2 Cytogenetical Investigations on Malformed Sheep

Three chromosomally distinct types of intersexes have been studied in sheep. The first type is referred to as male pseudohermaphrodites. The sheep resembled an overfat barren ewe with a degree of masculinization of bone and musculature. The external genitalia were female in appearance, but the internal genitalia were partly male and female. A 54,XY chromosomal complement was found in blood leucocytes and fibroblasts (Bruère, McDonald and Marshall, 1969a). The second type is referred to as a male-type freemartin (Gerneke, 1965; Bruère and McNab, 1968). The sheep have ambiguous external genitalia and testicular gonads but xx sex chromosomes were found in tissues other than blood and bone marrow, which showed xx/xy sex chromosome chimerism.

The third sheep intersex is found in rams which have a normal phenotype except for testicular hypoplasia. The sheep have an xxy sex chromosome complement similar to the chromatin-positive Klinefelter's syndrome in man (Bruère, Marshall and Ward, 1969b). Bruère *et al.* (1969b) suggested that non-disjunction of the sex chromosomes during spermatogenesis and the resultant fusion of an xy sperm and an ovum bearing normal chromosomes or *vice versa* occurred. This was previously suggested as the origin of Klinefelter's syndrome in man (Jacobs and Strong, 1959; Ferguson-Smith, 1961). It appears that the extra x chromosome inhibits testicular development and spermatogenesis more in the ram than in man (Bruère *et al.*, 1969b) although it seems to have less effect on the mental capacity of the ram (Kilgour and Bruère, 1970).

"Hour glass" testes with spermatocyte arrest, bilateral hypoplasia and cryptorchidism have been observed in rams with centric fusion translocations, though a definite cause and effect relationship has not been shown (Bruère and Mills, 1971; Long, 1978; Bruère and Ellis, 1979).

Two ewes and two rams with short upper jaws were studied and found to have a deletion of one arm of an autosome in a proportion of blood leucocytes for a mixoploid 54,xx or xy/54,xx or xy,?13-chromosomal complement (Luft, 1972). An acephalic-acardiac monster has been described with a high proportion of hypodiploid cells suggestive of a possible chromosomal aberration (Dunn and Roberts, 1972).

2.1.3 Taxonomic and Evolutionary Investigations in Sheep

Extensive chromosome studies have been applied to taxonomic and evolutinary investigations in sheep as well as other members of the Bovidae (Wurster and Benirschke, 1968; Dain, 1970; Nadler, 1971; Nadler, Lay and Hassinger, 1971; Evans *et al.*, 1973; Nadler, Hoffmann and Woolf, 1973; 1974; Bunch, Foote and Spillett, 1976; Henderson and Bruère, 1979). Although different types of wild and

domestic sheep have different diploid chromosome numbers (2n = 52, 54, 55, 56, 57 and 58) in all cases the fundamental number or the number of chromosome arms (Matthey, 1945) is 60 (Nadler *et al*., 1971; 1973). The fundamental numbers in the goat (*Capra hircus*), the ox (*Bos taurus*) and wild and domestic sheep have been shown to be remarkably constant, varying between 58 and 62 in the majority of species (Wurster and Benirschke, 1968). This suggested that the main mechanism of chromosomal evolution in the *Bovidae* was by a process of Robertsonian centric fusion (Wurster and Benirschke, 1968). This view is supported by Evans *et al* . (1973) and Schnedl and Czaker (1974) who demonstrated in their studies G-band homology between cattle, goat and domestic sheep and Bunch *et al* . (1976) who showed similar homologies between *Ammotragus lervia* (2n = 58), *Ovis vignei* (2n = 58), *Capra hircus* (2n = 60), *Ovis aries* (2n = 54) and several species of wild sheep with 2n = 54 including *Ovis orientalis*, *Ovis musimon*, *Ovis canadensis nelsoni* and *Ovis canadensis mexicana* . More recent studies reported by Henderson and Bruère (1979) have revealed that the nucleolus organizer regions of the sheep, aoudad, goat and cattle occur on chromosomes which have previously been shown to have homologous banding patterns (Evans *et al*., 1973; Nadler *et al*., 1974). It would appear that there has been a conservation of the number and location of nucleolus- organizer regions during the evolution of these members of the family *Bovidae* and the *Bovidae* karyotype would seem to be fairly stable apart from the gross morphological changes occurring by centric fusion (Henderson and Bruère, 1979).

2.2 ROBERTSONIAN TRANSLOCATIONS

2.2.1 Introduction

The transfer of a segment or fragment of one chromosome into another part of a different chromosome is termed a translocation (Bridges, 1923). Most translocations are

thought to occur by a chromosome breakage followed by the reunion of the non-related broken ends. Robertsonian translocations are the association of two acrocentric or telocentric chromosomes to form a metacentric or submetacentric chromosome. This was first described in insects by Robertson (1916).

The mechanism of formation of Robertsonian translocations is still unclear. The classic concept of Robertsonian fusion was described by White (1954). A break occurs in one chromosome close to the centromere in the long arm and another break in the second chromosome at the short arm or even "invisible" second arm. A translocation will result in a biarmed chromosome and a minute centric fragment which is subsequently lost. The centric fragment is comprised of the centromere from one and the chromosomal material from both acrocentric chromosomes. It is assumed that the loss of the centric fragment has little or no effect on the genome of the individual (White, 1954). Although there is now one chromosome less than normal in the resultant karyotype, the number of chromosome arms N.F. (Matthey, 1945) remains the same.

White (1954) also suggested that a translocation and a hypothetical 'donor' chromosome, which provides the necessary extra centromere and two telomeres could by a process of "dissociation" result in the formation of two new acrocentrics.

Other explanations have been proposed to the mechanism for the formation of Robertsonian translocations. Telocentric chromosomes may be formed by fission within the centromere of a metacentric or submetacentric chromosome. These telocentrics, in turn, may produce metacentric chromosomes by simple fusion (John and Hewitt, 1968; McFee and Banner, 1969). Hsu and Mead (1969) suggested that the 2 centromeres are present in the Robertsonian translocation but are not visible under the light microscope.

Recent evidence seems to indicate that Robertsonian translocations in man are formed by a break in the invisible second arms of the two acrocentrics and the formation of a dicentric translocation chromosome (Niebuhr, 1972; Hsu, Pathak and Chen, 1975; Lau and Hsu, 1977). One of the centromeres, for reasons unknown, may be suppressed and is no longer visible in light microscopy (Niebuhr, 1972; Lau and Hsu, 1977). The existence of a dicentric chromosome has been previously reported in Robertsonian translocations by Barnicot, Ellis and Penrose in 1963. The evidence from the electron microscope studies in man, mouse, chinese hamster, sheep and goat chromosomes supports the concept that 2 centromeres are present in Robertsonian translocations (Comings and Okada, 1970).

The large block of centric heterochromatin seen in all three translocations in sheep is similar to that described in one Robertsonian translocation of the goat (Evans *et al.*, 1973) and closely resembles the dicentric Robertsonian translocation now described in man (Niebuhr, 1972; Ferguson-Smith, 1973). Therefore, it would appear that the loss of centric heterochromatin in the formation of Robertsonian translocations in the sheep is minimal, so that effective genetic loss is unlikely. This fact has already been demonstrated in other species by Comings and Okada (1970); Chen and Ruddle (1971) and Comings and Avelino (1972).

In the MI and MIII Robertsonian translocations in sheep the centromeric block of heterochromatin is evenly distributed on either side of the centric constriction, whereas in the MII translocation the deep-staining heterochromatin is clearly exocentric in the majority of cells (Bruère, Zartman and Chapman, 1974). This adds further evidence that 'true' and apparent Robertsonian translocations could be formed by different mechanisms. Some could be truly dicentric chromosomes formed by the fusion of nonhomologous chromosomes with homologies at their nucleolar organiser regions (Ferguson-Smith, 1973), whereas others could have been formed in the manner originally described by White (1954).

Although the mechanism for the formation of Robertsonian translocations is still uncertain it is known that they occur frequently in the phylogeny of many groups of animals and are now considered to be a regular feature of karyotype alteration in the eukaryotes.

2.2.2 Robertsonian translocations in cattle, pigs, goats, sheep and dogs

Cattle, goats and sheep belong to the superfamily *Bovoidea*. The number of chromosome arms in the karyotypes of the species in the *Bovoidea* is strikingly constant. Wurster and Benirschke (1968) considered this to be evidence that the primary mechanism of karyotype evolution in the *Bovoidea* was the result of Robertsonian translocations. Subsequent studies by Buckland and Evans (1978) on G-banding of *Bovoidea* chromosomes has substantially confirmed this view. Assuming that centric fusions are important in mammalian karyotype evolution, then it is likely that a group of animals evolving from a high number of acrocentric chromosomes in their karyotype will have a high rate of karyotype evolution (Bengtsson, 1980). This would appear to be the case in the *Bovoidea*. This is supported by the banding studies by Evans *et al.* (1973) on goats, sheep and ox which have indicated that the sheep metacentrics result from Robertsonian fusions of chromosomes 1 and 3, 2 and 8, and 4 and 9 of a basic or ancestral bovid karyotype.

2.2.2.1 Cattle

The occurrence of a Robertsonian translocation in cattle was first reported by Gustavsson and Rockborn in 1964. Further studies by Gustavsson (1966; 1969) revealed that the translocation involved chromosomes 1 and 29. Some animals were found to be homozygous for the translocation. The 1/29 translocation has subsequently been reported in a variety of breeds from many different countries (Harvey, 1974a; 1974b; Pollock, 1974; Popescu and Boscher, 1974).

Other centric fusions have since been reported in cattle. A 2/4 translocation has been found in British Friesians (Pollock 1972; Pollock and Bowman, 1974). Bruère and Chapman (1973) reported finding a 11 or 12/15 or 16 translocation in a Simmental bull imported into New Zealand from Scotland. A similar translocation to the latter has been reported in the same line of Simmental by Harvey (1974) and Harvey and Logue (1975). It has been identified as a 14/20 translocation. Bongso and Basrur (1976) reported a 27/29 translocation in Canadian Guernseys, and Stranzinger and Förster (1976) a 1/25 translocation in German Piebalds.

Two other unidentified centric fusions have been reported in Blonde d'Aquitaine x Limousin (Darré, Berland and Quéinnec, 1974) and in Brown Swiss cattle (Tschudi, Zahner, Küpfer and Strampfli, 1977). Two dicentric fusions have also been reported in cattle. These were a dic 6/16 in a dexter cow (Eldridge, 1974) and a dic 3/4 in a Limousin (Popescu, 1977).

2.2.2.2 Pigs

Chromosome polymorphism has been reported in European wild pigs (McFee, Banner and Rary, 1966). Wild pigs are homozygous for a Robertsonian translocation between autosomal 14 and 17 in the domestic pig karyotype. Intermating experiments between pigs carrying the different chromosome numbers produced the expected ratios of progeny with the three chromsome numbers (McFee and Banner, 1969).

A male pseudohermaphrodite pig with xx sex chromosomes was also found to have a centric fusion for a 37,xx, +13/17 chromosome complement (Masuda, Okamoto and Waide, 1975).

2.2.2.3 Goats

Familial centric fusion translocations have been observed several times in phenotypically normal goats (Padeh, Wysoki, Ayalon and Soller, 1965; Soller, Wysoki and Padeh, 1966; Hulot, 1969; Popescu, 1972; Ricordeau, 1972). It has

not been determined if the translocations involve the same autosomes.

Soller *et al*. (1966) reported a familial centric fusion of two acrocentric autosomes in a family of Saanen goats. A similar translocation was observed in an apparently unrelated xx/xy pseudohermaphrodite (Padeh *et al*., 1965). A later study by Padeh, Wysoki and Soller (1971) found that heterozygous and homozygous animals born in a series of matings were phenotypically normal.

Evans *et al*. (1973) also reported a Robertsonian translocation in a goat and identified the individual chromosomes involved in the translocation.

2.2.2.4 Sheep

Early studies on sheep chromosomes, in a large number of sheep, did not reveal any chromosome polymorphism. From the extensive studies on domestic sheep (*Ovis aries*) by Bruère (1969); Bruère and Mills (1971); Bruère *et al*. (1972) and Bruère (1974) chromosome polymorphism has been found in a number of sheep.

A sterile ram was found with a translocation comprising of a large and a small autosome. Four phenotypically normal relatives also carried the translocation. This submetacentric translocation was named the Massey I translocation (t_1) by Bruère and Mills (1971).

In the same survey a Robertsonian translocation involving two of the large acrocentrics was found in a normal Romney ewe. The metacentric chromosome was named the Massey II translocation (t_2) (Bruère and Mills, 1971).

A third Robertsonian translocation (t_3) was found in the Drysdale breed of sheep by Bruère *et al*. (1972). The translocation formed from the fusion of one of the larger and one of the smaller acrocentrics.

The three translocations have been identified by a Giemsa-banding technique (Bruère *et al.*, 1974). The composition of the translocation chromosomes is t_1 (5:26); t_2 (8:11) and t_3 (7:25).

In the study by Bruère *et al.* (1972) of 327 sheep the overall incidence of chromosome polymorphism was found to be 26.3 percent. The Robertsonian translocation (t_1) was found in 12 (7.6 percent) of the 158 sheep examined from 5 pedigree flocks of Romney Marsh sheep in England (Bruère, Evans, Burtenshaw and Brown, 1978). The incidences of Robertsonian translocations in domestic sheep are given in Table 2.1.

The diploid chromosome number in members of the Genus *Ovis* varies between 52 and 58 (Schmitt and Ulbrich, 1968; Wurster and Benirschke, 1968; Nadler, 1971; Nadler *et al.*, 1973). In all cases the N.F. was 60, or 58 if only the autosomal arms were counted (Nadler *et al.*, 1973).

Besides domestic sheep with chromosome polymorphism the only other known member of the sub-family *Ovis*, Superfamily *Bovoidea*, with a chromosome number less than 54 is the Siberian Snow sheep, O. *nivicola alleni* (Nadler *et al.*, 1973; Korobitsyna, Nadler, Vorontsova and Hoffmann, 1974). The chromosome changes have passed through the heterozygous state in relatively recent times on the evolutionary scale.

2.2.2.5 Dogs

Centric fusion translocations have been described in phenotypically normal dogs (Ma and Gilmore, 1971; Larsen *et al.*, 1978). Centric fusion of chromosomes has also been reported in dogs with anatomical abnormalities shown to be unrelated to the translocation and in dogs with anatomical defects where a cause and effect relationship was equivocal (Shive, Hare and Patterson, 1965; Patterson, Hare, Shive and Luginbühl, 1966; Hare, Wilkinson, McFeely and Riser, 1967).

TABLE 2.1: INCIDENCE OF ROBERTSONIAN TRANSLOCATIONS IN DOMESTIC SHEEP

Author	Breed	Country	2n	Number Studied (Number with Translocation)	%	Translocation Type
Bruère, A.N. (1969)	Romney	N.Z.	53	6(4)		Massey I
Bruère, A.N. and Mills, R.A. (1971)	Romney "	N.Z. "	53 "	180(25) 2(2)	13.9	Massey I Massey II
Bruère, A.N.; Chapman, H.M. and Wyllie, D.R. (1972)	Drysdale "	N.Z. "	53 52	((76) (321 ((6)	23.7 1.9	Massey III " "
Bruère, A.N. (1972 unpublished)	Polled Drysdale	N.Z.	53	11(4)		" "
Bruère, A.N. (1973)	Romney	N.Z.	53	309(3)		Massey II
Bruère, A.N.; Chapman, H.M.; Jaine, P.M. and Morris, R.M. (1976)	Romney " "	N.Z. "	53 53 52 53 52	323(35) (128(24) ((4) (23(5) ((1)	10.5 22.6 26.1	Massey II Massey I & II Massey III
Bruère, A.N.; Evans, E.P.; Burtenshaw, M.D. and Brown, B.B. (1978)	Romney	England	53 52	(158(11) ((1)	7.6	Massey I

2.2.3 Robertsonian translocations in man

An extensive amount of research has been carried out on the human karyotype. This is due to the finding that a variety of clinical conditions are often associated with an abnormal karyotype.

Robertsonian translocations are the most common translocation reported in man and are present in 0.5 percent of the population (Court Brown, 1967). Only chromosomes in groups D and G are acrocentric (10 out of 46 chromosomes). Human acrocentric chromosomes have visible satellites, believed to consist of euchromatin on their short arms (Ferguson-Smith and Handmaker, 1961; 1963). Associations between the acrocentric chromosomes are very obvious in human metaphases. This phenomenon, called satellite association, was first reported by Ferguson-Smith and Handmaker (1961); Harnden (1961) and Ohno, Trujillo, Kaplan and Kinosita (1961) and observed in mitotic metaphases. The phenomenon of satellite association was also reported in meiosis by Ferguson-Smith (1964). There were a number of reports of a random association pattern of the D group chromosomes (Shaw, Craig and Riccuiti, 1969; Nakagome, 1969; Cuevas-Sosa, 1970). The analysis of satellite association in banded chromosomes found that individuals with non-random satellite association pattern are frequently present(Patil and Lubs, 1971; Cook, 1971, 1972; Nakagome, 1973). By means of analysis of variance, Jacobs, Mayer and Morton (1976) found that there was a highly significant heterogeneity among individuals for the satellite association tendencies of all the acrocentrics. However, Hansson (1979) found in his studies that there was a specific tendency for certain satellite associations between certain acrocentric chromosomes. For example, chromosome 13 had a specific tendency to associate with chromosome 14 and 15.

A Robertsonian translocation between two members of the D group of chromosomes (13, 14 and 15) D/D or t (Dq Dq)

is the most common. Between one in 800 (Ford, 1973) and one in 1000 (Jacobs, Buckton, Cunningham and Newton, 1974) individuals carries a balanced D/D translocation. A translocation between two members of the G group of chromosomes (21 and 22) G/G or t (21q 22q) or between members of both groups D/G or t (Dq Gq) are rather more rare. Evans, Canning, Hunter, Martsolf, Ray, Thompson and Hamerton (1978) found, in a survey of 14,059 newborn infants, 11 Robertsonian translocations involving chromosomes 13 and 14, one 14 and 15, and one 14 and 21. This confirms previous observations that chromosomes 13 and 14 are preferentially involved in the t (Dq Dq) class, while chromosomes 14 and 21 are the most commonly involved in t (Dq Gq) (Cohen, 1971; Hecht and Kimberling, 1971; Mikkelsen, 1973; Friedrich and Nielsen, 1974; Jacobs *et al.*, 1974; Jacobs, Frackiewicz, Law, Hilditch and Morton, 1975).

The D/D translocation is a metacentric chromosome, familial and carriers of it produce offspring which are either normal balanced carriers or trisomic for a D group chromosome. Most D/D translocations appear to be of no consequence to their carriers. Translocation carriers produce substantially fewer phenotypically and chromosomally abnormal offspring than expected on the basis of meiotic production and equal fertilizing capacities of normal and abnormal gametes (Hamerton, 1970). The reason for this could be failure of implantation or elimination through an early abortion of unbalanced zygotes. Impaired fertility is only occasionally noted in males (Chandley, Edmond, Christie, Gowans, Fletcher, Frackiewicz and Newton, 1975) and the great majority of balanced carriers do not have any increased frequency of spontaneous abortions (Nielsen and Rasmussen, 1976). However, occasional individuals are known to have had several or even numerous unexplained miscarriages (Wilson, 1971; von Koskull and Aula, 1974; Gahmberg, Pajunen and de la Chapelle, 1980). It has been suggested that the deletion of certain sequences from the chromosomes involved in the translocation may be less well tolerated

in certain genetic backgrounds (Gosden, Gosden, Lawrie and Mitchell, 1978).

Mikkelsen (1971) in reviewing the literature on the frequency of Robertsonian translocations in patients with Down's Syndrome (trisomy 21), found that 8 percent born to mothers under the age of 30 had a translocation. A trisomy 21 mongol has 47 chromosomes instead of the normal 46. However, a number of mongoloid individuals (2 to 5 percent) have 46 chromosomes with the extra chromosome in the form of a translocation (Hamerton, Canning, Ray and Smith, 1975). Between 25 and 50 percent of translocation mongols have inherited the translocation chromosome from a parent (Giannelli, Hamerton and Carter, 1965; Court Brown, 1967) while in the remainder the translocation has arisen *de novo*.

2.2.4 Robertsonian translocations in the mouse

The earlier reports of translocations in the mouse include those made by Ford, Hamerton, Barnes and Loutit, (1956); Ohno and Cattanach (1962); Bennett (1965); Chu and Russell (1965) and Evans, Lyon and Daglish (1967). The most significant report of translocations in mice was made by Gropp *et al*. in 1970. They reported that the tobacco mouse (M. *poschiavinus* Fatio, 1869), a wild commensal mouse of the Val poschiavo, differed from the grey house mouse (M. *musculus*) by seven Robertsonian translocations. The tobacco mouse was found to have 26 chromosomes including seven pairs of metacentrics as compared to 40, all acrocentrics, in M. *musculus*. M. *poschiavinus* and M. *musculus* were crossed resulting in F_1 hybrids with 33 chromosomes, including 7 metacentrics (Tettenborn and Gropp, 1970). The F_1 hybrids had the Robertsonian translocation in the heterozygous state and were found to be fully viable but with greatly reduced fertility.

The 7 translocations were individually isolated into separate mouse strains. Studies were carried out by Cattanach and Moseley (1973) on whether the translocations all lead to non-disjunction, and if so, whether they all contributed equally. They found that all seven of the translocations lead to non-disjunction in the heterozygous state. However, there was a large individual variation ranging from 6.0 (T6) to 29.5 (T4) percent. The levels of aneuploidy in individual heterozygotes need not necessarily correlate with those of the combined heterozygotes (White *et al.*, 1978) although this would appear to be the case in some tobacco mouse hybrids (Cattanach and Moseley, 1973). The high levels of aneuploidy were also consistent with high frequencies of zygotic loss (Tettenborn and Gropp, 1970; Cattanach and Moseley, 1973). The reduction in fertility reported in F_1 tobacco mouse x domestic mouse progeny is considered to be the result of the different genetic backgrounds of each species. The animals have been geographically isolated from each other and have developed some degree of reproductive isolation (Bruère and Ellis, 1979).

The proportion of aneuploid spermatocytes and oocytes which actually develop into functional gametes and form abnormal embryos has been the subject of many investigations (Tettenborn and Gropp, 1970; Döring, Gropp and Tettenborn, 1972; Ford, 1972; Ford and Evans, 1973; Cattanach and Moseley, 1973; Gropp, 1973; Oshimura and Takagi, 1975). Many of these studies indicated that there was little selection against aneuploid gametes, zygotes, or early blastocysts, and that this only becomes discernible around, or shortly after, the time of implantation. White *et al.* (1978) from their studies on triple translocation-carrying mouse heterozygotes, suggested that the limitations of the technique used or specific genetic factors, or both, may be responsible for those discrepancies. They also found that the segregation products in the embryos were balanced and demonstrated no preferential segregation of the translocations.

2.2.5 Phenotypic effects of Robertsonian translocations in domestic animals

Most reports indicate that male and female animals heterozygous for a Robertsonian translocation are phenotypically normal. There are a few reports of domestic animals having phenotypic changes due to a Robertsonian translocation, and these are summarized in Table 2.2. The relationship between the phenotypic change and the presence of a Robertsonian translocation is often not clear and it may have been coincidental that these were found together.

In Swedish cattle, although no phenotypic abnormalities were observed, it has been reported that there is an increased return to service of daughters of bulls heterozyous for the 1/29 translocation (Gustavsson, 1969; Gustavsson, 1971; Refsdal, 1976). The reduction in fertility is thought to result from the early death of embryos with unbalanced karyotypes. King *et al*. (1981) presented direct evidence for the occurrence of chromosomally unbalanced embryos sired by bulls carrying the 1/29 translocation, suggesting non-disjunction and the formation of hyperhaploid gametes with fertilization ability (King *et al*., 1980). A similar study by Popescu (1980) revealed the presence of two monosomic embryos in 13-day blastocysts collected from cows inseminated with semen from bulls carrying the 1/29 translocation. It has also been found that the frequency of degenerating embryos was significantly higher when the sires were heterozygous for the 1/29 translocation (Linares, King and Gustavsson, 1980). Meiotic studies in bulls heterozygous for the 1/29 translocation have revealed non-disjunction (Logue, 1977; Popescu, 1978) at a rate increased by 5.5 percent over normal bulls (Logue and Harvey, 1978). These estimates are in agreement with the observations of King *et al*. (1981) that 5.26 percent of the analysable embryos had unbalanced karyotypes and with decreases of 5 percent and 7 percent in 28- and 56-day non-return rates in heterozygous bulls (Dyrendahl and Gustavsson, 1979).

TABLE 2.2: ANIMALS WITH PHENOTYPIC ABNORMALITIES AND A ROBERTSONIAN TRANSLOCATION

Author and Date	Species	Number	Phenotypic abnormality	Karyotype
Gustavsson, I. and Rockborn, G. (1964)	Cattle	3	Lymphatic Leukaemia	59 chromosomes including 1/29 translocation
Rugiati, S. and Fedrigo, M. (1967) (1968)	Cattle "	1 2	Achondroplastic dwarfism Chondrodystrophic lesions	58,xy including 2(1/28) translocations 59,xx including (1/28) translocation
Herzog, A. and Höhn, H. (1971)	Cattle	1	C.N.S. abnormalities	59 chromosomes including translocation
Froget, J.; Coulon, J.; Nain, M.-C. and Dalbiez, J.-M. (1972)	Cattle	1	Crooked forelegs	59,xx including 1/29 translocation
Shive, R.J.; Hare, W.C.D.; and Patterson, D.F. (1965)	Dog	1	Congenital cardiac defect cleft upper lip	77,xy including submetacentric translocation chromosome
Hare, W.C.D.; Wilkinson, J.S.; McFeely, R.A. and Riser, W.H. (1967)	Dog	1	Bone chondroplasia	77,xx including subtelocentric translocation chromosome

Continued..

TABLE 2.2: CONTINUED

Author and Date	Species	Number	Phenotypic Abnormality	Karyotype
Padeh, B.; Wysoki, M.; Ayalon, N. and Soller, M. (1965)	Goat	1	Pseudohermaphrodite	xx/xy 59 chromosomes including translocation in xx cells
Bruère, A.N. (1969)	Sheep	1	Small abnormally shaped testicles associated with complete spermatocyte arrest	53,xy including translocation chromosomes
Bruère, A.N. and Mills, R.A. (1971)	Sheep	5	2 rams - monorchids 3 rams - "hour-glass" testes with spermatocyte arrest	53,xy including translocation chromosome
Bruère, A.N.; Chapman, H.M. and Wyllie, D.R. (1972)	Sheep	2	1 ram - atrophic testes and absence of epididymis on right 1 ewe - segmental aplasia of genitalia	53,xy including translocation chromosome 53,xx including translocation chromosome
Long, S.E. (1978)	Sheep	1	Testicular hypoplasia	53,xy including translocation chromosome
Bruère, A.N. and Ellis, P.M. (1979)	Sheep	13	Severely under shot (1) wry neck (1) Hour-glass testes (2) Freemartin sheep (2) Unilateral cryptorchids (6) Cleft palate (1)	Translocation carrying animals

2.3 THE CHROMOSOMES OF SHEEP AT MEIOSIS

The number and morphology of sheep chromosomes were initially investigated by studies using testicular material (Wodsedalek, 1922; Krallinger, 1931; Bruce, 1935; Butarin, 1933-34; 1935; Novikov, 1935; Pchakadze, 1936; Ahmed, 1940; Berry, 1941; Makino, 1943a; Bomsel-Helmreich, 1959). An estimate of the chromosome number was made by Pchakadze (1936) from a study of primary spermatocytes, and Wodsedalek (1922), Krallinger (1931), Berry (1941) and Bomsel-Helmreich (1959) confirmed their counts with spermatocyte material.

Early studies on meiotic chromosomes were made by Ahmed (1940) and Makino (1943a). More recently, Bruère (1969), Loir (1971) and Nadler *et al*. (1973) reported on their investigations into various stages of meiosis in the ram. However, these authors only examined a small number of cells and it was the studies by Chapman (1974), Logue (1977) Long (1978) and Bruère, Scott and Henderson (1981) that has given us a better understanding of meiosis and meiotic chromosomes in the ram.

A detailed description of chromosome morphology during meiosis of normal and Robertsonian translocation-carrying rams was given by Chapman (1974). Chiasmata were counted in 34 diakinesis-metaphase I cells from 11 rams with normal karyotypes. The mean total number of chiasmata per cell was 55.4 (Chapman and Bruère, 1977). The aneuploid secondary spermatocyte frequency of single heterozygous (9.1-17.2 percent) and normal and homozygous (0.0 percent) rams was made by Chapman (1974). Logue (1977) found that the mean number of chiasmata per cell was similar in both normal (51.2) and single heterozygous (50.9) rams. This was also the finding of Long (1978) who reported chiasmata counts of 54.3 and 54.5 in normal and single heterozygous (t_1) rams respectively. Logue (1977) and Long (1978) made

estimates of the aneuploid secondary spermatocyte frequency in normal (7.3 and 4.0 percent) and single heterozygous (7.0 and 6.1 percent) rams. Bruère *et al.* (1981) examined the levels of aneuploid frequency in the secondary spermatocytes from normal (0.0 percent) and triple heterozygous (5.4 percent) rams.

Edwards (1965) has described the maturation *in vitro* of ewe oocytes and Long (1974; 1977) and Long and Williams (1978; 1980) have examined the karyotypes of embryos and unfertilized eggs from ewes. The examination of preimplantation blastocytes in sheep failed to detect any abnormal karyotypes (Long, 1974; 1977). However, it was suggested that abnormal embryos may have been degenerating by the late preimplantation stage and that more accurate information would be obtained from an examination of earlier embryos. Consequently embryos and unfertilized eggs were collected 2 or 3 days post coitum from mature sheep with normal karyotypes (Long and Williams, 1978; 1980). The karyotypes of 89 of the 376 eggs and embryos collected were established. Thirteen of the 89 were considered to deviate from the normal. However, the nature of the technique is such that the possibility that the abnormal embryos were technical artefacts cannot be excluded (Long and Williams, 1980).

CHAPTER 3

MATERIALS AND METHODS

All materials and methods, used in this study of meiosis in the ram, are recorded and discussed in this chapter.

3.1 EXPERIMENTAL ANIMALS

3.1.1 Selection

Flocks of sheep carrying the three different translocations have been established by Professor A.N. Bruère at Massey University. In this study rams were used from these flocks.

Meiotic preparations were made from a total of 30 rams. Three had a normal karyotype and the remainder were translocation-bearing animals (Table 3.1).

TABLE 3.1: NUMBERS AND KARYOTYPES OF RAMS EXAMINED

Ram type as determined by leucocyte culture		Number of rams	Number of meiotic preparations
Normal	(54,xy)	3	3
Double heterozygote	(52,xy)	4	6
Triple heterozygote	(51,xy)	6	12
Single homozygote Single heterozygote	(51,xy)	3	6
Single homozygote Double heterozygote	(50,xy)	11	19
Double homozygote Single heterozygote	(49,xy)	3	6
Total		30	52

Individual rams were selected for this study on the basis of history, genital examination and mitotic karyotype.

3.1.1.1 History

The rams with a normal karyotype (2n = 54, xy) were healthy and genitally sound.

Inter-mating of sheep with different Robertsonian translocations has resulted in the production of progeny with an ever-decreasing number of chromosomes (Bruère and Ellis, 1979). Meiotic preparations were made on individual rams with karyotypes of 2n = 52, 51, 50 and 49.

3.1.1.2 Genital Examination

Rams were examined and found to be genitally sound (Bruère, 1970). Testicular size and tone were assessed by manual palpation. Chapman (1974) classified the testes she used in her meiotic studies as small, medium or large in size and their tone as soft and dull, firm and elastic, or firm and dull. Only those rams with testes of medium to large size and of good tone were used in the present study (the rams were assessed by manual palpation by Prof. Bruère)

The size of the testis is a reasonable guide to spermatogenesis in the ram and it is thought that increases in size are due mainly to increased activity of the seminiferous tubules (Watson, Sapsford and McCance, 1956; Voglmayr and Mattner, 1968).

3.1.1.3 Mitotic Karyotype

The karyotypes of the rams used in this study were determined by a whole blood leucocyte technique (Bruère and McLaren, 1967). Details of the materials used are given in Appendix I.

3.1.2 Method for Obtaining Testicular Material

Two methods, testicular biopsy and castration, are used for obtaining testicular material from the living animal.

3.1.2.1 Testicular biopsy

Charny (1940) was the first to describe the technique of testicular biopsy in man. There have subsequently been a number of papers describing the method in both man (Charny, 1963; Rowley and Heller, 1966; Hendricks, Lambird and Murphy, 1969; Wong, Straus and Warner, 1973; Colgan, Bedard, Strawbridge, Buckspan and Krotz, 1980) and the domestic animals (Sykes, Wrenn, Moore, Underwood and Sweetman, 1949; Byers, 1953; Knudsen, 1960; McDonald and Hudson, 1960; Galina, 1971; Eaglesome, Hare and Singh, 1979).

A review was made by Galina (1971) on the value of testicular biopsy in farm animals. Various workers have reported that the technique, used in the bull, results in decrease in sperm concentration, morphological changes in tubules and infections near the biopsy site (Sykes *et al.*, 1949; Gassner and Hill, 1955; Věžnik, 1962; Gordon, Barr, Herrigel and Paulsen, 1965). Other workers have reported that the technique has little effect on semen quality or testicular histology (Byers, 1953; McDonald, 1960; Knudsen, 1960). Stipančević (1966) has suggested that surgical trauma produced by biopsy in the ram caused inflammatory responses of the testicle which results in depressed spermatogenesis. Galina (1971) modified the technique and has reported an improved method suitable for rams, boars, bulls and stallions. More recently, Eaglesome *et al.* (1979) concluded from their work in the bull that their procedure was a safe and practical method of obtaining testicular tissue for meiotic analysis and the effects on the semen of the bull were transient. Logue and Harvey (1978) have also used the technique, without any complications, in their studies in the bull. Biopsy is widely used as a technique in human medicine for providing material for both histological and cytogenetical analysis (Colgan *et al.*, 1980). However, the method can only be used for the collection of small pieces of testicular material and for this reason it was not tried in the present work.

3.1.2.2 Castration

Unilateral castration was the method used in this study for obtaining testicular material. This method ensured that there was significant material for both histological and meiotic studies.

Samples from both testicles were utilized in the majority of rams. The second testis was removed from a few days up to a year after the first. Chapman (1974) found in a number of rams that the weight of the second testis was less than the first when the testes were removed within 10 days of each other. She reported that this did not appear to have any affect on the result, although it has previously been suggested by Gassner and Hill (1955) that surgical interference may adversly affect the spermatogenic function of the contralateral testis. In the present study it was observed that poor meiotic results were often found in the second testis when it was removed within 7 days of the first.

At first Chapman (1974) castrated the rams under a general anaesthetic. The rams were starved overnight prior to castration. The scrotum was clipped and cleansed with warm water containing detergent.

Pentobarbitone sodium, 60 mg/ml, was given intravenously to effect. Chapman (1974) used this method for the first 38 operations and then she castrated the rams under a local anaesthetic. This method was also used in this study. The ram was pre-medicated with 4 mg acetyl promazine intramuscularly and 1.8 ml of xylocaine was injected subcutaneously along the line of the intended incision (Appendix II).

For castration under local anaesthesia the ram was held firmly in an upright position on its hindquarters. Prior to surgery the scrotum was first disinfected with aqueous hibitane and then with hibitane tincture. After sterile

drapes were placed around the surgical site the ram was unilaterally castrated by the "open" method. An incision was made down to the tunica albuginea and then emasculators were applied across the spermatic cord and the testicle was removed. The blood vessels were tied off and the incision sutured.

After removal, the testis was taken immediately to the laboratory where small pieces were cut for histological examination and other sections were used in the meiotic preparations.

3.2 METHOD FOR OBTAINING MEIOTIC CELLS

Three techniques have been used for the preparation of chromosomes from testicular material.

3.2.1 Histological Sections

The early work on animal chromosomes used sectioned material preserved with various fixatives. These sections were made in meiotic studies in the sheep (Ahmed, 1940; Makino, 1943,a), goat (Makino, 1943,a), bull (Melander and Kundsen, 1953) and boar (Henricson and Bäckström, 1964). This technique gave disappointing results due to the difficulties of preservation and the resulting separation of the chromosomes.

3.2.2 Squash Preparations

A technique developed in 1952 by Makino and Nishimura established the making of satisfactory cytological preparations directly from mammalian gonads. The method they applied to animal material was the squash technique previously used in plant cytology. This technique was simpler and gave morphologically better chromosomes than those from paraffin sections used in the histological methods.

The squash technique has been modified and used by many authors. Meiotic chromosomes prepared by squash methods have included those in the cat (Ohno, Stenius, Weiler, Trujillo, Kaplan and Kinosita, 1962), bull (Melander and Kundsen, 1953; Kundsen, 1954; Treadwell, Kieffer and Cartwright, 1968; Gustavsson, 1969), sheep (Berry, 1941; Loir, 1971), man (Ford and Hamerton, 1956; Böök and Kjessler, 1964; Ohno, 1965) and rodents (Welshons, Gibson and Scandlyn, 1962; Brooks and Lengemann, 1967).

Well spread chromosomes at the diakinesis and metaphase stages were obtained by the teasing of the testis tubules in a hypotonic solution prior to fixation and squashing (Ford and Hamerton, 1956; Böök and Kjessler, 1964; Ohno, 1965).

The squash technique has a number of disadvantages. These include: -

1) Cells may be lost or damaged on removal of the cover-slip (Eicher, 1966).

2) Repeated observations under the microscope are required for control of squashing by thumb (Rothfels and Siminovitch, 1958).

3) Few good spermatogonial cells are found when hypotonic treatment is used (Ford and Hamerton, 1956).

4) Cells tend to cluster together (Ford and Hamerton, 1956).

5) Very few well-spread figures of a particular stage of meiosis are found in the limited number of slides which can be made in the 40 minute time limit suggested by Welshons *et al.*(1962).

6) Chromosome morphology is often poor, with the nuclei often damaged (Melander and Knudsen, 1953).

The squashing techniques are generally inferior as a chromosome spreading method to the air-drying methods commonly used today.

3.2.3 Air-dried Preparations

Well spread metaphases with superior chromosome morphology are produced by the air-drying of films of cells (Rothfels and Siminovitch, 1958).

The first air-drying method for male mammalian meiotic chromosomes was published in 1963 by Benirschke and Brownhill. Subsequently other air-drying methods were published (Evans, Breckon and Ford, 1964; Sasaki and Makino, 1965; Meredith, 1969; Williams, Hagen, Runyan and Lafferty, 1971). Modifications of these methods have been widely used for studies in man and domestic animals (Eicher, 1966; Hultén, Lindsten, Ming and Fraccaro, 1966; Ferguson-Smith, 1966; pers. comm. to Bruère; Hoo and Bowles, 1971).

The method of Evans *et al.* (1964) has been used successfully in cattle (Popescu, 1971) while modifications have been used, for example, in the dog (Eliasson, Gustavsson, Hultén and Lindsten, 1967), cattle (Gustavsson, 1969; Logue, 1975; 1977; Logue and Harvey, 1978), sheep (Logue, 1977; Long, 1978), goat (Logue, 1977) and mouse (Cattanach and Moseley, 1973; Boer and Groen, 1974; Markvong, Ward and Hsu, 1976; Evans, Beechey and Burtenshaw, 1978). Bruère (1969) used a simplification supplied by Ferguson-Smith (1966 pers. comm. to Bruère) of the method of Evans *et al.* (1964). Chapman (1974) in her meiotic studies in rams modified the method used by Bruère (1969). However, Nadler *et al.* (1973) used Meredith's (1969) method for meiotic preparations from rams and Datta (1970) studied meiosis in the goat by using the method of Benirschke and Brownhill (1963).

Evans *et al.*(1964) tried to overcome some of the technical difficulties produced by the "wave" of the spermatogenic cycle. By converting the contents of the tubules into a suspension, the clumps of spermatogonia and spermatocytes are separated into single cells and thoroughly mixed.

The suspension also allows advantage to be taken of the more even spreading of the cells on the slide afforded by the air-drying procedure (Rothfels and Siminovitch, 1958).

Meredith's (1969) method produced good spermatocyte metaphases in both the rat and Syrian hamster without employing either centrifugation or fragmentation of tubules. Benirschke and Brownhill (1963) and Meredith (1969) found that slides could be made successfully from tubules which have been stored in cold fixative for several weeks. This could be useful in the collection and subsequent examination of samples collected on expeditions. Williams *et al.*(1971) also considered that cell loss was reduced by not centrifuging at any stage.

A large number of good mitotic metaphases were seen when colchicine was injected intra-peritoneally prior to the sacrificing of animals for meiotic study (Eicher, 1966). There have been a number of other reports of the pre-treatment of animals with colchicine and these include: Benirschke and Brownhill (1963); Clendenin (1969); Tettenborn and Gropp (1970); Hoo and Bowles (1971); Döring *et al.* (1972) and Cattanach and Moseley (1973).

Colchicine has been widely used for the arrest of somatic cells at mitotic metaphase and it was tried in studies on meiotic chromosomes since it should also arrest germ cells at first and second meiotic metaphases (Clendenin, 1969). A marked increase in cells at first meiotic metaphase after one and two hour intervals of pre-treatment with colchicine was demonstrated by Clendenin (1969). His results also showed that a three hour pre-treatment increased second meiotic metaphase cell counts. The effects of pre-treatment with colchicine in rams ~~has~~ have not been studied since it would cause pain to the animal.

Sodium citrate and potassium chloride are the two most widely used hypotonic agents in meiotic studies. The hypotonic agents aid in the swelling of the cells, the disruption of the spindle and the dispersion of the chromosomes. The agents cause a spreading of the chromosomes by loosening the inter-chromosomal connections, which otherwise hold the chromosomes tightly together into a clump (Klášterská and Ramel, 1979). Several workers have reported that the use of potassium chloride as the hypotonic solution results in sharper definition of the chromosome outline and less disruption of the individual chromosome (Clendenin, 1969; Luciani, Devictor-Vuillet and Stahl, 1971). Hungerford (1971) reported that the structural integrity of the chromosomes appears to have been better preserved using potassium chloride than is the case with other hypotonic solutions. However, little difference in the effectiveness of the two hypotonic solutions has been found in the male meiotic studies in sheep (Chapman, 1974).

Eicher (1966) considered that inferior results on testicular material were produced when a fixative of 1 part acetic acid and 3 parts methyl alcohol was used. She reported that better results were obtained with a 1:1 mixture of acetic acid and methyl alcohol. However, there have been many reports of good meiotic results on testicular material using a 1:3 mixture of acetic acid and methyl alcohol.

3.2.4 Method used in this Study

The method used in this study was a modification of those of Evans *et al.* (1964) and Ferguson-Smith (1966, pers. comm. to Dr Bruère) and was developed for the ram by Bruère (1969) and Chapman (1974).

Immediately after the testis was removed from the ram it was taken to the laboratory. Sections, 3 to 4 mm in size, were cut from the testis and were placed in Bouin's

fluid in an universal bottle. Further sections were cut and were quickly washed in a watch-glass containing a few ml of warm (37°C) 2.2 percent sodium citrate. The pieces were then transferred to another watch-glass containing 2-3 ml of warm (37°C) 1.2 percent sodium citrate.

The pieces were chopped with a pair of fine scissors to form a fine suspension. The suspension was transferred to a graduated centrifuge tube which was kept in a water bath at 37°C. More hypotonic solution was added and the process was repeated until 10 ml of cell suspension was produced. A second piece of testis was cut and treated in the same manner. At least 2 centrifuge tubes of material were processed together. A glass rod was used to crush the larger fragments.

The centrifuge tubes were left in the water-bath (37°C) and incubated. After the tubules had been exposed to the hypotonic treatment for 20 minutes the centrifuge tubes were removed from the water-bath and spun for 10 minutes at 1000 r.p.m. The tubules have then been exposed to the hypotonic solution for a total of 30 minutes. The supernatant was carefully removed with a pipette and discarded. The cells were resuspended in the remaining 1-2 ml of hypotonic solution and 0.5 ml of freshly made, cold fixative (Methanol: glacial acetic acid, 3:1) was slowly added and mixed with the hypotonic. A further 8ml of fixative was added.

The centrifuge tubes were left at 4°C for 30 minutes. The cells were again centrifuged at 1000 r.p.m. for 10 minutes and the supernatant was discarded. The cells were resuspended in the 1 ml of fixative which is retained. A few drops of fresh fixative was added and the cells were left at 4°C. After 20 minutes the cells were again resuspended.

A few drops of cell suspension were pipetted from a height of about 10 cm onto cold, clean wet slides. The slides were air-dried and then placed on a warm (37°C) hot-plate until dry. Figure 3.1 illustrates diagrammatically the meiotic technique.

The number of the meiotic preparation and the number of the slide were recorded on the slide, using a diamond-tipped pencil. The materials used in making meiotic preparations are listed in Appendix III.

3.2.5 Discussion

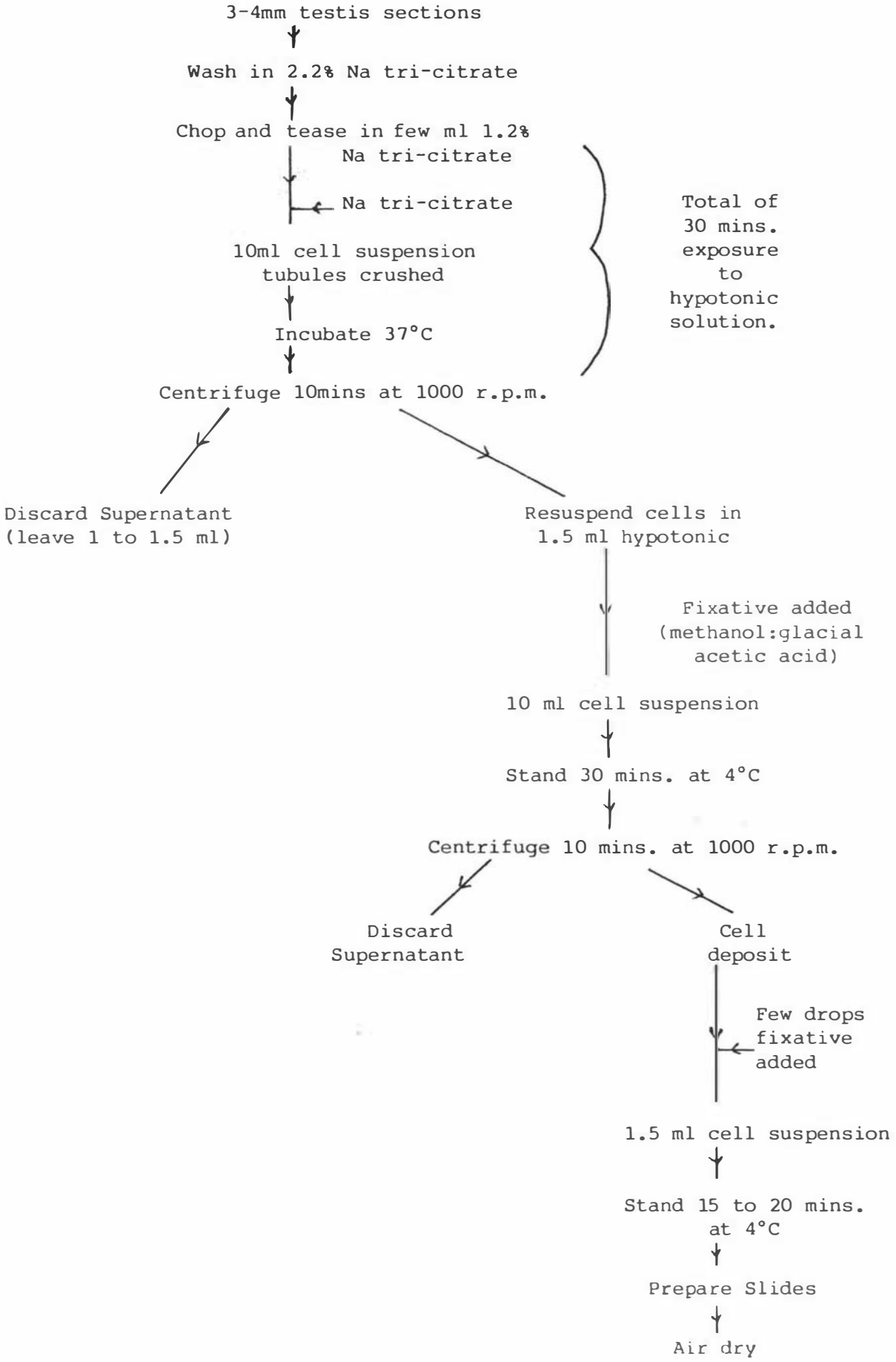
There are a number of reports in sheep of the use of an air-drying method rather than histological or squash techniques. Bruère (1969) and Nadler *et al.* (1973) studied sheep meiotic cells but the results from both studies were poor. Better results, using modifications of the air-drying method, were obtained by Chapman (1974), Logue (1977) and Long (1978) in their studies on male meiotic cells.

Many factors affect the quality and quantity of meiotic cells. These include: -

(a) The length of time between castration and the processing of the testicular material. For the best results the time interval between castration and the start of hypotonic treatment was kept to a minimum.

(b) Effect of time of year
When the spermatogenic activity increases with the onset of the breeding season (autumn) the testes increase in size and become firm and elastic. Most meiotic preparations were obtained during this time and they were usually good. During the non-breeding season the testes become smaller and lose their tone. Meiotic preparations made at this time were often poor.

FIGURE 3.1: FLOW-DIAGRAM OF TECHNIQUE USED IN THIS STUDY FOR THE PREPARATION OF MEIOTIC CHROMOSOMES



(c) Hypotonic fluid

The optimum concentration, exposure time and temperature at which the hypotonic solution acts, probably differ for the preparation of meiotic cells from different species (Ford and Evans, 1969).

The shortest exposure time consistent with good spreading of chromosomes should be used. An excessive exposure to hypotonic solution results in fewer good cells in the end preparations since it is thought that the meiotic and mitotic cells are more prone to rupture than non-mitotic cells. It is believed that autolysis begins in the lining cell and it has therefore been suggested that the cells should be exposed to the hypotonic solution at a low temperature. In the present study it was found that better results were obtained using the hypotonic solution at 37°C than at room temperature (16°C).

Ford and Evans (1969) and Chapman (1974) used fresh sodium citrate as their hypotonic solution. Only fresh hypotonic solution was used in this study although 3 to 4 day old sodium citrate has been tried by Chapman (1974) without any noticeable improvement.

Chapman (1974) tried hypotonic solutions ranging in concentration from 0.7 to 1.4 percent. She considered that the optimal concentration was 1.2 percent. Long (1978) used 1.0 percent sodium citrate as the hypotonic solution in her meiotic studies in sheep.

Chapman (1974) also tried various exposure times ranging from 5 to 55 minutes. She found that an exposure time of 30 minutes gave the best spreading and morphology of chromosomes. In the present study good results were obtained when the cells were exposed for 30 minutes to fresh 1.2 percent sodium citrate solution.

Potassium chloride has been widely used as the hypotonic solution (Hungerford, 1965, 1971; Clendenin, 1969; Luciani *et al.*, 1971; Long, 1978 and others). However, Chapman (1974) observed no improvement using potassium chloride instead of sodium citrate.

(d) Centrifugation

Chapman (1974) tried various times of centrifugation and she found that ten minutes was adequate. Long (1978) also used ten minutes for the time of centrifugation. This time was consequently used in this study.

After centrifugation the supernatant is discarded. This has become the routine practice since when the supernatant was processed few cells were seen on the slides made from these preparations (Chapman, 1974; Author).

(e) Fixation

This is considered to be the most critical step in obtaining satisfactory preparations (Long, pers. comm. 1979). There is a great number of possible variants such as the volume of supernatant retained; the volume, shape and density of the pellet of cells; the time interval between fixative changes; the number of changes of fixatives and the effects of temperature.

The most important variable is probably the speed of penetration of the fixative (Ford and Evans, 1969). Faster penetration is achieved when the cells are in suspension at the time the first fixative is added. The penetration is slower when the pellet of cells is larger in size. Ford and Evans (1969) considered that an optimum size of the pellet is about 2 mm in depth. They also reported that the cells were fixed better when they were in a pellet rather than in suspension.

A marked loss of cells occurred if the fixative was changed more than twice (Chapman, 1974; Author). As a consequence, only two changes of fixative were used.

(f) Preparation of slides

Improved spreading of chromosomes was achieved by using a dilute suspension rather than a dense suspension (Ford and Evans, 1969; Chapman, 1974). The same conclusion was drawn from the present studies.

(g) Stages visible

Although satisfactory preparations of cells in most stages of the meiotic cycle were found there were few cells in either 1st or 2nd anaphase. It is thought that the hypotonic solution arrests the cell division at the metaphase stage and there is a progressive reduction in cells at the 1st and 2nd anaphase (Ford and Evans, 1969).

Few spermatogonial metaphases were observed by Eliasson *et al*. (1969), Ford and Evans (1969) and Chapman (1974) in their meiotic preparations. Few spermatogonial metaphases were seen in the preparations made in this study.

3.3 STAINING TECHNIQUES

Initially all slides were stained with aceto-orcein (Appendix IV). The slides were stained in refiltered aceto-orcein for at least two hours. They were passed through two changes of 95 percent alcohol (5 seconds in each), dipped in absolute alcohol and then allowed to stand for 20 minutes in two consecutive dishes of xylol. The slides were permanently mounted using D.P.X.

Latterly slides were stained with 2 percent Giemsa for 10-15 minutes (Appendix V). This was a quick, and success-

ful, staining technique.

3.4 HISTOLOGICAL SECTIONS

Pieces of testis 5 mm thick were removed, immediately after castration, and placed in a fixative. The pieces were fixed in Bouin's fluid. After 24 hours the Bouin's fluid was replaced by 70 percent alcohol. The alcohol was discarded, and the tissue placed overnight in a Standon Elliott automatic tissue processor. The next morning, the tissue was embedded in paraffin blocks and cut into Sections 5μ thick. The sections were stained with H & E (Ehrlich's haematoxylin + 10% aqueous Eosin-y).

3.5 ANALYSIS OF RESULTS

3.5.1 Microscopy

All slides were scanned using the 10 x objective. Each cell which was to be examined more closely had its position on the slide read from a vernier scale and recorded.

When a cell was seen, it was examined under the 20 x objective. If the chromosomes were too contracted or not spread sufficiently the cell was rejected. The other cells were then examined under oil immersion with the 50 x objective. If the chromosomes were not entirely clear the cell was rejected.

The chromosomes in the diplotene, diakinesis and metaphase I cells were counted and the number recorded. The presence and position of the sex chromosomes were noted along with any other unusual features of the cell. The number of chromosomes and the number of chromosome arms were counted for each secondary spermatocyte. The type of sex chromosomes, and the number and type of translocation chromosome(s) when present, was recorded. The analysis of the cells is discussed in greater detail in Chapter 4 and 5.

All slides were labelled and filed in groups. Each group consisted of slides prepared from the one animal.

Cells were counted using a hand operated counter (Appendix VII). The cell was rejected if there was any doubt surrounding the number of chromosomes present. A number of cells from different stages of meiosis were photographed and karyotypes prepared. The histological sections of the testes of all rams were assessed for spermatogenic activity.

3.5.2 Photographic techniques

The wide field (15x) eye-pieces on the microscope were exchanged for one ordinary (10x) eye-piece, and one (10x) photographic eye-piece with a focusing sight and frame. Photographs were usually taken at 500x and sometimes at 1000 X. All photographs were taken on Copex-pan rapid film 37 ASA (Agfa-Gevaert) using a photoautomat (Wild) with fully-automatic exposure and built-in light meter (Appendix VI). A record was kept of each photograph taken.

The films were developed in the photographic unit as described in Appendix VIII. The negatives were labelled and filed in a book folder.

The negatives were enlarged (Appendix IX) and printed on Ilfobrom (Ilford, Australia Proprietary Ltd) Number 3 or 4, depending on whether the negative was light or dark (Appendix X).

Histological preparations were photographed on Pan F film (32 A.S.A. Ilford) (Appendix XI).

3.5.3 Karyotypes

The chromosomes on the print were carefully cut out avoiding any accidental omissions or duplications. The chrom-

osomes were glued to ruled sheets of cardboard and were arranged in decreasing order of size. The sex bivalent or sex chromosomes were placed last. The bivalents, or chromosomes were labelled and the karyotype was allowed to dry. An entire photograph of each cell was pressed onto the card which was labelled and filed.

CHAPTER 4

MEIOSIS IN THE NORMAL (54,XY) RAM

4.1 NOMENCLATURE OF MEIOTIC CHROMOSOMES

The notations used to describe single cells and to summarize the meiotic analysis in the ram follow the recommendations of the Paris Conference (1971) for human cytogenetics.

The stages of meiosis are indicated by abbreviations: M1 - first diakinesis and metaphase and M11 - second metaphase.

The abbreviations are followed by the total count of separate chromosomal elements. The sex chromosomes are indicated by either xy or xx when associated and x,y when separate. Either the absence or presence of chromosomal elements is denoted after the sex chromosomes by the abbreviation -A or +A.

Some primary spermatocyte samples are: -

M1, 27, xy	A primary spermatocyte at diakinesis or metaphase I with 27 chromosome elements including an xy bivalent.
M1, 28, x,y	A primary spermatocyte at diakinesis or metaphase I with 28 elements including x and y univalents.
M1, 26, xy, -A	A primary spermatocyte at diakinesis or metaphase I with 26 elements. An autosomal bivalent is missing.

4.2 LITERATURE REVIEW

4.2.1 Meiotic studies in male domestic animals with normal karyotypes

The meiotic chromosomes of domestic animals have been less extensively studied than those of either man or rodents. As a result most of the data on them is qualitative.

4.2.1.1 Horse

Makino (1943,b) described the morphology of meiotic chromosomes in two breeds of horse and recorded counts of 33 in both primary and secondary spermatocytes. It has since been found that the diploid chromosome number for the horse is 64 (Rothfels, ~~Alexrad~~ Axelrad, Siminovitch, McCulloch and Parker, 1959).

Relatively little meiotic work has been carried out in this species. Chandley, Jones, Dott, Allen and Short (1974) made limited meiotic studies on an Arab stallion. The most extensive meiotic study was carried out on 8 stallions by Scott and Long (1980). The other meiotic studies reported have been made on equine hybrids (Short, Chandley, Jones and Allen, 1974; Chandley, Short and Allen, 1975).

4.2.1.2 Cattle

Meiotic chromosomes in cattle have been studied by several authors including Krallinger (1931), Makino (1944), Melander and Knudsen (1953), Knudsen and Bryne (1960), Treadwell *et al*. (1968), Gustavsson (1969) and Popescu (1971). More recent meiotic studies have been made by Logue (1975, 1977) and Logue and Harvey (1978). The diploid chromosome number of 60 was found in spermatogonial metaphases (Krallinger, 1931; Makino, 1944; Melander and Knudsen, 1953; Knudsen and Bryne, 1960; Gustavsson, 1969; Popescu, 1971).

The various meiotic stages in the bull have been studied extensively (Gustavsson, 1969). Makino (1944) was the

first to describe the end-to-end association of the x and y chromosomes. Popescu (1971) suggested that a terminal chiasma may be involved in the union of the sex chromosomes. He found that 33.3 percent of the diakinesis-metaphase I cells examined had separated sex chromosomes. Chiasmata counts in the bull have been made by Popescu (1971) and Logue (1977). Popescu (1971) examined only 38 cells and estimated the chiasmata number per cell at 53.7 with the average number per bivalent of 1.8. Logue (1977) examined 325 cells and reported a chiasmata number per cell of 49.5.

4.2.1.3 Goat

Goat meiotic chromosomes were investigated by Makino (1943,a) and more recently by Datta (1970) and Logue (1977). Makino (1943,a) counted 30 bivalents at metaphase I including a sex bivalent. Datta (1970) from a study of 200 cells in all stages of meiosis confirmed this and concluded that meiosis in goats was similar to that found in other animals. Chiasmata counts were calculated from 15 cells by Datta (1970) who recorded the average number per cell at diplotene, diakinesis and metaphase I as 72, 54.2 and 49 respectively. Chiasmata counts from 20 cells were recorded by Logue (1977) and he found an average of 49.7 chiasmata per cell.

Makino (1943,a) found the x chromosome to have a tripartite structure and be connected at its end with the y chromosome. Datta (1970) made a different interpretation and proposed that the chiasma formation occurred between the short arm of the x chromosome and one of the arms of the y chromosome. Datta (1970) postulated that the gap between the two chromosomes sometimes seen at metaphase I was proof of the occurrence of chiasma terminalization.

4.2.1.4 Sheep

The early studies on meiotic chromosomes were made to determine the diploid chromosome number (Wodsedalek, 1922; Krallinger, 1931; Pchakadze, 1936). Ahmed (1940) and

Berry (1941) were the first to report that there were 27 bivalents at metaphase I. Makino (1943,a) confirmed this in his studies and he also gave the first description of the chromosomes in spermatogonial cells as well as in primary and secondary spermatocytes. Ahmed (1940) analysed an unreported number of cells and recorded the chiasmata counts from 25 cells.

A detailed description of meiosis in normal rams has been made by Chapman (1974). Chiasmata counts from 34 cells were recorded and a total of 309 cells in the diakinesis-metaphase I stages were analysed (Chapman and Bruère, 1977). There were 27 bivalents in diakinesis-metaphase I cells, which agreed with the previous study by Loir (1971) and is the same number as Nadler *et al*. (1973) counted in each of 45 cells from a wild ram.

Long (1975; 1978) also made a detailed examination of meiosis in a normal ram. Her findings were in agreement with the previous reports by Loir (1971) and Chapman (1974).

4.2.1.5 Pig

The early meiotic studies in the pig were reviewed by Bryden (1933). He was the first to report a haploid chromosome number of 19 for the domestic pig and he also studied the chromosome morphology of different stages in meiosis. However, Bryden (1933) did not report on the number of cells examined in his study, nor provide information on chiasmata counts.

The first description of the association between the sex chromosomes was given by Crew and Koller (1939). They described the pairing and non-pairing segments on the x chromosome and chiasma formation between the homologous sections of the x and y chromosomes. Crew and Koller (1939) made chiasmata counts on 36 cells from five boars. They found that the age of the boar had no effect on the chiasmata frequency and they calculated the average

number of chiasmata per bivalent of 2.16 at diplotene and 2.06 at metaphase I.

More recent meiotic studies in boars have been very limited (King, pers. comm. 1979).

4.2.1.6 Dog and Cat

A number of authors have studied the morphology of dog meiotic chromosomes (Minouchi, 1928; Ahmed, 1941; Eliasson *et al.*, 1967; Ford, 1969; Williams *et al.*, 1971). Chiasmata counts were first made in dogs by Ahmed (1941). He found in three breeds of dog that the total number of chiasmata per metaphase I cells varied between 78.2 and 82.6. He also reported that the morphology of the sex bivalent was not constant.

Williams *et al.* (1971) carried out extensive meiotic studies on preparations from 150 dogs. They observed, in pachytene cells, non-homologous associations in bivalents, usually appearing as end-to-end attachments. Eliasson *et al.* (1967) observed separated x and y chromosomes in 16 (17.4 percent) of the 92 diakinesis-metaphase I bivalents examined. Williams *et al.* (1971) also observed separated x and y chromosomes. The x and y chromosomes were usually associated terminally (Eliasson *et al.*, 1967; Williams *et al.*, 1971) and the union might involve the short arm of the x chromosome (Eliasson *et al.*, 1967).

Studies on the meiotic chromosomes of cats are limited. Koller (1941) studied the chromosomes during meiosis and he counted an average of 2.7 chiasmata per bivalent at diakinesis and 2.4 at metaphase I. Three types of sex bivalent at meiotic metaphase were identified by Koller (1941).

4.2.2 Meiotic studies in "normal" men

The meiotic chromosomes of man have been investigated far

more than the meiotic chromosomes of male domestic animals.

The diploid chromosome number for man of 46 was originally found by Tjio and Levan (1956). This finding was confirmed by Ford and Hamerton (1956) who observed 46 chromosomes in spermatogonial metaphases and a total of 23 bivalents in primary spermatocytes. There has since been a large number of meiotic studies in men with a normal karyotype (46,xy) and normal spermatogenesis (Eberle, 1963; 1966; Böök and Kjessler, 1964; Sasaki and Makino, 1965; McIlree, Tulloch and Newsam, 1966, b; Falek and Chiarelli, 1968; Fraccaro, Hultén and Lindsten, 1968; Luciani, 1970; Luciani, Carlon and Stahl, 1970; McDermott, 1971; Ferguson-Smith, 1972; Hultén and Lindsten, 1973; Skakkabaek, Bryant and Philip, 1973; Hultén, 1974). These studies have provided a great amount of information on the behaviour and morphology of meiotic chromosomes in normal men. Studies on the meiotic chromosomes in men with various clinical conditions including infertility have been of increasing interest (Kjessler, 1966; McIlree, Price, Court Brown, Tulloch, Newsam and MacLean, 1966,a ; Chandley, 1970; Hultén, Eliasson and Tillinger, 1970; Skakkebaek *et al.*, 1973; Miklos, 1974; Chandley *et al.*, 1975; Chandley Maclean, Edmond, Fletcher and Watson, 1976).

The C-banding studies of Hultén (1971; 1974); Falek, Chen and Chan (1972); Bobrow, Madan and Pearson (1972); Chandley and Fletcher (1973) and Chandley, Senanez and Fletcher (1976) and the fluorescent techniques of Caspersson, Hultén, Lindsten and Zech (1971) and Hultén (1974) have greatly advanced the understanding of meiosis in man and have enabled the individual bivalents to be identified by their banding patterns.

Chiasmata were usually counted in cells at the diakinesis stage. Estimates of chiasmata frequency per cell are given in Table 4.1. The number of chiasmata per bivalent varied from one to five reported by Ford and Hamerton (1956) and

TABLE 4.1: CHIASMATA FREQUENCIES IN MAN

Author	Number of Cells Counted	Average number Chiasmata/cell
Ford, C.E. and Hamerton, J.L. (1956)	23	55.9
McIlree, M.E. *et al.* (1966,b)	96	49.8 - 58.3
Eberle, P. (1966)	4 60	55.7 (Diplotene) 44.0 (Diakinesis)
Falek, A. and Chiarelli, B. (1968)	8	49-58 (55.3 Average)
Luciani, J.M. (1970)	88	47.5
Chandley, A.C.; Christie, S.; Fletcher, J. Frackiewicz, A. and Jacobs, P.A. (1972)	93	49
Ferguson-Smith, M.A. (1972)	849	51.11 ± 2.97*
McDermott, A. (1973)	518	53.7
Skakkebaek, N.E. *et al.* (1973)	57	51.2
Hultén, M. (1974)	41	50.61 ± 3.87*

* Standard deviation

Luciani (1970) and one to eight chiasmata in bivalents at diplotene (Eberle, 1966). As the bivalents pass from the diakinesis to the metaphase I stage they contract and there is a reduction in the number of chiasmata.

4.3 MEIOSIS IN THE NORMAL RAM (54, XY)

4.3.1 Introduction

Meiotic preparations were obtained from 3 rams (*Ovis aries*). An example of a mitotic karyotype from a normal ram is shown in plate 4.1. The rams used aged from one to four years and were genitally and physically sound. Histological examination of the testes from these rams is reported in Appendix XIII. The rams are identified by a number and a letter, and the year of its birth was indicated (79 = 1979). So for example an ear tag could be read as A1/79.

The technique used in this study gave a predominance of cells in the diakinesis-metaphase I stages. A lesser number of cells were recorded in the spermatogonial and secondary spermatocyte stages.

4.3.2. Results of analyses of spermatogonial and primary spermatocyte cells

4.3.2.1 Spermatogonial cells

Eight spermatogonial cells were seen in the meiotic preparations from the three rams. Four cells were recorded in both ram A2/79 and ram B51/79. The modal number was 54. No hyperploid cells were seen and only one hypomodal cell was counted and chromosome loss in this cell probably occurred during slide preparation. No polyploid cells were seen in the preparations from the three rams. Two examples of spermatogonial cells are shown in plates 4.2 and 4.3.

PLATE 4.1: Mitotic karyotype from a normal ram (54,xy). There are six large metacentric chromosomes, an acrocentric x chromosome and a submetacentric y chromosome.
(Aceto-orcein).

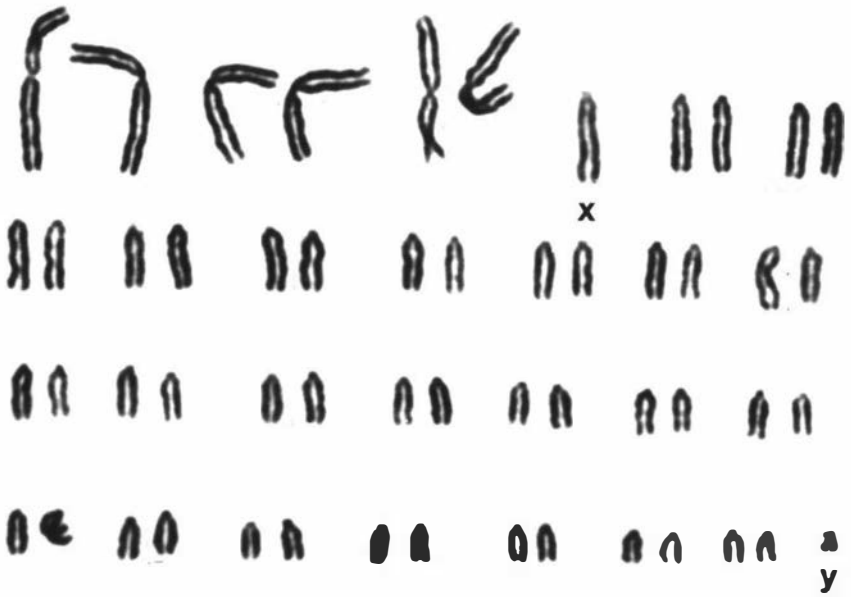

x
a
y

PLATE 4.2: Spermatogonial metaphase from a normal ram. Chromatid separation is evident in some chromosomes. Sex chromosomes are identified. (Giemsa x 2,000)

PLATE 4.3: Spermatogonial metaphase from a normal ram. Sex chromosomes are identified. (Giemsa x 2,000)


X
y


X
y

4.3.2.2 Diakinesis-metaphase I

The chromosomes in cells at the diakinesis-metaphase I stages were easy to count as the bivalents were well separated and they rarely overlapped each other. However, it was difficult to determine the transition stage and the two stages were therefore combined for the analysis of the chromosome counts.

Chromosome counts on cells at diakinesis-metaphase I from the 3 normal rams (2n = 54, xy) are given in Table 4.2. A total of 396 cells were counted and this total included 96 (24.2 percent) polyploid cells.

The analysis of the non-polyploid cells is given in Table 4.2. The percentage of cells with 27 chromosomal bodies was 88.3 percent. Two hypermodal cells with 28 chromosomal bodies were recorded. Thirty three hypomodal cells, with one or more chromosomal bodies missing, were recorded.

The modal cell had 27 chromosomal bodies comprising of 26 autosomal bivalents and a sex bivalent. The 26 autosomal bivalents were comprised of three large bivalents and 23 small bivalents. The large bivalents were formed from the pairing of the metacentric chromosomes and the small bivalents were formed from the pairing of acrocentric chromosomes. Representative cells are seen in plates 4.4 to 4.6 and Figure 4.1.

Two of the 300 non-polyploid cells had 28 chromosomal bodies. They were comprised of 26 autosomal bivalents and separated x and y chromosomes. Twentyfour of the sex bivalents were associated with one of the small autosomal bivalents. The percentage of cells from the 3 rams with the association varied from 5.0 to 8.6 percent.

The analyses of the polyploid cells are given in Table 4.3. Of the 96 cells recorded 68 were tetraploid, 16 were hexaploid and 8 were octaploid. Four cells were not

TABLE 4.2: CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS - METAPHASE I

Normal Rams (2n = 54, xy)

Ram Identification	Number of Chromosome bodies/cell				Total	Number of Polyploid Cells	Total Including Polyploid Cells	Analysis of cells excluding polyploids. Number of cells with:					Total Number Cells Analysed	Number of cells with xy associated with autosomal bivalent	x and y separated	
	<25	26	27	28				27,xy	28,xy	26,xy-A	25,xy-2A	Other Aberrations			Number	%
A1/79	6	9	90	1	106	59	165	90	1	9	4	1-22,xy-5A 1-21,xy-6A	106	8	1	0.9
A2/79	1	2	17	0	20	5	25	17	0	2	1		20	1	0	0
B51/79	8	7	158	1	174	32	206	158	1	7	0	5-24,xy-3A 1-24,-xy-2A 1-23,xy-4A 1-22,xy-5A	174	15	1	0.6
Total	15	18	265	2	300	96	396	265	2	18	5	10	300	24	2	0.7

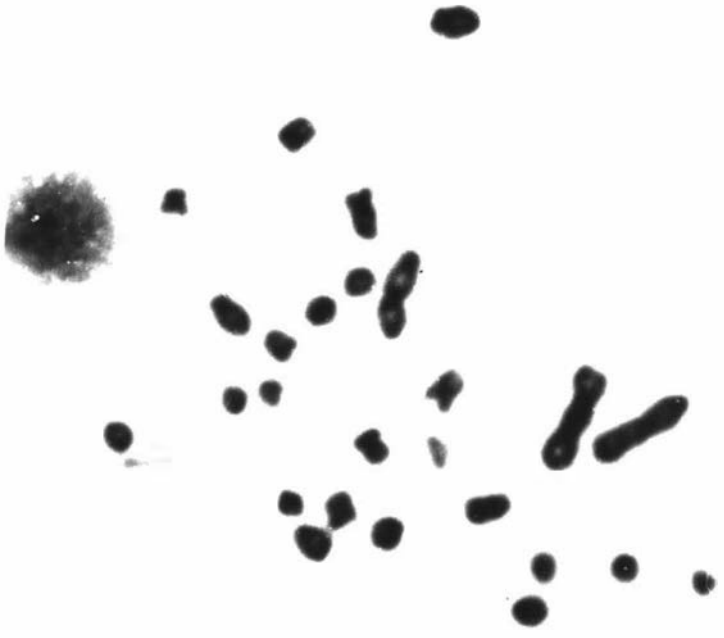
TABLE 4.3: NORMAL RAMS (54,XY) POLYPLOID CELLS AT DIAKINESIS-METAPHASE I

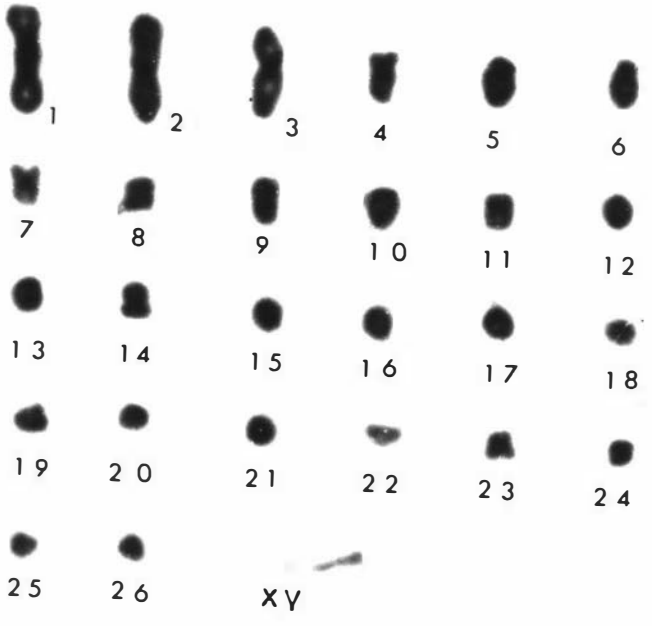
Ram Identification	Number of Cells showing chromosome complement			N.A.	Total	4n			6n		8n		N.A.	Total
	4n	6n	8n			54,2xy	53,2xy	<53,2xy	81,3xy	<81,3xy	108,4xy	<108,4xy		
A1/79	38	13	6	2	59	24	3	11	6	7	2	4	2	59
A2/79	5	0	0	0	5	3	1	1	0	0	0	0	0	5
B51/79	25	3	2	2	32	16	4	5	2	1	1	1	2	32
Total	68	16	8	4	96	43	8	17	8	8	3	5	4	96

N.A. = Not analysed

PLATE 4.4: Cell in diakinesis from a normal ram B51/79. There are 27 bivalents including three large autosomal bivalents, 23 small acrocentric bivalents and a sex bivalent.
(Giemsa x 2,500)

FIGURE 4.1: Karyotype of cell illustrated in Plate 4.4.




1
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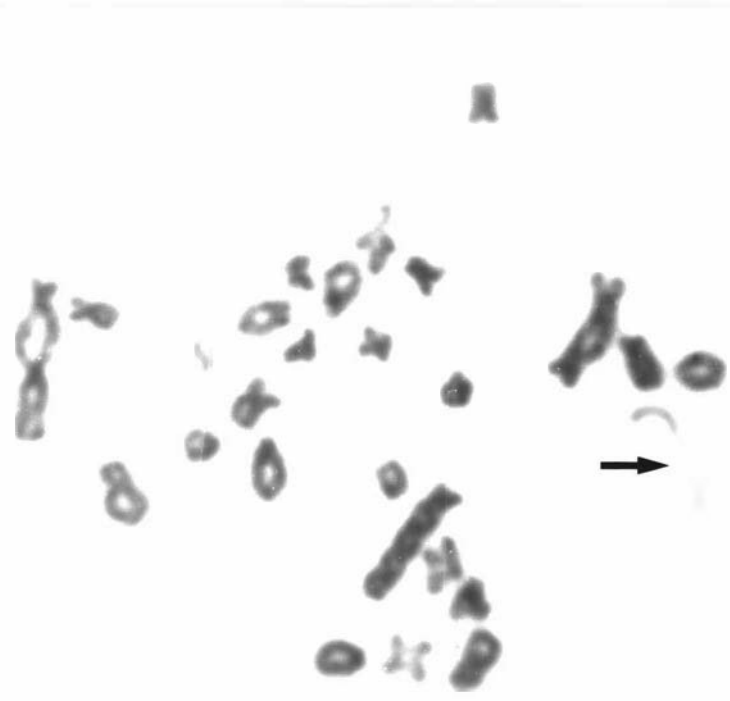
23
24
25
26
XY

PLATE 4.5: Cell in diakinesis from a normal ram, B51/79. There are 27 bivalents including an xy bivalent (arrowed).
(Giemsa x 2,500).

PLATE 4.6: Polyploid cell in diakinesis from a normal ram. There are 54 bivalents including two XY bivalents (arrowed).
(Giemsa x 2,500).



analysed. None of the cells were seen with univalent chromosomal bodies.

4.3.3 Analysis of secondary spermatocytes

4.3.3.1 Introduction

The method of analysis of chromosomes of second metaphase was that used by Chapman (1974) based on the system of Tettenborn and Gropp (1970).

Problems can arise in counting metaphase II chromosomes because of their contracted nature and the fact that not infrequently a single chromatid from a metacentric or acrocentric chromosome could be confused easily with the two chromatids of an acrocentric chromosome still held together at the centromere. For this reason Tettenborn and Gropp (1970) suggested that the counts of total chromosome arms and the number of chromosomes should be made. All acrocentric chromosomes were counted as having one chromosome arm. Metacentric and submetacentric chromosomes were counted as having two chromosome arms and the x and y chromosomes were both counted as having one chromosome arm. However, the y chromosome is known to be a small submetacentric chromosome.

The haploid chromosome complement for the normal ram (2n = 54, xy) consists of 23 acrocentrics, 3 metacentrics and one sex chromosome, a total of 27 chromosomes or 30 chromosome arms.

The haploid number for the secondary spermatocyte of a normal ram was scored as $\frac{27,x}{30}$ or $\frac{27,y}{30}$.

A euploid cell is a cell that has a balanced set of chromosomes whilst an aneuploid cell is a cell that has either more or less than the normal haploid number of chromosomes. Euploid secondary spermatocytes are therefore those having a fundamental number divided by two (N.F./2) of 30 and

aneuploid secondary spermatocytes are those with a N.F./2 of greater than, or less than 30.

A polyploid cell is a cell whose chromosome complement is an exact multiple of the basic haploid number characteristic of the species (Swanson, Merz and Young, 1967).

The author used the following terminology to describe the polyploid secondary spermatocyte cells. Diploid cells had 2n = 54 chromosomes; triploid 2n = 81 chromosomes and tetraploid 2n = 108 chromosomes.

4.3.3.2 Results - analysis of metaphase II cells

The morphology of the chromosomes in the MII figures was found to be similar to that described previously by Chapman (1974). The metacentric chromosomes and the sex chromosomes, x and y, were easily distinguished and they appeared similar to the corresponding chromosomes found in mitotic cells.

The y chromosome was the smallest chromosome visible in the haploid set and it was often seen to be lightly stained. The x chromosome was distinctive as a large acrocentric chromosome with 2 chromatid arms which remained more or less in apposition.

One hundred and three metaphase II figures were recorded in the 3 rams with a karyotype of 2n = 54, xy. This total included 19 polyploid cells of which 3 could not be analysed (N.A.).

The analyses of the metaphase II cells from normal rams are recorded in Tables 4.4 and 4.5.

Eighty (95.2 percent) of the 84 non-polyploid metaphase II figures examined were euploid. There were no hypermodal cells and 4.8 percent of the cells were hypomodal or aneuploid. The hypomodal cells are probably caused by

TABLE 4.4: DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS AT MII IN NORMAL RAMS (2n = 54,XY)

Ram Identification		27,X / 30	27,Y / 30	Total	24,X / 27	25,Y / 28	26,X / 29	26,Y / 29	Total	Total MII/Ram
A1/79	Number of Cells	3	5	8	1	1	0	0	2	10
A2/79		9	10	19	0	0	1	0	1	20
B51/79		29	24	53	0	0	0	1	1	54
Total		41	39	80	1	1	1	1	4	84

TABLE 4.5: CHROMOSOME COUNTS FROM POLYPLOID CELLS AT METAPHASE II IN NORMAL RAMS

(2n = 54,XY)

Ram Identification	$\frac{54}{60}$,X,Y	$\frac{81}{90}$,X,X,Y	$\frac{108}{120}$,2(XY)	N.A.	Total	Total No. of MII cells Counted	% Polyploid MII cells
A1/79	1	1	0	2	4	14	28.6
A2/79	3	0	0	0	3	23	13.0
B51/79	9	1	1	1	12	67	17.9
Total	13	2	1	3	19	104	18.3

N.A. = Not analysed

chromosome loss during slide preparation.

Nineteen polyploid cells were recorded. Two cells were triploid and 1 was tetraploid and the remainder were diploid (13 cells). None of the cells were either hypoploid or hyperploid. The polyploid cells appeared to be true polyploids rather than two or three cells lying close together. The percentage of polyploid cells recorded in the 3 normal rams was 18.3 percent. A high number of MII figures were analysed and counted in ram, B51/79. Twelve of the 67 figures recorded in this ram were polyploid. It would therefore seem that the mean percentage of polyploid cells provides a reasonable estimate of the incidence of polyploidy in normal rams.

It was found in the 3 normal rams that there was no departure from the expected 1:1 ratio of male:female metaphase II cells (χ^2 not significant).

Plates 4.7 and 4.8 and Figures 4.2 and 4.3 show representative MII figures from normal rams.

4.3.4 Cell distribution at meiosis in normal rams

Cell distributions in each of the categories, spermatogonial metaphase, diakinesis and metaphase I and metaphase II are shown in Table 4.6.

4.4 DISCUSSION

4.4.1 Introduction

An A- type spermatogonial sperm cell produces two daughter cells one of which divides four times to produce 16 primary spermatocytes and the other becomes a dormant A- type cell during part of the seminiferous cycle and thus ensures the renewal of the spermatogonial population (Ortavant, 1959). The primary spermatocytes are the cells which undergo the meiotic division. The primary spermatocytes at

TABLE 4.6: DISTRIBUTION OF CELLS IN SPERMATOGONIAL METAPHASE (SM), DIAKINESIS AND METAPHASE I (MI) AND METAPHASE II (MII) IN NORMAL RAMS (54,XY)

Polyploid cells included in counts are recorded in brackets after counts.

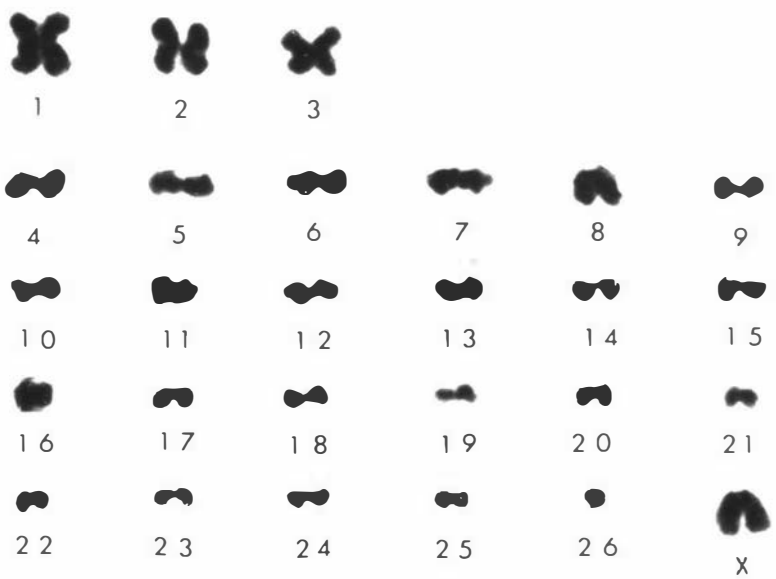
Ram Identification	Sm	MI	MII	Total	MII/MI ratio
A1/79	0(0)	165(59)	14(4)	179(63)	0.1
A2/79	4(0)	25(5)	23(3)	52(8)	0.9
B51/79	4(0)	166(32)	66(12)	276(44)	0.4
Total	8(0)	396(96)	103(19)	507(115)	0.3 *

* Mean ratio

The proportion of cells in each category varied between rams. Relatively few spermatogonial cells were seen.

PLATE 4.7: Metaphase II cell from a normal ram B51/79. There are 30 chromosome arms and 27 chromosomes including three metacentric chromosomes and an X chromosome.
(Giemsa x 3,000)

FIGURE 4.2: Karyotype of cell illustrated in Plate 4.7.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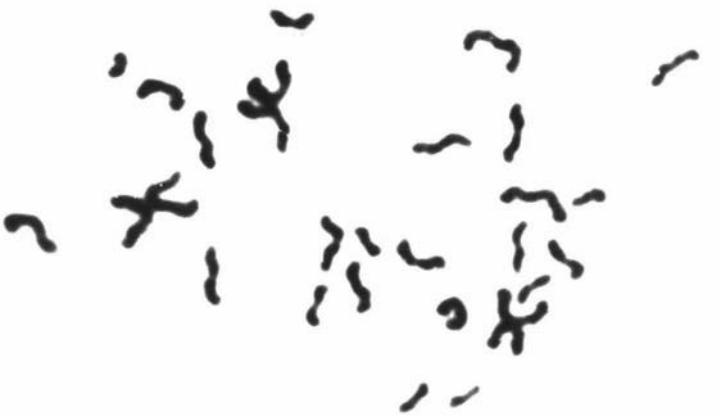
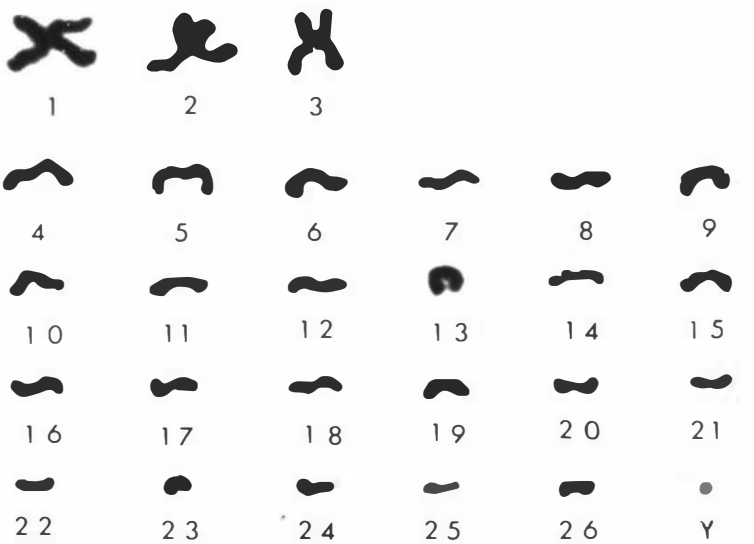
X

PLATE 4.8: Metaphase II cell from a normal ram B51/79. There are 27 chromosomes including three meta-centrics and a Y chromosome which is more lightly stained than the autosomes. (Giemsa x 3,000)

FIGURE 4.3: Karyotype of cell illustrated in Plate 4.8. There are 30 chromosome arms. The chromatid arms of the three metacentric chromosomes are slightly separated and could be confused with one of the large acrocentric chromosomes.

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25
26
Y

metaphase I in a normal ram each have 27 bivalents or tetrads. At the first meiotic division 27 dyads pass to each pole. The two daughter secondary spermatocytes undergo the second meiotic division and at anaphase II 27 monads pass to each of the four haploid nuclei formed.

4.4.2 Cell distribution at meiosis in normal rams

Few spermatogonial cells were observed in the meiotic preparations from the three normal rams. A similar result was found by Chapman (1974) in her studies on normal rams. It was also similar to the results found in studies on other species (Ford and Hamerton, 1956; McIlree *et al.*, 1966,b; Eliasson *et al.*, 1967; Ford and Evans, 1969; Skakkebaek *et al.*, 1973).

The observed ratio of metaphase II to metaphase I cells in the 3 normal rams was 0.3. This was considerably less than the theoretically expected ratio of 2.0. However, it is similar to the ratio (0.3) reported by Chapman (1974) in her studies on normal rams. The true proportion of metaphase II cells in the meiotic preparations is underestimated since a greater number of metaphase II cells than metaphase I cells were discarded as unsuitable for analysis. In contrast, Skakkebaek *et al.* (1973), in their studies on normal men, counted all the metaphase I and metaphase II cells observed and they reported a higher MII/MI ratio of 0.9. Therefore, this ratio was a more accurate reflection of the true proportions of metaphase I and II cells.

A number of the primary spermatocytes fail to pass through anaphase I and mature into secondary spermatocytes. This failure in spermatocyte maturation is seen in the MII/MI ratio and it provides useful information on the spermatogenic activity in the testes of sub-fertile and infertile individuals.

4.4.3 Non-association of sex chromosomes

The incidence of cells with dissociation of the x and y chromosomes (0.7 percent) in this study was similar to that reported by Chapman (1974) in her studies on normal rams (1.2 percent). These levels were considerably lower than those reported in the dog (17.4 percent) (Eliasson *et al.*, 1967), man (15 percent) (Skakkebaek *et al.*, 1973) and horse (16.1 percent) (Scott and Long, 1980).

The cause of the non-association of the sex chromosomes has been of considerable speculation. Ford and Hamerton (1956) thought that although a number of the x and y dissociations could be due to precocious non-disjunction most could be accounted for by the rupture of the terminal connection between the chromosomes during slide preparation. Ford and Evans (1964) and Winsor, Ferguson-Smith and Shire (1978) also thought that the dissociation of the x-y bivalent often occurred during slide preparation. Aternatively it has been suggested that as the separation of the sex chromosomes tended to occur towards the later stages of metaphase I it would seem likely that precocious non-disjunction of the chromosomes could have occurred (Hultén *et al.*, 1966; Fraccaro *et al.*, 1968; Skakkebaek *et al.*, 1973; Chapman, 1974).

Although the cause of non-association of sex chromosomes is uncertain it has been observed that the low incidence of univalency in normal rams was subsequently reflected in the absence of hypermodal secondary spermatocytes. This suggested that the observed univalency in normal rams may have reflected the early separation of the x-y bivalent during diakinesis (Skakkebaek *et al.*, 1973). Or possibly these cells with dissociated sex chromosomes were selected against at anaphase I and failed to mature into secondary spermatocytes.

4.4.4 Metaphase II

The results from this study and the earlier meiotic studies on normal rams by Chapman (1974); Logue (1977) and Long (1978) are summarized in Table 4.7.

It is seen from Table 4.7 that, as previously reported for normal sheep by Chapman (1974), no hyperhaploid cells were found in the 84 cells evaluated from the 3 normal rams in this study. In contrast, Logue (1977) and Long (1978) both reported 2 hyperhaploid cells from their studies on normal rams. Higher proportions of cells with 29 chromosome arms were also recorded in the studies by Logue (1977) and Long (1978). Although it is possible that these cells could have been true aneuploid secondary spermatocytes it was more likely that they were either produced during the preparation of the slides or as a result of chromosome loss due to lagging at first anaphase. The evidence suggests that there are either very few or no aneuploid secondary spermatocytes produced during spermatogenesis by normal rams. It has been previously suggested by Chapman (1974) that few or no aneuploid secondary spermatocytes are produced by normal and balanced homozygous rams (t_1t_1 and t_3t_3).

The results from the normal rams are compared to the findings from the translocation - carrying rams in Chapter 5 of this thesis.

4.4.5 Polyploidy

Poikiloploidy is a term that includes diploid, aneuploid and polyploid cells, all of which may be present in the same animal. It should first be noted that in this study individual variations in the percentages of polyploid cells were observed.

The percentage of apparent polyploid secondary spermatocytes in the normal rams of this study and in the study by Chapman

TABLE 4.7: DISTRIBUTION OF EUPLOID AND ANEUPLOID MII FIGURES IN NORMAL RAMS (Pooled Data)

Author	No. of Rams	Number of Cells	N.F./2 = <28 N	<28 %	28 N	28 %	29 N	29 %	30 N	30 %	31 N	31 %	>31 N	>31 %	Total
Chapman (1974)	10		6	9.7	2	1.6	3	4.8	52	83.9	0	0	0	0	62
Logue (1977)	2		0	0	3	5.5	8	14.5	42	76.4	2	3.6	0	0	55
Long (1978)	1		0	0	2	2.0	10	10.0	86	86.0	2	2.0	0	0	100
Author	3		1	1.2	1	1.2	2	2.4	80	95.2	0	0	0	0	84
Total	16		7	2.3	7	2.3	23	7.7	260	86.4	4	1.3	0	0	301

(1974) was higher than the reported values in other species. However, it should be mentioned that in this study only 104 secondary spermatocytes were counted and in the study by Chapman (1974) only 87 secondary spermatocytes were recorded.

The primary spermatocyte polyploid cells were predominantly tetraploid and diploid cells were the most common among the secondary spermatocytes. Cells of higher ploidy were rarely seen. These observations were similar to those reported by Sasaki (1964), Sasaki and Makino (1965), Hultén *et al*, (1970), Pogosianz and Brujako (1971) and Chapman (1974).

The cause of the polyploidy is uncertain. It may be artifactual, due to overlapping of cells as suggested by Hultén *et al*. (1970), Ford and Evans (1971) and Lin, Tsuchida and Morris (1971). Disturbances of cytokinesis in spermatogonial cells (Lin *et al*., 1971; Pogosianz and Brujako, 1971), endomitosis and fusion of spermatogonial cells (Fechheimer, 1961; Pogosianz and Brujako, 1971) have all been suggested as causes of polyploidy. It is likely that the fate of these cells is similar to that of the aneuploid cells, since recent studies on human, mouse and rabbit spermatozoa indicate that there is little evidence for the existence of high numbers of polyploid spermatozoa (Carothers and Beatty, 1975). The few polyploid spermatocytes that mature into polyploid spermatozoa could be one of the causes of triploid zygotes (Sasaki and Makino, 1965; Sumner, 1971; Jonasson, Therkelsen, Lauritsen and Lindsten, 1972; Niebuhr, 1974) and therefore contribute towards embryonic loss, as most triploids are aborted (McFeely, 1967; Carr, 1971; Niebuhr, 1974).

CHAPTER 5

MEIOSIS IN ROBERTSONIAN TRANSLOCATION CARRIER RAMS

5.1 INTRODUCTION

Three different Robertsonian translocations are known in sheep (Bruère, 1969; Bruère and Mills, 1971; Bruère *et al.*, 1972). The mating experiments carried out by Prof. A.N. Bruère at Massey University have resulted in the production of sheep with all combinations of the three types of Robertsonian translocations (Bruère, 1974; Bruère and Chapman, 1974; Bruère and Ellis, 1979). This includes sheep which have the three different centric fusions in the homozygous state (Bruère and Ellis, 1979). The description and nomenclature of the Robertsonian translocations of sheep have been based on the recommendations of the Paris Conference (1971) and is summarized in Table 5.1.

TABLE 5.1: NOMENCLATURE FOR ROBERTSONIAN TRANSLOCATIONS IN DOMESTIC SHEEP

	Massey I	Massey II	Massey III
Breed of origin	Romney	Romney	Drysdale
Chromosomes involved in translocation	5;26	8;11	7;25
Type of translocation Chromosome	Submetacentric	Metacentric	Submetacentric
Heterozygote designation detail	53,xy,-5,-26,+ t(5q 26q)	53,xy,-8,-11,+ t(8q 11q)	53,xy,-7,-25,+ t(7q 25q)
	or	or	or
Short	53,xy,t_1	53,xy,t_2	53,xy,t_3

The most detailed meiotic examination of translocation-carrying rams, to date, was carried out at Massey University by Chapman (1974). She analysed 1,008 MII figures from three different types of heterozygous Robertsonian translo-

cation-carrying rams (53,xy,t_1 ; 53,xy,t_2; 53,xy,t_3) and 225 MII figures from homozygous Robertsonian translocation-carrying rams (52,xy,t_1t_1;52,xy,t_3t_3) and rams with a normal karyotype (54,xy). She also studied the chromosome morphology during meiosis of the Robertsonian translocation-carrying rams (Chapman and Bruère, 1977).

The other meiotic studies have only been carried out on the t_1 Robertsonian translocation-carrying rams. Logue (1977) studied the meiosis in 5 heterozygous (t_1) rams. Chiasma counts and non-disjunction frequencies were also made by Long (1978) on three rams heterozygous for the t_1 translocation and one ram homozygous for the t_1 translocation. These rams were sent over to Britain by Prof. A.N. Bruère, Massey University.

This present work reports on the meiotic studies carried out on rams with various combinations of all three types of Robertsonian translocations. The identification, numbers and karyotypes of the rams studied are summarized in Table 5.2.

TABLE 5.2: ROBERTSONIAN-CARRYING RAMS FROM WHICH MEIOTIC CHROMOSOMES STUDIED

52,xy	51,xy	2n = 50,xy	49,xy
B147/75 - t_1t_3	B120/77 - $t_1t_1t_3$	B128/77 - $t_1t_1t_2t_3$	B38/79 - $t_1t_2t_2t_3t_x$
B 63/77 - t_1t_2	B154/77 - $t_1t_3t_3$	B141/77 - "	B92/79 - "
B166/78 - t_2t_3	B117/78 - $t_2t_3t_3$	B137/78 - $t_1t_2t_2t_3$	B196/76/79 - $t_1t_2t_2t_3t_3$
B6/79 - t_2t_x	B7/76 - $t_1t_2t_3$	B42/79 - "	
	B86/76 - "	B49/79 - "	
	B111/77 - "	B118/79 - "	
	B44/78 - "	B147/79 - "	
	B47/78 - "	B121/78 - $t_1t_2t_3t_x$	
	B101/78 - "	B2/79 - "	
		B32/79 - "	
		B140/77 - $t_1t_2t_3t_3$	

$t_x = t_1$ or t_3

5.2 RESULTS

5.2.1 Spermatogonial Metaphases

A number of spermatogonial cells were analysed from the translocation-carrying rams and are given in Table 5.3.

A total of 126 spermatogonial metaphases were analysed from the 27 translocation-carrying rams. Eleven (8.7 percent) of the cells were hypomodal. No hypermodal metaphases were seen, but 8 polyploid cells (6.4 percent) were recorded. The modal chromosome numbers for the individual rams were found in the remainder of the cells analysed.

The karyotypes of all the rams studied were determined from leucocyte cultures. Rams with a normal karyotype have 54 chromosomes. The translocation-carrying rams used in this study have a decreased chromosome number, as a result of the centric fusions present in the karyotype. For example, the ram B196/76, (49,xy,t_1 t_2 t_2 t_3 t_3), has 6 metacentric chromosomes, 2 translocation metacentrics (t_2 t_2), 3 translocation submetacentrics (t_1 t_3 t_3), 36 acrocentrics, 2 sex chromosomes. The karyotypes representative of the rams studied in this work are shown in Plates 5.1 to 5.4.

The morphology of the chromosomes in the spermatogonial metaphases was similar to that seen in the somatic mitotic metaphases. Plates 5.5 to 5.8 show some examples of spermatogonial metaphases.

5.2.2 Diakinesis - Metaphase I Stages

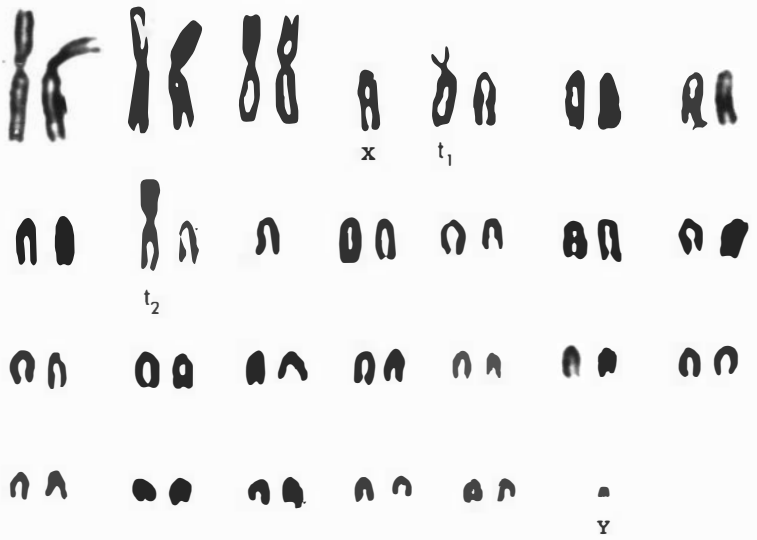
A total of 2,864 cells at the diakinesis-metaphase I stage were counted from the translocation-carrying rams. This total included 404 polyploid cells. Karyotypes were made of 63 diakinesis-metaphase I cells.

TABLE 5.3: DISTRIBUTION OF CHROMOSOME COUNTS IN SPERMATOGONIAL CELLS IN TRANSLOCATION-CARRYING RAMS

Karyotype of Ram	Ram Identification	Number of cells with chromosome number <2n-1	2n-1	2n	2n+1	≥2n+2	Number of Polyploid Cells	Total
52,xy,t_1t_3	B147/75			2			0	2
52,xy,t_1t_2	B63/77			4			0	4
52,xy,t_2t_3	B166/78			5			1	6
52,xy,t_2t_x	B6/79	3		5			0	8
Total		3	0	16	0	0	1	20
51,xy,$t_1t_1t_3$	B120/77			3			0	3
51,xy,$t_1t_3t_3$	B154/77						0	0
51,xy,$t_2t_3t_3$	B117/78			2			1	3
51,xy,$t_1t_2t_3$	B7/76						0	0
51,xy,$t_1t_2t_3$	B86/76			6			0	6
51,xy,$t_1t_2t_3$	B111/77	4		4			0	8
51,xy,$t_1t_2t_3$	B44/78						0	0
51,xy,$t_1t_2t_3$	B47/78			9			0	9
51,xy,$t_1t_2t_3$	B101/78	1		4			0	5
Total		5	0	28	0	0	1	34
50,xy,$t_1t_2t_2t_3$	B137/78			3			1	4
50,xy,$t_1t_2t_2t_3$	B42/79			2			0	2
50,xy,$t_1t_2t_2t_3$	B49/79	1		11			1	13
50,xy,$t_1t_2t_2t_3$	B118/79			2			2	4
50,xy,$t_1t_2t_2t_3$	B147/79	1		11			0	12
50,xy,$t_1t_1t_2t_3$	B128/77	1		20			2	23
50,xy,$t_1t_1t_2t_3$	B141/77			2			0	2
50,xy,$t_1t_2t_3t_x$	B121/78			3			0	3
50,xy,$t_1t_2t_3t_x$	B2/79						0	0
50,xy,$t_1t_2t_3t_x$	B32/79			2			0	2
50,xy,$t_1t_2t_3t_3$	B140/77			3			0	3
Total		3	0	59	0	0	6	68
49,xy,$t_1t_2t_2t_3t_3$	B196/76			3			0	3
49,xy,$t_1t_2t_2t_3t_x$	B38/79			1			0	1
49,xy,$t_1t_2t_2t_3t_x$	B92/79						0	0
Total		0	0	4	0	0	0	4

PLATE 5.1: Karyotype of a male Robertsonian translocation double heterozygote. The translocation sub-metacentric chromosome (t_1), the translocation metacentric chromosome (t_2) and the sex chromosomes are identified.
(Giemsa)

PLATE 5.2: Karyotype of a male Robertsonian translocation triple heterozygote. The translocation sub-metacentric chromosomes ($t_1 + t_3$), the translocation metacentric chromosome (t_2) and the sex chromosomes are identified.
(Giemsa)


X
t1
t2
Y

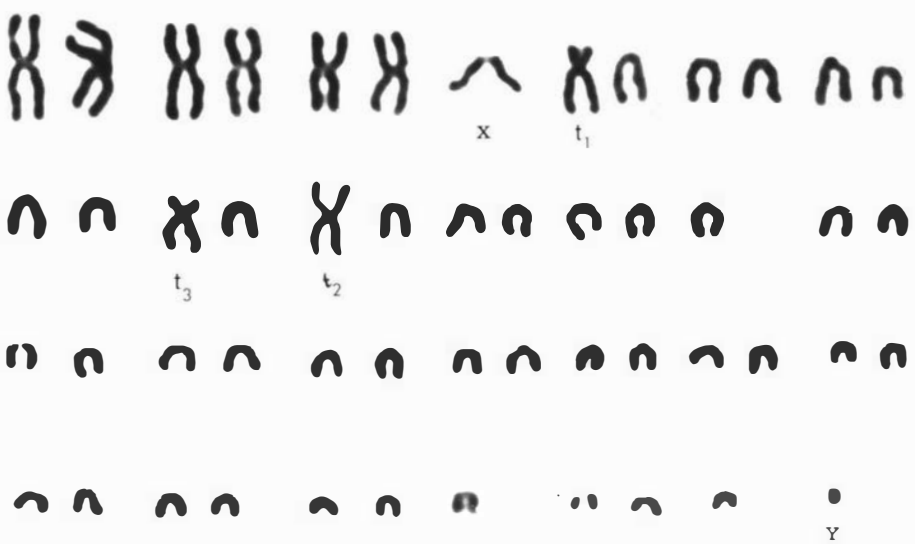

X
t1
t3
t2
Y

PLATE 5.3: Karyotype of a 50,xy,$t_1t_2t_3t_3$ ram. The translocation chromosomes and the sex chromosomes are identified.
(G-banded).

PLATE 5.4: Karyotype of a 49,xy,$t_1t_2t_2t_3t_3$ ram. The translocation chromosomes and the sex chromosomes are identified.
(Giemsa).


X
t1
t3 t3
t2
Y

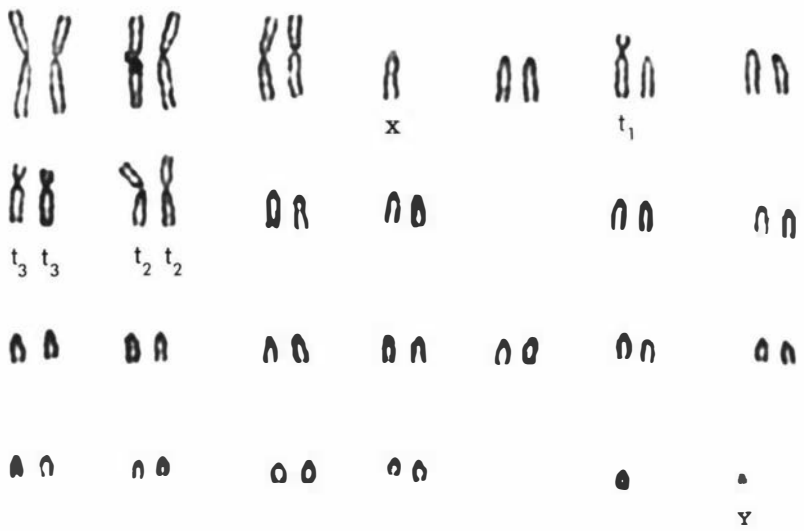
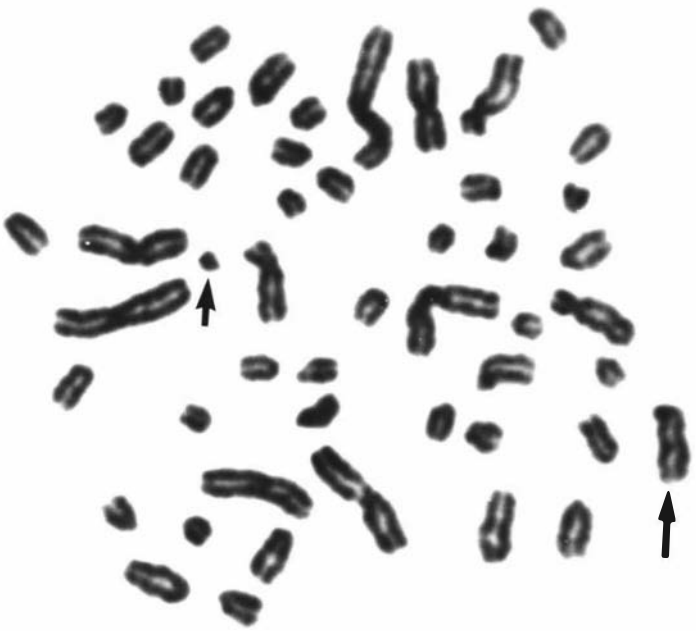

X
t1
t3 t3
t2 t2
Y

PLATE 5.5: Diploid spermatogonial metaphase from B147/75 (52,xy,t_1 t_3). The small arrow points to the Y chromosome.
(Giemsa x 1,750).

PLATE 5.6: Diploid spermatogonial metaphase from B47/78 (51,xy,t_1 t_2 t_3). Large arrows point to the translocation chromosomes and the small arrow points to the Y chromosome.
(Giemsa x 1,750).

PLATE 5.7: Diploid spermatogonial metaphase from B121/78 (50,xy,t_1 t_2 t_3 t_x). The X chromosome (large arrow) and the Y chromosome (small arrow) are identified.
(Giemsa x 1,750).

PLATE 5.8: Tetraploid spermatogonial metaphase from B128/77 (50,xy, $t_1t_2t_2t_3$). The arrows point to the Y chromosomes.
(Giemsa x 1,750).



5.2.2.1 Double heterozygous rams

Chromosome counts on cells at diakinesis-metaphase I from the 4 double heterozygous rams are given in Table 5.4. A total of 299 cells were counted and this total included 30 (10.0 percent) polyploid cells.

The analyses of non-polyploid cells are given in Table 5.4. The majority of cells, 97 percent, had 25 chromosomal bodies. Only one hypermodal cell, with 26 chromosomal bodies, was recorded. Seven hypomodal cells, with one chromosomal body missing, were recorded.

The modal cell had 25 chromosomal bodies comprising of 22 autosomal bivalents, a sex bivalent and 2 trivalents. The 22 autosomal bivalents were comprised of three large bivalents and 19 small bivalents. The large bivalents were formed by the pairing of the metacentric chromosomes and the small bivalents were formed from the pairing of acrocentric chromosomes. The trivalent is comprised of the translocation chromosome and its two acrocentric homologues. Representative cells are seen in Plates 5.9 and 5.10 and Figures 5.1 and 5.2.

One of the 269 non-polyploid cells had 26 chromosomal bodies. It comprised of 22 autosomal bivalents, 2 trivalents and separated X and Y chromosomes. Eleven of the sex bivalents were associated with one of the small autosomal bivalents. The percentage of cells from the 4 rams in which the association was recorded varied from 0 to 5.1 percent.

Chiasmata counts were made on 15 diakinesis-metaphase I cells from 3 of the double heterozygous rams and the results are summarized in Table 5.5. The average number of chiasmata per cell was 54.5 with a range from 52 to 58. The average number of chiasmata per element was 2.3.

The analyses of the polyploid cells are given in Table 5.6. Thirty cells were recorded. Of these, 27 were tetra-

TABLE 5.4: ROBERTSONIAN TRANSLOCATION DOUBLE HETEROZYGOUS RAMS: CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS-METAPHASE I STAGES

Ram Identification	Number of Chromosome Bodies / Cells				Total	Number of Polyploid Cells	Total Including Polyploid Cells	Analysis of Cells excluding Polyploids				Total Number Cells Analysed	Number of cells with xy associated with Autosomal Bivalent	X and Y separated	
	23	24	25	26				25,xy	26,x,y	24,xy-A	23,xy-2A			No.	%
B147/75	0	0	9	0	9	1	10	9	0	0	0	9	0	0	0
B63/77	0	0	8	0	8	3	11	8	0	0	0	8	1	0	0
B166/78	0	0	38	1	39	3	42	38	1	0	0	39	2	1	2.6
B6/79	0	7	206	0	213	23	236	206	0	7	0	213	8	0	0
Total	0	7	261	1	269	30	299	261	1	7	0	269	11	1	0.4

TABLE 5.5: FREQUENCY AND DISTRIBUTION OF CHIASMATA IN TRANSLOCATION-CARRYING RAMS

Karyotype	No. of Rams	Total Number Counted		Distribution of Chiasmata Number. Average Number of elements with given Number of Chiasmata							Average Number of Chiasmata per Element	Total Chiasmata Per cell		Total Terminal Chiasmata		Terminalization Coefficient	
		Cells	Elements	1	2	3	4	5	6	7		Ave.	Range	Ave.	Range	Ave.	Range
52,xy,$t_x t$	3	15	360	4.2	14.4	1.7	2.1	1.3	0.3	0	2.3	54.5	52-58	21.8	18-26	0.4	0.35-0.5
51,xy,$t_1 t_2 t_3$	3	13	299	3.6	12.3	2.5	3.2	1.2	0.1	0.1	2.4	55.5	50-60	22.1	18-25	0.4	0.3-0.5
50,xy,$t_1 t_2 t_3 t_x$	3	23	529	3.1	13.6	2.7	2.4	0.8	0.3	0.1	2.4	54.2	49-58	22.3	18-27	0.4	0.3-0.5
49,xy,$t_1 t_2 t_2 t_3 t_x$	2	9	207	4.1	12.9	2.2	1.9	1.6	0.3	0	2.3	53.9	52-57	21.9	18-25	0.4	0.3-0.6

TABLE 5.6: CHROMOSOME COUNTS FROM POLYPLOID CELLS AT DIAKINESIS-METAPHASE I STAGES IN TRANSLOCATION-CARRYING RAMS

Karyotype	No. of Rams	Number of cells Showing Chromosome Complement			N.A.	Total	Analysis of Polyploid Cells							
							4n			6n		8n	N.A.	Total
		4n	6n	8n			>4n	4n	<4n	6n	<6n	8n		
52,xy,$t_x t$	4	27	2	1	0	30	0	24	3	2	0	1	0	30
52,xy,$t_x t_x t$	3	16	5	1	0	22	0	13	3	5	0	1	0	22
51,xy,$t_1 t_2 t_3$	6	200	39	9	3	251	3	167	30	20	19	9	3	251
50,xy,$t_1 t_2 t_3 t_x$	6	46	2	2	0	50	0	39	7	1	1	2	0	50
50,xy,$t_1 t_2 t_2 t_3$	5	25	5	0	0	30	0	22	3	5	0	0	0	30
49,xy,$t_1 t_2 t_2 t_3 t_x$	3	17	2	2	0	21	0	17	0	2	0	2	0	21
Total	27	331	55	15	3	404	3	282	46	35	20	15	3	404

N.A. = Not Analysed

PLATE 5.9: Cell in diakinesis from B147/75 (52,xy,t_1t_3). There are three large autosomal bivalents, two trivalents, 19 small acrocentric bivalents and a sex bivalent.
(Giemsa x 2,500).

FIGURE 5.1: Karyotype of cell illustrated in Plate 5.9.

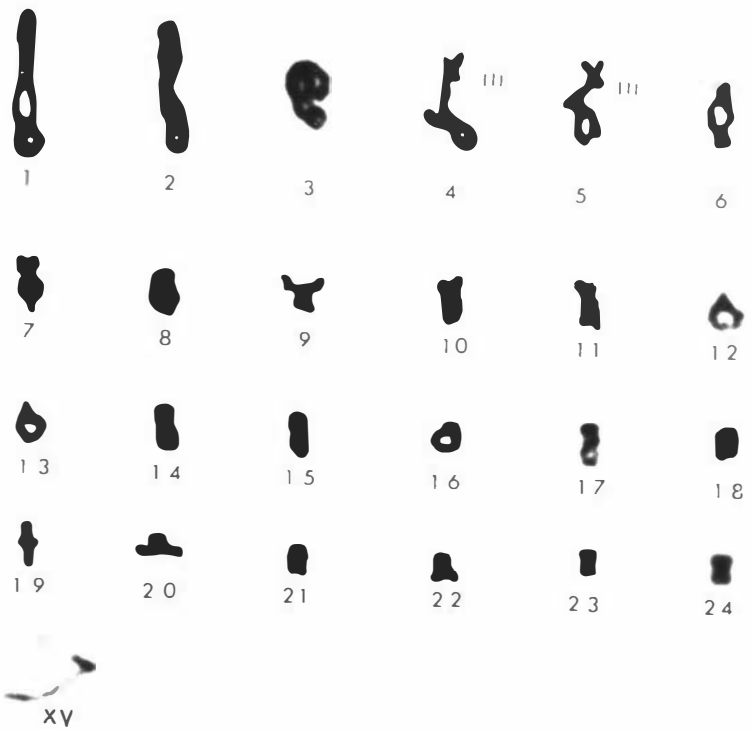

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PLATE 5.10: Cell in diakinesis from B6/79 (52,xy,t_2 t_x). There are three large autosomal bivalents, two trivalents, 19 small acrocentric bivalents and a sex bivalent.
(Giemsa x 3,000).

FIGURE 5.2: Karyotype of cell illustrated in Plate 5.10.


III
III
XY

ploid, 2 were hexaploid and one was octaploid. None of the cells were seen with univalent chromosomal bodies.

5.2.2.2 Triple translocation-carrying rams

Chromosome counts on cells at diakinesis-metaphase I from the 3 triple translocation (51,xy,$t_x t_x t$) rams are given in Table 5.7. A total of 158 cells were counted and this total included 22 (13.9 percent) polyploid cells.

The analyses of non-polyploid cells are given in Table 5.7. The majority of cells, 90.4 percent, had 25 chromosomal bodies. Only one hypermodal cell was recorded while 12 hypomodal cells, with either one or more chromosomal bodies missing, were seen.

The modal cell had 25 chromosomal bodies, comprising of 23 autosomal bivalents, a sex bivalent and a trivalent. The 23 autosomal bivalents comprised of four large bivalents and 19 small bivalents. A representative cell is seen in plate 5.11.

One of the 136 non-polyploid cells had 26 chromosomal bodies, with separated X and Y chromosomes. Seven of the sex bivalents were associated with one of the small autosomal bivalents. The percentage of cells from the 3 rams in which the association was recorded varied from 0 to 6.2 percent.

The analyses of the polyploid cells are given in Table 5.6. Twenty two cells were recorded. Of these, 16 were tetraploid, 5 were hexaploid and one was octaploid. A polyploid cell is seen in Plate 5.12.

5.2.2.3 Triple heterozygous rams

Chromosome counts on cells at diakinesis-metaphase I from the 6 triple heterozygous rams are given in Table 5.8. A total of 1,585 cells were counted and this total included 251 (15.8 percent) polyploid cells.

TABLE 5.7: ROBERTSONIAN TRANSLOCATION RAMS (51,XY,$t_x t_x t$): CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS-METAPHASE I STAGES

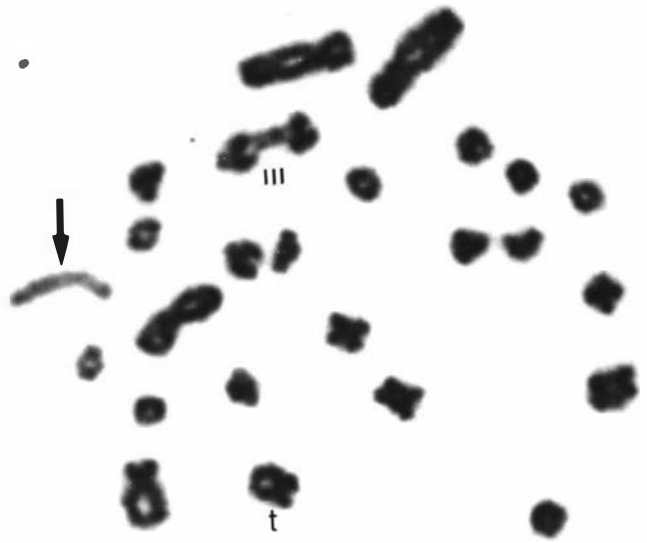
Ram Identification	Number of Chromosome Bodies/Cell				Total	Number of Polyploid Cells	Total Including Polyploid Cells	Analysis of Cells Excluding polyploids				Total Number Cells Analysed	Number of Cells with Xy Associated with Autosomal Bivalent	X and Y Separated	
	≤23	24	25	26				25,xy	26,x,y	24,xy-A	≤23,xy-2A			No.	%
B120/77	1	2	61	1	65	9	74	61	1	2	0	65	4	1	1.5
B154/77	4	4	50	0	58	8	66	50	0	4	3	58	3	0	0
B117/78	0	1	12	0	13	5	18	12	0	1	0	13	0	0	0
Total	5	7	123	1	136	22	158	123	1	7	3	136	7	1	0.7

TABLE 5.8: ROBERTSONIAN TRANSLOCATION TRIPLE HETEROZYGOUS RAMS: CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS-METAPHASE I STAGES

Ram Identification	Number of Cells Showing Chromosome Complement						Total	Number of Polyploid Cells	Total Including Polyploid Cells	Analysis of cells excluding Polyploids				Total Number Cells Analysed	Number of cells with xy associated with Autosomal Bivalent	X and Y Separated	
	<22	22	23	24	25	26				24,xy	≥25,x,y	23,xy-A	≤22,xy-2A			No.	%
B7/76	0	8	17	235	1	0	261	95	356	235	1	17	8	261	5	1	0.4
B86/76	0	0	2	54	0	0	56	8	64	54	0	2	0	56	2	0	0
B111/77	5	5	43	668	2	1	724	110	834	668	3	43	10	724	40	3	0.4
B44/78	0	0	0	1	0	0	1	1	2	1	0	0	0	1	0	0	0
B47/78	0	0	10	152	4	0	166	27	193	152	4	10	0	166	4	4	2.4
B101/78	2	0	4	120	0	0	126	10	136	120	0	4	2	126	4	0	0
Total	7	13	76	1230	7	1	1334	251	1585	1230	8	76	20	1334	55	8	0.6

PLATE 5.11: Cell in diakinesis from B117/78 (51,xy,$t_2t_3t_3$). There are three large autosomal bivalents, a trivalent (III), a translocation bivalent (t), 19 small autosomal bivalents and a sex bivalent (arrowed).
(Giemsa x 2,000).

PLATE 5.12: Tetraploid cell in diakinesis from B117/78 (51,xy,$t_2t_3t_3$). There are two trivalents (III), two translocation bivalents (t) and two sex bivalents (arrowed).
(Giemsa x 2,000)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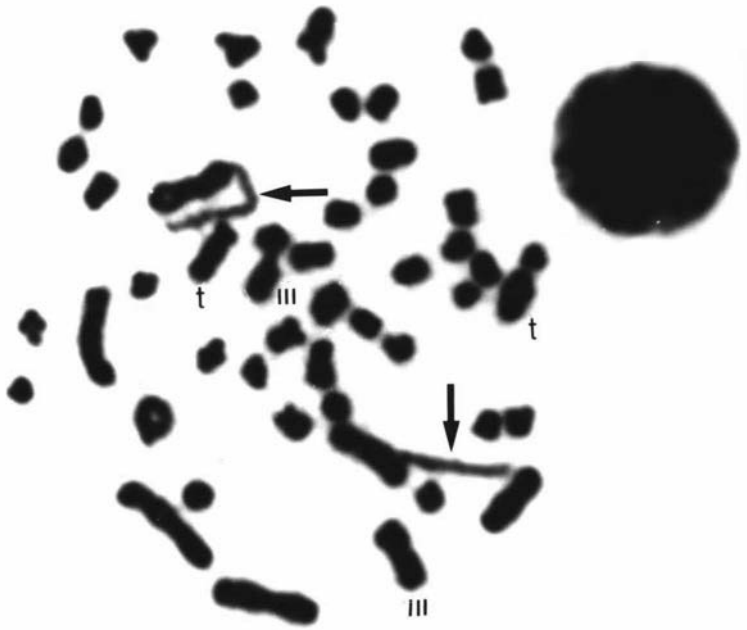
t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The analyses of non-polyploid cells are given in Table 5.8. The majority of cells, 92.2 percent, had 24 chromosome bodies. Eight hypermodal cells were recorded and 96 hypomodal cells, with either one or more chromosomal bodies missing, were counted.

The modal cell had 24 chromosomal bodies comprising 20 autosomal bivalents, a sex bivalent and three trivalents. The 20 autosomal bivalents comprised of 3 large bivalents and 17 small bivalents. The trivalents comprised of the translocation chromosomes and their acrocentric homologues. A representative cell is seen in Plate 5.13 and Figure 5.3.

Six of the 1,334 non-polyploid cells had 25 chromosomal bodies, with a separated X and Y chromosome. One of the cells was seen with an extra autosomal body and separated X and Y chromosomes. Fifty five of the sex bivalents were associated with one of the small autosomal bivalents. The percentage of cells from the 4 rams in which the association was recorded varied from 0 to 5.5 percent.

Chiasmata counts were made on 13 diakinesis-metaphase I cells from 3 of the triple heterozygous rams and the results are summarized in Table 5.5. The average number of chiasmata per cell was 55.5 with a range of 50 to 60. The average number of chiasmata per element was 2.4.

The analyses of the polyploid cells are given in Table 5.6. Of the 248 cells analysed 200 were tetraploid (Plate 5.14 and Figure 5.4), 39 were hexaploid and 9 were octaploid. Three cells were not analysed. Three tetraploid cells were seen with an extra(s) chromosomal body. These cells could have been broken hexaploid cells.

5.2.2.4 Multiple translocation-carrying rams ($50,xy,t_1t_2t_3t_x$)

Chromosome counts on cells at diakinesis-metaphase I from the 6 multiple translocation-carrying rams ($50,xy,t_1t_2t_3t_x$)

PLATE 5.13: Cell in diakinesis from B111/77 (51,xy,t_1 t_2t_3). There are three large autosomal bivalents, three trivalents, 17 small autosomal bivalents and a sex bivalent. (Giemsa x 2,000.)

FIGURE 5.3: Karyotype of cell illustrated in Plate 5.13.

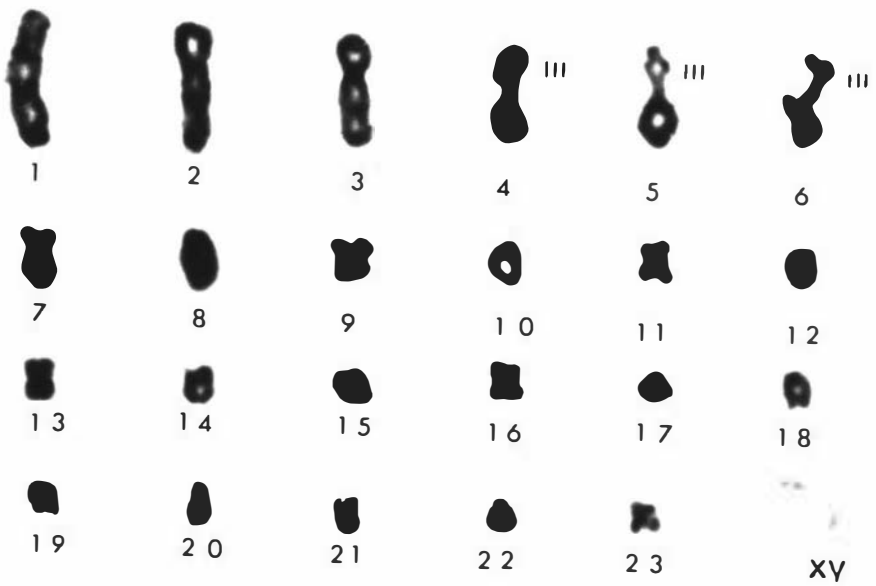
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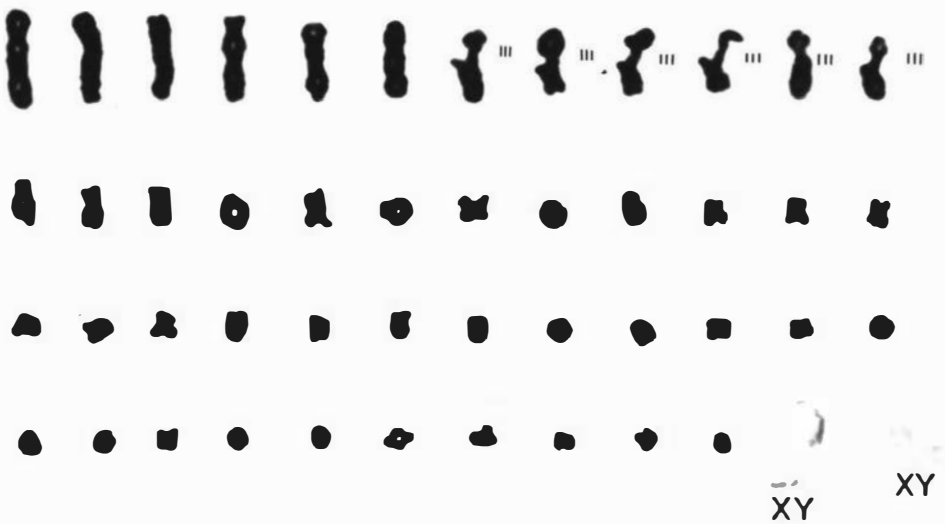
PLATE 5.14: Tetraploid cell in diakinesis from B111/77 (51,xy,t_1 t_2 t_3). There are six trivalents and two sex bivalents.
(Giemsa x 2,000).

FIGURE 5.4: Karyotype of cell illustrated in Plate 5.14.


XY
XY

are given in Table 5.9. A total of 411 cells were counted and this total included 50 (12.2 percent) polyploid cells.

The analyses of non-polyploid cells are given in Table 5.9. The majority of cells, 93.9 percent, had 24 chromosomal bodies. Three hypermodal cells and 19 hypomodal cells, with either one or more chromosomal bodies missing, were recorded.

The modal cell had 24 chromosomal bodies comprising 21 autosomal bivalents, a sex bivalent and 2 trivalents. The 21 autosomal bivalents were comprised of 4 large bivalents and 17 small bivalents. A representative cell is seen in Plate 5.15 and Figure 5.5.

Three of the 361 non-polyploid cells had 25 chromosomal bodies (Plate 5.16 and Figure 5.6). The cells had separated X and Y chromosomes. Nineteen of the sex bivalents were associated with one of the small autosomal bivalents. The percentage of cells from the 6 rams in which the association was recorded varied from 0 to 6.9 percent.

Chiasmata counts were made on 23 diakinesis-metaphase I cells from 3 of the multiple translocation-carrying rams and the results are summarized in Table 5.5. The average number of chiasmata per cell was 54.2 with a range from 49 to 58. The average number of chiasmata per element was 2.4.

The analyses of the polyploid cells are given in Table 5.6. Fifty cells were recorded. Of these, 46 were tetraploid, 2 were hexaploid and 2 were octaploid.

5.2.2.5 Multiple translocation-carrying rams (50,xy,$t_1t_2t_2t_3$)

Chromosome counts on cells at diakinesis-metaphase I from the 5 multiple translocation-carrying rams (50,xy,$t_1t_2t_2t_3$) are given in Table 5.10. A total of 218 cells were counted and this total included 30 (13.8 percent) polyploid cells.

TABLE 5.9: MULTIPLE TRANSLOCATION-CARRYING RAMS (50,XY,$t_1t_2t_3t_x$): CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS-METAPHASE I STAGES

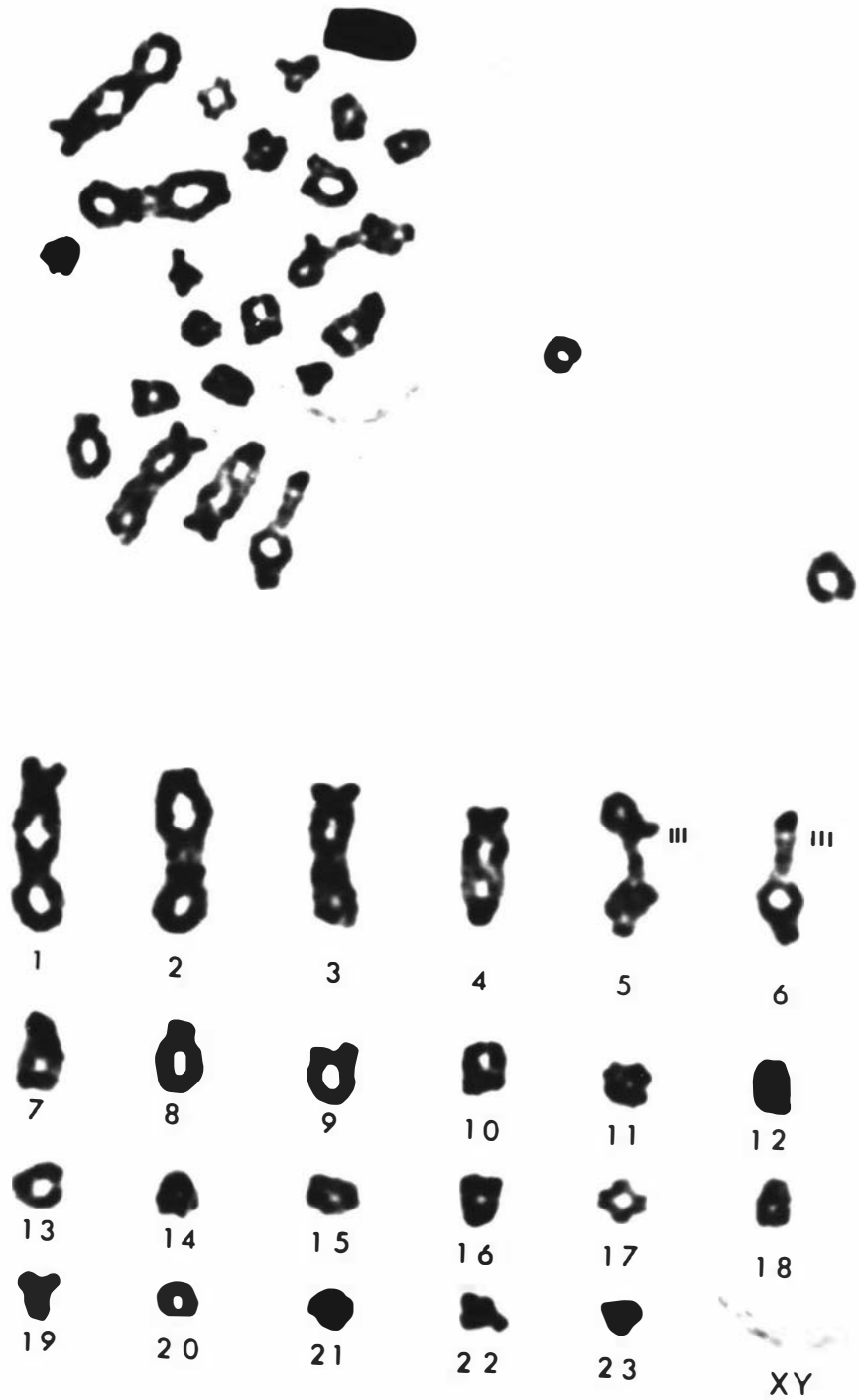
Rams Identification	Number of Chromosome Bodies/Cells				Total	Number of Polyploid Cells	Total Including Polyploid Cells	Analysis of cells Excluding Polyploids					Total Number Cells Analysed	Number of Cells with XY Associated with Autosomal Bivalent	X and Y Separated	
	<22	23	24	25				24,xy	25,x,y	23,xy-A	≤22,xy-2A	23,-xy			No.	%
B140/77	1	1	32	0	34	2	36	32	0	1	1	0	34	1	0	0
B128/77	0	12	180	2	194	28	222	180	2	11	0	1	194	11	2	1.0
B141/77	1	0	16	0	17	4	21	16	0	0	1	0	17	0	0	0
B121/78	1	2	26	0	29	5	34	26	0	2	1	0	29	2	0	0
B2/79	0	0	14	0	14	4	18	14	0	0	0	0	14	0	0	0
B32/79	0	1	71	1	73	7	80	71	1	1	0	0	73	5	1	1.4
Total	3	16	339	3	361	50	411	339	3	15	2	1	361	19	3	0.8

TABLE 5.10: MULTIPLE TRANSLOCATION-CARRYING RAMS (50,XY,$t_1t_2t_2t_3$): CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS-METAPHASE I STAGES

Rams Identification	Number of Chromosome Bodies/Cells				Total	Number of Polyploid Cells	Total Including Polyploid Cells	Analysis of cells Excluding Polyploids				Total Number Cells Analysed	Number of Cells with xy Associated with Autosomal Bivalent	X and Y Separated	
	22	23	24	25				24,xy	25,x,y	23,xy-A	22,xy-2A			No.	%
B137/78	0	0	19	0	19	2	21	19	0	0	0	19	0	0	0
B42/79	0	1	86	1	88	5	93	86	1	1	0	88	3	1	1.1
B49/79	0	0	24	0	24	4	28	24	0	0	0	24	0	0	0
B118/79	0	1	12	0	13	7	20	12	0	1	0	13	1	0	0
B147/79	0	1	42	1	44	12	56	42	1	1	0	44	3	1	2.3
Total	0	3	183	2	188	30	218	183	2	3	0	188	7	2	1.1

PLATE 5.15: Cell in diakinesis from B128/77 (50,xy,$t_1t_1t_2t_3$). There are three large autosomal bivalents, two trivalents, a translocation bivalent, 17 small acrocentric bivalents and a sex bivalent.
(Giemsa x 2,000).

FIGURE 5.5: Karyotype of cell illustrated in Plate 5.15. The fourth largest bivalent is formed by the paired translocation chromosomes.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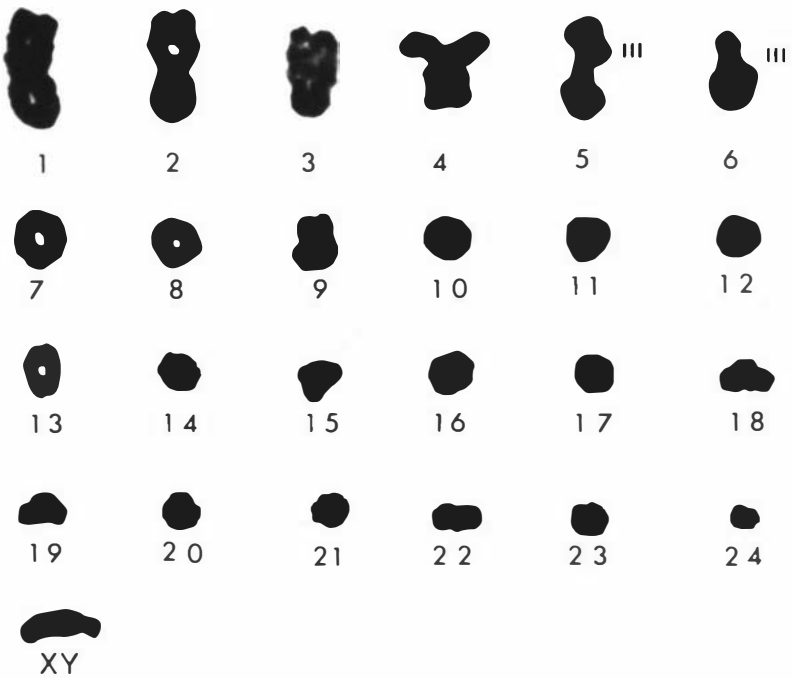
XY

PLATE 5.16: Cell in diakinesis from B32/79 (50,xy,$t_1t_2t_3t_x$). There are three large autosomal bivalents, two trivalents, a translocation bivalent, 17 small acrocentric bivalents, a sex bivalent and an extra element which is probably a univalent (I).
(Giemsa x 2,000).

FIGURE 5.6: Karyotype of cell illustrated in Plate 5.16. The fourth largest bivalent is the translocation bivalent.


I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XY

The analyses of non-polyploid cells are given in Table 5.10. The majority of cells, 97.3 percent, had 24 chromosomal bodies. Two hypomodal cells and three hypomodal cells, with either one or more chromosomal bodies missing, were recorded.

The modal cell had 24 chromosomal bodies comprising of 20 autosomal bivalents, a sex bivalent and 2 trivalents. A representative cell is seen in plate 5.17 and Figure 5.7.

Two of the 218 non-polyploid cells had 25 chromosomal bodies. The cells had separated X and Y chromosomes. Seven of the sex bivalents were associated with one of the small autosomal bivalents. The percentage of cells from the 4 rams in which the association was recorded varied from 0 to 6.8 percent.

The analyses of the polyploid cells are given in Table 5.6. Thirty cells were recorded. Of these, 25 were tetraploid, and 5 were hexaploid.

5.2.2.6 Multiple translocation-carrying rams (49,xy,$t_1t_2t_2t_3t_x$)

Chromosome counts on cells at diakinesis-metaphase I from the 3 multiple translocation-carrying rams (49,xy,$t_1t_2t_2t_3t_x$) are given in Table 5.11. A total of 193 cells were counted and this total included 21 (10.9 percent) polyploid cells.

The analyses of non-polyploid cells are given in Table 5.11. The majority of cells, 95.3 percent, had 24 chromosomal bodies. No hypermodal cells were recorded. Eight hypomodal cells, with either one or more chromosomal bodies missing, were recorded.

The modal cell had 24 chromosomal bodies comprising 22 autosomal bivalents, a sex bivalent and a trivalent. The 22 autosomal bivalents were comprised of 5 large bivalents and 17 small bivalents. Representative cells are seen in Plates

TABLE 5.11: MULTIPLE TRANSLOCATION-CARRYING RAMS (49,XY,t_1 t_2 t_2 t_3 t_x): CHROMOSOME COUNTS FROM PRIMARY SPERMATOCYTE CELLS AT DIAKINESIS-METAPHASE I STAGES

Ram Identification	Number of Chromosome Bodies/Cell				Total	Number of Polyploid Cells	Total Including Polyploid Cells	Analysis of Cells Excluding Polyploids				Total Number Cells Analysed	Number of Cells with Xy Associated with Autosomal Bivalent	X and Y Separated	
	<22	23	24	25				24,xy	25,x,y	23,xy-A	≤22,xy-2A			No.	%
B196/76	3	1	48	0	52	1	53	48	0	1	3	52	2	0	0
B38/79	0	0	71	0	71	9	80	71	0	0	0	71	3	0	0
B92/79	1	3	45	0	49	11	60	45	0	3	1	49	1	0	0
Total	4	4	164	0	172	21	193	164	0	4	4	172	6	0	0

PLATE 5.17: Cell in diakinesis from B42/79(50,xy,$t_1t_2t_2t_3$). There are three large autosomal bivalents, two trivalents, a translocation bivalent, 17 small acrocentric bivalents and a sex bivelent. (Giemsa x 2,000).

FIGURE 5.7: Karyotype of cell illustrated in Plate 5.17. The translocation bivalent is the fourth largest autosomal bivalent.

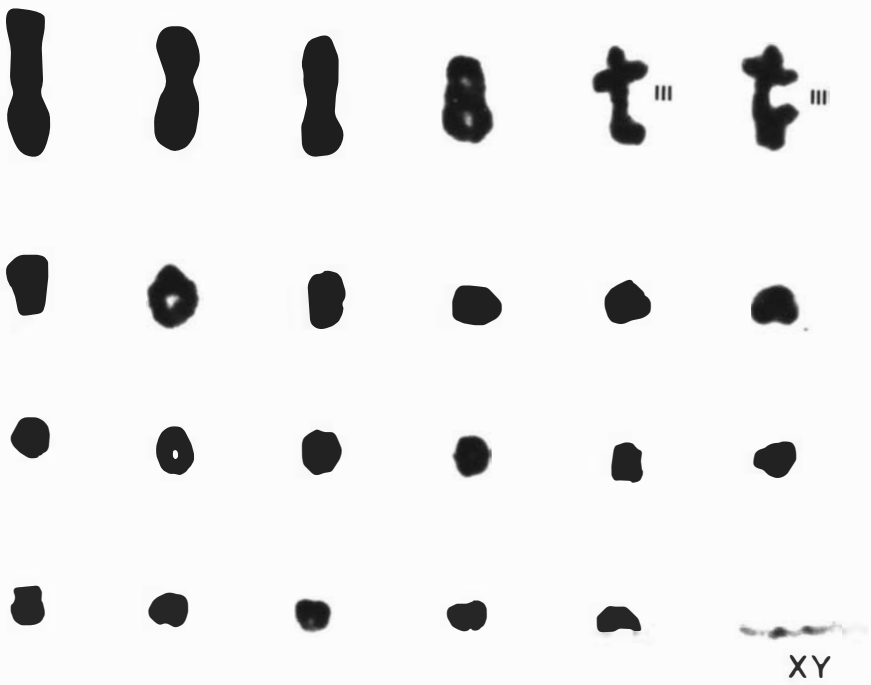
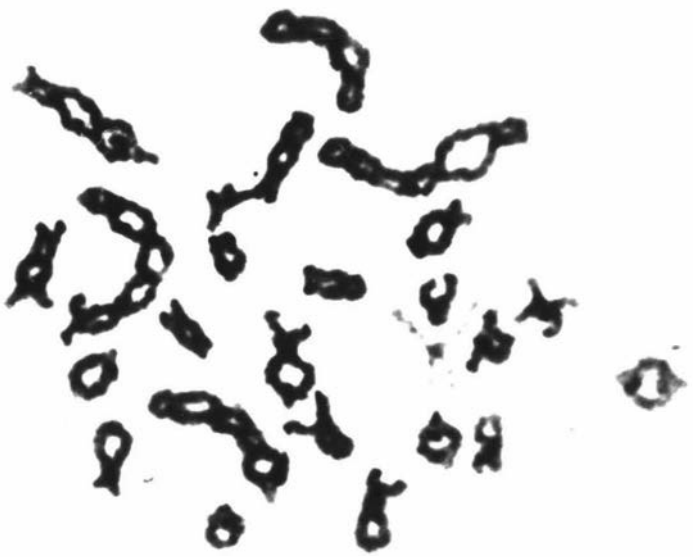

XY

PLATE 5.18: Cell in diakinesis from B92/79 (49,xy,$t_1t_2t_2t_3t_x$) There are three large autosomal bivalents, a trivalent, two translocation bivalents, 17 small acrocentric bivalents and a sex bivalent. (Giemsa x 2,500).

FIGURE 5.8: Karyotype of cell illustrated in Plate 5.18. The translocation bivalents are the fourth and fifth largest autosomal bivalents.



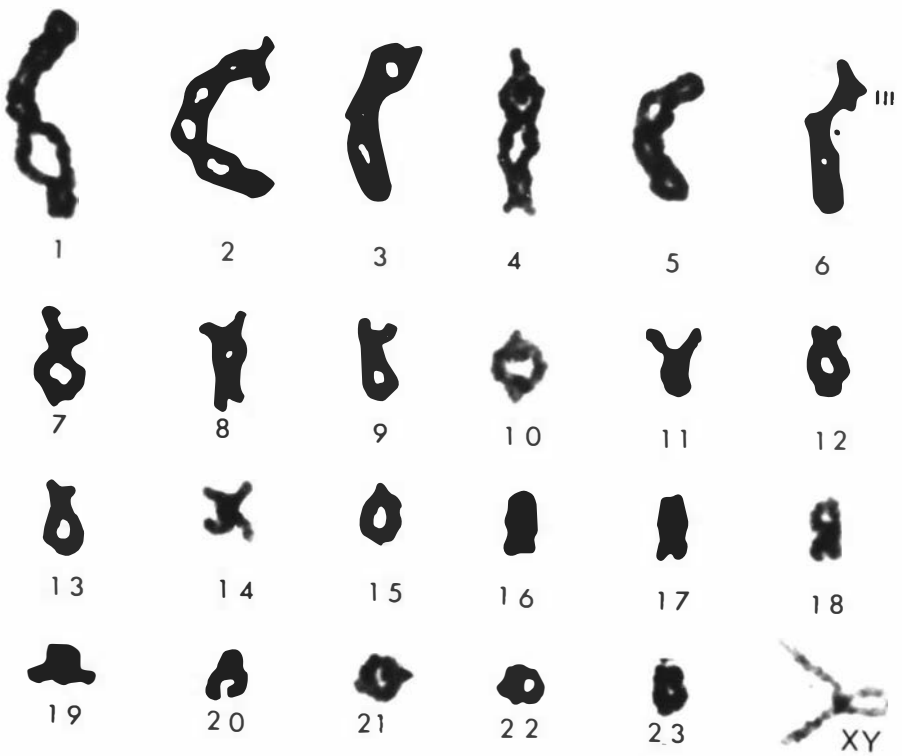

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PLATE 5.19: Cell in diakinesis from B196/76 (49,xy,$t_1t_2t_2t_3t_3$). There are a trivalent, two translocation bivalents and a sex bivalent.
(Giemsa x 2,000).

FIGURE 5.9: Karyotype of cell illustrated in Plate 5.19. The translocation bivalents are the fourth and fifth largest autosomal bivalents.


III
XY

5.18 and 5.19 and Figures 5.8 and 5.9.

None of the cells had separated X and Y chromosomes. Six of the sex bivalents were associated with one of the small autosomal bivalents. The percentage of cells from the 4 rams in which the association was recorded varied from 2 to 4.2 percent.

Chiasmata counts were made on 9 diakinesis-metaphase I cells from 2 of the multiple translocation-carrying rams and the results are summarized in Table 5.5. The average number of chiasmata per cell was 53.9 with a range from 52 to 57. The average number of chiasmata per element was 2.3.

The analyses of the polyploid cells are given in Table 5.6. Twenty one cells were recorded. Of these, 17 were tetraploid, 2 were hexaploid and 2 were octaploid. Representative cells are seen in Plates 5.20 to 5.22 and Figure 5.10.

5.2.3 Discussion

The percentage values of hypomodal, modal and hypermodal cells from the translocation-carrying rams included in this study are given in Table 5.12.

The percentage of hypermodal cells recorded in the translocation-carrying rams ~~are~~ was similar to that found in the normal rams. There are few hypermodal cells but a greater number of hypomodal cells. Most of the hypermodal cells have resulted either from the separation of the sex chromosomes or to a lesser extent by the separation of an autosomal bivalent. None of the cells in this study or in the earlier studies by Chapman (1974); Long (1975) and Logue (1977) were seen with a separation of the chromosomes involved in the trivalent. A lower percentage of hypomodal cells and a higher percentage of modal cells are seen in the

TABLE 5.12: PERCENTAGE OF HYPOMODAL, MODAL AND HYPERMODAL CELLS AT DIAKINESIS-METAPHASE I IN ROBERTSONIAN TRANSLOCATION CARRYING RAMS

	% Hypomodal	% Modal	% Hypermodal
*53,xy,t_1	16.4	82.7	0.9
*53,xy,t_2	13.3	85.2	1.5
*53,xy,t_3	16.5	82.2	1.3
*52,xy,t_1t_1	20.1	77.3	2.6
*52,xy,t_3t_3	9.5	90.5	0.0
52,xy,t_xt	2.6	97.0	0.4
51,xy,t_xt_xt	8.8	90.5	0.7
51,xy,$t_1t_2t_3$	7.2	92.2	0.6
50,xy,$t_1t_2t_3t_x$	5.3	93.9	0.8
50,xy,$t_1t_2t_2t_3$	1.6	97.3	1.1
49,xy,$t_1t_2t_2t_3t_x$	4.7	95.3	0.0
[54,xy	11.0	88.3	0.7]

* Data from Chapman (1974)

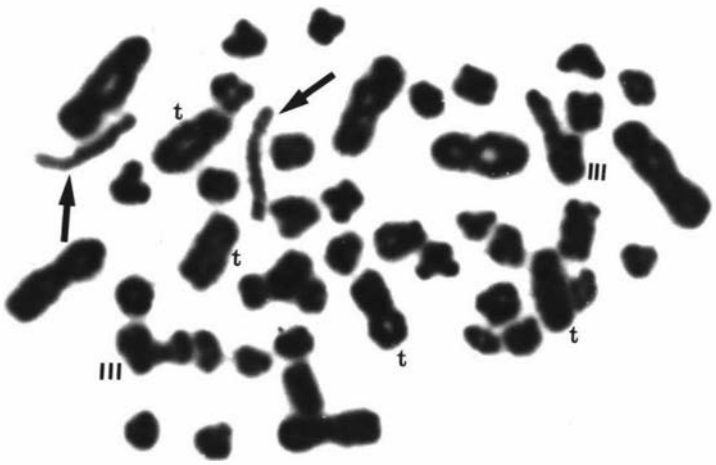
PLATE 5.20: Tetraploid cell in diakinesis from B196/76 (49,xy,t_1 t_2 t_2 t_3 t_3). There are two trivalents, four translocation bivalents and two sex bivalents. One of the sex bivalents is associated with a small autosomal bivalent(a).
(Giemsa x 2,000).

FIGURE 5.10: Karyotype of cell illustrated in Plate 5.20. The trivalents (III) are identified.


a
III
III
XY
XY

PLATE 5.21: Tetraploid cell in diakinesis from B38/79 (49,xy,$t_1t_2t_2t_3t$). There are two trivalents (III), four translocation bivalents(t) and two sex bivalents (arrowed).
(Giemsa x 2,000).

PLATE 5.22: Hexaploid cell in diakinesis from B38/79 (49,xy,$t_1t_2t_3t_3t$). There are 72 chromosomal elements including three trivalents (III), six translocation bivalents and three sex bivalents (arrowed).
(Giemsa x 2,000).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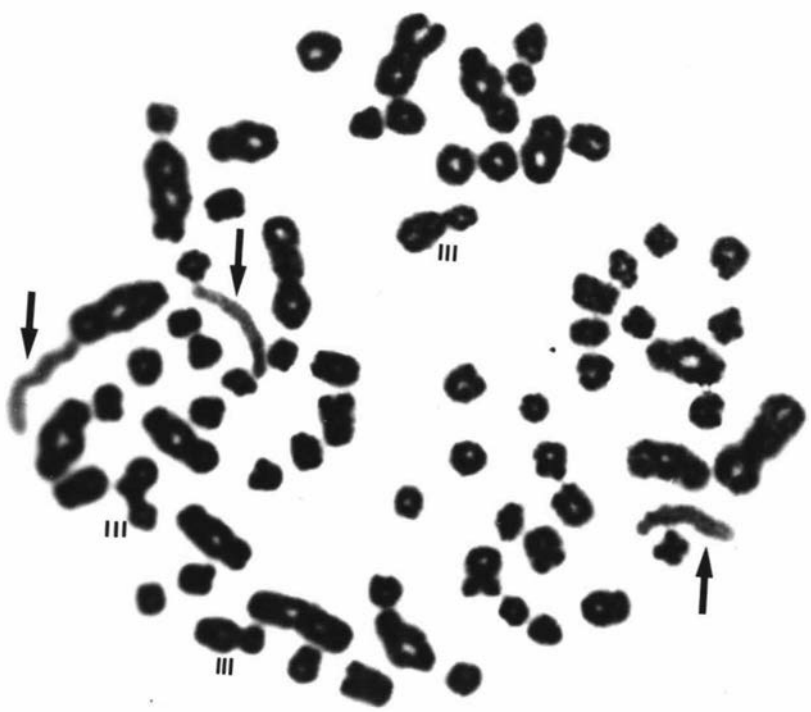
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translocation-carrying rams included in this study as compared to the single heterozygous and homozygous rams studied by Chapman (1974) as seen in Table 5.12. This discrepancy in the two results is probably a result of technical differences in the method of meiotic preparation rather than a true difference between translocation-carrying rams with various karyotypes.

Associations between sex bivalents and small autosomal bivalents in the Robertsonian translocation-carrying rams are summarized in Table 5.13. The levels observed in the translocation rams ranged from 3.5 to 5.3 percent. The levels found in the translocation rams were similar to those of the normal rams examined. The results in this study are similar to those found by Chapman (1974) in her studies of single heterozygous (3.4 to 4.7 percent) and homozygous (3.6 percent) rams.

The sex chromosomes were separated in from 0.0 to 1.1 percent of the diakinesis-metaphase I cells studied in the translocation-carrying rams (Table 5.13). This was comparable to the level of 0.7 percent seen in the normal rams. The levels found in the translocation-carrying rams in this study were within the range found by Chapman (1974) in her studies on single heterozygous and homozygous (0.5 to 1.5 percent) rams.

The causes of non-association of sex chromosomes may be due to a number of factors including the preparation technique, the age of the animals and the degree of condensation of the chromosomes (Winsor *et al.*, 1978). The non-association of the sex chromosomes could result in random segregation at the first meiotic division and as a consequence could be one of the causes of sex chromosome aneuploidy. It is likely that a number of the cells in this study with x-y dissociation are produced during slide preparation and it can be assumed that the actual number of univalent bodies found in diakinesis-metaphase I cells is much lower.

TABLE 5.13: THE NUMBER OF SEPARATED SEX CHROMOSOMES AND ASSOCIATIONS BETWEEN SEX BIVALENTS AND SMALL AUTOSOMAL BIVALENTS IN DIAKINESIS-METAPHASE I CELLS FROM ROBERTSONIAN TRANSLOCATION-CARRYING RAMS

Ram Karyotype	Total Number of cells counted	Number of cells with separated sex chromosomes	% of cells with separated sex chromosomes	Number of cells with xy associated with autosomal Bivalent	% of cells with xy associated
*53,xy,t_1	445	2	0.5	21	4.7
*53,xy,t_2	974	13	1.3	43	4.4
*53,xy,t_3	473	5	1.1	16	3.4
*52,xy,t_1t_1	194	3	1.5	7	3.6
*52,xy,t_3t_3	84	0	0.0	3	3.6
52,xy,t_xt	269	1	0.4	11	4.1
51,xy,t_xt_xt	136	1	0.7	7	5.1
51,xy,$t_1t_2t_3$	1334	8	0.6	55	4.1
50,xy,$t_1t_2t_3t_x$	361	3	0.8	19	5.3
50,xy,$t_1t_2t_2t_3$	188	2	1.1	7	3.7
49, x y,$t_1t_2t_2t_3t_x$	172	0	0.0	6	3.5
[54,xy	300	2	0.7	24	8.0]

* Data from Chapman (1974)

A high percentage of diakinesis-metaphase I cells with x-y dissociation has been found in conjunction with sterility in mice (Beechey, 1973) and man (McIlree *et al*., 1966). Chandley *et al*. (1976) found a strong correlation between a high percentage of diakinesis-metaphase I cells with x-y dissociation and a low percentage of MII values in infertile men. It was suggested that these cells with x-y dissociation do not usually proceed beyond metaphase I (Chandley *et al*., 1976) and the association between the sex chromosomes in some species was necessary for normal spermiogenesis (Beechey, 1973). However, a more recent investigation by Hultén and Palmer (1980), on 49 infertile and 20 control men, has indicated that there is no clear evidence that MI univalance *per se* induces disturbance of subsequent spermatogenesis.

The chiasma counts were very similar in all the translocation-carrying rams examined (Table 5.5). The average number of chiasmata per element was almost identical (2.3 and 2.4) for the four different groups of translocation rams. The average number of chiasmata per cell found in normal rams by Chapman (1974) and Long (1978) was comparable to the numbers found in the translocation-carrying rams in this study. This agrees with the findings of Logue and Harvey (1978) who reported that a 1/29 translocation heterozygous bull had a similar chiasma count to that found in normal bulls. Hultén and Lindsten (1973) in their review of meiosis in man found the majority of translocation cases reported had a count similar to that of normal men. This differed from the earlier report by Chandley *et al*. (1972) who found that the chiasma counts in men with a Robertsonian translocation present had a below average number.

The heterozygous carrier of Robertsonian translocations has a trivalent formation, involving non-homologous chromosomes, at the diakinesis-metaphase I stage. This was first reported in a ram heterozygous for a Massey I Robertsonian translocation by Bruère (1969). The morphology of the

the bivalents in rams heterozygous for the Massey I, II and III translocations has been extensively studied by Chapman (1974). The morphology of the trivalents in the diakinesis-metaphase I cells in this study were similar to those described by Chapman and Bruère (1977). There have been many reports of trivalent formation in other species (Kjessler, 1964; Evans *et al.*, 1967; White and Tjio, 1967; Gustavsson, 1969; Hultén and Lindsten, 1970; Chandley *et al.*, 1972). Bivalent formation is characteristic of homozygous carriers for a Robertsonian translocation. The morphology of the translocation bivalent in this study was similar to that described by Chapman (1974).

5.3 ANALYSIS OF SECONDARY SPERMATOCYTES

Metaphase figures in secondary spermatocytes from Robertsonian translocation-carrying rams were analysed in a manner similar to that already described in Chapters 3.5.1 and 4.3.3.

5.3.1 Statistical Analysis

The results from the secondary spermatocyte counts were, where relevant, analysed using the standard method for the Chi-squared (χ^2) test with the correction for continuity where required (Snedecor and Cochran, 1967). If necessary, counts from neighbouring classes were combined to ensure that the smallest expected number was at least one (Cochran, 1954). The null hypothesis was rejected when probabilities of less than 0.05 ($P < .05$) were obtained.

5.3.2 Theoretical Considerations

5.3.2.1 Rams heterozygous for a Robertsonian translocation

The Robertsonian translocation in the heterozygous state is found as a trivalent in the diplotene, diakinesis and metaphase I stages of meiosis. The trivalent comprises the translocation and its two homologous acrocentric

chromosomes. The orientation of chromosomes forming the trivalent at first metaphase and the segregation of them at anaphase I is crucial to determining the chromosome complement of the secondary spermatocytes.

There are, in theory, four possible types of segregation and eight possible segregation products, if it is assumed that there is no chance of either chromosome dissociation or association. The range of possible segregation products ~~are~~ is summarized in Table 5.14.

Alternate segregation of the chromosomes of the trivalent lead to the formation of balanced metaphase II cells and subsequently to balanced gametes. One will be a balanced translocation carrier $\frac{26,t,x}{30}$ or $\frac{26,t,y}{30}$ and the other will be a normal $\frac{27,x}{30}$ or $\frac{27,y}{30}$ gamete.

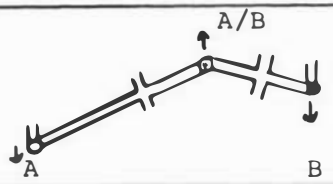
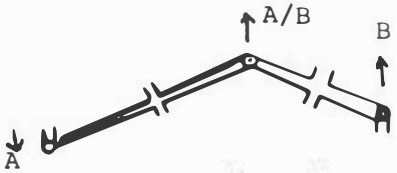
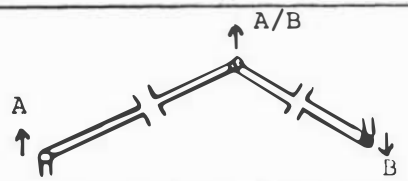
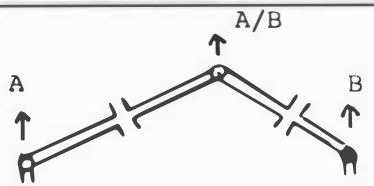
Partial non-disjunction can occur whereby the translocation and one acrocentric homologue pass to the same pole producing gametes carrying either a duplication (n + 1) or deficiency (n - 1) of chromosome material.

Total non-disjunction is the third possibility whereby the translocation and its two acrocentric homologues pass to the same pole producing gametes either with a duplication (n + 2) or deficiency (n - 2) of chromosomes.

5.3.2.2 Ram homozygous for a Robertsonian translocation

The Robertsonian translocation in the homozygous state is present as a bivalent in the diplotene, diakinesis and metaphase I stages of meiosis. The segregation products of the homozygous translocations are either normal gametes with $\frac{26,t,x}{30}$ or $\frac{26,t,y}{30}$ or total non-disjunction results in $\frac{27,tt}{32}$ or $\frac{25}{28}$ gametes.

TABLE 5.14: ROBERTSONIAN TRANSLOCATION HETEROZYGOTE: TYPES OF SEGREGATION AT MI AND PRODUCTS FROM TRIVALENT

Type of Segregation	Segregation	Products	Meta	Acro	t	X or Y	Total*
Alternate		A/B	3/6	21/21	1/2	1/1	26/30
		+					53/60
		A + B	3/6	23/23	0	1/1	27/30
Partial non-disjunction		A/B + B	3/6	22/22	1/2	1/1	27/31
		+					53/60
		A	3/6	22/22	0	1/1	26/29
		A/B + A					
		+					53/60
		B	"	"	"	"	"
Total non-disjunction		A/B+A+B	3/6	23/23	1/2	1/1	28/32
							53/60
		—	3/6	21/21	0	1/1	25/28

Key * $\frac{\text{Total number chromosomes}}{\text{Total number chromosome arms}}$

meta = metacentric chromosomes

acro = acrocentric chromosomes

t = translocation chromosome

5.3.3 Results

A total of 1,757 secondary spermatocytes were counted from 27 translocation-carrying rams. A total of 1,556 non-polyploid and 108 polyploid secondary spermatocytes were counted and analysed. A further 93 polyploid cells were recorded but were unable to be analysed. These cells were recorded under the heading N.A. (not analysed). The number of secondary spermatocytes counted and karyotyped from the translocation-carrying rams is summarized in Table 5.15.

5.3.3.1 Double heterozygous rams

A total of 417 secondary spermatocytes were counted from the four double heterozygous rams. Included in this total were 395 non-polyploid cells. The number of chromosome arms in the non-polyploid cells is given in Table 5.16. The number of chromosomes in the cells counted is not given in Table 5.16 as there would be insufficient space to record all the various chromosome combinations. For example, cells with 30 chromosome arms and an x chromosome may have 25, 26 or 27 chromosomes in total. The majority of non-polyploid cells, 369 (93.4 percent), had the modal number of 30 chromosome arms. Seven hypermodal cells, including 5 from ram B6/79, were recorded. A greater number of hypomodal cells (19) was counted.

Diploid cells were recorded (Table 5.17) but no cells with higher ploidy were observed. The percentage of polyploid cells varied from 4.9 to 9.1 percent. Five of the 10 polyploid cells analysed had a euploid chromosome arm complement (60).

The number and percentage of euploid and aneuploid cells recorded from the 4 rams is summarized in Table 5.18. The number of euploid cells was far in excess of the number of aneuploid cells. A modal number of 30 chromosome arms was recorded in 93.4 percent of the 395 cells analysed. The frequency of aneuploid cells was 6.6 percent and this total included 4.8 percent hypomodal cells and 1.8 percent

TABLE: 5.15: COUNTS OF MII CELLS AND NUMBER OF MII CELLS KARYOTYPED FROM ROBERTSONIAN TRANSLOCATION-CARRYING RAMS

	Ram Identification		MII Cells	Polyploid MII Cells	Total Including Polyploids	Number of Karyotypes
52,XY,t$_x$t	B147/75		25	2	27	3
	B63/77		10	1	11	2
	B166/78		70	4	74	7
	B6/79		290	15	305	20
	Total	Number of Cells	395	22	417	32
51,XY,t$_x$t$_x$t	B120/77		33	4	37	3
	B154/77		0	0	0	0
	B117/78		43	6	49	4
	Total		76	10	86	7
51,XY,t,t$_x$t$_x$	B7/76		8	5	13	2
	B86/76		24	0	24	4
	B111/77		163	54	217	9
	B44/78		2	0	2	1
	B47/78		101	10	111	12
	B101/78		118	8	126	8
	Total		416	77	493	36
50,XY,t,t$_x$t$_x$t$_x$	B128/77		150	17	167	12
	B140/77		28	2	30	3
	B141/77		20	3	23	3
	B121/78		30	4	34	4
	B2/79		25	5	30	2
	B32/79		67	8	75	1
	Total	Number of Cells	320	39	359	25
50,XY,t,t$_x$t$_x$t$_x$	B137/78		24	2	26	3
	B42/79		58	4	62	3
	B49/79		9	2	11	1
	B118/79		16	3	19	2
	B147/79		63	18	81	3
	Total		170	29	199	12
49,XY,t,t$_x$t$_x$t$_x$t$_x$	B196/76		18	1	19	2
	B38/79		54	8	62	2
	B92/79		107	15	122	9
	Total		179	24	203	13
	Total - All Rams		1556	201	1757	125

TABLE 5.16: DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS AT MII IN DOUBLE HETEROZYGOUS RAMS

Ram Identification		Alternate Segregation		Partial Non-disjunction				Non-disjunction				Other Cell Counts					Total
		30,x	30,y	29,x	29,y	31,x	31,y	28,x	28,y	32,x	32,y	25,y	26,-	26,x	28,-	31,xy	
B147/75	$t_1 t_3$	17	7	1	0	0	0										25
B63/77	$t_1 t_2$	5	5	0	0	0	0										10
B166/78	$t_2 t_3$	44	20	2	1	1	1	1									70
B6/79	$t_2 t_2$	142	129	2	4	2	2	2	2			1	1	1	1	1	290
Total		208	161	5	5	3	3	3	2	0	0	1	1	1	1	1	395

TABLE 5.17: CHROMOSOME COUNTS FROM POLYPLOID CELLS AT METAPHASE II IN TRANSLOCATION-CARRYING RAMS

Karyotype	No. of Rams	<2n-2	2n-1	2n	2n+1	3n	N.A.	Total	% of MII Counted that are Polyploid	Range of Polyploidy (%)
52,xy,$t_x t$	4	2	2	6	0	0	12	22	5.6	4.9 - 9.1
51,xy,$t_x t_x t$	3	1	2	3	0	0	4	10	11.6	0.0 - 12.2
51,xy,$t_1 t_2 t_3$	6	8	6	23	0	0	40	77	15.6	0.0 - 38.5
50,xy,$t_1 t_2 t_3 t_x$	6	3	4	19	0	0	13	39	10.9	6.7 - 16.7
50,xy,$t_1 t_2 t_2 t_3$	5	0	3	11	0	1	14	29	14.6	6.5 - 22.2
49,xy,$t_1 t_2 t_2 t_3 t_x$	3	1	3	10	0	0	10	24	11.8	5.3 - 12.9
Total	27	15	20	72	0	1	93	201	11.4	0.0 - 38.5

N.A. = Not Applicable

Table 5.18: DISTRIBUTION OF EUPLOID (NF/2 = 30) AND ANEUPLOID (NF/2< or >30) MII FIGURES IN TRANSLOCATION-CARRYING RAMS

Karyotype	No. of Rams	<28		28		29		30		>31		Total
		N	%	N	%	N	%	N	%	N	%	
52,xy,$t_x t$	4	3	0.8	6	1.5	10	2.5	369	93.4	7	1.8	395
51,xy,$t_x t_x t$	3	1	1.3	0	0	3	4.0	71	93.4	1	1.3	76
51,xy,$t_1 t_2 t_3$	6	2	0.5	8	1.9	32	7.7	363	87.3	11	2.6	416
50,xy,$t_1 t_2 t_3 t_x$	6	4	1.3	2	0.6	8	2.5	299	93.4	7	2.2	320
50,xy,$t_1 t_2 t_2 t_3$	5	1	0.6	3	1.8	2	1.2	159	93.5	5	2.9	170
49,xy,$t_1 t_2 t_2 t_3 t_x$	3	7	3.9	3	1.7	6	3.4	159	88.8	4	2.2	179
Total	27	18	1.2	22	1.4	61	3.9	1420	91.3	35	2.2	1556

hypermodal.

Two estimates of aneuploid frequency were made based on the method of Cattanach and Moseley (1973). The first estimate gave the total proportion of cells with 29 or 31 chromosome arms and the second was calculated by doubling the frequency of cells with a chromosome arm count of 31. Because of technical factors there are always more hypomodal cells than hypermodal cells so that the former frequency is an over-estimate of the number of aneuploid secondary spermatocytes. The latter is probably more accurate. The aneuploid frequencies were calculated on the rams from which more than 20 secondary spermatocytes were counted. The frequencies for the three rams are given in Table 5.19.

TABLE 5.19: ANEUPLOID FREQUENCIES (52,XY,t_xt)

	29 + 31 %	31 x 2 %
B147/75	4.0	0.0
B166/78	7.1	5.7
B6/79	3.8	3.4

The frequency of aneuploidy varied between rams but in each case the estimation of aneuploid frequency based on the hypermodal cell counts was similar to the estimate based on the 29 + 31 chromosome arm counts.

The distribution and association of the translocations and sex chromosomes in the euploid secondary spermatocytes are shown in Table 5.20. Although there was no overall deviation from the expected 1:1:2:2:1:1 ratio (χ^2_5 not significant), there was a significant excess of - t,x cells (χ^2_1 = 6.46; 0.01 < P < 0.02). When the euploid and aneuploid cells were grouped together there was an overall deviation from the expected segregation ratio (χ^2_5 = 12.73; 0.02 < P < 0.05). As before, when the euploid cells were

TABLE 5.20: ASSOCIATION OF TRANSLOCATION CHROMOSOMES WITH SEX CHROMOSOMES AT MII (EUPLOID CELLS) IN DOUBLE HETEROZYGOUS RAMS

* Aneuploid cells are recorded in brackets

Ram Identification	+t, t, y	+t, t, x	+t, y	+t, x	-t, y	-t, x
B147/75	2	5	4	8	1	4
B63/77	2	1	1	2	2	2
B166/78	4	11	14(1)	18(1)	2(1)	15(3)
B6/79	37(1)	30	59(7)	68(4)	29(1)	42(3)
Total	45(1)	47	78(8)	96(6)	34(2)	63(6)

χ^2_5 = not significant (1:1:2:2:1:1 ratio)

considered separately, there was a significant excess of -t,x cells (χ^2_1 = 8.50; P < 0.01).

The distribution of sex chromosomes in the MII cells is also given in Table 5.20. A significantly greater number of the euploid MII cells had an X-chromosome (χ^2_1 = 5.73; P < 0.05). In all cells (euploid + aneuploid) there was also a significant excess of X-carrying to Y-carrying cells (χ^2_1 = 5.64; P < 0.05).

The numbers of euploid MII cells with respective chromosome number are given in Tables 5.21 and 5.22. For the double heterozygotes (t_2t_x) it is seen that there was no significant deviation from the expected 1:1:1:1 ratio. The number of euploid cells with chromosome numbers of 25,t_2t and 26,t_2 was identical. The translocation, t_2, was found in 170 (50.1%) of the 339 cells counted. Only 24 euploid MII cells were counted in the double heterozygote (t_1t_3). The segregation ratio was close to the expected 1:2:1 ratio. Two examples of euploid metaphase II cells from double heterozygotes are shown in Plates 5.23 and 5.24 and Figures 5.11 and 5.12.

5.3.3.2 Triple translocation-carrying rams (51,xy,t_xt_xt)

A total of 86 secondary spermatocytes were counted from the 2 triple translocation-carrying rams. Included in this total were 76 non-polyploid cells. The number of chromosome arms in the non-polyploid cells is given in Table 5.23. The majority of non-polyploid cells, 71 (93.4 percent), had the modal chromosome arm count of 30. Five (6.6 percent) of the cells were aneuploid. Of these, four were hypomodal and one cell was hypermodal.

Ten (11.6 percent) of the secondary spermatocytes examined were polyploid (Table 5.17). Six of the cells were diploid and of these cells three had the diploid chromosome arm complement (60). The other 4 polyploid cells were not analysed. The percentage of polyploid cells in the two

TABLE 5.21: NUMBER OF EUPLOID (NF/2 = 30) METAPHASE II FIGURES WITH RESPECTIVE CHROMOSOME NUMBER RECORDED FROM THE DOUBLE HETEROZYGOTES (52,xy,t_2t_x)

Karyotype	25,t_2t_x	26,t_x	26,t_2	27	Total
Expected number	84.75	84.75	84.75	84.75	339
Observed number	85	77	85	92	339

χ^2_3 = not significant (1:1:1:1 ratio)

TABLE 5.22: NUMBER OF EUPLOID (NF/2 = 30) METAPHASE II FIGURES WITH RESPECTIVE CHROMOSOME NUMBER RECORDED FROM THE DOUBLE HETEROZYGOTE (52,xy,t_1t_3)

Karyotype	25,t_1t_3	26,t_x	27	Total
Expected number	6	12	6	24
Observed number	7	12	5	24

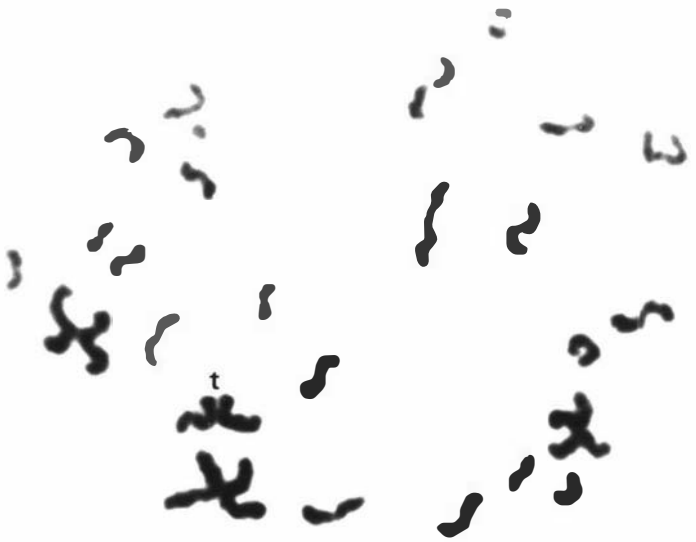
χ^2_2 = not significant (1:2:1 ratio)

TABLE 5.23: DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS AT MII IN TRIPLE TRANSLOCATION RAMS (51,xy,t_x,t_x,t)

Ram Identification	Alternate Segregation		Partial Non-disjunction				Non-disjunction				Other Cell Counts	Total
	30,x	30,y	29,x	29,y	31,x	31,y	28,x	28,y	32,x	32,y	27,x	
B120/77	16	15	0	1	0	0					1	33
B154/77	0	0	0	0	0	0						0
B117/78	19	21	0	2	1	0						43
Total	35	36	0	3	1	0	0	0	0	0	1	76

PLATE 5.23: Metaphase II cell from B147/75 (52,xy,t_1 t_3). There are 26 chromosomes including a translocation submetacentric (t) and a Y chromosome. (Giemsa x 3,000).

FIGURE 5.11: Karyotype of cell illustrated in Plate 5.23. There are 30 chromosome arms. The translocation is the fourth largest chromosome.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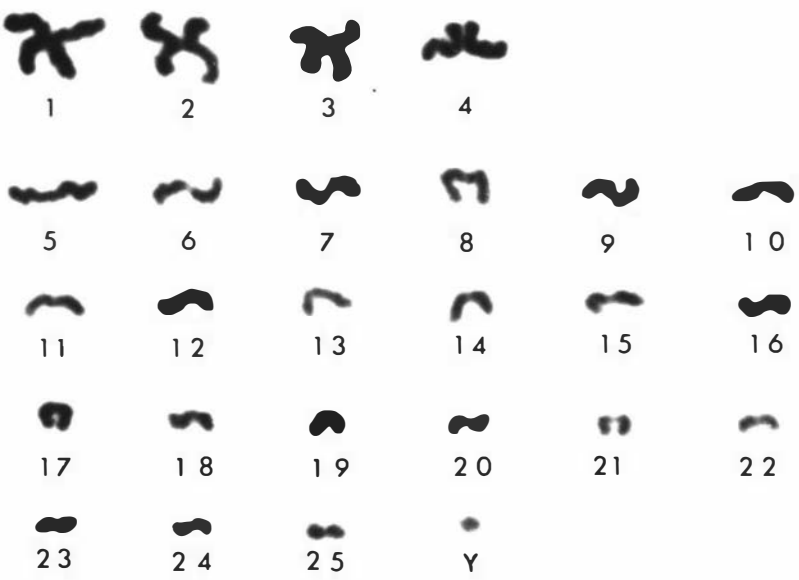
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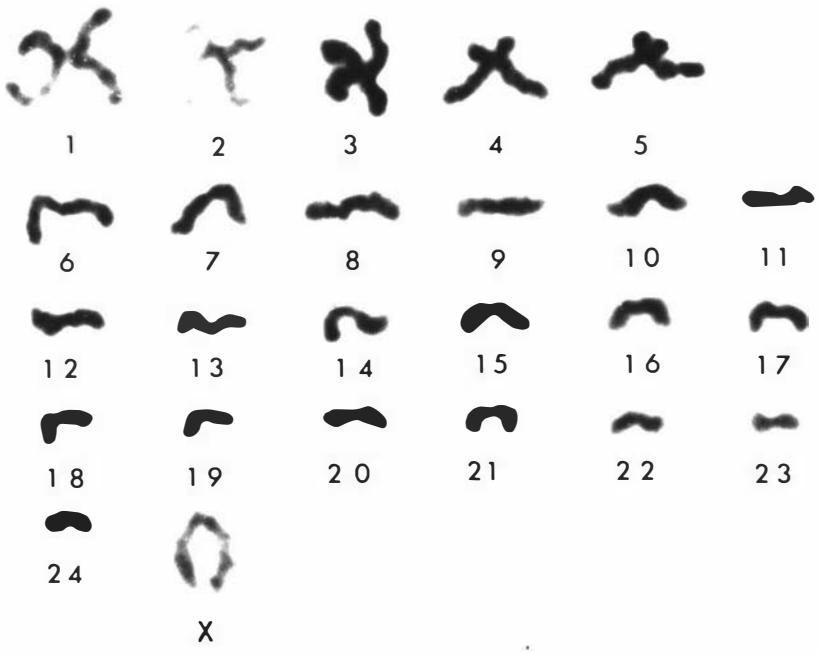
PLATE 5.24: Metaphase II cell from B147/75 (52,xy t_1t_3).
There are 25 chromosomes including two translocation submetacentrics (t) and an X chromosome. (Giemsa x 3,000).

FIGURE 5.12: Karyotype of cell illustrated in Plate 5.24. There are 30 chromosome arms.


t
t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rams was 10.8 and 12.2 percent.

The number and percentage of euploid and aneuploid cells recorded from the 2 rams is given in Table 5.18. The number of euploid cells was far in excess of the number of aneuploid cells. Therefore the modal chromosome number of arms was 30. Only one hypermodal cell was recorded, in ram B117/78.

The aneuploid frequencies of the two rams are given in Table 5.24.

TABLE 5.24: ANEUPLOID FREQUENCIES (51, XY,$t_x t_x t$)

	29 + 31 %	31 x 2 %
B120/77	3.0	0.0
B117/78	7.0	4.7

The frequency of aneuploidy varied between the two triple translocation-carrying rams.

The distribution and association of the translocations and sex chromosomes in the euploid cells are shown in Table 5.25. There was no overall deviation from the 1:1:1:1 ratio expected for each of the four possible cell classes produced by alternate segregation. When the euploid and aneuploid cells were grouped together the overall deviation was closer to the expected 1:1:1:1 ratio. None of the individual groups departed significantly from the numbers expected for each class.

The distribution of sex chromosomes in the secondary spermatocytes was also given in Table 5.25. There was no significant deviation from the expected 1:1 ratio of X and Y carrying cells.

TABLE 5.25: ASSOCIATION OF TRANSLOCATION CHROMOSOMES WITH SEX CHROMOSOMES AT MII (EUPLOID CELLS) IN TRIPLE TRANSLOCATION-CARRYING RAMS

* Aneuploid cells are recorded in brackets

Ram Identification	+t,t,y	+t,t,x	+t,y	+t,x
B120/77	8(2)	6	5	10
B154/77	0	0	0	0
B117/78	11	9(1)	10(2)	10
Total	19(2)	15(1)	15(2)	20

χ^2_3 = not significant (1:1:1:1 ratio)

The numbers of euploid MII cells with respective chromosome number are given in Tables 5.26 and 5.27. There was no significant deviation in the two rams from the expected 1:1 ratio (χ^2_1 not significant). Two examples of euploid metaphase II cells from triple translocation-carrying rams are shown in Plates 5.25 and 5.26 and Figures 5.13 & 5.14.

5.3.3.3 Triple heterozygous rams

A total of 493 secondary spermatocytes were counted from the 6 triple heterozygous rams. Included in this total were 416 non-polyploid cells. The distribution of chromosome arm counts in the non-polyploid cells is given in Table 5.28. The majority of the non-polyploid cells, 363 (87.3 percent), had 30 chromosome arms. The number of hypomodal cells recorded was 42 compared to only 11 cells that were hypermodal.

Diploid cells were recorded (Table 5.17) but no cells with higher ploidy were observed. Forty of the polyploid cells were not analysed. The percentage of polyploid cells varied from 0 to 24.9 percent in rams in which more than 20 secondary spermatocytes were recorded.

The number and percentage of euploid and aneuploid cells recorded from the 6 rams are summarized in Table 5.18. The percentage of euploid cells ranged from 78.5 to 95.8 percent in rams in which more than 20 cells were counted. The frequency of hypomodal cells, counted in the 6 rams, was 9.1 percent while only 2.6 percent were hypermodal. Of the 11 hypermodal cells recorded 7 were from ram B111/77. This was the equivalent to 4.3 percent of the total number of cells counted in the ram.

The estimates of the aneuploid frequencies are given in Table 5.29.

It was seen that the two rams B86/76 and B111/77 had similar aneuploid frequencies (31 x 2%) of 8.3 and 8.6 percent respectively. The 2 rams, B47/78 and B101/78, had lower

TABLE 5.26: NUMBER OF EUPLOID (NF/2 = 30) METAPHASE II FIGURES WITH RESPECTIVE CHROMOSOME NUMBER RECORDED FROM THE TRIPLE TRANSLOCATION RAM (51,xy,$t_1t_1t_3$)

Karyotype	25,t_1t_3	26,t_1	Total
Expected numbers	14.5	14.5	29
Observed numbers	14	15	29

χ^2_1 = not significant (1:1 ratio)

TABLE 5.27: NUMBER OF EUPLOID (NF/2 = 30) METAPHASE II FIGURES WITH RESPECTIVE CHROMOSOME NUMBER RECORDED FROM THE TRIPLE TRANSLOCATION RAM (51,xy,$t_2t_3t_3$)

Karyotype	25,t_2t_3	26,t_3	Total
Expected numbers	20	20	40
Observed numbers	20	20	40

χ^2_1 = not significant (1:1 ratio)

TABLE 5.28: DISTRIBUTION OF CHROMOSOME COUNTS AT MII IN TRIPLE HETEROZYGOUS RAMS

Ram Identification	Alternate Segregation		Partial Non-disjunction				Non-disjunction				Other cell counts					Total
	30,x	30,y	29,x	29,y	31,x	31,y	28,x	28,y	32,x	32,y	27,-	27,x	27,y	28,-	29,-	
B7/76	4	4	0	0	0	0										8
B86/76	9	14	0	0	1	0										24
B111/77	66	62	11	6	4	3	2	3			1		1	1	3	163
B44/78	0	1	1	0	0	0										2
B47/78	40	52	2	4	1	1	1									101
B101/78	48	63	4	1	1	0						1				118
Total	167	196	18	11	7	4	3	3	0	0	1	1	1	1	3	416

PLATE 5.25: Metaphase II cell from B120/77 (51,xy,$t_1t_1t_3$). There are 26 chromosomes including one translocation submetacentric and a Y chromosome. (Giemsa x 3,000).

FIGURE 5.13: Karyotype of cell illustrated in Plate 5.25. There are 30 chromosome arms.


t_1

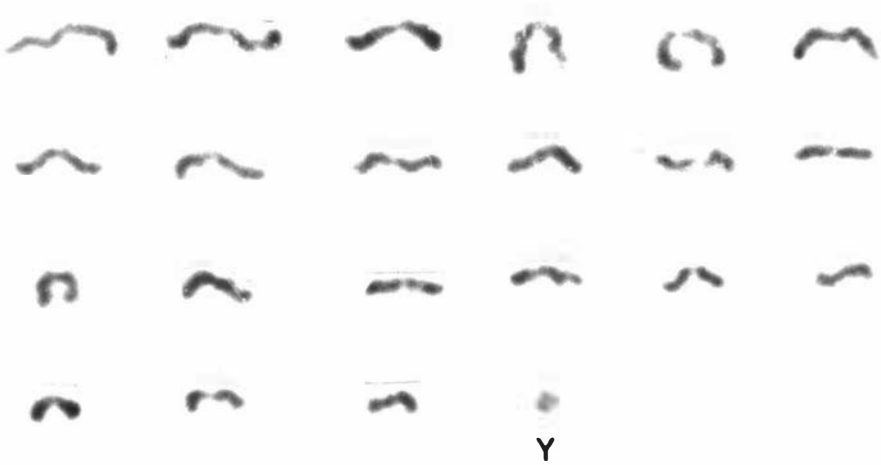
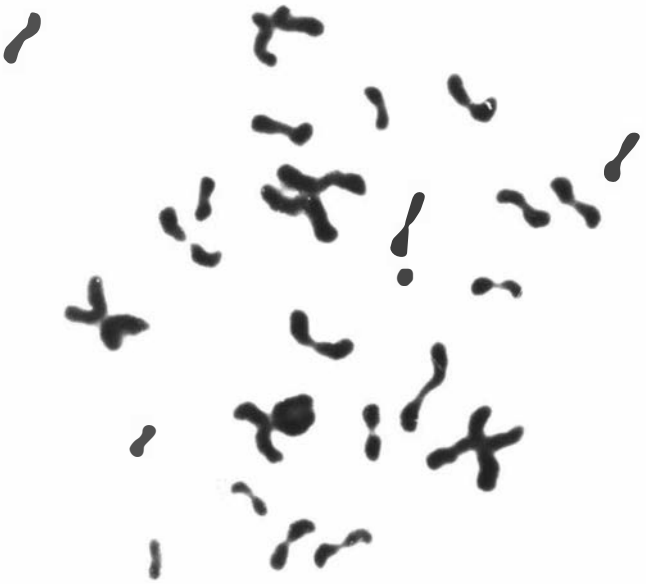
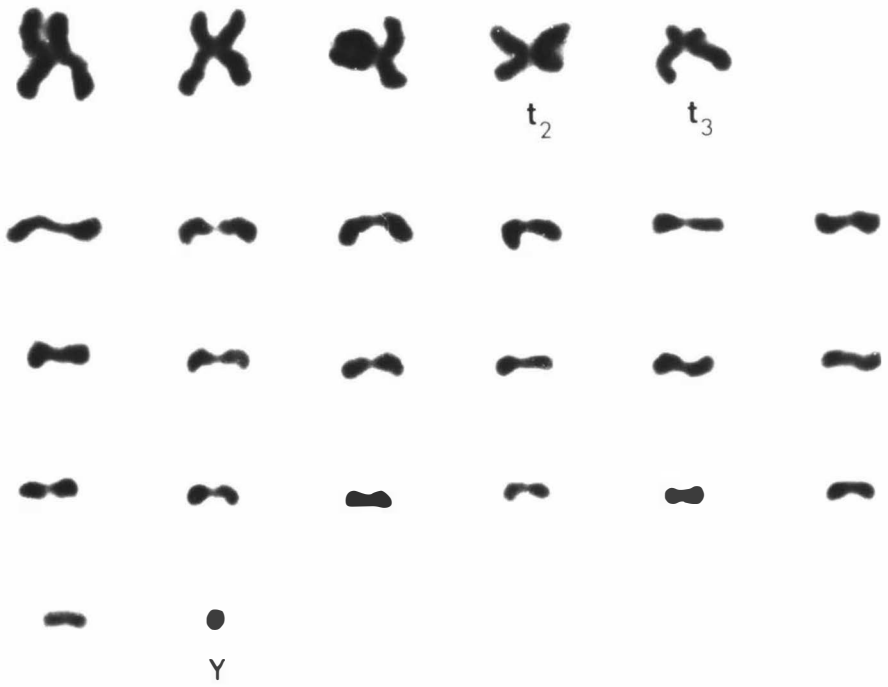

Y

PLATE 5.26: Metaphase II cell from B117/78 (51,xy,$t_2t_3t_3$). There are 25 chromosomes including one translocation submetacentric, one translocation metacentric and a Y chromosome. (Giemsa x 2,500).

FIGURE 5.14: Karyotype of cell illustrated in Plate 5.26. There are 30 chromosome arms.




t2
t3
Y

aneuploid frequencies. The overall aneuploid frequency of the triple heterozygous rams was 5.3 percent.

TABLE 5.29: ANEUPLOID FREQUENCIES (51,XY,$t_1t_2t_3$)

	29 + 31%	31 x 2%
B86/76	4.2	8.3
B111/77	16.6	8.6
B47/78	7.9	4.0
B101/78	5.1	1.7

The distribution of the translocations and sex chromosomes in the euploid cells is shown in Table 5.30. There was no overall deviation from the 1:1:2:2:2:2:1:1 ratio expected for each of the eight possible cell classes. There was a significant deficit of +t,t,x cells (χ^2_1 = 4.23; $P < 0.05$). However, when the euploid and aneuploid cells were grouped together there was an overall deviation from the ratio (χ^2 = 19.23; $P < 0.01$) expected for each cell class. A significant excess of +t,y cells (χ^2_1 = 4.22; $P < 0.05$) and deficit of +t,t,x cells (χ^2_1 = 4.73; $P < 0.05$) were observed in the grouped data.

The distribution of sex chromosomes in the secondary spermatocytes was also given in Table 5.30. There was no significant deviation from the expected 1:1 ratio of X and Y carrying cells.

The number of euploid MII cells with respective chromosome number is given in Table 5.31. It was seen that there was a significant overall deviation from the expected 1:1:2:2:1:1 ratio (χ^2_1 = 20.07; $P < 0.01$). A significant excess of 27 MII cells (χ^2_1 = 7.24; $P < 0.01$) and a deficit of 25,t_1t_3 MII cells (χ^2_1 = 12.56; $P < 0.01$) were observed. These two groups, with a combined χ^2 value of 19.80, contributed 98.7 percent of the total χ^2 value.

TABLE 5.30: ASSOCIATION OF TRANSLOCATION CHROMOSOMES WITH SEX CHROMOSOMES AT MII (EUPLOID CELLS) IN TRIPLE HETEROZYGOUS RAMS.

* Aneuploid cells are recorded in brackets

Ram Identification	+t,t,t,y	+t,t,t,x	+t,t,y	+t,t,x	+t,y	+t,x	-t,y	-t,x
B7/76	0	0	1	1	2	1	1	2
B86/76	3	1	6	2	5	4	0	2(1)
B111/77	6	5(3)	22(3)	17(4)	23(8)	33(5)	11(2)	11(5)
B44/78	0	0	1	0(1)	0	0	0	0
B47/78	7	8	16(2)	10(1)	22(3)	13(1)	7	9(2)
B101/78	7	7	23	14	23	16(5)	10(1)	11(1)
Total	23	21(3)	69(5)	44(6)	75(11)	67(11)	29(3)	35(9)

χ^2_7 = not significant (1:1:2:2:2:2:1:1 ratio)

TABLE 5.31: NUMBER OF EUPLOID (NF/2 = 30) METAPHASE II FIGURES WITH RESPECTIVE CHROMOSOME NUMBER RECORDED FROM THE TRIPLE HETEROZYGOUS RAMS

Karyotype	24,$t_1t_2t_3$	25,t_1t_3	25,t_2t_x	26,t_x	26,t_2	27	Total
Expected numbers	45.375	45.375	90.75	90.75	45.375	45.375	363
Observed numbers	44	21	92	93	49	64	363

χ^2_5 = 20.07; $P < 0.01$ (1:1:2:2:1:1 ratio)

TABLE 5.32: NUMBERS OF PROGENY WITH RESPECTIVE CHROMOSOME NUMBER RECORDED FROM MATINGS OF TRIPLE HETEROZYGOUS RAMS AND 83 EWES OF NORMAL KARYOTYPE

Karyotype	51,$t_1t_2t_3$	52,t_1t_3	52,t_2t_x	53,t_x	53,t_2	54	Total
Expected numbers	10.375	10.375	20.75	20.75	10.375	10.375	83
Observed numbers	10	3	24	24	4	18	83

χ^2_5 = 13.50; $0.01 < P < 0.02$ (1:1:2:2:1:1 ratio)

The number of progeny with respective chromosome number from the matings of normal ewes x triple heterozygous rams is given in Table 5.32. A significant overall deviation from the expected 1:1:2:2:1:1 ratio (χ^2_5 = 13.50; 0.01 < P < 0.02) was observed. A significant excess of progeny with a normal karyotype (χ^2_1 = 4.89; P < 0.05) and a deficit of progeny with a 52, t_1t_3 karyotype (χ^2_1 = 4.56; P < 0.05) were observed.

Four examples of euploid and aneuploid metaphase II cells from triple heterozygotes are shown in Plates 5.27 to 5.30 and Figures 5.15 and 5.16.

5.3.3.4 Multiple translocation-carrying rams (50,xy,$t_1t_2t_3t_x$)

A total of 359 secondary spermatocytes were counted from the 6 rams. Included in this total were 320 non-polyploid cells. The distribution of chromosome arm counts is given in Table 5.33. The majority of non-polyploid cells, 93.4 percent, had the modal number of 30 chromosome arms. Fourteen of the cells were hypomodal and only 7 cells were hypermodal.

Diploid cells were recorded (Table 5.17) and no other ploidy was observed. One of the polyploid cells was not analysed. The percentage of polyploid cells varied from 6.7 to 16.7 percent.

The number and percentage of euploid cells recorded from the 6 rams is given in Table 5.18. The number of cells with the modal chromosome count ranged from 84.0 to 95.5 percent with a mean for the 6 rams of 93.4 percent. The frequency of hypomodal cells was 4.4 percent (Plates 5.31 Figure 5.17) and 2.2 percent were hypermodal (Plate 5.32 and Figure 5.18).

The estimates of aneuploid frequencies are given in Table 5.34.

TABLE 5.33: DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS AT MII IN MULTIPLE TRANSLOCATION RAMS (50,xy,$t_1t_2t_3t_x$)

Rams Identification	Alternate Segregation		Partial Non-disjunction				Non-disjunction				Other Cell Counts				Total
	30,x	30,y	29,x	29,y	31,x	31,y	28,x	28,y	32,x	32,y	25,y	26,x	27,x	27,y	
B128/77	70	73	2	1	2	1		1							150
B140/77	14	12	0	1	0	1									28
B141/77	10	9	0	0	0	0							1		20
B121/78	10	16	1	0	0	1	1							1	30
B2/79	9	12	1	0	0	1					1	1			25
B32/79	34	30	1	1	1	0									67
Total	147	152	5	3	3	4	1	1	0	0	1	1	1	1	320

PLATE 5.27: Metaphase II cell from B47/78 (51,xy,t_1 $t_2$$t_3$). There are 26 chromosomes including one translocation metacentric (t_2) and a Y chromosome (arrowed). (Giemsa x 3,000).

PLATE 5.28: Metaphase II cell from B47/78 (51,xy,$t_1$$t_2$$t_3$). There are 25 chromosomes including one translocation submetacentric (t), one translocation metacentric (t_2) and a Y chromosome (arrowed). (Giemsa x 3,000).

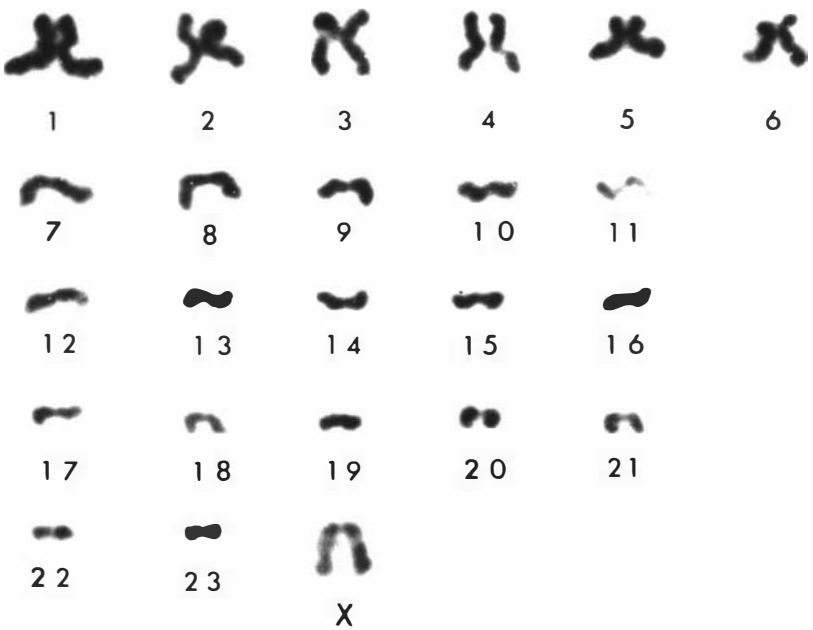

t_2


t
t_2

PLATE 5.29: Metaphase II cell from B47/78 (51,xy,$t_1t_2t_3$).
There are 24 chromosomes including two translocation submetacentrics (t), one translocation metacentric (t_2) and an X chromosome.
(Giemsa x 3,000).

FIGURE 5.15: Karyotype of cell illustrated in Plate 5.29.
There are 30 chromosome arms.


t
t
t2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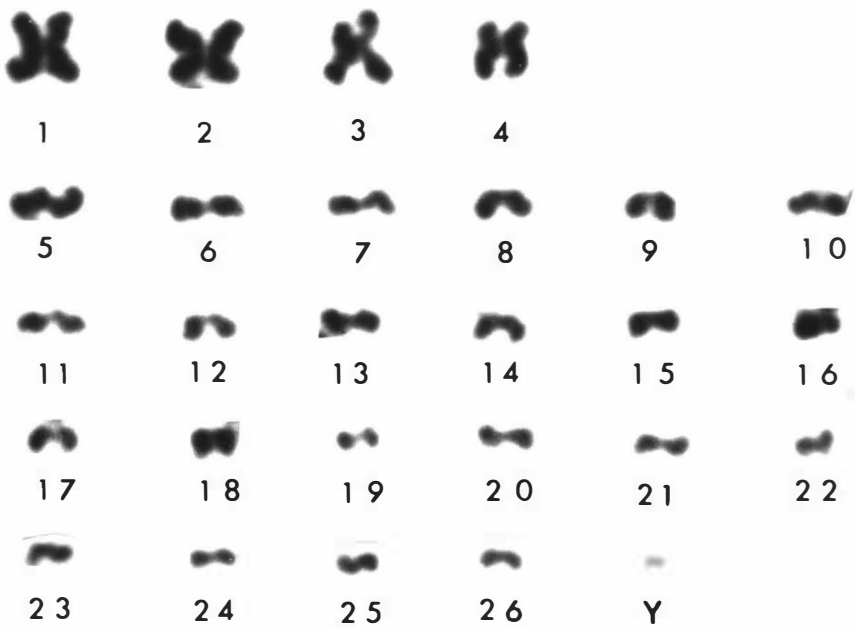
X

PLATE 5.30: Metaphase II cell from B47/78 (51,xy,$t_1t_2t_3$).
There are 31 chromosome arms and 27 chromosomes including a translocation metacentric chromosome (t_2) and a Y chromosome.
(Giemsa x 3,000).

FIGURE 5.16: Karyotype of cell illustrated in Plate 5.30.


t_2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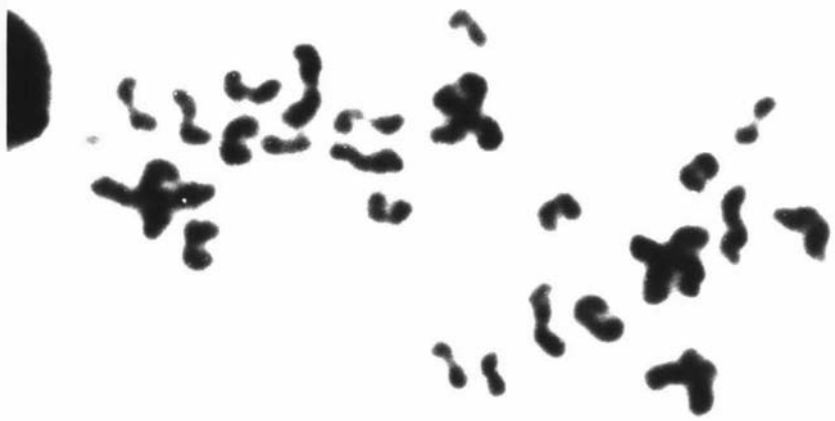
Y

PLATE 5.31: Metaphase II cell from B128/77 (50,xy,$t_1t_1t_2t_3$). There are 29 chromosome arms and 25 chromosomes including one translocation submetacentric and a Y chromosome.
(Giemsa x 3,000).

FIGURE 5.17: Karyotype of cell illustrated in Plate 5.31. The translocation submetacentric(t) is identified.



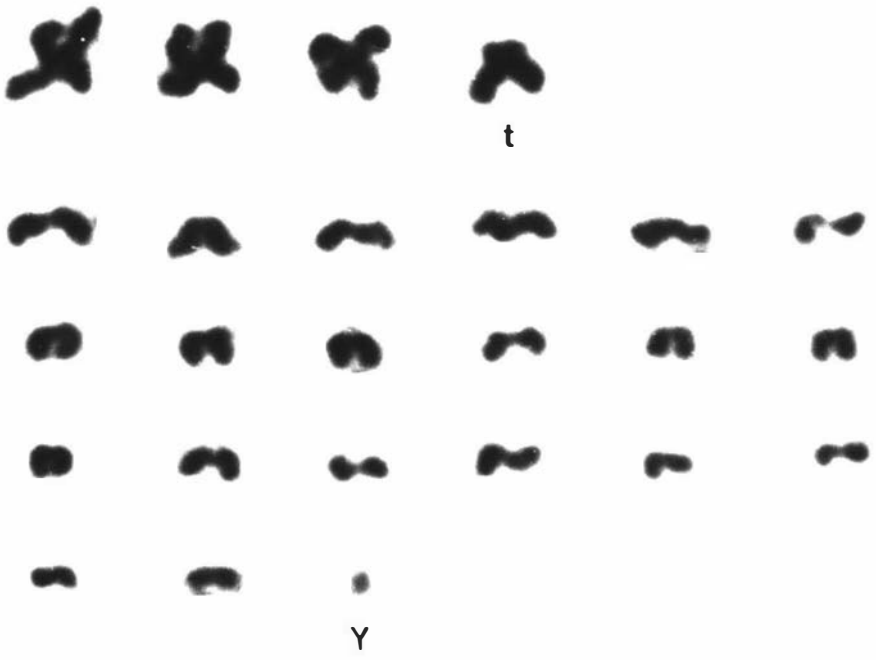
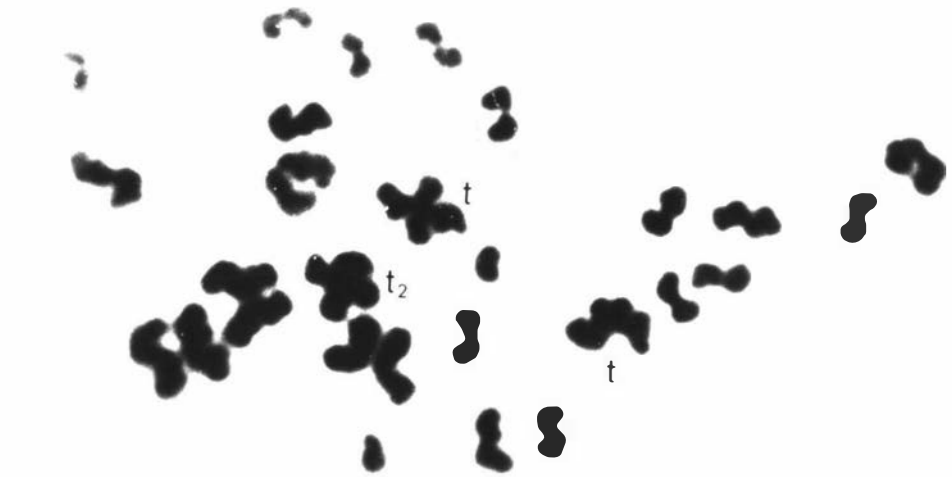

t
Y

PLATE 5.32: Metaphase II cell from B128/77 (50,xy,$t_1t_1t_2t_3$). There are 31 chromosome arms and 25 chromosomes including two translocation submetacentrics (t), one translocation metacentric (t_2) and an X chromosome. (Giemsa x 3,000).

FIGURE 5.18: Karyotype of cell illustrated in Plate 5.32.


t
t_2
t

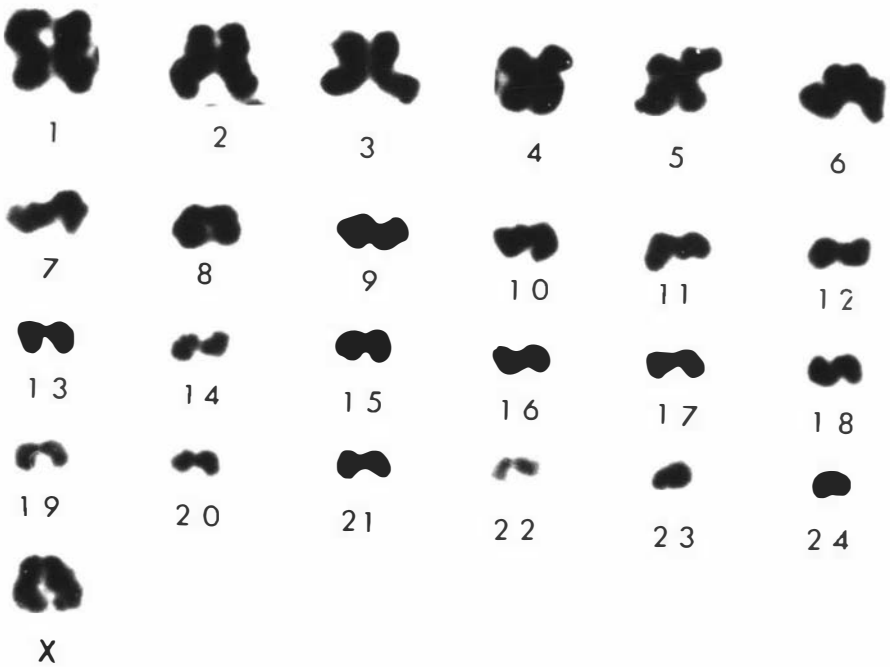

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TABLE 5.34: ANEUPLOID FREQUENCIES (50,xy,$t_1t_2t_3t_x$)

	29 + 31%	31 x 2%
B128/77	4.0	4.0
B140/77	7.1	7.1
B141/77	0.0	0.0
B121/78	6.7	6.7
B2/79	8.0	8.0
B32/79	4.5	3.0

The aneuploid frequency (31 x 2%) varied from 0.0 to 8.0 percent with a mean frequency of 4.4 percent.

The distribution of the translocations and sex chromosomes in the euploid cells is shown in Table 5.35. There ~~were~~ was no overall deviation from the 1:1:2:2:1:1 ratio expected for each of the six possible cell classes. None of the groups departed significantly from the numbers expected for each class. When the aneuploid and euploid cells were grouped together there was no overall deviation from the 1:1:2:2:1:1segregation ratio. However, there was a significant deficit of $+t_1t_2t_3$,x cells (χ^2_1 = 4.32; P < 0.05).

The number and distribution of the sex chromosomes in the euploid and aneuploid secondary spermatocytes are also shown in Table 5.35. There was no significant deviation from the expected 1:1 ratio of x and y carrying cells.

The number of euploid secondary spermatocytes with respective chromosome number is given in Table 5.36. There was no significant departure from the expected 1:1:1:1 ratio.

5.3.3.5 Multiple translocation-carrying rams (50,xy,$t_1t_2t_2t_3$)
A total of 199 secondary spermatocytes were counted from 5 rams. Included in this total were170 non-polyploid cells.

TABLE 5.35: ASSOCIATION OF TRANSLOCATION CHROMOSOMES WITH SEX CHROMOSOMES AT MII (EUPLOID CELLS) IN MULTIPLE TRANSLOCATION RAMS (50,xy, $t_1t_2t_3t_x$)

* Aneuploid cells are recorded in brackets

Ram Identification	$+t_1t_2t_3$,y	$+t_1t_2t_3$x	+tt,y	+tt,x	+t,y	+t,x
B128/77	23(1)	9(1)	29(1)	39(2)	20(1)	21(1)
B140/77	2(1)	3	4(1)	9	6	2
B141/77	3	2	3	5	3	3(1)
B121/78	3(1)	1	7(1)	4(2)	5	2
B2/79	3(1)	1	4	5(1)	2	2(1)
B32/79	10	8	13(1)	12(2)	7	12
Total	44(4)	24(1)	60(4)	74(7)	43(1)	42(3)

X^2_5 = not significant (1:1:2:2:1:1 ratio)

TABLE 5.36: NUMBER OF EUPLOID (NF/2 = 30) METAPHASE II FIGURES WITH RESPECTIVE CHROMOSOME NUMBER RECORDED FROM THE MULTIPLE TRANSLOCAION (50,xy,$t_1t_2t_3t_x$) RAMS

Karyotype	24,$t_1t_2t_3$	25,t_1t_3	25,t_2t_x	26,t_x	Total
Expected numbers	71.75	71.75	71.75	71.75	287
Observed numbers	68	57	77	85	287

χ^2_3 = Not significant (1:1:1:1 Ratio)

TABLE 5.40: NUMBER OF EUPLOID (NF/2 = 30) METAPHASE II FIGURES WITH RESPECIVE CHROMOSOME NUMBER RECORDED FROM THE MULTIPLE TRANSLOCATION (50,xy,$t_1t_2t_2t_3$) RAMS

Karyotype	24,$t_1t_2t_3$	25,t_2t_x	26,t_2	Total
Expected numbers	38.75	77.5	38.75	155
Observed number	31	66	58	155

χ^2_2 = 11.99; $P < 0.01$ (1:2:1 Ratio)

The number of chromosome arms in the non-polyploid cells is given in Table 5.37. The majority of the non-polyploid cells, 158 (92.9 percent), had the modal number of 30 chromosome arms. Six hypermodal cells, including 5 from rams B147/79, were recorded and an equivalent number of hypomodal cells were seen in the five rams.

The chromosome counts of the polyploid cells are given in Table 5.17. Twenty eight of the cells were diploid and one cell was triploid. The percentage ranged from 6.5 to 22.2 percent with a mean of 14.6 percent.

The number and percentage of euploid and aneuploid cells recorded from the 5 rams is given in Table 5.18. The percentage of euploid cells ranged from 88.8 to 100 percent. The frequency of aneuploid cells in the 5 rams was 6.5 percent. Included in this total were 3.6 percent hypomodal cells and 2.9 percent hypermodal cells (Plate 5.33 and Figure 5.19).

The estimates of aneuploid frequency were made on the three rams from which at least 20 cells were counted and are given in Table 5.38.

TABLE 5.38: ANEUPLOID FREQUENCIES (50,xy,$t_1t_2t_2t_3$)

	29 + 31%	31 x 2%
B137/78	0.0	0.0
B42/79	3.4	3.4
B147/79	7.9	12.7

The aneuploid frequency ranged from 0.0 to 12.7 percent with a mean of 5.9 percent.

The distribution of the translocations and sex chromosomes in the euploid cells is shown in Table 5.39. There was an overall deviation from the 1:1:2:2:1:1 ratio (χ^2_5 = 13.23;

TABLE 5.37: DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS AT MII IN MULTIPLE TRANSLOCATION RAMS ($50,xy,t_1t_2t_2t_3$)

Ram Identification	Alternate Segregation 30,x	30,y	Partial Non-disjunction 29,x	29,y	31,x	31,y	Non-disjunction 28,x	28,y	32,x	32,y	Other Cell Counts 27,x	Total
B137/78	13	11	0	0	0	0						24
B42/79	28	27	0	1	0	1	1					58
B49/79	3	6	0	0	0	0						9
B118/79	10	5	0	0	0	0	1					16
B147/79	28	27	1	0	1	4	1				1	63
Total	82	76	1	1	1	5	3	0	0	0	1	170

TABLE 5.39: ASSOCIATION OF TRANSLOCATION CHROMOSOMES WITH SEX CHROMOSOMES AT MII (EUPLOID CELLS) IN MULTIPLE TRANSLOCATION RAMS (50,xy,$t_1t_2t_2t_3$)

* Aneuploid cells are recorded in brackets

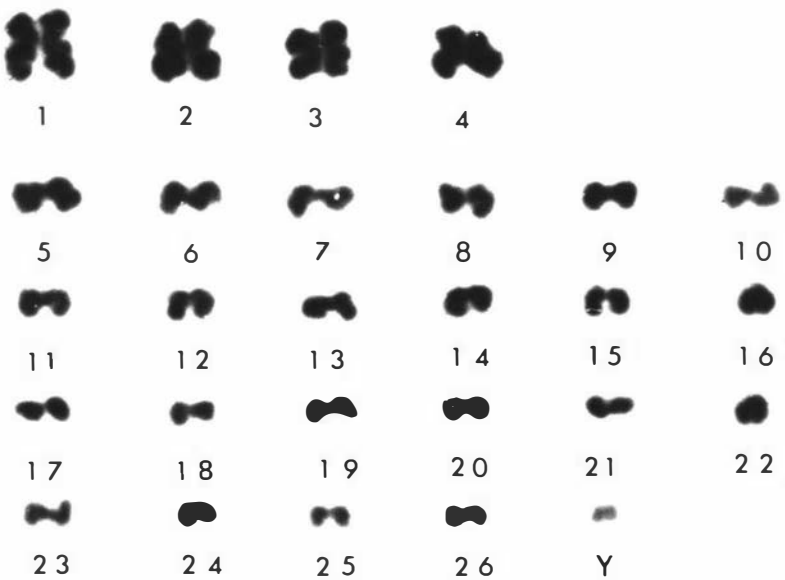
Ram Identification	$+t_1t_2t_3$,y	$+t_1t_2t_3$,x	$+t_2t$,y	$+t_2t$,x	$+t_2$y	$+t_2$x
B42/79	7(1)	5	10(1)	11	10	12(1)
B49/79	3	1	2	0	1	2
B118/79	3	0	1	5	0	3(1)
B137/79	0	2	6	7	5	4
B147/79	5	5	9(2)	15	14(2)	7(4)
Total	18(1)	13	28(3)	38	30(2)	28(6)

χ^2_5 = 13.23; 0.01 < P < 0.02 (1:1:2:2:1:1 ratio)

PLATE 5.33: Metaphase II cell from B147/79 (50,xy,t_1 t_2 t_2 t_3). There are 31 chromosome arms and 27 chromosomes including a translocation metacentric chromosome (t_2) and a Y chromosome. (Giemsa x 3,000).

FIGURE 5.19: Karyotype of cell illustrated in Plate 5.33.


t_2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26
Y

0.01 < P < 0.02) expected for each of the six possible cell classes. A significant excess of euploid cells with $+t_2$,y was recorded (χ^2_1 = 5.29; P < 0.05). When the euploid and aneuploid cells were grouped together there was an increased overall deviation from the 1:1:2:2:1:1 ratio (χ^2_5 = 18.51; P < 0.01). An excess of cells with $+t_2$,Y (χ^2_1 = 5.41; P < 0.05) and $+t_2$, X (χ^2_1 = 7.64; P < 0.01) ~~were~~ was recorded.

The distribution of sex chromosomes in the secondary spermatocytes was also seen in Table 5.39. There was no significant difference in the number of cells with X and Y chromosomes.

The number of euploid MII cells with respective chromosome number is given in Table 5.40. A significant deviation from the expected 1:2:1 segregation ratio (χ^2_2 = 11.99; P < 0.01) was found. This deviation has resulted from the very significant excess of euploid cells with a 26,t_2 karyotype (χ^2_1 = 9.07; P < 0.01).

5.3.3.6 Multiple translocation-carrying rams (49,xy,$t_1t_2t_2t_3t_x$)

A total of 203 secondary spermatocytes were counted from the three rams. Included in this total were 179 non-polyploid cells. The number of chromosome arms in the non-polyploid cells is given in Table 5.41. The majority of the non-polyploid cells, 159 (88.8 percent), had the modal number of 30 chromosome arms. Four hypermodal cells, including 3 from ram B92/79, were recorded and 16 hypomodal cells were counted.

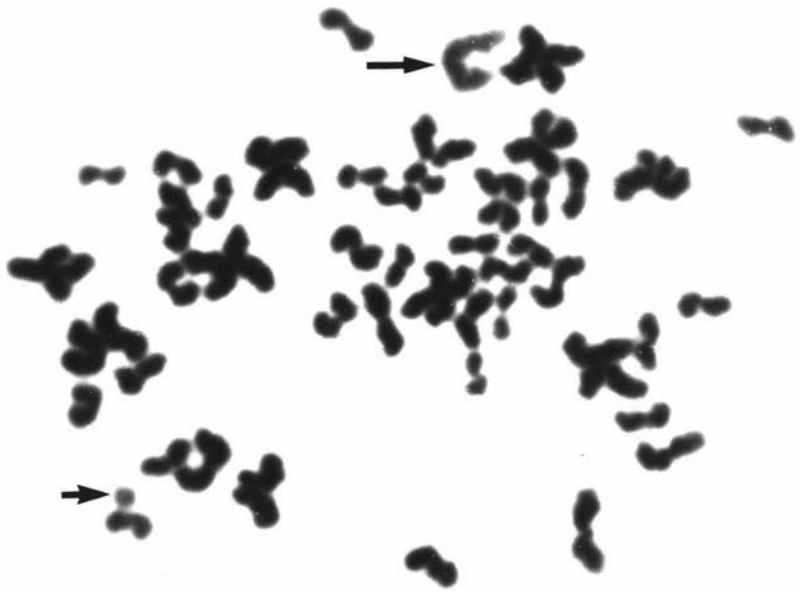
Diploid cells were recorded (Table 5.17) but no cells with higher ploidy were observed (Plate 5.34). The percentage of polyploid cells varied from 5.3 to 12.9 percent.

The number and percentage of euploid and aneuploid cells recorded from the 3 rams is given in Table 5.18. The percentage of euploid cells ranged from 83.3 to 94.4 percent with a mean of 88.8 percent. The frequency of aneuploid

TABLE 5.41: DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS AT MII IN MULTIPLE TRANSLOCATION RAMS ($49,xy,t_1t_2t_2t_3t_x$)

Ram Identification	Alternate Segregation		Partial Non-disjunction				Non-disjunction				Other cell counts				Total
	30,x	30,y	29,x	29,y	31,x	31,y	28,x	28,y	32,x	32,y	25,-	25,x	25,y	27,x	
B196/76	6	9	0	1	0	0						1	1		18
B38/79	25	26	0	0	1	0	1					1			54
B92/79	58	35	4	1	2	1	2				1	1		2	107
Total	89	70	4	2	3	1	3	0	0	0	1	3	1	2	179

PLATE 5.34: Diploid metaphase II cell from B92/79 (49,xy, $t_1 t_2 t_2 t_3 t_x$). There are 60 chromosome arms. The sex chromosomes are indicated, X chromosome (large arrow) and Y chromosome (small arrow). (Giemsa x 3,000).



cells was 11.2 percent. Of these 9.0 percent were hypomodal cells and 2.2 percent were hypermodal cells (Plate 5.35 and Figure 5.20).

The estimates of aneuploid frequency were made on the two rams from which at least 20 cells were analysed and are given in Table 5.42.

TABLE 5.42: ANEUPLOID FREQUENCIES (49,xy,$t_1t_2t_2t_3t_x$)

	29 + 31%	31 x 2%
B38/79	1.8	3.7
B92/79	7.4	5.6

The aneuploid frequency ranged from 3.7 to 5.6 percent with a mean for the 3 rams of 4.5 percent.

The distribution of the translocations and sex chromosomes in the euploid cells is shown in Table 5.43. There was no overall deviation from the 1:1:1:1 ratio expected for each of the four possible cell classes. None of the groups departed significantly from the numbers expected for each class. However, when the euploid and aneuploid cells were grouped together there was a significant deviation from the 1:1 segregation ratio (χ^2_3 = 11.23; 0.01 < P < 0.02). This has resulted from the very significant excess of +t_2t,x cells (χ^2_1 = 7.12; P < 0.01).

The distribution of sex chromosomes in the secondary spermatocytes is also seen in Table 5.43. A significantly greater number of the cells (aneuploid and euploid) had an x-chromosome (χ^2_1 = 4.72; P < 0.05). There was no significant difference between the number of euploid cells with x and y chromosomes.

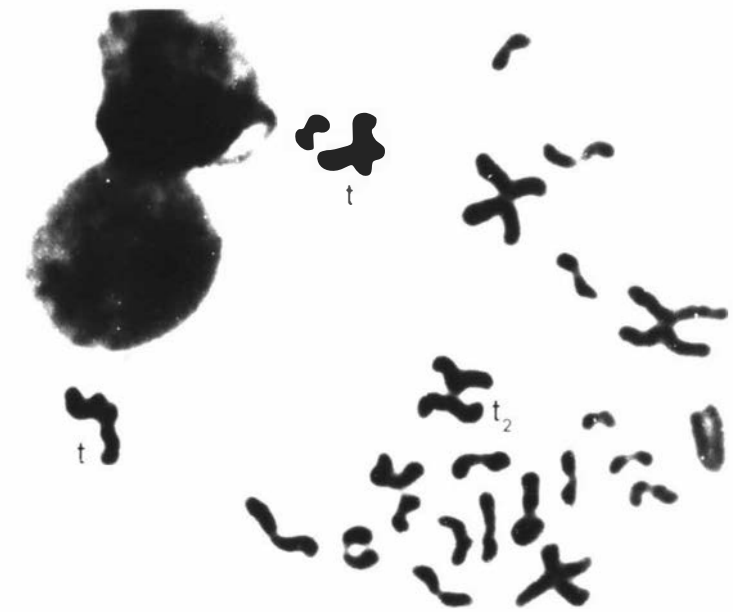
The number of euploid secondary spermatocytes with respective chromosome number is given in Table 5.44. There was a significant deviation from the 1:1 ratio, (χ^2_1 = 4.99; 0.01 < P < 0.02). It was seen that 59.2 percent (50 percent

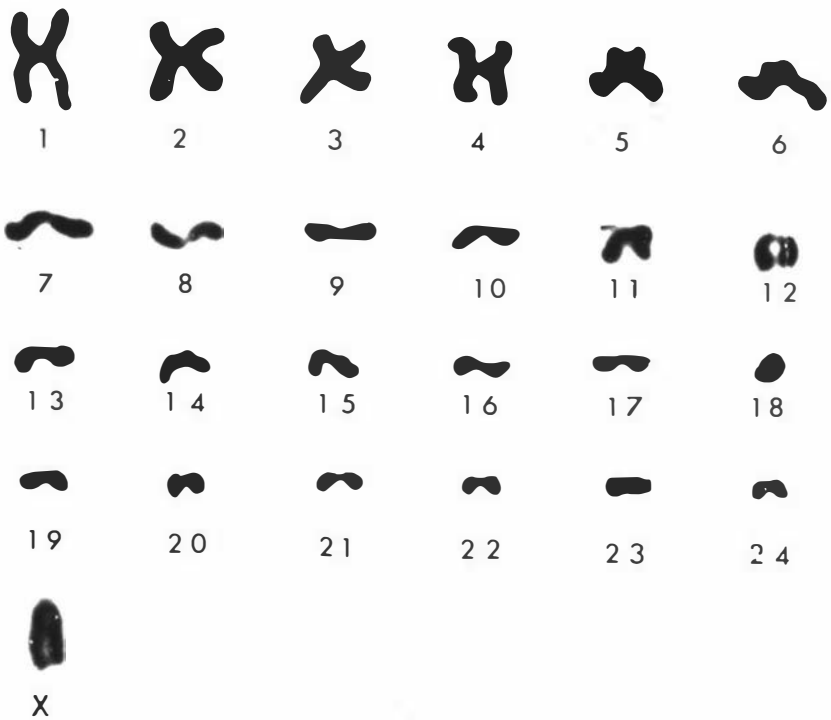
TABLE 5.43: ASSOCIATION OF TRANSLOCATION CHROMOSOMES WITH SEX CHROMOSOMES AT MII (EUPLOID CELLS) IN MULTIPLE TRANSLOCATION RAMS (49,xy,$t_1t_2t_2t_3t_x$)

* Aneuploid cells are recorded in brackets

Ram Identification	+ $t_1t_2t_3$,y	+ $t_1t_2t_3$,x	+ t_2t,y	+ t_2t,x
B196/76	4	2	5(1)	4
B38/79	9	11(2)	17	14
B92/79	16(1)	22(1)	19(1)	34(9)
Total	29(1)	35(3)	41(2)	52(9)

X_3^2 = not significant (1:1:1:1 ratio)


t
t
t2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23
24
X

PLATE 5.35: Metaphase II cell from B38/79 (49,xy,$t_1t_2t_2t_3t_x$). There are 31 chromosome arms and 25 chromosomes including two translocation submetacentrics (t), one translocation metacentric (t_2) and an X chromosome. (Giemsa x 3,000).

FIGURE 5.20: Karyotype of cell illustrated in Plate 5.35.

was expected) of the cells had a karyotype of 25,t_2t.

TABLE 5.44: NUMBER OF EUPLOID (NF/2 = 30) METAPHASE II FIGURES WITH RESPECTIVE CHROMOSOME NUMBER RECORDED FROM THE MULTIPLE TRANSLOCATION RAMS (49,XY,$t_1t_2t_2t_3t_x$)

Karyotype	24,$t_1t_2t_3$	25t_2t_x	Total
Expected numbers	78.5	78.5	157
Observed numbers	64	93	157

χ^2_1 = 4.99; 0.01 < P < 0.02 (1:1 ratio)

5.3.4 Discussion of results from secondary spermatocyte analysis

5.3.4.1 Introduction

The examination of metaphase II figures is an accurate method of determining the incidence of malsegregation of chromosomes at anaphase I.

The segregation analysis of different chromosomes by the examination of the karyotypes of progeny is also an important method in the study of meiotic disjunction. The analysis of the chromosomes from secondary spermatocytes has been limited due to the difficulty in obtaining cytologically unequivocal metaphase preparations for the accurate determination of chromosome number (Chapman and Bruère, 1975). This can be seen in the reports by Luciani (1970) and Skakkebaek *et al*. (1973) in man; Gustavsson (1969) in the bull; Datta (1970) in the goat and Makino (1943,a) in the sheep. The use of colchicine in living mice by Tettenborn and Gropp (1970); Döring *et al*. (1972) and Cattanach and Moseley (1973) enabled them to analyse a large number of metaphase II cells. This procedure has not been widely used in domestic animals for humane reasons.

The majority of the early meiotic studies ~~were~~ was reported on the chromosome morphology of the secondary spermatocytes (Makino 1943,a; Böök and Kjessler, 1964; Sasaki and Makino, 1965; Luciani, 1970; Pogosianz, 1970; McDermott, 1971; Popescu, 1971). However, the more recent studies have reported on the chromosome morphology and the number of chromosomes in the secondary spermatocytes (Chapman, 1974; Hultén, 1974; Logue, 1977; Logue and Harvey, 1978; Long, 1978; Bruère *et al.*, 1981; King *et al.*, 1981).

The first report of metaphase II analysis in the sheep by Makino (1943,a) found a haploid number of 27 chromosomes with the two types of secondary spermatocyte, one with an x and the other a y chromosome. The more recent studies by Chapman (1974); Logue (1977) and Long (1978) have analysed the metaphase II figures and counted the number of chromosomes and chromosome arms from both sheep with a normal karyotype and sheep which were either single heterozygous or homozygous for a translocation. The main conclusion drawn from their studies was that it appeared that there was an excess of secondary spermatocytes without a translocation to those cells carrying a translocation in heterozygous rams.

5.3.4.2 Non-disjunction and aneuploidy

The percentages of hypomodal, modal and hypermodal cells at MII in the rams examined in this study are given in Table 5.45. For comparison, the results from the earlier meiotic studies by Chapman (1974) are included in Table 5.45.

The normal and translocation-carrying rams in this study had euploid percentage distributions of over 85 percent. This agreed closely with the results from the normal and single homozygous rams studied by Chapman (1974). However, she found that the single heterozygous rams had lower

TABLE 5.45: PERCENTAGE OF HYPOMODAL, MODAL AND HYPERMODAL CELLS AT MII IN NORMAL AND ROBERTSONIAN TRANSLOCATION-CARRYING RAMS

Karyotype	Hypomodal cells	Modal cells	Hypermodal Cells
*53,xy,t_1	27.5	66.9	5.6
*53,xy,t_2	41.1	54.4	4.5
*53,xy,t_3	36.8	54.0	9.2
*52,xy,t_1t_1	14.1	85.9	0.0
*52,xy,t_3t_3	8.7	91.3	0.0
*[54,xy	16.1	83.9	0.0]
52,xy,t_1t_3	4.0	96.0	0.0
52,xy,t_2t_x	4.9	93.2	1.9
51,xy,$t_1t_1t_3$	6.1	93.9	0.0
51,xy,$t_2t_3t_3$	4.7	93.0	2.3
51,xy,$t_1t_2t_3$	10.1	87.3	2.6
50,xy,$t_1t_2t_2t_3$	3.5	93.5	3.0
50,xy,$t_1t_2t_3t_3$	3.6	92.8	3.6
50,xy,$t_1t_1t_2t_3$	6.6	91.0	2.4
50,xy,$t_1t_2t_3t_x$	2.9	95.3	1.8
49,xy,$t_1t_2t_2t_3t_x$	9.0	88.8	2.2
[54,xy	4.8	95.2	0.0]

* Data from Chapman (1974)

euploid percentage distributions of between 54.0 and 66.9 percent. Correspondingly the percentage distributions of hypomodal cells in the single heterozygous rams (27.5 to 41.1 percent) were considerably higher than the normal rams and the other translocation-carrying rams studied (2.9 to 16.1 percent). Likewise, the percentage distributions of hypermodal cells in the single heterozygous rams (4.5 to 9.2 percent) were higher than the normal rams (0.0 percent) and the other translocation rams (0.0 to 3.6 percent).

This difference between the single heterozygous rams and rams with other karyotypes suggested that the single heterozygotes had an inherently higher percentage of aneuploid cells. Although this could indeed be the case it was also possible that the difference could be technical in nature. This would seem very likely when the results of Logue (1977) and Long (1978) on the aneuploid frequency of single heterozygous rams were considered. Long (1978) found that 27 (16.6 percent) of the 163 cells seen in the three single (t_1) heterozygous rams were aneuploid. This percentage of aneuploid cells was in between that found in the translocation-carrying rams in this study and the percentage found in the single heterozygous rams studied by Chapman (1974).

In all rams studied the percentage of hypermodal cells was less than 10 percent. The normal rams in this study and in the study of Chapman (1974) had no hypermodal cells. This was also the case in the single homozygous rams studied by Chapman (1974). This was probably due to the balanced nature of the homozygous translocation bivalent. It was likely that the translocation bivalent, like any other bivalent, would undergo regular segregation during meiosis I. The translocation trivalent was more likely to undergo either partial or total non-disjunction since it is comprised of a translocation chromosome and the 2 homologous acrocentric chromosomes.

The aneuploid frequencies for the rams studied in this work are summarized in Table 5.46. For comparison, the aneuploid frequencies of the normal and translocation-carrying rams studied by Chapman (1974) are given in Table 5.47.

A large variation in the estimates of aneuploid frequency was found using the three different methods. Because of technical factors there are always more hypomodal cells than hypermodal cells. Therefore the two estimates which used the number of cells with 29 chromosome arms was an over-estimate of the number of aneuploid secondary spermatocytes. The estimate calculated by doubling the frequency of cells with a chromosome arm count of 31 was probably an under-estimate of the number of aneuploid secondary spermatocytes. However, the latter method probably gave a more accurate value for the aneuploid frequency than the other two methods. For this reason this method will be used in the discussing and comparing of the aneuploid frequencies of the normal and translocation-carrying rams.

The aneuploid frequency of the normal rams in this study (0.0 percent) was, as expected, the same frequency found by Chapman (1974) in her studies on normal and homozygous rams.

The mean level of aneuploid frequency in the double (3.5 percent) and triple heterozygous (5.3 percent) rams was significantly higher than the zero level found in the normal and homozygous (t_1t_1 and t_3t_3) rams. Therefore, as with the single heterozygotes (Chapman and Bruère, 1975), double and triple heterozygosity for the Robertsonian translocations is a factor leading also to the increased frequency of aneuploid secondary spermatocytes. However, the level found in the double and triple heterozygous rams was comparable to the levels reported for single heterozygous rams (Chapman and Bruère, 1975; Logue, 1977; Long, 1978) and was unexpectedly not even approximately equal to the sum of the individual levels in the single heterozygous animals,

TABLE 5.46: ANEUPLOID FREQUENCIES FOR NORMAL AND TRANSLOCATION-CARRYING RAMS FROM THOSE ANIMALS FOR WHICH AT LEAST TWENTY MII CELLS WERE ANALYSED

Karyotype	Ram	29 + 31%	31 x 2%	$\frac{\Sigma \text{ 29,31 classes}}{\Sigma \text{ 29,30,31 classes}} \times 100\%$
52,xy,t_1t_3	B147/75	4.0	0.0	4.0
52,xy,t_2t_3	B166/78	7.1	5.7	7.2
52,xy,t_2t_x	B6/79	3.8	3.4	3.9
51,xy,$t_1t_2t_3$	B86/76	4.2	8.3	4.2
	B111/77	16.6	8.6	17.4
	B47/78	7.9	4.0	8.0
	B101/78	5.1	1.7	5.1
51,xy,$t_1t_1t_3$	B120/77	3.0	0.0	3.1
51,xy,$t_2t_3t_3$	B117/78	7.0	4.6	7.0
50,xy,$t_1t_2t_2t_3$	B137/79	0.0	0.0	0.0
	B147/79	7.9	12.7	8.2
	B42/79	3.4	3.4	3.5
50,xy,$t_1t_1t_2t_3$	B128/77	4.0	4.0	4.0
	B141/77	0.0	0.0	0.0
50,xy,$t_1t_2t_3t_3$	B140/77	7.1	7.1	7.1
50,xy,$t_1t_2t_3t_x$	B121/78	6.7	6.7	7.1
	B2/79	8.0	8.0	8.7
	B32/79	4.5	3.0	4.5
49,xy,$t_1t_2t_2t_3t_x$	B38/79	1.8	3.7	1.9
	B92/79	7.5	5.6	7.9
54,xy	Grouped	1.2	0.0*	1.2*

*Calculation based on pooled data

TABLE 5.47: ANEUPLOID FREQUENCIES FOR t_1, t_2 AND t_3 RAMS (HETEROZYGOTES) AND NORMAL AND HOMOZYGOUS RAMS FROM THOSE ANIMALS FOR WHICH AT LEAST TWENTY MII CELLS WERE ANALYSED(1)

Karyotype	Ram	29 + 31%	31 x 2%	$\frac{\Sigma 29, 31 \text{ classes}}{\Sigma 29, 30, 31 \text{ classes}} \times 100\%$
53,xy,t_1	B53/69	29.3	6.9	28.2*
	A1/82	23.7	13.6	
	B8/72	19.0	0.0	
53,xy,t_2	B25/72	24.6	13.1	35.5*
	B87/72	31.8	10.6	
	B68/72	33.8	9.2	
	B66/72	25.9	0.0	
53,xy,t_3	B13/72	29.6	16.5	38.7*
	B76/72	47.5	22.2	
52,xy,t_1t_1	B55/70	6.1	0.0	6.6
52,xy,t_3t_3	A134	8.7	0.0	8.7
54,xy	Grouped	4.8*	0.0*	5.4*

* calculation based on pooled data

(1) Chapman (1974)

which were 6.9 - 13.6 per cent (t_1), 9.2 - 13.1 percent (t_2) and 16.5 - 20.2 percent (t_3). In this respect the multiple translocation-carrying sheep appear to differ from the tobacco mouse (M. *poschiavinus*) hybrids (Cattanach and Moseley, 1973) but are similar to the artificially evolved triple translocation-carrying mouse heterozygotes described by White *et al.* (1978). The individual variation in aneuploid spermatocyte frequency and the lack of evidence for an increase in aneuploid spermatocyte frequency in double and triple heterozygous rams further suggests that the rate of non-disjunction is genetically determined and is not a function which can be attributed entirely to the presence of translocations *per se* (Bruère *et al.*, 1981).

The aneuploid frequencies of the multiple translocation-carrying rams (4 and 5 translocations) were comparable to the levels found in the double and triple heterozygotes. The aneuploid spermatocyte frequency was less than 9 percent in all the rams examined, except for ram B147/79 (12.7 percent). In 15 of the 21 rams, in which more than 20 cells were analysed, the frequency was less than 6 percent. The overall frequency for the translocation-carrying rams examined was 4.5 percent. This level of aneuploid frequency is comparable to the previous reports in translocation-carrying rams (Chapman and Bruère, 1975; Logue, 1977; Long, 1978) and to the reports in bulls heterozygous for the 1/29 translocation (Logue, 1977; Logue and Harvey, 1978).

There was a significant excess of hypomodal to hypermodal cells ($\chi^2 = 9.30$; $P < 0.01$). In particular the triple heterozygous ram, B111/77, had 20 hypomodal and 7 hypermodal cells. The difference between this ram and the other rams could be due to technical variations in the meiotic technique.

The number of cells with hypomodal and hypermodal chromosome arm counts in the rams in this study is compared in Table 5.48 to the results from the single heterozygous rams (Chapman, 1974) and the single heterozygous mice (Cattanach

TABLE 5.48: NUMBER OF CELLS WITH HYPER-AND HYPOMODAL CHROMOSOME ARM COUNTS

Author		Number of Chromosome Arms 19	21	χ^2_1
Cattanach & Moseley (1973)	T_1	63	37	6.25; P < 0.05
	T_2	66	29	13.64; P < 0.001
	T_3	58	27	10.59; P < 0.01
	T_4	81	59	3.15; P > 0.05
	T_5	56	16	21.13; P <0.001
	T_6	49	12	21.25; P < 0.001
	T_7	48	24	7.35; P < 0.01
		Number of Chromosome Arms 29	31	
Chapman (1974)	t_1	33	9	12.60; P < 0.001
	t_2	84	15	46.71; P < 0.001
	t_3	83	25	26.27; P < 0.001
Author	$t_x t$	10	7	
	$t_x t_x t$	3	1	
	$t_1 t_2 t_3$	32	11	9.30; P < 0.01
	$t_1 t_2 t_3 t_x$	8	7	
	$t_1 t_2 t_2 t_3$	2	5	
	$t_1 t_2 t_2 t_3 t_x$	6	4	
	All rams	61	35	6.51; P < 0.05
	" $-t_1 t_2 t_3$	29	24	0.30; P > 0.05

and Moseley, 1973). It is evident that a reduction in the proportions of hypomodal and hypermodal cells in the meiotic preparations in this study has resulted from the rejection of cells with ambiguous chromosomes. As a consequence the aneuploid frequencies of the rams in this study are closer to the true levels of non-disjunction than the estimates given in earlier studies.

5.3.4.3 Cause of aneuploidy in Robertsonian translocation heterozygotes

The formation of aneuploid secondary spermatocytes in structured heterozygotes is the result of the trivalent associations undergoing irregular segregation and resulting in the metacentric chromosome and one acrocentric going to one pole and the remaining acrocentric to the other. Gametes may then be produced with one extra or one missing chromosome arm and give rise to trisomic and monosomic zygotes respectively (Tettenborn and Gropp, 1970). In the present study it was observed that a number of aneuploid secondary spermatocytes were produced by the heterozygous-carrying rams. However, the level of aneuploid spermatocyte frequency in the double and triple heterozygous rams was less than the levels reported by Chapman (1974) in single heterozygous rams. Therefore the presence of the translocations is only one factor leading to the increased frequency of aneuploid secondary spermatocytes.

5.3.4.4 Fate of aneuploid cells and effects of heterozygosity

Although the fate of the aneuploid secondary spermatocytes is still unclear the evidence from different species suggests there are several possibilities.

(1) They may degenerate during spermatogenesis (Roosen-Runge, 1973; Bunch and Foote, 1977), or

(2) They may mature into aneuploid sperm which are subsequently selected against in the uterus (Gabriel,

1977; Fechheimer, pers. comm. 1979).

(3) They may develop into mature sperm capable of fertilizing an ovum (Döring *et al.*, 1972; Ford, 1972; 1975). Ford (1975) concluded from his studies in mice, that the gross genome unbalance did not prejudice the capacity of a spermatid to mature into a functional sperm, with the same chance of effecting fertilization as a normal sperm.

(4) The aneuploid sperm fertilizes the ovum, but the aneuploid zygote dies before implantation (Evans *et al.*, 1967; Gropp *et al.*, 1970; Cattanach and Moseley, 1973; King *et al.*, 1981).

(5) Post-implantation death, recognizable as abortions may occur. The abortuses could be monosomic or trisomic embryos or foetuses (Waxman, Arakaki and Smith, 1967; Tettenborn and Gropp, 1970; Cattanach and Moseley, 1973; Popescu, 1980).

(6) A number of the aneuploid zygotes may survive to term and produce individuals which are monosomic (Say, Tuncbilek, Yamak and Balei, 1970) or trisomic for a particular chromosome (Griffen and Bunker, 1964; White, Tjio, van de Water and Crandall, 1972; White, Tjio, van de Water and Crandall, 1974; Tschudi *et al.*, 1977).

The effects of the heterozygous translocation on the individual male animal are observed in various ways in different species. The effect in mice is to reduce the fertility (Evans *et al.*, 1967; White and Tjio, 1967; Tettenborn and Gropp, 1970; Cattanach and Moseley, 1973; Baranov and Dyban, 1975). In man, the D/D translocation may have little effect on the fertility of the individual (Chandley *et al.*, 1975; Nielsen and Rasmussen, 1976) or it may have a great effect (Palmer, Morris, Thompson and Nance, 1973; van Koskull and Aula, 1974; Gahmberg *et al.*, 1980). The number of progeny of male D/G translocation carriers which are chromosomally unbalanced is considerably

less than would be expected (Ferguson-Smith, 1972). This could be because there is a selection against aneuploid spermatozoa at fertilization (Hamerton, 1968) or there could be a failure of implantation or early abortion of unbalanced zygotes.

In Swedish cattle, although no phenotypic abnormalities were observed it was claimed that daughters of bulls heterozygous for the 1/29 translocation showed an increased return to service (Gustavsson, 1969, 1971). This claim was based on statistical evidence only and the extrapolation was made that this relative reproductive inefficiency was probably due to an abnormal karyotype in some of the daughters. Gustavsson (1969) further claimed from the deviating non-return rates within 273 days of pregnancy and the difference observed in heifers between the non-return rates within 56 and 273 days, that the reduced fertility was due to an increased rate of embryonic death. Close examination of Gustavsson's data by Bruère (1974) indicated that of 11 bulls whose daughters were investigated the progeny of one showed a better than expected conception rate, while the data from seven was not significant and that from the translocation homozygote barely significant. Additionally, Gustavsson (1969) commented that it was evident the conception rate at first service and the percent non-returns to service at 56 days deviated significantly from the mean values in both positive and negative directions when single sires and daughter groups were considered. He also conceded that there was nothing to indicate a lowered fertility in the sires heterozygous for the 1/29 translocations when considering the conception rate at first service. Gustavsson (1969) neither produced cytological evidence of reduced fertility in these bulls nor of embryonic death in the translocation heifers. He did not take into account sire relationships nor any of the inherited factors which could have affected the results.

The more recent meiotic studies by Dyrendahl and Gustavsson (1979); Popescu (1980) and King *et al*. (1981) have been claimed to support the view that 1/29 translocation heterozygous cattle have a reduced fertility which was similar to the level found in the 1/29 heterozygous females. However, only 4 chromosomally abnormal embryos were karyotyped and the exact origin of the aneuploidy was not determined (Popescu, 1980; King *et al*., 1981). Consequently there is no conclusive evidence for the claim that the fertility in translocation-bearing cattle is reduced.

To date there have been no reports of lambs with unbalanced karyotypes associated with Robertsonian translocations (Bruère and Ellis, 1979). Similarly there have been no reports of monosomic or trisomic cattle (Gustavsson, 1969, 1971; Refsdal, 1976) or goats (Soller *et al*., 1966; Popescu, 1972; Riccordeau, 1972) associated with Robertsonian translocations.

Extensive breeding experiments have shown that the fertility of single, double and triple heterozygous translocation sheep is at least as good as that recorded for the New Zealand data on Romney flocks (Bruère and Ellis, 1979). This suggests that significant numbers of unbalanced zygotes were not found. This view is supported by the fact that the majority of the ewes conceived to their first service suggesting that only chromosomally balanced spermatozoa were involved in fertilization. The absence of increased numbers of phenotypic anomalies and the normal conception rates suggest that it is highly unlikely that embryos trisomic for any of the 3 translocations have either been formed or developed (Bruère and Ellis, 1979). This was confirmed in one study on t_1 blastocysts by Long (1977) which showed no evidence of trisomy in 102 13-18 day blastocytes and an equal segregation of t_1 and non-translocation homologues. More recently studies by Long and Williams (1980) in early embryos of sheep with normal karyotypes found that 4 out of 89 (4.7 percent) embryos collected

and analysed, 2 or 3 days postcoitum, were trisomic. The extra chromosome in each case was an acrocentric. It was tentatively identified as an autosome but definite identification was not possible. They concluded that trisomic individuals may arise as a result of non-disjunction at the first or second ~~meiotic~~ meiotic division in the formation of an ovum or spermatozoa. It would therefore seem that there is little or no evidence to suggest that, in translocation-carrying sheep, aneuploid gametes are either formed or take place in fertilization and their elimination during spermatogenesis is highly probable (Bruère, 1974; 1975; Bruère and Chapman, 1974; Long, 1978). It has previously been suggested by Roosen-Runge (1973) that cell loss in spermatogenesis is species-specific and that in some species chromosomally abnormal cells are removed before maturation is complete. A similar conclusion has been reached by Bunch and Foote (1977) to explain the absence of aneuploid progeny from kara-tau (Argali) *Ovis ammon nigromontana* (2n = 56) x mouflon *Ovis musimon* (2n = 54) hybrids.

5.3.4.5 The segregation at meiosis of the translocation chromosome

The translocation chromosome in the heterozygous state should, in theory, segregate evenly during meiosis resulting in the production of equal numbers of sperm with and without the translocation. However, Chapman (1974) and Long (1978) found in the single heterozygous rams a significant excess of secondary spermatocytes without the translocation chromosome. This finding was also reported by Logue and Harvey (1978) in their studies on bulls heterozygous for the 1/29 Robertsonian translocation. They found a highly significant difference between the number of presumptively intact cells that included the translocation and the number which lacked one. Logue and Harvey (1978) assumed this discrepancy could be due to misidentification of the separated chromatids. In other words the translocation meta-or submetacentric chromosome was, on occasion, mistakenly counted as 2 acro-

centric chromosomes. The findings in this study differ from the previous reports in translocation-carrying rams (Chapman, 1974) and bulls (Logue and Harvey, 1978).

The results from this study suggest that the number of translocation chromosomes that are mistaken for 2 acrocentric chromosomes is dependent on a number of factors. These include the quality of the meiotic preparations and the number and chromosomal morphology of the heterozygous translocation chromosomes present in the karyotype of the ram. The results from the rams heterozygous for the metacentric translocation (t_2) show that of the 1029 secondary spermatocytes analysed 520 cells were found to have a translocation chromosome. This figure (50.5 percent) was slightly, but insignificantly greater than the number of cells one would have expected to carry the translocation (X^2_1 not significant). In the rams heterozygous for the submetacentric translocation (either t_1 or t_3) 176 of the 368 secondary spermatocytes analysed had a translocation chromosome. This figure (47.8 percent) was slightly, but insignificantly, less than the expected number of cells with the translocation (χ^2_1 not significant).

It was seen that rams heterozygous for the two submetacentric translocations (t_1 and t_3) had a significant deficit of secondary spermatocytes with the karyotype of $25,t_1t_3$. For example, 21 cells with a karyotype of $25,t_1\,t_3$ were counted in the triple heterozygous rams ($51,xy,t_1t_2t_3$). This was significantly less than the expected number of cells (45.375) with this karyotype (χ^2_1 = 12.56; $P < 0.001$). In contrast there was a significant excess of cells with no translocations (χ^2_1 = 4.36; $P < 0.05$).

It would appear that the submetacentric translocation is, on occasion, being misidentified as two acrocentric chromosomes. This might explain the significant deficit in the number of $25,t_1\,t_3$ secondary spermatocytes as there is a higher possibility of a mistaken identification with the

presence of two submetacentric chromosomes. However, it is possible, for reasons unknown, that there could be a selective disadvantage against the cells with two submetacentric translocations. From the matings of triple heterozygous rams and ewes of normal karyotype this would be a possibility since there was also a significant deficit of progeny with a 52,t_1t_3 karyotype. However, it would be very premature to confirm or speculate on the selective disadvantage of the 25,t_1 t_3 sperm and the advantage of the 27,sperm from the limited mating figures obtained to date.

It is worth noting that it would seem the metacentric translocation (t_2) and the submetacentric translocations (t_1 and t_3) are seldom mistaken for two acrocentric chromosomes. This is in strong contrast to the results found in the single heterozygous rams (Chapman, 1974) and heterozygous 1/29 bulls (Logue and Harvey, 1978). A number of the translocations must have been analysed and counted as 2 acrocentrics since it would only require a slight displacement of the two chromatid arms for a wrong identification to be made. In the present study only cells of a high quality were analysed and a cell with the slightest ambiguity was discarded. For this reason it is believed that the number of wrongly identified translocations was probably greatly reduced.

5.3.4.6 Univalent sex chromosomes

Separated sex chromosomes in diakinesis and metaphase I cells have been observed in the preparations from both normal and Robertsonian translocation-carrying rams (Chapman 1974; Author). However, there was no evidence of sex chromosome aneuploidy in the secondary spermatocytes although this would have been expected because of the presence of the two univalents at first anaphase. Similar findings have been reported in mice (Ohno, Kaplan and Kinosita, 1959; Ford and Evans, 1964; Lin *et al.*, 1971). The

proportion of cells at diakinesis in which the x and y have separated may vary depending on the preparation technique, age of the animals and the degree of condensation of the chromosomes (Winsor *et al.*, 1978). The technique may have been a factor in the formation of some of the separated sex chromosomes. The fate of these cells is uncertain. However, it has been suggested that the cells do not proceed beyond metaphase I (Beechey, 1973; Chapman, 1974) and this would indeed seem to be the case from the results in this thesis.

5.3.4.7 Cell distributions at meiosis in translocation carrying rams

Cell distributions in each of the categories, spermatogonial metaphase, diakinesis and metaphase I and metaphase II are shown in Table 5.49.

The number of cells counted in each category varied between rams. Few spermatogonial cells were seen. The average ratio of metaphase II to metaphase I cells was 0.6 with a range of zero (no metaphase II cells observed) to 2.7. The ratio in the majority of rams examined was less than the theoretically expected ratio of 2:1. This discrepancy was probably partly caused by the technique used for meiotic preparations.

Chapman (1974) found an average MII/MI ratio of 0.5 in the single heterozygous rams. This was comparable to the ratio of 0.3 found in the triple heterozygous rams. However, in the double heterozygous rams a considerably higher ratio of 1.4 was found. Ratios of 0.9 and 1.0 were found in the rams with 4 and 5 translocations respectively. The overall ratio, excluding ram B111/77 ($51,xy,t_1 t_2 t_3$) was 0.8. The MII/MI ratio increased as refinements were made to the meiotic technique.

TABLE 5.49: DISTRIBUTION OF CELLS IN SPERMATOGONIAL METAPHASE (SM), DIAKINESIS AND METAPHASE (MI) AND METAPHASE II (MII) IN ROBERTSONIAN TRANSLOCATION-CARRYING RAMS * Polyploid cells included in counts are recorded in brackets

Ram Identification	Ram Karyotype	SM	MI	MII	Total	MII/MI Ratio
B147/75	52,xy,t_1t_3	2(0)	10(1)	27(2)	39(3)	2.7
B63/77	52,xy,t_1t_2	4(0)	11(3)	11(1)	26(4)	1.0
B166/78	52,xy,t_2t_3	6(1)	42(3)	74(4)	122(8)	1.8
B6/79	52,xy,t_2t_x	8(0)	236(23)	305(15)	549(38)	1.3
B120/77	51,xy,$t_1t_1t_3$	3(0)	74(9)	37(4)	114(13)	0.5
B154/77	51,xy,$t_1t_3t_3$	0(0)	66(8)	0(0)	66(8)	0.0
B117/78	51,xy,$t_2t_3t_3$	3(1)	18(5)	49(6)	70(12)	2.7
B7/76	51,xy,$t_1t_2t_3$	0(0)	356(95)	13(5)	369(100)	0.1
B86/76	51,xy,$t_1t_2t_3$	6(0)	64(8)	24(0)	94(8)	0.4
B111/77	51,xy,$t_1t_2t_3$	8(0)	834(110)	217(54)	1059(164)	0.3
B44/78	51,xy,$t_1t_2t_3$	0(0)	2(1)	2(0)	4(1)	1.0
B47/78	51,xy,$t_1t_2t_3$	9(0)	193(2)	110(10)	313(37)	0.6
B101/78	51,xy,$t_1t_2t_3$	5(0)	136(10)	126(8)	267(18)	0.9
B137/78	50,xy,$t_1t_2t_2t_3$	4(1)	21(2)	26(2)	51(5)	1.2
B42/79	50,xy,$t_1t_2t_2t_3$	2(0)	93(5)	62(4)	157(9)	0.7
B49/79	50,xy,$t_1t_2t_2t_3$	13(1)	28(4)	11(2)	52(7)	0.4
B118/79	50,xy,$t_1t_2t_2t_3$	4(2)	20(7)	19(3)	43(12)	1.0
B147/79	50,xy,$t_1t_2t_2t_3$	12(0)	56(12)	81(18)	149(30)	1.4
B140/77	50,xy,$t_1t_2t_3t_3$	3(0)	36(2)	30(2)	69(4)	0.8
B128/77	50,xy,$t_1t_1t_2t_3$	23(2)	222(28)	167(17)	412(47)	0.8
B141/77	50,xy,$t_1t_1t_2t_3$	2(0)	21(4)	23(3)	46(7)	1.1
B121/78	50,xy,$t_1t_2t_3t_x$	3(0)	34(5)	34(4)	71(9)	1.0
B2/79	50,xy,$t_1t_2t_3t_x$	0(0)	18(4)	30(5)	48(9)	1.7
B32/79	50,xy,$t_1t_2t_3t_x$	2(0)	80(7)	75(8)	157(15)	0.9
B196/76	49,xy,$t_1t_2t_2t_3t_3$	3(0)	53(1)	19(1)	75(2)	0.4
B38/79	49,xy,$t_1t_2t_2t_3t_x$	1(0)	80(9)	62(8)	143(17)	0.8
B92/79	49,xy,$t_1t_2t_2t_3t_x$	0(0)	60(11)	121(14)	181(25)	2.0

CHAPTER 6

CONCLUSIONS

The first aim of this study was to compare and contrast the primary spermatocytes from normal and translocation - carrying rams. The number of sex bivalents associated with small autosomal bivalents, the percentage of cells with non-association of the sex bivalents and the percentage of hypermodal cells was similar in both the normal and translocation-carrying rams. Furthermore, the results from the rams with 2, 3, 4 or 5 translocations were remarkably similar. The translocations seem to have little or no effect on the stability of the chromosomes in the primary spermatocytes. It should be re-emphasized that the number of chiasmata in the diakinesis-metaphase I cells in the multiple translocation-carrying rams was similar to that found in normal rams (Chapman, 1974; Logue, 1977; Long, 1978) and single heterozygous and homozygous rams (Chapman, 1974; Logue, 1979). This is in agreement with the findings of Logue and Harvey (1978) in their studies in normal and heterozygous 1/29 bulls.

The meiotic studies carried out on heterozygous translocation rams have clearly shown that the translocation trivalent can undergo partial non-disjunction at anaphase I (Chapman and Bruère, 1975; Logue, 1977; Long, 1978; Bruère *et al*., 1981; Author). This contrasts to the zero level of non-disjunction found in normal and balanced translocation-carrying rams (Chapman and Bruère, 1975; Bruère *et al*., 1981; Author). The fate of the aneuploid spermatocyte cells, produced by the heterozygous translocation rams, is still uncertain. However, it has been suggested that the aneuploid cells fail to mature into sperm and are selected against or degenerate during spermatogenesis. This suggestion was previously proposed by Roosen-Runge (1973) and supported by Bunch and Foote (1977). Further evidence for this suggestion comes from the results of

an extensive breeding programme carried out at Massey University that has shown the translocation-carrying sheep to have good conception rates and an average breeding performance (Bruère and Ellis, 1979). It would appear that the presence of translocation chromosomes has little or no effect on the fertility of the sheep. This finding differs from the reports in the F_1 of the tobacco mouse x domestic mouse (Gropp *et al.*, 1970) and the Apennine mouse x laboratory mouse (Capanna, Gropp, Winking, Noack and Civitelli, 1976). This finding in sheep also differs markedly from the reports of reduced fertility found in cattle with a 1/29 translocation (Gustavsson, 1969; Dyrendahl and Gustavsson, 1979; Popescu, 1980; King *et al.*, 1981). The development of an animal population with a number of different centric fusions may produce genetic differences which reduce fertility when such animals are mated back to others of the original or normal karyotype, as demonstrated by Gropp *et al.* (1970) and Capanna *et al.* (1976) in the two mouse crosses. In the early studies by Gustavsson (1969) only limited statistical evidence was produced suggesting that the daughters of translocation-bearing bulls of the Swedish Red breed show an increased return to service due to an increased level of zygotic aneuploidy. More recently Popescu (1980) and King *et al.* (1981) have collected preimplantation embryos from normal cows sired by bulls heterozygous for the 1/29 translocation. However, only 2 trisomic and 2 monosomic embryos were karyotyped and the exact origin of the trisomy or monosomy was not determined by Popescu (1980) and King *et al.* (1981). Therefore the evidence for a reduction in fertility in translocation-bearing cattle is inconclusive and further studies would be required to verify their claim.

A number of studies have reported on the uneven proportions of segregation products at second metaphase in heterozygous rams (Chapman and Bruère, 1975; Long, 1978; Bruère *et al.*, 1981; Author). What could be the reason for

this? The most likely explanation, proposed by Logue and Harvey (1978), is that certain chromosomes are misidentified due to the separation of their chromatids. This suggestion could only partly explain the occurrence of unbalanced segregation ratios found in the present study since cells were discarded if there was any ambiguity in the identification of the chromosomes. Another possible explanation is that certain segregation products are preferentially selected against during spermatogenesis and are eliminated in the same manner and at the same time as the aneuploid cells. However, it would be premature to speculate on this possibility and further studies on the chromosome complements of mature sperm are required to ascertain whether certain cells are selected against or degenerate during spermatogenesis. The evidence from the mating experiments showed that an even distribution of segregation products was recorded in the progeny of all combinations of translocation ewes x translocation rams in those groups in which sufficient animals were available for statistical analysis (Bruère and Ellis, 1979). Although the distribution of segregation products in the progeny of normal ewes x triple heterozygous rams was irregular it was probably only a reflection of the small number of progeny analysed.

The successful breeding of sheep with 50, 49 and 48 chromosomes confirm the remarkable stability of the sheep chromosomes (Bruère and Ellis, 1979). This is reflected in the cytological similarities of the chromosomes of bovids which in general have uniform G-bands and C-bands (Evans *et al* ., 1973; Buckland and Evans, 1978) and in four species, sheep (*Ovis aries*), cattle (*Bos taurus*), goat (*Capra hircus*) and aoudad (*Ammotragus lervia*), the nucleolar organizer regions (NORS) are homologous (Henderson and Bruère, 1979). From the breeding programme (Bruère and Ellis, 1979) and the meiotic studies (Chapman, 1974; Bruère *et al*., 1981; Author) it now seems clear that the presence of at least 3 stable centric fusions (Robertsonian translocations) in domestic sheep, even in triple heterozygous

animals, is in no way detrimental to either the individual or the population. The mere persistence of such a polymorphic system both naturally for probably 100 years or more (Bruère *et al*., 1978) and experimentally gives weight to the argument that future investigations of such systems in domestic animals should be to determine their genetic meaning (Bruère and Ellis, 1979). The only other known member of the sub-family *Ovis* with a chromosome number less than 54 is the Siberian snow sheep O. *nivicola alleni* (Matschie). This species provides an example of a chromosome change having passed through the heterozygous state becoming fixed in the homozygous state. A similar process of chromosome change, by centric fusions, could be occurring in the domestic sheep. Therefore in no way should the translocation-carrying sheep be eliminated arbitrarily from our animal populations because of the claims of reduced fertility reported in other translocation-carrying animals.

APPENDICES

APPENDIX I: MATERIALS FOR BLOOD LEUCOCYTE CULTURE

(a) For each culture:

Sterilized 1 x 10cc syringe + 20g x 2" needle

10 ml Venoject tube with sodium heparin (Jintan Terumo Co. Ltd., Tokyo, Japan) + 20g x 1" needle universal jar and lid.

Adapter for venoject tube

Methylated spirits and swabs

Media TC 199 (Glaxo Laboratories (N.Z.) Ltd., Palmerston North, New Zealand)

20 cc syringe + 18 g x 1" needle

Phytohaemagglutinin (Wellcome Reagents Ltd., Wellcome Research Laboratories, Beckenham, Kent, England).

5 ml syringe + 18 g x 1" needle.

Centrifuge (Bench centrifuge Watson Victor Ltd., Australia and New Zealand).

Incubator set at 37°C.

(b) To harvest cultures:

For each culture - 1 graduated centrifuge tube

1 pasteur pipette + rubber bulbs

Colchicine C Aqua-Colchin, 1 ml contains 0.5 mg of colchicine) Park, Davis and Company, Sydney, Australia.

Fixative - 15 ml methanol + 5 ml glacial acetic acid (For each culture).

0.075 m KCl (Hungerford, 1965).

To make basic solution hypotonic KCl: Add 7.46g KCl to 100 ml glass distilled H_2O.

To make working solution KCl: Take 7.5 ml of basic solution and make up to 100 ml with glass distilled water.

Water bath at 37°C (Qualtex/contherm water bath, Manufacturing Laboratory Supplies Ltd.,, Lower Hutt, New Zealand).

Beaker for Supernatant

Cleaned slides (3/culture in beaker iced water)

Bunsen Burner.

Hot plate.

Centrifuge.

Refrigerator.

(c) To mount slides:

Materials: Cover glasses (Matsunami 22 x 60 No.1, Chance Propper Ltd., Smethwick, Warley, England).

D.P.X. (D.P.X. Mountant, BDH Chemicals Ltd., Poole, England).

Glass rod, xylol, absolute alcohol and swabs.

(1) Cover glass should be soaked in alcohol and dried with swab.

(2) Place drop of D.P.X. on cover slip.

(3) Remove slide to be mounted from xylol and invert over cover slip.

(4) Turn right side up and gently express any air bubbles.

(5) Leave to dry overnight.

APPENDIX II: MATERIALS USED IN ANAESTHESIA OF RAM AND PREPARATION OF SCROTAL AREA:

Acetyl promazine (2 mg/ml).
Boots Pure Drug Co. Ltd., Nottingham, England.

Xylocaine containing HCl 20 (mg/ml) and adrenaline 1:80,000. Astra Pharaceuticals (Australia) Pty Ltd.

Hibitane (chlorhexidine) 1% solution: 60 ml distilled H_2O + 15 ml 5% concentrate solution Hibitane

(Imperial Chemical Industries Ltd., Cheshire, England).

Hibitane tincture:

7 fluid oz. 5% concentrate solution

10.5 fluid oz. distilled H_2O

52.5 fluid oz. methylated spirits

APPENDIX III: MATERIALS FOR MEIOTIC PREPARATIONS

Tri-Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)

2.2% and 1.2% solutions made in 100 ml flask with glass distilled water.

Watch-glasses - (x 2).

Forceps.

Scalpel blades (x 2).

Graduated centrifuge tubes (x 4).

Glass rod.

Water bath at 37°C (See Appendix I).

Centrifuge (See Appendix I).

Alarm clock.

Pasteur pipettes, one for fixative, one for hypotonic solution.

Coplin jars (x 3)

Ice bath

Slides (3" x 1", .8 to 1 mm thickness). Up to 10 for each centrifuge tube of material processed.

Fixative, 5 ml glacial acetic acid + 15 ml absolute alcohol in universal jar (for each pair centrifuge tubes of material).

APPENDIX IV: ACETO-ORCEIN STAIN:

Materials

Synthetic Orcein (G.T. Gurr Ltd., London, England)	8g
Glacial acetic acid	240 ml
Glass distilled water	160 ml

Method

(1) Heat acetic acid to 100°C.

(2) Add orcein powder and stir well. Keep at 90°C for 1 hour.

(3) Cool and add glass distilled water.

(4) Filter before use.

APPENDIX V: GIEMSA STAIN:

Materials

Giemsa powder (Allied Chemical Corporation New York)	3.75 g
Glycerine	247.5 ml
Methyl alcohol	247.5 ml

Method

(1) Dissolve Giemsa powder in glycerine at 60°C for 2 hours.

(2) Add methyl alcohol.

(3) For use, a 2% solution was made with pH 6.8 buffered water (Buffer tablets pH 6.8, Geo. W. Wilton & Co. Ltd., Lower Hutt, New Zealand: 1 tablet to 100 ml distilled water.

APPENDIX VI: EXAMINATION OF HISTOLOGICAL AND CHROMOSOME PREPARATIONS

Wild Mka 5 microscope with "photoautomat" (Wild Heerbrugg Ltd., Switzerland).

APPENDIX VII: COUNTING CELLS:

Hand operated Tally counter (English Numbering Machines Ltd., England).

APPENDIX VIII: DEVELOPING FILM:

(1) The film is developed in D76 (Kodak) or 1 D 11 (Ilford) for 6 minutes at 20°C.

(2) The film is then fixed in Universal fixer for 2-5 minutes, and washed in running tap water for at least 30 minutes.

(3) Finally, the film is rinsed with a wetting agent and hung up to dry.

APPENDIX IX: ENLARGER:

Focomat 11c, Leitz, Germany.

APPENDIX X: DEVELOPING PRINTS:

(1) The prints were developed in 1 part Dektol diluted with 3 parts water at 20°C for 2 minutes.

(2) The prints were briefly immersed in a stop bath of a very weak acetic acid solution.

(3) The prints were then put in the hypo bath for 5 minutes, with occasional agitation.

(4) The final step is washing in the water bath for at least 5 minutes, and then drying.

APPENDIX XI: HISTOLOGY PHOTOGRAPHS - DEVELOPING FILM:

(1) Develop film in Microdol-X at 20°C for 9 minutes

(2) "Stop bath" 2 minutes.

(3) Fix, wash 20 minutes and glaze.

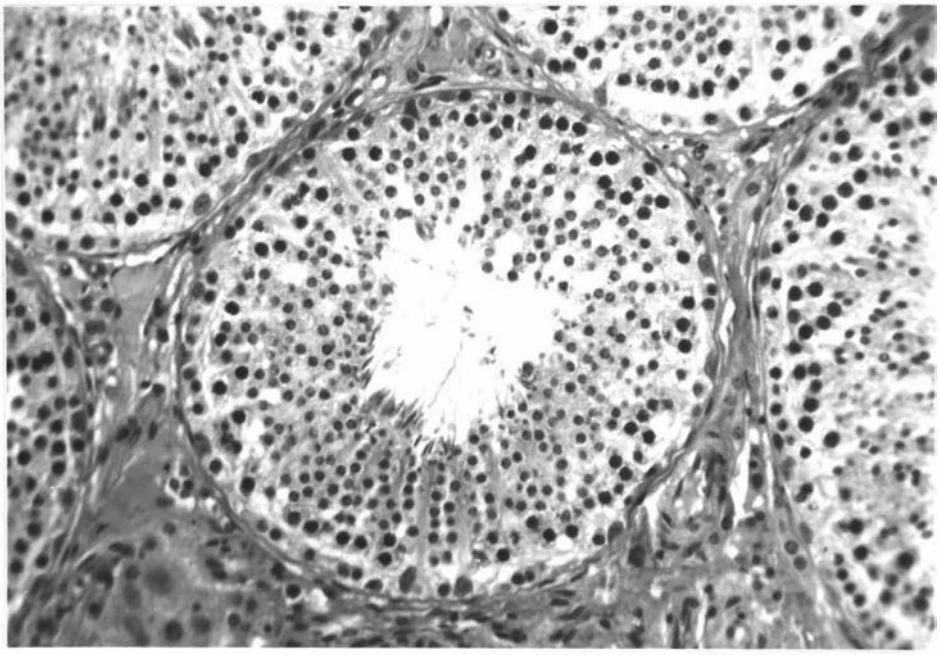
APPENDIX XII: WASHING OF LABORATORY GLASSWARE:

All glassware was soaked for 24 hours in Decon 75 concentrate (approximately 5 ml in 10 litres), rinsed in tapwater, washed in Pyroneg, rinsed, drained and dried.

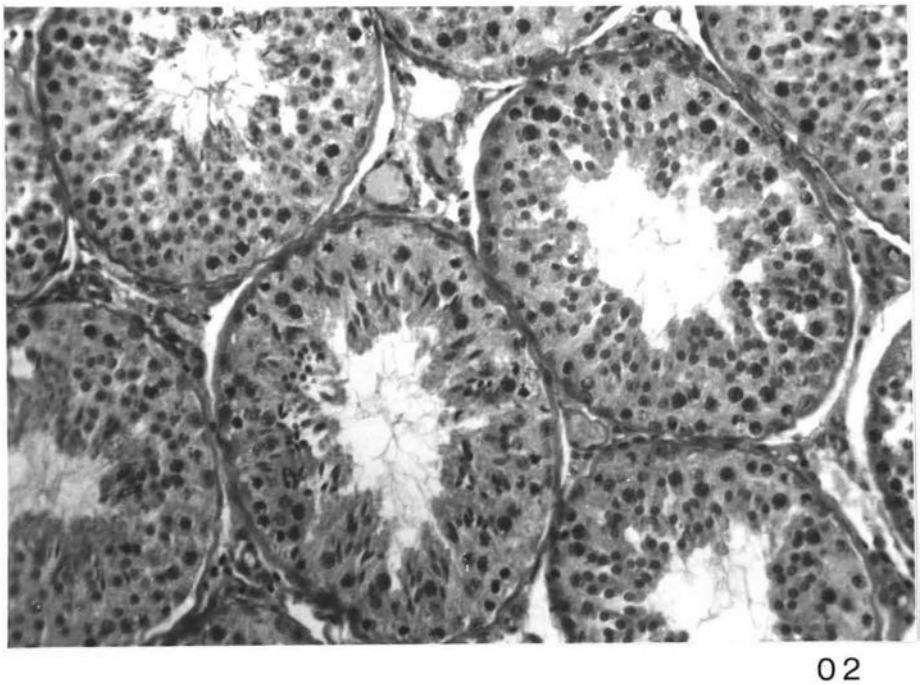
Universal jars and lids used in blood leucocyte cultures were rinsed in 1 N HCl then given 3 rinses with glass distilled water before being dried.

APPENDIX XIII: EXAMINATION OF HISTOLOGICAL SECTIONS FROM NORMAL AND ROBERTSONIAN TRANSLOCATION-CARRYING RAMS

Histological sections of the testes of all rams, both normal and translocation-carrying, were examined microscopically. No differences were detected between these samples. In all rams a normal spermatogenic process was observed. Photomicrographs of the testis section are shown in Plates 01 and 02.



01



02

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Aneuploid spermatocyte frequency in domestic sheep heterozygous for three Robertsonian translocations

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Summary. In an analysis of the chromosomes in 332 metaphase II figures from 3 triple heterozygous rams (51,xy,$t_1t_2t_3$) and a further 84 figures from 3 normal rams (54,xy), there were no hypermodal cells in the normal rams but 9 such cells were found in the triple heterozygotes, giving a mean aneuploid frequency of 5·42% which was similar to the levels previously found in the single heterozygotes. Forty hypomodal cells were found in the 6 rams of which a number would have lost chromosomes due to lagging at anaphase I. Individual variation in the aneuploid metaphase II frequency was observed in the triple heterozygotes.

A significant surplus of secondary spermatocytes of normal karyotype and a deficit of 25,t_1t_3 were found in the metaphase II figures from the triple heterozygotes. There was also a significant increase of normal progeny and a deficit of 52,t_1t_3 and 53,t_2 progeny from the matings of triple heterozygous rams and normal ewes. It is possible that the significantly uneven distribution of segregation products in the triple heterozygotes might have been the result of either cell selection or degeneration during spermatogenesis.

Introduction

Extensive studies have been made on the high frequency of centric fusions (Robertsonian translocations) in several species of animals (Bruère, 1974b). From one such study there is little or no evidence to suggest that the centric fusions have any significant effect on the reproductive fitness of translocation-carrying sheep (Bruère, 1974a; Bruère & Chapman, 1974; Bruère & Ellis, 1979). Although significant levels of hypermodal secondary spermatocytes have been found in single heterozygous rams (t_1, 5·63%; t_2, 4·5%; t_3, 9·2%, Chapman & Bruère, 1975; 6·1% in t_1 only, Long, 1978), it is believed that these cells do not mature into aneuploid gametes.

From the mating studies with the single translocation heterozygous sheep no unequal number of progeny of the various segregation categories was recorded consistently. However, a significant excess of non-translocation-carrying secondary spermatocytes was found in meiotic studies of the singly heterozygous rams (Chapman, 1974; Chapman & Bruère, 1975; Long, 1978). The studies on the tobacco mouse hybrid suggest that the effect of multiple heterozygosity would be to increase the aneuploid frequency significantly above that of the single heterozygotes. Therefore, if the same effect should be found in the ram an aneuploid frequency significantly above that of single heterozygotes would be expected in the triple heterozygotes. Variations in the levels of aneuploid secondary spermatocyte frequency have been reported in the tobacco mouse (Cattanach & Moseley, 1973) and in sheep (Chapman & Bruère, 1975) with different centric fusions. However, unlike sheep, the high levels of aneuploidy in the tobacco mouse hybrids are consistent with high frequencies of zygotic loss and reduction of fertility of

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the F_1 animals (Gropp, Tettenborn & Von Lehmann, 1970; Cattanach & Moseley, 1973). It has also been reported that the aneuploid secondary spermatocytes of one translocation-carrying strain of mice did not appear to form viable gametes (Baranov & Dyban, 1976).

Although the significance of centric fusions and their method of formation is not fully understood (John & Freeman, 1975; Kurnit, Brown & Maio, 1978) it would appear that their effect on the fertility is species-specific. The present report describes chromosomes of the F_1 animals from the breeding of triple heterozygous rams and normal ewes. It also examines the individual variations in the level of aneuploid metaphase II frequency in the spermatocytes of normal and triple heterozygous rams.

Materials and Methods

The three triple heterozygous rams used were derived from the mating experiments reported previously (Bruère & Ellis, 1979). The karyotypes of the rams were determined by a whole blood leucocyte technique (Bruère & McLaren, 1967) and testicular material was obtained from testes after surgical castration under local anaesthesia. The meiotic preparations were made using the technique developed for the ram by Chapman (1974). The meiotic preparations were stained with aceto-orcein for 2–3 h or with Giemsa for 10–15 min. Samples were also taken from the testes for histological examination. The sections were cut at 5 μm and stained with haematoxylin and eosin. As described previously (Chapman & Bruère, 1975) ambiguity can occur in counting metaphase II chromosomes because of their contracted nature. All chromosome arms were counted as well as the chromosome number. Acrocentric chromosomes were counted as having one chromosome arm, while metacentrics and submetacentrics were counted as having two chromosome arms. The X and the Y chromosomes were both counted as having one chromosome arm, although the latter chromosome is submetacentric.

The haploid chromosome complement for the sheep consists of 23 acrocentrics, 3 metacentrics and one sex chromosome, a total of 27 chromosomes or 30 chromosome arms.

The haploid number for the secondary spermatocyte of a normal ram was scored as 27X/30 or 27Y/30; euploid secondary spermatocytes were NF/2 = 30, and aneuploid secondary spermatocytes had an NF/2 of > or < 30.

Three estimates of aneuploid frequency were made, two were based on the method of Cattanach & Moseley (1973). The first estimate gave the total proportion of cells with 29 or 31 chromosome arms, and the second was calculated by doubling the frequency of cells with a chromosome arm count of 31. Because of technical factors there are always more hypomodal cells than hypermodal cells so that the former frequency is an overestimate of the number of aneuploid secondary spermatocytes. The latter is probably more correct.

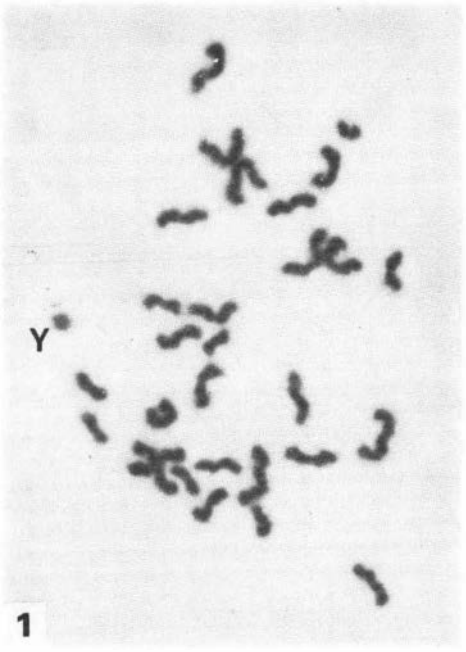
A third estimate of aneuploid frequency was made by using the following formula: $\sum$ 29,31 classes $\times$ 100/$\sum$29,30,31 classes.

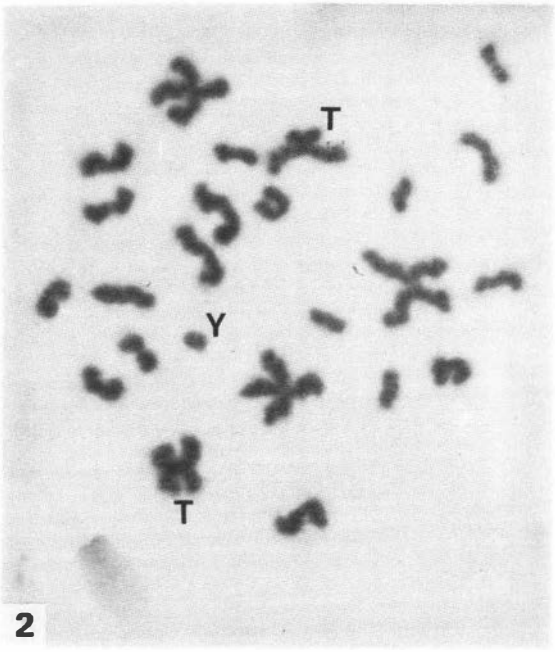
Between 1975 and 1979 our breeding programme with translocation-carrying sheep involved mating triple heterozygous rams to ewes with a normal karyotype, 2n = 54. The karyotypes of the progeny were determined from leucocyte blood cultures.

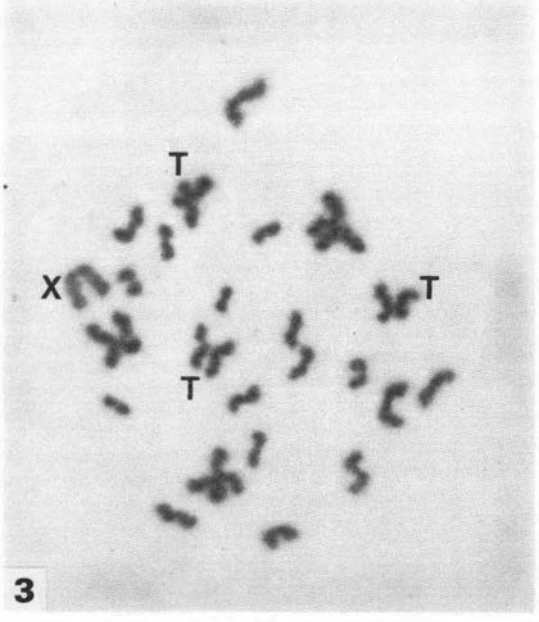
Results

An analysis was carried out on 84 secondary spermatocytes from 3 normal rams and 332 secondary spermatocytes from 3 triple heterozygous rams (Table 1; Pl. 1, Figs 1–4). For comparison with the spermatocytes from the triple heterozygotes the 84 spermatocytes from the normal rams were combined with 62 spermatocytes from normal rams previously reported (Chapman & Bruère, 1975) and 99 spermatocytes from t_1t_1 rams and 23 spermatocytes from

PLATE 1







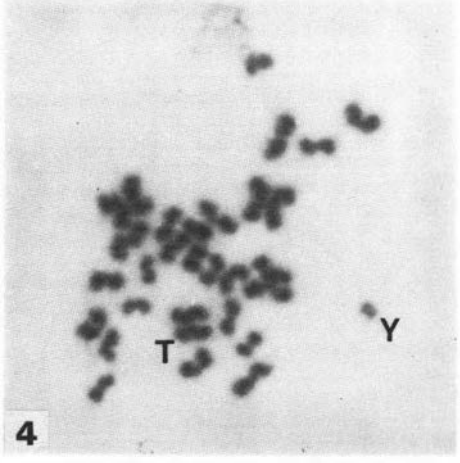


Fig. 1. Metaphase II cell with 30 chromosome arms and 27 chromosomes, including 3 metacentrics and a Y chromosome.

Fig. 2. Metaphase II cell with 30 chromosome arms and 25 chromosomes, including 2 translocation (T) chromosomes (t_2t_x) and a Y chromosome.

Fig. 3. Metaphase II cell with 30 chromosome arms and 24 chromosomes, including 3 translocation (T) chromosomes ($t_1t_2t_3$) and an X chromosome.

Fig. 4. Metaphase II aneuploid cell with 31 chromosome arms and 27 chromosomes, including a translocation (T) metacentric and a Y chromosome.

(*Facing p.* 62)

t_3t_3 rams. As described (Chapman & Bruère, 1975) no hypermodal cells were observed in 268 cells from the normal rams or the homozygous translocation-carrying rams. However, 9 aneuploid (hypermodal) cells were observed in the triple heterozygotes.

Table 1. Distribution of euploid (NF/2 = 30) and aneuploid (NF/2 < or >30) metaphase II figures in normal and triple heterozygous rams 51,xy,$t_1t_2t_3$

		NF/2						
Karyotype	Ram	<28	28	29	30	31	>31	Total
54,xy	B51/79	0	1	0	53	0	0	54
	A1/79	1	1	0	8	0	0	10
	A2/79	0	0	1	19	0	0	20
51,xy,$t_1t_2t_3$	B47/78	0	1	4	80	1	0	86
	B101/78	0	0	3	79	1	0	83
	B111/77	2	6	20	128	7	0	163

The aneuploid spermatocyte frequencies of the individual rams were analysed and are given in Table 2 and the combined data are presented in Table 3. The mean aneuploid frequency 31 × 2 (%) for the three 51,xy,$t_1t_2t_3$ rams was 5·42%. In the metaphase II cells of the 51,xy,$t_1t_2t_3$ rams the X chromosome was present in 162 cells and the Y chromosome in 163 cells, while 41 X bearing cells and 42 Y bearing cells were seen in the metaphase II figures of the normal rams. In neither case does this depart from the expected ratio.

Table 2. Aneuploid frequencies for the normal and triple heterozygous rams

Karyotype	Ram	29 + 31(%)	31 × 2(%)	$\frac{\Sigma 29,31 \text{ classes}}{\Sigma 29,30,31 \text{ classes}} \times 100\%$
54,xy	B51/79	0·00	0·00	0·00
	A1/79	0·00	0·00	0·00
	A2/79	5·00	0·00	5·00
51,xy,$t_1t_2t_3$	B47/78	5·81	2·33	5·88
	B101/78	4·82	2·41	4·82
	B111/77	16·56	8·59	17·42

Table 3. Aneuploid frequencies for the normal single translocation-carrying rams (53,xy,t_x), homozygous (52,xy,t_1t_1 and 52,xy,t_3t_3) rams and triple translocation-carrying rams (51,xy,$t_1t_2t_3$)

Karyotype	No. of rams	Total no. of cells	29 + 31(%)	31 × 2(%)	$\frac{\Sigma 29,31 \text{ classes}}{\Sigma 29,30,31 \text{ classes}} \times 100\%$
†54,xy	13	146	2·74	0·00	2·94
*52,xy,t_1t_1	1	99	6·06	0·00	6·59
*52,xy,t_3t_3	1	23	8·70	0·00	8·70
*53,xy,t_1	6	160	26·26	11·25	28·19
*53,xy,t_2	4	331	29·91	9·06	35·48
*53,xy,t_3	5	326	34·05	17·18	38·68
51,xy,$t_1t_2t_3$	3	332	10·84	5·42	11·15

* Data from Chapman & Bruère (1975).
† Data from this study and that of Chapman & Bruère (1975).

The segregation pattern of the progeny produced by the matings of 51,xy,$t_1t_2t_3$ rams and ewes of normal karyotype (2n = 54) is given in Table 4. There was a significant deviation from the expected 1 : 1 : 2 : 2 : 1 : 1 ratio of the products because of a significant deficit of 52,t_1t_3 and 53,t_2 progeny and a surplus of 54 progeny. The segregation pattern of the euploid secondary

spermatocytes from the triple heterozygous rams (Table 5) also showed a significant deviation from the expected segregation ratio of the products, with a deficit of 25,t_1t_3 figures and a surplus of 27 figures being observed.

Table 4. Numbers of progeny with respective chromosome number recorded from matings of triple heterozygous rams and 83 ewes of normal karyotype

Karyotype	51,$t_1t_2t_3$	52,t_1t_3	52,t_2t_x	53,t_x	53,t_2	54	Total
Expected numbers	10·375	10·375	20·75	20·75	10·375	10·375	83
Observed numbers	10	3	24	24	4	18	83

$\chi^2_5 = 13{\cdot}50$; $0{\cdot}01 < P < 0{\cdot}02$ (1:1:2:2:1:1 ratio).

Table 5. Number of euploid (NF/2 = 30) metaphase II figures with respective chromosome number recorded from the triple heterozygous rams

Karyotype	24,$t_1t_2t_3$	25,t_1t_3	25,t_xt_2	26,t_x	26,t_2	27	Total
Expected numbers	35·875	35·875	71·75	71·75	35·875	35·875	287
Observed numbers	35	14	72	74	40	52	287

$\chi^2_5 = 19{\cdot}96$; $P < 0{\cdot}01$ (1:1:2:2:1:1 ratio).

Discussion

It is seen from Table 1 that, as previously reported for homozygous translocation sheep and normal sheep (Chapman & Bruère, 1975), no hyperhaploid cells were found in the 84 cells evaluated from the 3 normal rams. In the present study only one cell from the normal rams was found to have 29 chromosome arms. This cell may not have been a true aneuploid secondary spermatocyte but rather the result of a chromosome loss due to lagging at the first anaphase. This and previous evidence suggest that there are either very few or no aneuploid secondary spermatocytes produced during spermatogenesis by either the normal ram or homozygous translocation-carrying rams.

The data from Table 2 show that there was a considerable variation in the levels of aneuploid metaphase II frequency between the 3 triple heterozygous rams. This variation ranged from 5·81–2·33% in Ram B47/48 to 16·6–6·6% in Ram B111/77, the mean level being 5·42% and is consistent with the individual variations in aneuploid metaphase II frequencies which have been found in all the single heterozygous rams studied so far (Chapman & Bruère, 1975; Long, 1978). This is similar to results from comparable studies on translocation-carrying mice (White & Tjio, 1967; Cattanach & Moseley, 1973; White, Crandall, Raveche & Tjio, 1978).

The mean level of aneuploid frequency in the triple heterozygote rams was significantly higher than the zero level found in the homozygous (t_1t_1 and t_3t_3) and normal rams (Table 3). Therefore, as with the single heterozygotes (Chapman & Bruère, 1975), triple heterozygosity for the Robertsonian translocations is a factor leading also to the increased frequency of aneuploid secondary spermatocytes. However, the level found in the triple heterozygotes was comparable to the levels reported for single heterozygous rams (Chapman & Bruère, 1975; Logue, 1977; Long, 1978) and was unexpectedly not even approximately equal to the sum of the individual levels in the singly heterozygous animals, which were 6·90%–13·56% (t_1), 9·23%–13·11% (t_2) and 16·5%–20·20% (t_3). In this respect the multiple translocation-carrying sheep appear to differ from the tobacco mouse (*M. poschiavinus*) hybrids (Cattanach & Moseley, 1973) but are

similar to the artificially evolved triple translocation-carrying mouse heterozygotes described by White *et al.* (1978). The individual variation in aneuploid spermatocyte frequency and the lack of evidence for an increase in aneuploid spermatocyte frequency in triple heterozygous rams further suggest that the rate of non-disjunction is genetically determined and is not a function which can be attributed entirely to the presence of translocations *per se*.

There was a significant excess of normal secondary spermatocytes and a deficit of t_1t_3 secondary spermatocytes in the triple heterozygotes as well as a significant excess of normal progeny and a deficit of t_1t_3 progeny from the matings of the triple heterozygous rams and normal ewes. Again, this differs from the results for triple heterozygous mice described by White *et al.* (1978) in which the segregation products in the embryos were balanced and demonstrated no preferential segregation of the translocations. However, it is probably premature, with the limited data available, to speculate on the significance of this correlation in sheep. In earlier meiotic studies, a significant excess of normal balanced secondary spermatocytes was found in single heterozygous rams (Chapman & Bruère, 1975; Long, 1978) but subsequent mating data demonstrated a balanced segregation pattern (Bruère & Ellis, 1979). Although it is possible that a process of cell selection during spermatogenesis results in the production of gametes in proportions which are significantly different from the expected ratio (1 : 1 : 2 : 2 : 1 : 1), it has previously been suggested by Ortavant (1958) and Roosen-Runge (1973) that the chromosomally abnormal cells in the ram and translocation-carrying rams (Bruère, 1974a; Chapman & Bruère, 1975) are removed before maturation is complete. Further studies on the other multiple translocation-carrying rams might indicate that certain cells are selected against or degenerate during spermatogenesis.

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