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Meiotic configurations in female trisomy 21 foetuses

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Summary. Analysis of the meiotic configurations formed by the three No 21 chromosomes in oocytes from two trisomy 21 foetuses was undertaken using a spreading technique. Light microscope analysis of the first gave limited resolving power, such that over half the oocytes could not be classified as to presence or absence of trivalent or bivalent plus univalent. In the second, investigated at the electron microscope level, all 65 cells analysed were informative and precise detail of meiotic pairing in trivalents could be obtained. Two principal forms of trivalent occurred, one in which pairing was initiated at opposite ends of the three No 21's, each initiation point involving only two of the three homologous lateral elements; the other in which pairing was initiated by all three elements at the same end, a triple synaptonemal complex being formed. Only in one oocyte out of the 65 analysed at EM level, however, did triple pairing occur along the entire length of the No 21 trivalent. All others showed splitting into bivalent and univalent at some point along the structure. Unpaired regions within trivalents and all univalents were consistently seen to be thickened and dark staining with silver over the whole period from pachytene to diplotene. This contrasted with the desynapsing lateral elements of previously paired synaptonemal complexes which appeared thin by comparison at diplotene. The significance of the thickening remains, as yet, obscure.

Introduction

Current work in our laboratory is concerned with the evaluation of human female meiosis using spreading techniques applied to prophase oocytes obtained from the ovaries of aborted foetuses (Speed, in preparation). Compared with methods which have been used in the past for human oocyte prophase analysis, e.g. squashing (Baker 1963; Manotaya and Potter 1963), sectioning for electron microscopy (Baker and Franchi 1967) and air-drying (Luciani and Stahl 1971), spreading affords greater resolution of the synaptic events and the study of meiotic prophase is greatly facilitated. This is especially so when investigation is carried out at the electron microscope level.

Although spreading techniques have been used extensively in the past to study meiotic prophase in male mammals (see Moses 1977, 1980 for review), and in human males (Solari 1980; Moses et al. 1975), oocyte spreading is a more recent development (Speed 1982; Moses et al. 1982; Speed and Chandley 1983) and has mainly involved mice.

Among the human foetal specimens received in our laboratory, following induced abortion, have been two which were pre-natally diagnosed as trisomy 21. In the first specimen, meiotic analysis of the aneuploid oocytes obtained from the ovaries was made exclusively at light microscope level. In the second, however, extensive data were obtained with the higher resolving power of the electron microscope. The findings for the two sets of data clearly illustrate the need for the latter type of investigation in order to establish unambiguously the pairing detail in the aneuploid configurations.

Only one previous account of meiosis in a human trisomy 21 foetal ovary, analysed by spreading, has been given (Wallace and Hultén 1983). Analysis by air-drying has, however, been carried out at pachytene in three female trisomy 21 foetuses (Luciani et al. 1976) and in a trisomy 18 foetus (Luciani et al. 1978). In males, the trivalents seen in air-dried pachytene preparations from a Down's syndrome adult individual were described some years ago by Hungerford et al. (1970) while, more recently, an analysis by spreading and electron microscopy was carried out by Johannisson et al. (1983).

Materials and methods

Single ovaries obtained from two prostaglandin-induced trisomy 21 foetuses were collected in phosphate-buffered saline. The somatic karyotype of each had previously been determined following amniocentesis. The first specimen, (Ovary No 29, 20 weeks gestation) was processed to obtain microspread oocytes according to the method of Fletcher (1979). These were examined only at the light microscope (LM) level. The second (Ovary No 55, 21 weeks gestation), was used exclusively to prepare grids for electron microscope (EM) analysis (Speed and Chandley 1983). Silver staining was used in both cases.

Results

Total numbers of oocytes analysed from each of the specimens, and the percentage distributions of different types of meiotic pairing configuration observed, are given in Table 1. Because of the greater resolution afforded by EM analysis, cells of Ovary 55 could be classified into those showing a trivalent, those showing a bivalent plus univalent, and those appearing to be normal and diploid. No cells with three univalent No 21s were seen. Such a precise classification could not be made, however, for Ovary 29 because, at LM level, accurate counting of the silver-stained elements was impossible. Only when an apparent univalent or trivalent occupied a peripheral location

Table 1. Total numbers of oocytes analysed and percentage distributions of meiotic pairing configurations observed for the two trisomy 21 foetuses. Comparative findings for light microscope (Ovary 29) and electron microscope (Ovary 55)

	Light microscope (Ovary 29)		Electron microscope (Ovary 55)	
	n	%	*n*	%
Trivalent A – |||		1	1.5
B – ||| or |||	12	28.6	7	10.8
C – |||		14	21.5
Bivalent plus univalent	8	19.0	39	60.0
Diploid	Not discernible		4	6.2
Uninformative	22	52.4	0	0.0
Total no cells	42		65	

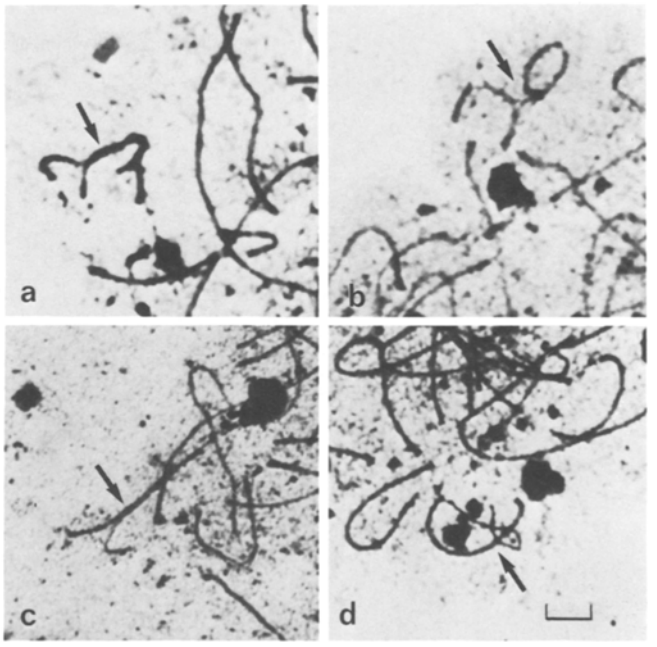

Fig. 1a–d. Meiotic configurations involving the three No 21 chromosomes *(arrows)*, light microscope level, silver staining. **a–c** Probable trivalents. **d** Possible bivalent plus univalent. Bar = 2 μm

in the nucleus could a classification be attempted. For over half of the oocytes examined therefore, a decision regarding the pairing mode of the three No 21s was not made: they remained grouped under the general heading of "uninformative". That trivalents are more easily recognised at LM level than a bivalent plus univalent can be seen from Table 1. The frequency of oocytes containing trivalents for Ovary 29 (scored at LM level) (28.6%) was only slightly lower than that recorded for Ovary 55 (scored in the EM) (33.8%). Some examples of the configurations scored as "trivalents" at LM level are shown in Fig. 1a–c and a possible trivalent or bivalent plus univalent in Fig. 1d. The abnormal structures show what are interpreted to be the three No 21s in association, but whether paired in whole or in part by a synaptonemal complex, or simply associated or twisted around one another over certain segments, could not be ascertained at this level of resolution.

In the EM analysis of Ovary 55, however, three types of trivalent could be identified and the pairing patterns unambiguously interpreted. They are given in diagrammatic form in Table 1.

Type A showed complete pairing of the three No 21s in a triple synaptonemal complex along the entire length of the trivalent.

Type B showed triple pairing at one (or other) end (centromeres could not be identified but clues were gained in some cells from silver-stained NOR material which served to indicate the short arm of the acrocentrics), or occasionally, interstitially. The length of the triple synapsed region varied from cell to cell, the remaining elements splitting into a single axial core and a synaptonemal complex. The unpaired sections of the extra No 21 exhibited marked thickening and dense staining with silver.

Type C showed pairing starting from the two ends, but involving only two of the three No 21 elements. This represents classical two-by-two pairing. As in Type B, asynaptic segments showed thickening and dark staining.

Of the three types, the rarest was Type A, only one example of which was found in the 65 oocytes examined on grids. Unfortunately, breakage of the film coating the EM grid prevented a photograph of this one cell from being taken. Type B trivalents were found in about one-third of trivalent-containing oocytes. Figure 2a shows a Type B trivalent in which triple pairing was confined to a short segment at one end of the configuration. The presence of nucleolar material in close proximity suggested this was the short arm and centromeric end. In Fig. 2b, triple pairing extends along about one-third of the total length, with separation into synaptonemal complex and axial core then occurring. Thickening and splitting in the unpaired axial core can be seen, especially in the terminal portion. In Figs. 2c and d, triple pairing occurred interstitially. Again evidence of splitting and thickening was found in the unpaired axial cores.

Type C trivalents were the most common, however, and were found in two-thirds of the trivalent-containing oocytes. Examples are shown in Figs. 2e and f. In the complex configuration shown in Fig. 2f, it appeared that the free axial elements had managed to establish a secondary association with the two regions of synaptonemal complex and in these segments thickening in the single core had not occurred. The presence of NOR material in this cell indicated the centromeric end of one of the axial elements and a switch of pairing partner was also observed at one point.

By far the most common type of oocyte in Ovary 55 was, however, that showing bivalent plus univalent: 60% of cells fell into this category. In the majority, the univalent was observed as a free-lying axial element not in association with any other element (Fig. 3a). In a proportion (11/39), however, it was seen to be in close proximity to a nucleolar synaptonemal complex of similar length and which, being the shortest in the complement, was interpreted to be the No 21 bivalent (Fig. 3b). The thickened nature of unpaired segments previously described for the trivalents also characterized whole univalents (Figs. 3a and b). Splitting in the short arm region of the univalent was also observed (Fig. 3b). The fact that thickening did not characterize axial cores which had previously been paired normally in

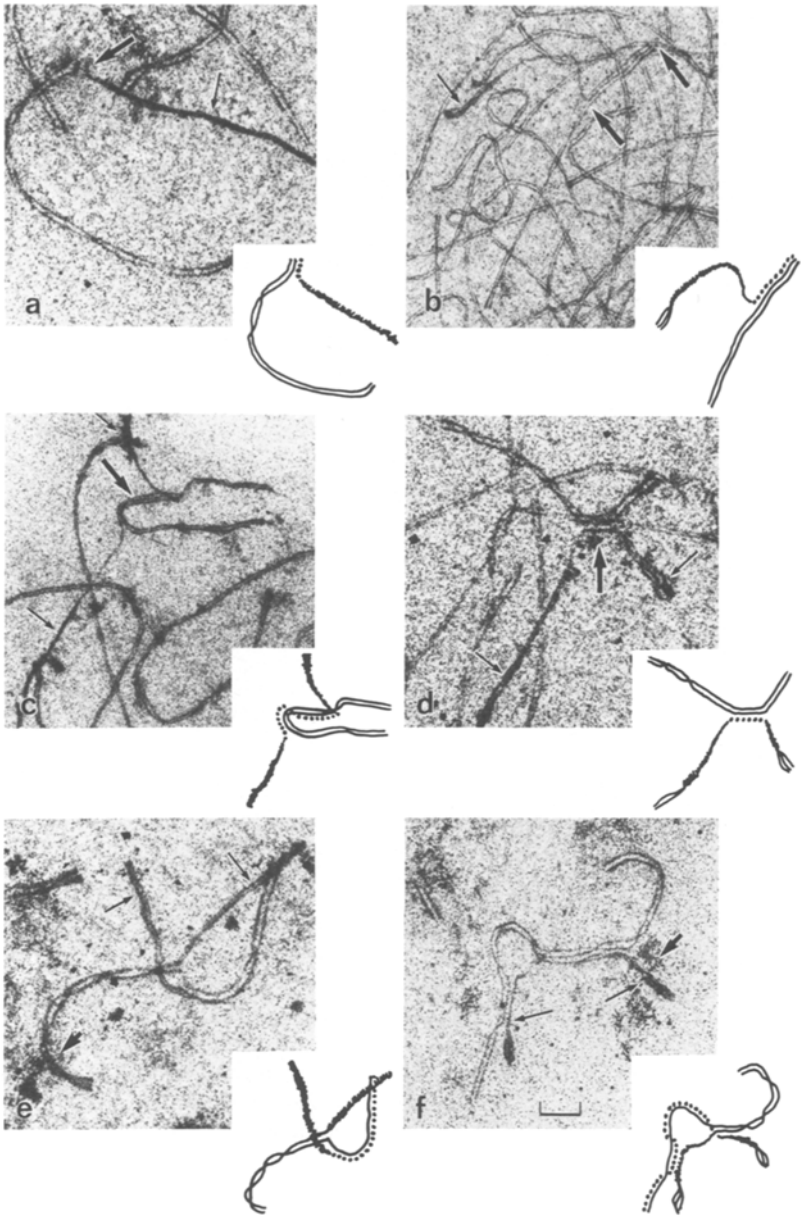

Fig. 2a–f. Trivalent configurations seen at electron microscope level, silver staining. **a** Small terminal pairing segment *(arrow)* between three lateral elements, unpaired segment thickened *(small arrow)*. **b** Three lateral elements paired for one third of their length *(arrows)*. Unpaired section becomes thickened and split *(small arrow)*. **c–d** Interstitial triple pairing *(arrow)*. Unpaired regions of one lateral element appear thickened *(small arrows)*. **e–f** Pairing between two of the three lateral elements. Unpaired regions appear thickened *(small arrows)*. Centromeric regions bearing NOR material may be paired or unpaired *(arrow heads)*. = 1 μm

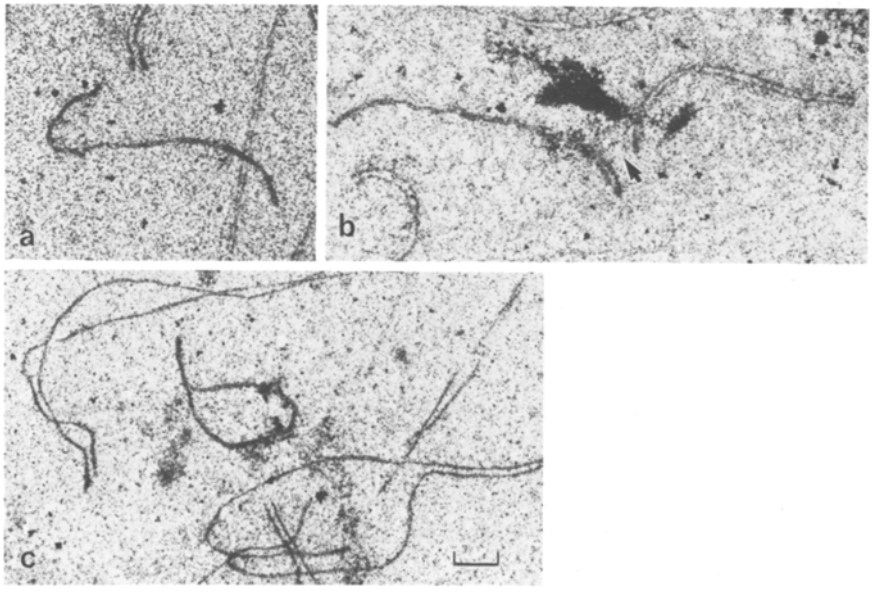

Fig. 3a–c. Univalent chromosome No 21, electron microscope, silver staining. **a** Univalent 21 thickened in comparison with lateral elements of fully paired synaptonemal complexes. **b** Univalent 21 in association with bivalent No 21 at NOR bearing centromeric end *(arrow head)*. **c** Univalent 21 at diplotene still appears thicker than desynapsing lateral elements of previously normally paired synaptonemal complexes. Bar = 1 μm

synaptonemal complex formation is illustrated in Fig. 3c. Here the thickened axial core of an unpaired univalent at diplotene contrasts strikingly with the desynapsing lateral elements of the surrounding bivalents which remain thin.

The remaining 6.2% of oocytes analysed in Ovary 55 gave what appeared to be a normal diploid count of 23 synaptonemal complexes. No evidence was found of univalent or trivalent structures.

Discussion

This study of oocytes from two trisomy 21 foetuses has emphasised the necessity for electron microscopic examination in the evaluation of meiotic pairing in aneuploid oocytes. Human oocytes in general are difficult to analyse because the synaptonemal complexes tend to be consistently long throughout meiotic prophase and much longer than those normally seen in spermatocytes. They are therefore difficult, if not impossible, to count accurately, especially at LM level. In the present study it was found that identification of a univalent or trivalent at LM level depended largely on whether or not it occupied a peripheral location in the nucleus. More than half the oocytes were uninformative on this point and therefore could not be categorized. Indeed, the spreading study of Ovary 29 at LM level gave little more by way of detail than probably could have been obtained in an air-drying investigation (Luciani et al. 1976). When Ovary 55 was analysed using the EM, however, the data were very different, with no oocyte being classified as "uninformative". All could be analysed with regard to type of configuration (i.e. trivalent or bivalent plus univalent) as well as to the precise nature of pairing within trivalents.

As Wallace and Hultén (1983) reported, triple pairing occurs in the trivalents of trisomy 21 oocytes and appears to be a relatively common feature. The present more extensive EM studies showed that about one-third of all trivalents in Ovary 55 demonstrated this phenomenon, at least over short segments: only one oocyte out of 65, however, showed complete triple pairing along its entire length. Wallace and Hultén (1983) have emphasised the importance of the observation in view of the classical viewpoint that only two-by-two pairing characterises the trivalents of primary trisomics (Sybenga 1975) and triploids (Darlington 1965). Triple pairing over short segments has been noted before, however, at EM level, in triploid chickens (Comings and Okada 1971), triploid *Coprinus* (Rasmussen et al. 1981), in the primary spermatocyte configurations of translocation trisomic mice (de Boer and van Beek 1982) and in trivalents from a Down's syndrome male (Johannisson et al. 1983).

The other feature of interest at the EM level was the observed thickening of unpaired regions of trivalents and univalents. The thickening appeared to characterize asynaptic elements: it was not found in the desynapsing lateral elements of synaptonemal complexes which had previously been paired normally. Thickening of the unpaired portion of the X and Y axes is a consistent feature at meiotic prophase in spermatocytes prepared by spreading (Tres 1977; Solari 1980), and its occurrence has also been noted in a variety of autosomal situations, both in oocytes and spermatocytes, where pairing has failed (unpublished observations). Others too have commented on the phenomenon (Johannisson et al. 1983). It appears to be a good morphological criterion for the recognition of asynapsis, but what its significance is in terms of chromosomal behaviour and germ-cell function remains to be elucidated. More data are needed from further trisomy 21 ovarian specimens to ascertain the extent of variation from one to another with regard to relative proportions of trivalent, bivalent plus univalent and apparently diploid complements. Wallace and Hultén (1983) reported that 40% of their trisomy 21 oocytes, examined at LM level, contained a conspicuous trivalent. In this study, the frequencies were 28.6% in Ovary 29 (LM) and 33.8% in Ovary 55 (EM). An apparently normal and diploid complement was found in 6.2% of oocytes in Ovary 55, but, with the limited resolution offered by light microscope analysis, information on this point could not be obtained for Ovary 29. Studies at diakinesis/MI in adult male Down's individuals (Hultén and Lindsten 1970; Kjessler and de la Chapelle 1971; Schröder et al. 1971) have also given evidence for this type of diploid/trisomic mosaicism in the germ line. Elimination of the extra No 21 in a minority of germ cells seems to occur therefore in both sexes, and in the present study, it would seem that in oocytes this takes place prior to the pachytene stage. The only report of a pachytene analysis in a male Down's individual (Johannisson et al. 1983) gave the puzzling impression of "disappearance" of the extra No 21 from the majority of cells during prophase, with its subsequent reappearance at diakinesis/MI. It was suggested by the authors that the extra No 21 was intimately associated with the sex bivalent, and thus "hidden", at the pachytene stage.

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