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Combined immunocytogenetic and molecular cytogenetic analysis of meiosis I oocytes from normal human females

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Summary

The microspread oocytes of three fetuses, two of 16 weeks gestation and one of 15 weeks gestation, were labelled with a combination of anti-lateral element antiserum and a human centromere labelling auto-immune serum. The anti-lateral element serum was found to label both asynapsed axial elements and synapsed lateral elements strongly. Nuclei were found from leptotene to diplotene in all three fetuses. The use of the human auto-immune serum led to the observation of 'staggered centromeres' and 'centromeric associations' as well as tightly clustered centromeres in 'stellar nuclei'. Nuclei displaying various aberrant features were detected. The use of antibody-labelled microspread oocytes as substrates for fluorescence *in situ* hybridisation (FISH) was found to be reliably successful only with repetitive (centromeric and telomeric) probes.

Key words: Fetal ovaries, Human, Meiosis, Oocytes, Synaptonemal complex

Introduction

The female requirement for gametes is dramatically lower than that of the male, and a relatively brief wave of meiotic divisions, which are restricted to fetal life and arrest prior to the completion of the first meiotic division, produces adequate numbers of oocytes to satisfy mammalian fecundity. The plentiful and persistent meiotic divisions that continue throughout a male mammal's adult life, necessary in order to furnish adequate spermatozoa to complete the mammoth task of locating scarce oocytes, makes specimens relatively straightforward to obtain.

This restriction of the first meiotic division to fetal life in females complicates the logistics and ethics of studying the events involved in humans. These complications have meant that events that give rise to human female gametes are poorly understood and have been studied in only a handful of cases by various methods including squashing (e.g. Baker, 1963), air drying (e.g. Stahl & Luciani, 1972), serial sectioning (Bojko, 1983, 1985) and microspreading at the level of either the light (e.g. Wallace & Hultén, 1985) or electron microscope (e.g. Speed, 1985). Recently fluorescence *in situ* hybridisation (FISH) techniques have been applied against squashed human oocytes using whole-chromosome paints in studies of the pairing behaviour of the X in normal females and the pairing behaviour of the 18 in trisomy 18 fetuses (Cheng & Gartler, 1994; Cheng *et al.*, 1995).

The synaptonemal complex (SC) is a proteinaceous structure found only in meiotic prophase I along the interface of synapsed chromosomes; the term 'zipper-like' is favoured by authors as it describes both the appearance and function of the SC (e.g. Loidl, 1994; Heyting, 1996). The first components of the SC to appear are the axial elements, proteinaceous rods (diameter 50 nm; Westergaard & Von Wettstein, 1972) that form the cores of chromosomes. In humans only short fragments of axial element appear in zygotene, narrowly preceding the onset of synapsis (Speed, 1988; Speed & Chandley 1990). Chromatin is anchored via loops that associate with the axial elements only at

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their bases (Moens & Pearlman, 1988). SC formation (synapsis) involves the close apposition of the two axial elements at a distance of 100 nm (Westergard & Von Wettstein, 1972), and the formation of transverse filaments, perpendicular to the axial elements, and the central element (diameter 20 nm; Westergaard & Von Wettstein, 1972) which runs parallel to and between the axial elements (which following synapsis are referred to as lateral elements). Zygotene nuclei contain SCs that are incomplete, whilst pachytene nuclei contain full-length SCs. The end of the pachytene substage is characterised at an ultrastructural level by the dissolution of the SC and the loss of central element proteins. Lateral element proteins are, however, retained in diplotene nuclei along the length of homologues (e.g. Moens & Spyropoulos, 1995).

In the work described here, human prophase I oocytes have been studied with immunocytogenetic techniques using antibodies that label axial/lateral elements and centromeres, and molecular cytogenetic techniques with various DNA probes in order to evaluate the ability of these techniques to obtain new data in this relatively neglected area.

Materials and methods

Microspreading

The ovaries of three fetuses were used for this study. In each case termination was conducted for social reasons and the fetuses were assumed to be chromosomally normal. One fetus was 15 weeks gestation (15 wks), the other two were 16 weeks (16 wks A and 16 wks B).

Fetuses were delivered after cervigen-induced termination in the early evening and ovaries removed the next morning. Ovaries were kept in Earle's balanced salt solution (EBSS) culture medium at room temperature for approximately 5 h prior to microspreading. After chopping with springbow scissors in EBSS, the pieces were teased apart with scalpel blades to produce a cell suspension. Microspreading was conducted according to the method described by Hultén *et al.* (1992) with modifications. One drop of suspension was mixed with five drops of a 0.3% solution of lipsol on a clean microscope slide and allowed to stand for 30 min. Ten drops of 1% ultrapure formaldehyde (TAAB), 0.04% SDS were added for 20 min, after which the slides were briefly dipped in distilled water and air dried.

Slides were then blocked in three changes of PBT (phosphate-buffered saline (PBS), 0.15% bovine serum albumin (BSA), 0.1% Tween 20) for approximately 30 min. A combination of antisera A1 and GS was then applied at concentrations of 1:1000 and 1:5000 respectively overnight. Serum A1 was raised against rat SCs in rabbits and predominantly recognises SCP3, a lateral element component (Lammers *et al.*, 1994). Serum GS was obtained from a patient with scleroderma and has been shown to label centromeres (Earnshaw & Rothfield, 1985). Slides were then given 5 min washes in PBT and the secondary fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody (Sigma) combined with a tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-human IgG antibody (Sigma) were applied, each at a concentration of 1:500 in PBT for 2–4 h at room temperature.

After three 5 min washes in 4 × SSC 0.05% Tween 20, followed by a brief rinse in distilled water, air-dried slides were stored at −70 °C.

Microscopy

Upon removal from the −70 °C freezer, sides were stained with 4′,6-diamidino-2-phenylindole (DAPI) in 2 × SSC and studied with a Zeiss Axioskop epifluorescence microscope fitted with a Pinkel number 1 filter set and a cooled charged coupled device (CCD) (Photometrics). Images were captured and manipulated on a Power Macintosh 8100/80 with Smartcapture software (Digital Scientific). The positions of nuclei chosen for subsequent FISH analysis were recorded using an England Finder (Graticules).

In situ hybridisation

Suitable slides were de-stained in three changes of 4 × SSC, 0.05% Tween 20 and passed through an ethanol series (70%, 85%, 100%, 2 min each) at room temperature and air dried. Slides pre-warmed to 65 °C were then placed in 70% formamide 2 × SSC at 70 °C for 2 min, passed through an ethanol series at −20 °C, air dried and warmed to 37 °C. Hybridisation fluid, consisting of either 20 µl biotinylated whole chromosome 1 paint (Cambio), 20 µl of biotinylated 'all human telomeres' probe (Oncor), 20 µl of chromosome 21 contig probe (Cambio), or 18 µl of biotinylated all human centromeres (Cambio) probe mixed with 2 µl of digoxigenin (Boehringer Mannheim) labelled pUC 1.77 (chromosome 1 heterochromatin) DNA, was heated to 70 °C for 6 min, and immediately applied to the slides beneath 24 × 50 mm coverslips and sealed, with hybridisation occurring overnight at 37 °C.

Stringency washes and probe detection

Slides were washed in three changes of 50% formamide 2 × SSC at 45 °C for 5 min each, once in 2 × SSC at 45 °C for 5 min, once in 4 × SSC 0.05% Tween 20 at 45 °C for 5 min and once in 4 × SSC 0.05% Tween 20 at room temperature for 5 min.

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Table 1 Nucleus staging

	Fetus		
	15 wks	16 wks A	16 wks B
Leptotene: partially polymerised axial elements	6 (5.2%)	14 (14%)	0
Leptotene: fully polymerised axial elements	5 (4.3%)	6 (6%)	10 (8.5%)
Zygotene	45 (39.1%)	19 (19%)	33 (28.2%)
Pachytene	20 (17.4%)	38 (38%)	21 (17.9%)
Diplotene	3 (2.6%)	3 (3%)	6 (5.1%)
Condensed, speckled chromosomes	10 (8.7%)	0	28 (23.9%)
Degenerate	24 (20.9%)	19 (19%)	18 (15.4%)
Other[a]	2 (1.7%)	1 (1%)	1 (0.9%)
Total	115	100	117

[a] Other nuclei contained axial/lateral elements but did not fit into any classical prophase stage.

The biotinylated probes were detected with incubation in either Texas-red–avidin (Vector Laboratories) 1:200 in 4 × SSC, 0.05% Tween 20, 5% non-fat milk or AMCA–avidin (Vector Laboratories) in the same medium for 20 min at room temperature, followed by a 20 min incubation in biotinylated anti-avidin (Vector Laboratories) 1:100 in 4 × SSC, 0.05% Tween 20, 5% non-fat milk at room temperature for 20 min and a final incubation in Texas-red–avidin or AMCA–avidin for 20 min. After each incubation slides were washed for 3 × 3 min in 4 × SSC 0.05% Tween 20. Digoxigenin-labelled probes were detected with anti-digoxigenin fab fragments–rhodamine (Boehringer Mannheim) 3:200 in 4 × SSC, 0.05% Tween 20, 5% non-fat milk at room temperature for 20 min followed by 3 × 3 min washes in 4 × SSC, 0.05% Tween 20. Slides were counterstained in DAPI (0.2 μg/ml) in 2 × SSC, briefly rinsed in water and Vectorshield (Vector Laboratories) applied beneath a coverslip.

Microscopy was conducted as described above, using England Finder references to re-locate nuclei.

Results

Immunocytogenetics

Microspread human oocytes were labelled with a combination of antisera A1 and GS. Antiserum A1 strongly labels both the asynapsed axial elements and the synapsed lateral elements of human prophase I nuclei. Serum GS strongly labels the centromeres of microspread oocytes. Around 1000 nuclei were observed, of which images of 692 nuclei were stored as computer files. In order to determine accurately the proportion of nuclei at each stage of prophase I of meiosis, approximately 100 images of nuclei from each of the three fetuses were collected without any pre-selection such as nucleus quality. The results of this quantitative study are shown in Table 1.

During the course of the quantitative study the axial and lateral elements of the 15 wks and 16 wks A fetus were found to be clear and sharp. Morphologically well-preserved nuclei were also observed from the 16 wks B fetus; however, many nuclei from this fetus contained poorly defined, 'fuzzy' elements. Quantitation of certain features has therefore been restricted to the 15 wks and 16 wks A fetuses.

Leptotene oocytes

Leptotene nuclei of two distinct types were observed during this study. The first consisted of nuclei in which the newly forming axial elements appeared highly fragmented. Short lengths of axial element interpreted as representing telomeric regions were present, and usually less fragmented in appearance than the rest of the axial element material. These telomeric regions were often present in pairs which were in register, giving the impression that they are about to synapse (Plate 1*a*, yellow arrows, opposite p. 32). Such leptotene nuclei were seen for the 15 wks and 16 wks A fetuses (Table 1). The second type of leptotene nuclei, seen in all three fetuses, contained fully formed axial elements for all chromosomes (Table 1). The axial elements of such nuclei were highly variable, with some nuclei containing long untraceable elements (Plate 1*e*) whilst others contained relatively short elements with a thickened appearance (Plate 1*c*); in a few rare cases the axial elements were not only thickened, but also nodular in appearance (Plate 1*f*). Two leptotene nuclei with fully formed axial elements showed evidence of homologue co-localisation and possibly alignment (Plate 1*c*, yellow arrows). Some of these co-localised bivalents were associated at their centromeres; in some cases

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this 'centromeric association' had clearly resisted the mechanical stress imposed during the microspreading procedure (Plate 1*c*, green arrows).

Zygotene oocytes

Zygotene nuclei, by definition those nuclei in which homologues are actively synapsing, generally contained homologues that were synapsed in terminal regions with interstitial asynapsis. Small 'terminal forks' were usually visible in such nuclei (Plate 1*b*, yellow arrows). The telomeres of early zygotene nuclei were often localised in a single region forming a classical 'bouquet' configuration (Plate 1*b*) – a configuration that had usually disappeared by late zygotene. The axial elements of asynapsed interstitial regions in early zygotene nuclei formed clear 'pairing forks', but attempts to trace such elements fully generally revealed that they are incomplete in these asynapsed regions (Plate 1*b*). By late zygotene all axial elements are fully formed, and interstitial asynapsed regions are visible as 'balloons' of asynapsis. Synapsed and asynapsed axial elements are clearly distinguishable in zygotene nuclei on the grounds of labelling intensity and thickness. Some exceptional zygotene nuclei were observed in which a very small degree of synapsis was present in a nucleus that contained apparently fully formed axial elements (Plate 1*d*).

Pachytene oocytes

Pachytene nuclei contained 23 fully synapsed and distinct bivalents. Observation of pachytene nuclei labelled with antisera A1 and GS led to a number of interesting observations, in particular regarding the centromeres of these nuclei. Centromeres of two or three bivalents were often seen in physical association, such associations frequently showing signs of surviving the physical stresses imposed during the microspreading procedure and therefore distinguishable from the chance co-localisation of centromeres that may occur during a cell's collapse (e.g. Plate 2*a*, yellow arrow; between pp. 32 & 33). Centromeric associations were seen for all three fetuses. They were observed at a rate of 48 of 279 (17%) nuclei for the 15 wks fetus, and at a rate of 42 of 204 (20%) for the 16 wks fetus A. Whilst these centromeric associations were detected in all stages from leptotene to diplotene (Tables 2, 3) they are predominantly a pachytene feature, with 28 of 48 (58.3%) nuclei with centromeric associations from the 15 wks fetus, and 29 of 42 (69%) nuclei with centromeric associations from the 16 wks A fetus being in pachytene.

Some bivalents showed two distinct regions of labelling with antiserum GS separated by a region of normally labelled synaptonemal complex, of lengths up to approximately 1.6 μm (Fig. 2*b*, white arrows).

Table 2 Centromeric features of 15 wks fetus

	Centromeric associations	Staggered centromeres
Leptotene	4 (8.3%)	0
Zygotene	11 (22.9%)	1 (4.2%)
Late zygotene	3 (6.3%)	2 (8.3%)
Pachytene	28 (58.3%)	21 (87.5%)
Diplotene	1 (2.1%)	0
Degenerate	1 (2.1%)	0
Total	48/279	24/279

Table 3 Centromeric features of 16 wks fetus A

	Centromeric associations	Staggered centromeres
Leptotene	1 (2.4%)	0
Zygotene	4 (9.5%)	0
Late zygotene	7 (16.7%)	8 (20.5%)
Pachytene	29 (69%)	28 (71.8%)
Diplotene		2 (5.1%)
Degenerate	1 (2.4%)	1 (2.6%)
Total	42/204	39/204

The telomeres of such bivalents are in register. These 'staggered centromeres' were seen in 24 of 279 (8.6%) nuclei from the 15 wks fetus, and 39 of 204 (19.1%) nuclei from the 16 wks fetus A. This feature was also observed in a small number of zygotene and diplotene nuclei (Tables 2, 3). 'Staggered centromeres' are not usually seen in more than two or three bivalents per nucleus.

Two pachytene nuclei were observed in which the region adjacent to the centromere on the long arm of a large metacentric bivalent (probably chromosome 1) was left asynapsed when all other bivalents were fully synapsed. The axial elements of this asynapsed region were of different lengths, with one straight and the other buckling out distinctly (Plate 2*b*, yellow arrow).

Diplotene oocytes

Diplotene nuclei, in which synapsis of bivalents has started to relax, have traditionally been considered difficult to distinguish from zygotene nuclei, and human oocytes labelled with antisera A1 and GS are no exception.

Nuclei in which at least two regions of terminal asynapsis were present combined with interstitial asynapsis were considered to be diplotene; examples are shown in Plate 2*c* and *d*. Other features that help to distinguish diplotene nuclei from zygotene nuclei are the absence of a bouquet, and the absence of the

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small terminal pairing forks shown in Plate 1*b* (yellow arrows). The axial elements of desynapsing regions continue to label with serum A1 and can be distinguished from regions that remain synapsed on the basis of labelling intensity and thickness.

Z-cells

Nuclei containing fragmentary axial elements or synaptonemal complexes, termed 'Z cells' (Beaumont & Mandl, 1962), and regarded as degenerative were observed in high numbers for all three fetuses (Table 1).

Other oocyte categories

The labelling of nuclei with sera A1 and GS led to the observation of a number of nuclei that did not fit into any of the classical meiotic stages. Some of these nuclei were seen on several occasions and have been grouped into novel categories. One such category is the 'Stellar' nucleus, characterised by the presence of a central cluster of centromeres (Plate 3, between pp. 32 & 33). Three 'stellar' nuclei were observed from the 15 wks fetus and one from the 16 wks B fetus. In one nucleus the clustering of the centromeres was fairly loose (Plate 3*a*); in this nucleus the axial elements have a disjointed and angular appearance. In the other three nuclei, the clustering of the centromeres was dramatic, with axial elements emanating from a very tight central cluster of centromeres to create a spectacular 'stellar' effect. In the nucleus shown in Plate 3*b* the axial elements are clearly organised into 'diplotene-like' pairs, with sites of apparent crossing over (chiasmata) present. A similarly dramatic nucleus is seen in Plate 3*c* (the only nucleus from 16 wks fetus B), but the axial elements of this nucleus, whilst desynapsed, are present in tight pairs with numerous bridges linking them. In one case (Plate 3*d*) a spectacular central cluster of centromeres could be seen, whilst antibody A1 produced only a speckled labelling pattern, with no linear elements present.

A second, relatively common category of nucleus that did not conform to classical meiotic stages were those containing condensed chromatin, which was divided into apparent chromosomal domains, that were labelled by antibody A1 in a speckled pattern (Plate 4, between pp. 32 & 33). These nuclei were observed from the 15 wks and 16 wks B fetuses. Often much of the chromatin is clumped together in the centre of these nuclei, but a few of the chromatin domains are usually visible at the periphery of the nucleus, clearly illustrating that each domain has a single centromere and a chromosomal appearance. Good examples are seen in Plate 4*a*. The number of distinct centromeres visible in these nuclei is usually close to 46 (between 42 and 46 can be counted in the examples shown in Plate 4). The labelling pattern produced by antibody A1 in these nuclei is variable, in some cases consisting of little more than spots (Plate 4*a*, *b*) while in others, short linear structures can be resolved (Plate 4*c*, *d*).

Many nuclei conformed to classical meiotic staging but were regarded as aberrant for various reasons (Plate 5, between pp. 32 & 33). Such nuclei are of interest as they illustrate the range of features that can be detected immunocytogenetically in microspread human oocytes. An example of terminal non-homologous synapsis was detected in a nucleus (from the 16 wks fetus A) in which interstitial 'buckling' of a single axial element (Plate 5*a*, yellow arrow) resulted in non-homologous synapsis of the remainder of the bivalent. The 'non-buckled' axial element protruded beyond its partner by a length equal to the length of the buckle (Plate 5*a*, white arrow). Other small regions of both terminal and interstitial asynapsis are visible in this nucleus, suggesting it may be an early diplotene nucleus. A nucleus (from the 16 wks fetus A) was observed in which the synaptonemal complexes present had an unusual aberrant appearance (Plate 5*b*). This nucleus again had numerous regions of both interstitial and terminal asynapsis, suggesting that it was a diplotene nucleus, but the still fully synapsed complexes had 'rods' (Plate 5*b*, yellow arrows) and loops (Plate 5*b*, white arrows) of axial element material protruding from what were otherwise SCs of normal appearance. An apparently normal late zygotene nucleus (from the 16 wks fetus A) was observed in which a peripheral univalent, probably chromosome 21, could be seen (Plate 5*c*, white arrows). Univalents can be distinguished on the basis of weaker labelling with antibody A1 and a thinner appearance than bivalents. In the example shown it is unclear whether the univalent has a partner in the mass of synaptonemal complexes with which it has failed to pair, or is the result of an aneuploid condition, such as trisomy, indicative of germline mosaicism. A nucleus was observed with a late diplotene-like appearance, but with elements that were angular in appearance with nodules (Plate 5*d*). As very few nuclei were observed in such a late phase it is unclear whether this nucleus is aberrant or truly representative of late diplotene nuclei.

Immunocytogenetics and FISH

Combined immunocytogenetics and FISH was attempted against microspread oocytes labelled with sera A1 and GS using whole-chromosome paints, repeated and single copy probes. Nuclei were observed prior to hybridisation, at which time labelling with both antisera A1 and GS was visible, and post-hybridisation when labelling with antiserum A1

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remained visible, combined with the hybridisation pattern of the DNA probe.

Hybridisation against SC-associated chromatin using whole-chromosome paints proved difficult in all three fetuses, and was successfully achieved in only exceptional nuclei from the 16 wks A fetus with a whole-chromosome 1 paint (Plate 6*a*,*b*, opposite p. 33). In the example shown in Plate 6*b* the bivalent is fully painted (yellow arrows) with the exception of a small region of the short arm; the twisted and convoluted chromosome 1 bivalent can therefore be traced from telomere to telomere. Confirmation that the painted bivalent is indeed chromosome 1 comes from the clearly distinguishable large heterochromatic block that is visible due to its differential DAPI staining qualities (red arrows).

Hybridisation using single-copy probes, like paints, also proved difficult in all three fetuses, with success in only exceptional nuclei from the 16 wk fetus A hybridised with the chromosome 21 contig probe. Hybridisation patterns in the handful of nuclei in which hybridisation was successfully conducted consisted of either a single or double spots in close proximity to the small, acrocentric 21 bivalent.

Hybridisation against SC-associated chromatin using repeated sequences (chromosome 1 heterochromatic, pan-centromeric probe, and pan-telomeric probe), in contrast to single-copy probes and paints, proved universally successful in the fetuses (15 wks and 16 wks A) in which it was attempted. Telomeric probes produce signals in the form of spots that are very closely associated with SCs, whilst the heterochromatic probe and pan-centromeric probe hybridise to loops that associate with SCs only at their bases, resulting in dramatic 'flare-like' signals (Plate 6*d*,*f*). The hybridisation of the chromosome 1 heterochromatic probe confirmed that the regions of asynapsis occasionally seen on a large metacentric chromosome do indeed correspond to the chromosome 1 het block (Plate 6*c* yellow arrow, Plate 6*d*). Hybridisation of the pan-centromeric probe confirmed that the spots labelled by serum GS correspond to regions of alpha satellite repeats, but also that the 'staggered centromeres' identified with serum GS correspond to identical double regions of alpha satellite repeats (Plate 6*e*,*f*, yellow arrows).

Discussion

Microspread human oocytes have been studied using a combination of anti-lateral element antibodies (Lammers *et al.*, 1994) and a human autoimmune serum that is known to label centromeres (Earnshaw & Rothfield, 1985). Nuclei were observed in all stages from leptotene to diplotene.

Leptotene nuclei of two distinct types were observed during this study: one in which only short fragments of lateral element material was present, and one in which fully formed axial elements were present. Leptotene oocytes were not recognised at all in light microscope studies of silver-stained microspread oocytes by Wallace & Hultén (1985), with zygotene the earliest stage recognised. Speed (1988) in an electron microscope study of microspread oocytes concluded that 'classical leptotene does not exist as such in human oocytes', with synapsis rapidly following the appearance of small, terminal regions of axial elements, when only a very small overall length of SC is present. The first type of leptotene nucleus described, seen in the 15 wks and 16 wks A fetuses and containing short fragments of lateral element material, appears to conform with the observation of Speed (1988), particularly as what appear to be telomeric regions are usually present in close pairs that are apparently about to synapse (Plate 1*a*, yellow arrows). The second type of leptotene nucleus described, seen in all three fetuses, in which fully formed axial elements are present, may correspond to 'multiply asynaptic' cells described in both human spermatocytes (Speed & Chandley, 1990) and oocytes (Speed, 1985, 1988). Speed (1988) speculated that nuclei with asynapsis were destined for atresia and they were characterised in his silver-stained preparations by unpaired, thickened axial elements. Apparently co-localised homologues in two such nuclei (Plate 1*c*, arrows) suggest that homologue co-localisation and synapsis are separate and independent processes in human females, as has been seen for human males (Barlow & Hultén, 1996). An alternative interpretation of the type of nuclei shown in Plate 1*c* was offered by Bojko (1985) in studies of serially sectioned oocytes. A nucleus containing two axial elements involved in a centromeric association (fig. 10e in Bojko, 1985) that appear remarkably similar to the two axial elements associated by their centromeres in Plate 1*c* (top left, green arrow) was reconstructed by Bojko (1985) and interpreted to be a mid-diplotene nucleus. Other bivalents observed in the same nucleus by Bojko (1985) were seen also to be in pairs and to either closely approach each other or form short fragments of SC. Given the similarities between the bivalent reconstructed by Bojko (1985) and the bivalent in Plate 1*c* it seems probable that these two cells were at the same stage. However, it seems extremely unlikely that the nucleus in Plate 1*c* could be a diplotene nucleus, as the majority of the axial elements do not have a closely associated homologous partner, and are in general present as single entities. Whilst Bojko's data are unrivalled in terms of spatial accuracy, they were limited to only 32 cells, making challenging the construction of an accurate chrono-

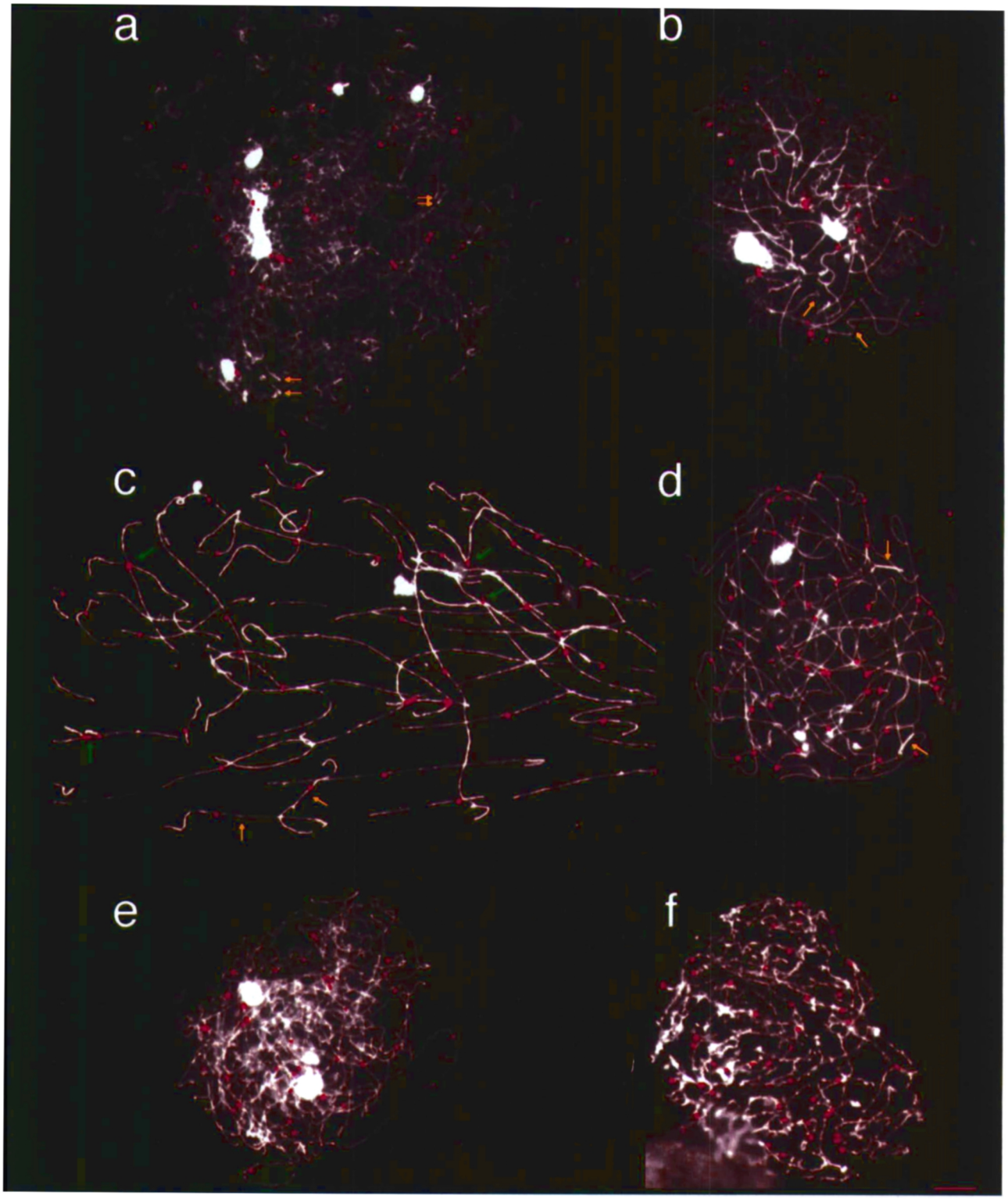

Plate 1. Microspread human oocytes labelled with antibody A1 (anti-lateral element – white) and GS (centromeres – red). (*a*) Leptotene nucleus containing newly polymerising axial elements. Structures interpreted to represent telomeric regions (arrows) frequently occur in pairs. (*b*) Zygotene nucleus showing clustering of telomeres to form a classical 'bouquet' configuration. The presence of 'forks' at the terminus of the synaptonemal complexes (SCs) (arrows) clearly illustrates that synaptic initiation is sub-telomeric. Two asynapsed elements can be seen splaying as 'pairing forks' from the interstitial end of the SCs into the mass of other asynapsed elements. Asynapsed interstitial axial elements have a speckled, discontinuous appearance. (*c*) Leptotene nucleus containing apparently fully formed, unsynapsed axial elements. There is no evidence of any synapsis in this nucleus; some elements show strong evidence of alignment with their homologues (e.g. yellow arrows), with some of these aligned homologues associated by their centromeres (green arrows). The axial elements of this nucleus appear relatively short and fattened. (*d*) Early zygotene nucleus containing two short regions of synapsis. The unsynapsed regions are apparently fully formed. (*e*) Leptotene nucleus containing fully formed unsynapsed axial elements. The axial elements of this nucleus are relatively long and impossible to trace (compare with those in *c*). (*f*) Leptotene nucleus containing apparently fully formed axial elements lacking any evidence of synapsis. The axial elements of this nucleus have a thickened, nodular appearance. Scale bar represents 10 μm.

(*Facing p.* 32)

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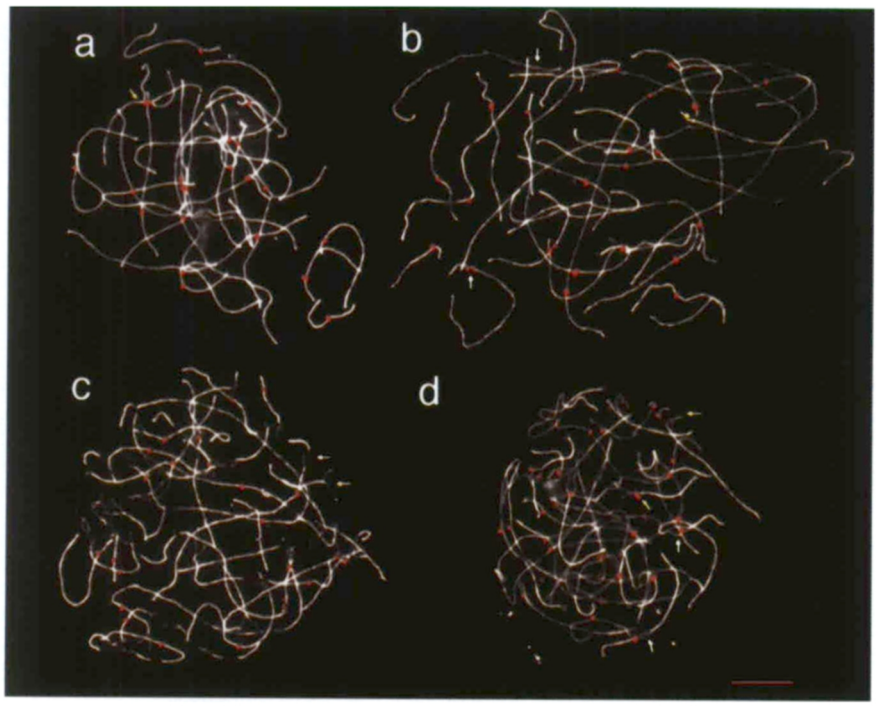

Plate 2. Microspread human oocytes labelled with antibody A1 (anti-lateral element – white) and GS (centromeres – red). (*a*) Pachytene nucleus showing full synapsis of all bivalents. Two bivalents are involved in a 'centromeric association' (arrow). The visible tension on these two bivalents, the result of them being dragged in opposite directions during the spreading procedure, indicates that this 'centromeric association' is a genuine feature and not merely a coincidental co-localisation. (*b*) Pachytene nucleus showing full synapsis of all bivalents with the exception of a single bivalent that shows a small region of asynapsis adjacent to the centromere (yellow arrow). Two bivalents show labelling of two distinct centromeric regions (white arrows), indicating the presence of some non-homologous synapsis within these bivalents. (*c*), (*d*) Diplotene nuclei in which bivalents have desynapsed both terminally (yellow arrows) and interstitially (white arrows). Scale bar represents 10 μm.

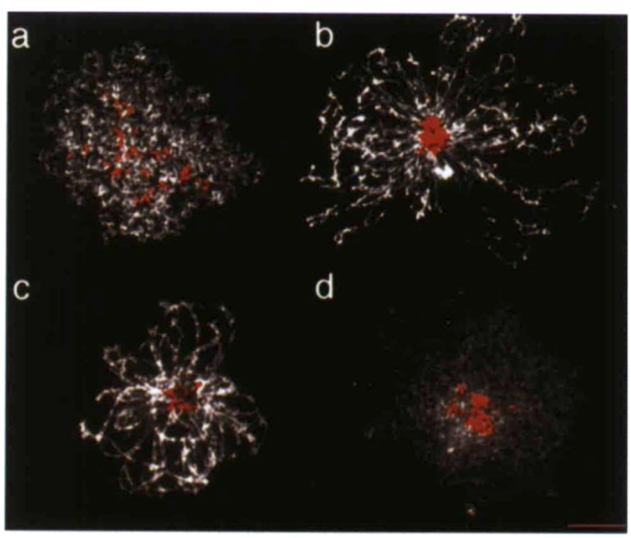

Plate 3. Microspread oocytes with 'stellar' appearance labelled with antibody A1 (anti-lateral element – white) and GS (centromeres – red). (*a*) Nucleus in which axial element material has a disjointed, angular appearance. The centromeres are loosely clustered towards the centre of the nucleus. (*b*) Nucleus with dramatic 'stellar' appearance. The centromeres are tightly clustered in the centre of the nucleus, with axial elements emanating from this central point to the periphery of the nucleus. The axial elements are arranged in pairs in a 'diplotene-like' configuration. (*c*) Nucleus with similar 'stellar' appearance to that shown in (*b*). The axial elements are again arranged in pairs but in this case remain closely associated with many 'bridges' joining them. (*d*) Nucleus with a central dramatic clustering of centromeres, but with only remnants of anti-lateral element labelling throughout the nucleus.

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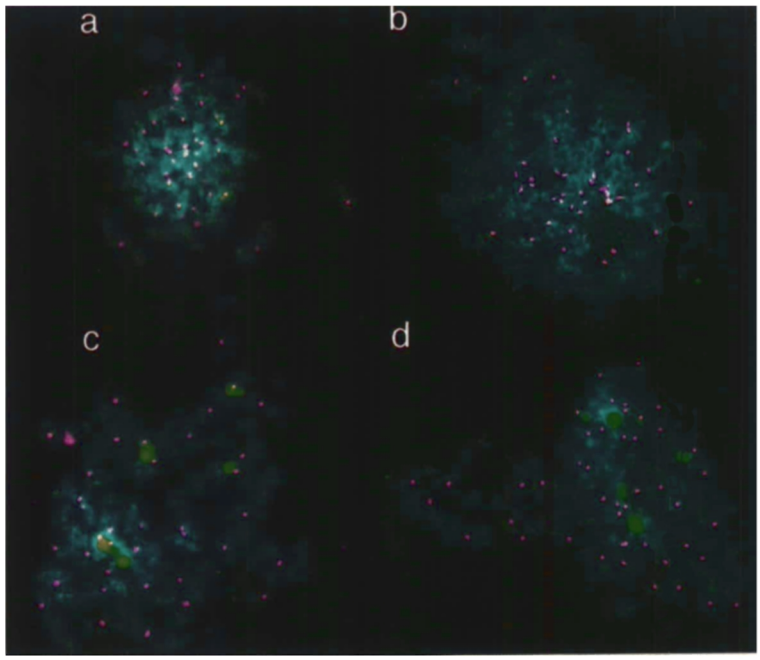

Plate 4. Microspread ovarian nuclei, showing distinct chromosome domains, labelling with antibody A1 (green), and 42–46 separate centromeres. (*a*), (*b*) Nuclei in which 42 (*a*) and 46 (*b*) separate centromeres are visible. Poor spreading has resulted in the clustering of the majority of the chromosomes; a few peripheral chromosomes are clearly visible. Note the speckled labelling of antibody A1. (*c*), (*d*) Nuclei in which 43 (*c*) and 44 (*d*) separate centromeres are visible; distinct chromosomal regions are visible. The antibody A1 labelling pattern of these nuclei shows evidence of angular and disjointed linear structures. Scale bar represents 10 μm.

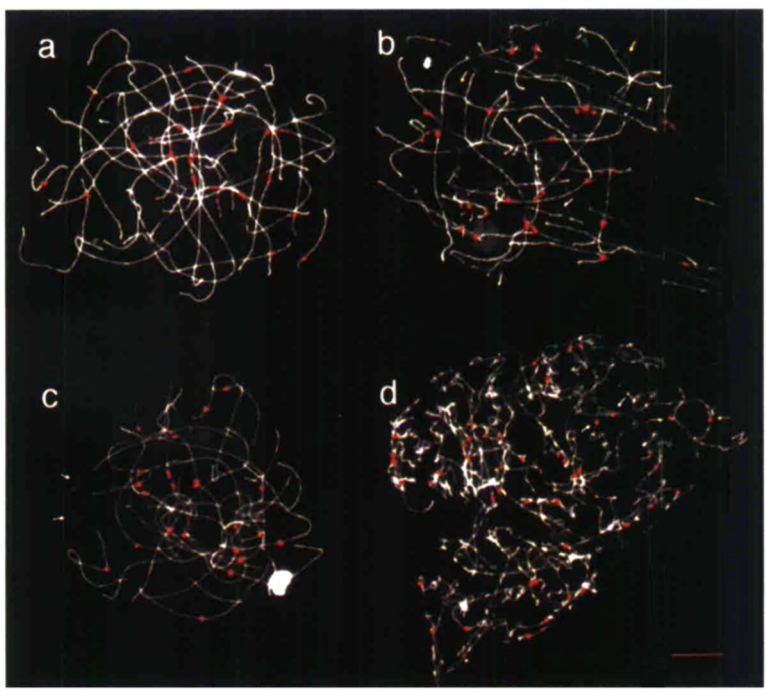

Plate 5. Microspread aberrant human oocytes labelled with antibody A1 (anti-lateral element – white) and GS (centromeres – red). (*a*) Oocyte demonstrating a region of terminal non-homologous asynapsis. The exclusion of a small loop of axial element from the synaptonemal complex (yellow arrow) results in the telomeres of this bivalent being out of register with a short region of axial element protruding, asynapsed beyond the end of the other (white arrow). (*b*) Oocyte with aberrant synaptonemal complexes. Both loops (white arrows) and rods (yellow arrows) can be seen protruding from the SCs of this nucleus. (*c*) Late zygotene nucleus in which a peripheral univalent chromosome (probably 21) can be seen (arrows). Such univalents can be distinguished on the basis of their thinner appearance and weaker labelling than bivalents. (*d*) Diplotene oocyte; examples of homologous centromeres are arrowed. The lateral elements of this nucleus have an angular, wrinkled appearance.

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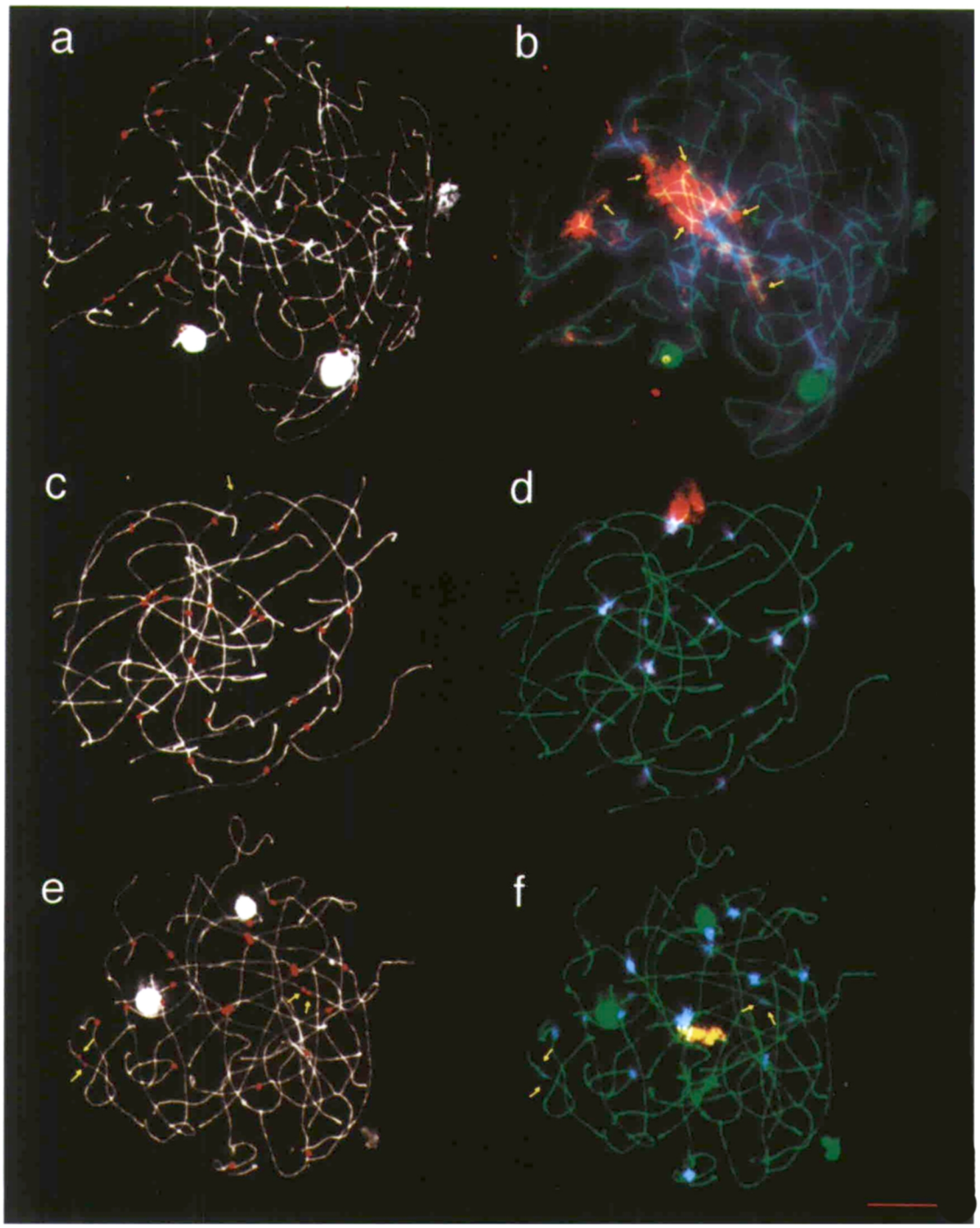

Plate 6. Pachytene nuclei used for fluorescence *in situ* hybridisation (FISH) experiments. (*a*) Pre-FISH image of microspread pachytene oocyte labelled with antibody A1 (anti-lateral element – white) and GS (centromeres – red). (*b*) Same nucleus as (*a*) labelled with antibody A1 (green), DAPI (blue) and whole chromosome 1 paint (red). The convoluted chromosome 1 bivalent can be followed throughout the nucleus (yellow arrows). The identity of this bivalent is confirmed by the presence of its large heterochromatic block (red arrows). Scale bar represents 10 μm. (*c*) Pre-FISH image of microspread pachytene oocyte labelled with antibody A1 (anti-lateral element – white) and GS (centromeres – red). Note the presence of a small region of interstitial asynapsis (arrow). (*d*) Same nucleus as (*c*) labelled with antibody A1 (green), pan-centromeric probe (blue) and chromosome 1 heterochromatic probe (red). This image shows that the region of asynapsis corresponds to the chromosome 1 heterochromatic block (*e*) Pre-FISH image of microspread pachytene oocyte labelled with antibody A1 (anti-lateral element – white) and GS (centromeres – red). Note the presence of two bivalents showing two staggered centromeric regions. (*f*) Same nucleus as (*e*) labelled with antibody A1 (green), pan-centromeric probe (blue) and chromosome 1 heterochromatic probe (red). The pan-centromeric probe confirms that the staggered centromeres do represent two separate regions of alpha satellite DNA (yellow arrows). Scale bar represents 10 μm.

(*Facing p.* 33)

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logical framework into which she could fit all the nuclei reconstructed. Leptotene nuclei in which a full complement of long thin axial elements are present (Plate 1*e*) were not described by Speed (1985, 1988), but such nuclei presumably represent an intermediate stage between nuclei in which only fragments of axial element material are present and nuclei in which short fattened axial elements are present. The nuclei observed during this study in which the axial elements were unpaired, thickened and nodular (Plate 1*f*) may represent a step down the degenerative path proposed by Speed (1988) for asynaptic cells.

Zygotene nuclei observed during this study, in common with leptotene nuclei, were of two types: those in which only the axial elements of synapsed telomeric regions of bivalents were fully formed, with the interstitial regions speckled and incomplete (Plate 1*b*) and those in which a full complement of axial elements was present with a small degree of synapsis (Plate 1*d*). The latter were considered by Speed (1985, 1988) and Speed & Chandley (1990) to be 'multiply asynaptic' and therefore destined for degeneration. The former type, which usually displays a 'bouquet' configuration, an apparently universal feature of eukaryotic meiosis (Scherthan, 1996), seems far more likely to represent the normal situation (Plate 1*b*). Such nuclei illustrate exactly what Speed meant in his statement 'classical leptotene does not exist as such in human oocytes', as the partially formed interstitial regions of zygotene nuclei show unequivocally that a full complement of axial elements does not exist prior to the onset of synapsis. The occurrence of small pairing forks in telomeric regions of early zygotene nuclei demonstrates that in the human female synaptic initiation is subtelomeric (Plate 1*b*, yellow arrows). This may contribute to the less terminal location of chiasmata in human females than males (Hultén *et al.*, 1978), with terminal forks not seen in males (e.g. Speed & Chandley, 1990).

This is the first study of microspread human oocytes in which centromeric regions could be accurately and reliably identified in all nuclei. This additional information has allowed the identification of 'centromeric associations' and 'staggered centromeres'. 'Centromeric associations' were demonstrated in air-dried human pachytene spermatocytes after C-banding or G11 staining by Driscoll *et al.* (1979), who observed multiple connections, capable of extending long distances across the nucleus. 'Centromeric associations' were observed by Wallace & Hultén (1985) who noted 'two or three pairs of bivalents were joined by short lengths of fibrillar material' in six pachytene human oocytes. They speculated that these attachments may link centromere regions but could not relate them to any structural features with certainty. Antiserum GS shows that the 'fibrillar material' not only links centromeric regions, but is composed of centromeric proteins. Centromeric clustering was demonstrated in mouse and human in preleptotene spermatocytes by Schertan *et al.* (1996) using centromeric (major satellite or alpha satellite) and telomeric probes against paraffin-embedded testis sections. These clusters consisted of centromeres pressed tightly against the nuclear envelope, and presumably represent the stage during which 'centromeric associations' form, some of which clearly persist until pachytene. Electron microscopic reconstruction of serially sectioned human spermatocytes has also produced evidence of centromeric associations; Holm & Rasmussen (1977) described fusion of the centromeric heterochromatin of bivalents 1, 9 and 16 as well as fusion between centromeric heterochromatin of two or more bivalents. The observation of leptotene nuclei with full complements of asynapsed axial elements (multiply asynaptic cells) with centromeric associations between possible homologues (Plate 1*c*, green arrows) suggests that centromeric regions may have a role in co-localisation of homologues prior to synapsis. Examples of meiocytes in which 'centromeric associations' exist in plants that bear a striking resemblance to those of human females (compare the centromeres of *Allium fistulosum* in Jones & Albini (1988) with those in Plate 2*a*), indicate that 'centromeric associations' are highly conserved. The demonstration of 'centromeric associations' in human lymphocyte metaphases (Schmid *et al.*, 1975) raises the possibility that such features are a general characteristic of all nuclei, and are of no particular significance to meiotic prophase.

The presence of 'staggered centromeres' in pachytene bivalents of human oocytes, in which single bivalents show two separate centromeric regions separated by a short distance of normal SC, indicate that within apparently normal bivalents in which telomeres are in register there exist regions of what must be non-homologous synapsis. FISH data from the pan-centromeric probe confirm unquivocally that serum GS accurately labels the sites of alpha satellite repeats (Plate 6*e,f*), and that the labelling pattern is not artefactual. The presence of non-homologously synapsed regions in human oocytes is well documented (Garcia *et al.*, 1987; Speed, 1985, 1988), but these cases all involve the synapsis of non-homologous chromosomes or self-synapsis, which is markedly different from the presence of non-homologous synapsis within homologous bivalents. The size of these regions of non-homologous synapsis, i.e. whether they affect large regions of bivalents or are restricted to centromeric regions (where they are visible), or whether non-homologous synapsis exists in bivalents with centromeres 'in register' in regions where it would not be visible, is

unclear. It has been shown in elegant cytogenetic observations of mice heterozygous for a tandem duplication that the progress of synapsis from initiation sites is dependent upon homology and is blocked by lack of homology in zygotene (Moses & Poorman, 1981). It was also shown in this and other studies (e.g. Moses, 1977; Saadallah & Hultén, 1986) that the restriction of homology is not lasting and that by pachytene heterosynapsis may occur with the formation of normal-appearing SCs. It is possible that regions of heterosynapsis resulting in staggered centromeres arise in human females due to the presence of asynaptic segments at pachytene that are eliminated without continuous homology checks, allowing synapsis to proceed 'out of register'. However, the observation of a small number of zygotene nuclei with staggered centromeres (Tables 2, 3) suggests that they may arise as the result of heterosynapsis without previous homosynapsis, a phenomenon that has been demonstrated in human spermatocytes (Saadallah & Hultén, 1986; Gabriel-Robez *et al.*, 1988) and oocytes (Speed, 1988). As meiotic recombination is known to occur only between homologous chromosomal segments, the presence of regions of non-homologous synapsis within bivalents raises the question of whether the distribution of chiasmata is affected by this phenomenon.

The presence of what were initially suspected, and later confirmed using FISH, to be asynapsed chromosome 1 heterochromatic blocks in two nuclei from the 16 wks A fetus is a confirmation of what has been observed in light microscope studies of microspread silver-stained oocytes (Wallace & Hultén, 1985), although these authors also observed the heterochromatic blocks of chromosome 9 and 16 asynapsed. Asynapsed heterochromatic blocks of chromosomes 1, 9 and 16 have been observed during electron microscopic studies of microspread silver-stained spermatocytes (Solari, 1980). Speed (1988) studied the oocytes of a fetus whose somatic karyotypes showed dramatically different amounts of centromeric heterochromatin for chromosome 16. Four per cent of the oocytes of this fetus contained an unpaired region looped out in the central region of one SC. Speed concluded that the polymorphism may have been the cause of the localised synaptic disruption (Speed 1988). A similar polymorphism for the heterochromatic block of chromosome 1 may be the reason for the presence of asynapsed 1 het blocks with asynapsed axial elements of clearly different lengths from two nuclei from this fetus (Plate 2*b*, yellow arrow). Unfortunately fetal somatic karyotypes were not available to confirm this. Speed (1988) concluded that only early nuclei would contain such asynapsed regions, based on evidence from mice heterozygous for a tandem duplication. In the SCs of these mice 'buckles' of asynapsis were present in early nuclei, but eliminated from later ones through the process of synaptic adjustment (Moses & Poorman, 1981).

The use of antisera A1 and GS has led to the observation of a spectacular and previously undescribed category of nucleus: the 'stellar nucleus' (Plate 3). The presence of axial/lateral element material within these nuclei indicates that they are prophase I nuclei, but they clearly do not fit into the classically recognised prophase I stages. The most striking feature of stellar nuclei is the tight central cluster of centromeres – compelling evidence that centromeres can have kinetic properties during the prophase of meiosis. The small number of 'stellar' nuclei observed and their highly unusual appearance suggests that they are not part of the usual meiotic progression, but are more likely to represent some form of degenerative phase. In two examples (Plate 3*b*,*c*) the axial elements had a 'diplotene'-like appearance, with axial/lateral elements present in pairs that are intimately linked at various points. Atresia of diplotene nuclei has been recognised as a major degenerative route in previous reports. Baker (1963), in a study of both squashed and wax-embedded oocytes, described atretic diplotene cells that were characterised by contraction of the chromatin material and wrinkling of the nuclear membrane material, with the highest concentration of such cells found in full-term and early postnatal specimens. Kurilo (1981), in a study of both spread and paraffin-embedded oocytes, described atretic diplotene nuclei with decreased nuclear volume, contours in the cells membrane and condensed chromosomes. The implosive appearance of 'stellar' nuclei, and the wrinkled appearance of their axial/lateral elements, seem to correlate well with Baker's and Kurilo's descriptions. The observation of a nucleus with a tight central cluster of centromeres but only speckled labelling from antibody A1 (Plate 3*d*) supports the notion that 'stellar' nuclei are degenerative.

An alternative explanation of stellar nuclei is that they represent dictyotene nuclei – a stage that has generally not been recognised in studies of human oocytes (e.g. Baker & Franchi, 1967) or for which there are only vague descriptions. A diffuse late diplotene stage was the latest stage recognised by Wallace & Hultén (1985) and dictyotene nuclei were thought to contain dispersed lateral elements and a fine background of chromatin threads by Speed (1985).

A class of nucleus frequently observed during this study from the 15 wks and 16 wks B fetuses are those containing condensed chromatin, which was divided into apparent chromosomal domains that were labelled by antibody A1 in a speckled pattern. The speckling varied between simple dots (Plate 4*a*,*b*) and the presence of some fragmented linear structures

(Plate 4*c*,*d*). Such nuclei have not been described in any previous studies of microspread human oocytes; however, strikingly similar nuclei have been described during studies of squashed, air-dried and wax-embedded material. Stahl & Luciani (1972) were the first to identify such highly condensed chromosomes in a study of squashed and air-dried oocytes. They regarded these highly condensed chromosomes as representing a pre-meiotic 'prochromosome' stage, and claimed to recognise 'spiralisation', 'full spiralisation' and 'despiralisation' phases, with the 'despiralisation' phase leading into leptotene. Stahl & Luciani (1972) compared their 'prochromosomes' with pre-leptotene spiralised chromosomes that have been described in plants (Walters, 1970, and references therein). Similar chromosomes were observed by Therman & Sarto (1977) in a study of squashed oocytes, though their interpretation was somewhat different. They concluded that the last pre-meiotic metaphase chromosomes do not unravel, but appear as 'prochromosomes' in the next interphase. 'Prochromosomes' were recognised as a genuine phase by Kurilo (1981) in a study of the squashed and wax-embedded oocytes of 97 fetuses. Kurilo (1981) saw strongly condensed chromosomes as compact irregular structures that were present 4 weeks prior to leptotene chromosomes. Subsequent studies provide ambiguous evidence as to whether 'prochromosomes' are a genuine feature of human female meiosis. Wallace & Hultén (1985) did not recognise 'prochromosomes' in microspread preparations but saw them in orcein squashes of several ovaries. Cheng & Gartler (1994) and Cheng *et al.* (1995) in FISH painting studies of squashed human fetal oocytes made no direct reference to 'prochromosomes', and referred to leptotene chromosomes as diffuse 'with no indication of typical chromosome configuration'. With their ability to easily identify chromosomal domains these authors would be ideally placed to spot 'prochromosomes'. Schertan *et al.* (1996), on the other hand, did observe compacted chromosome territories during a study of wax-embedded human spermatocytes, although they also made no direct reference to 'prochromosomes'. If a genuine 'prochromosome' stage exists during human female meiosis, and this is far from clear, it is plausible that the condensed, speckled chromosomal domains shown in Plate 4 represent prochromosomes. The 'speckling' pattern produced by antibody A1 for these nuclei may represent labelling of axial element proteins that would form an early leptotene pattern (Plate 1*a*) upon completion of the 'despiralisation' phase. An alternative explanation for these nuclei is that they represent a degenerative pathway; the lack of evidence of any pairing in these nuclei with chromatin present in individual domains, would mean that they represent degeneration prior to any synaptic stage. The 'multiply asynaptic' cells observed during this study and regarded by Speed (1985, 1988) as being degenerate, could be the source of such degenerative nuclei. Apoptotic somatic cells are known to have highly condensed chromatin (reviewed by Jacobson *et al.*, 1997).

The observation of microspread oocytes labelled with antisera A1 and GS has allowed the identification of nuclei displaying various types of aberration. The ability of any method of study to distinguish such aberrant nuclei from the normal population is crucial in determining the usefulness of the technique. The nucleus shown in Plate 5*a* contains a bivalent in which a 'buckled' axial element has resulted in the terminal region of the bivalent synapsing non-homologously and one axial element protruding beyond the other. Non-homologous synapsis has been described as a common feature of human oocytes. Speed (1985) recorded non-homologous synapsis consisting of synapsis between lateral elements from two non-homologues, or self-pairing of a univalent at a rate of 4.4%. In a later study, Speed (1988) described non-homologously synapsed bivalents, consisting of simple self-synapsis at a rate of 1.8%, and synapsis of non-homologous axes at a rate of 7.6%; he also observed triple associations of axes in 4.3% of oocytes from normal fetuses. Fewer numbers of nuclei containing non-homologous synapsis and fewer types of non-homologous synapsis were observed during this study than were observed by Speed (1988). Clearly the resolution of Speed's electron microscope studies was far greater than this light microscopic study, allowing him to identify a far greater range of non-homologously synapsed nuclei. The loss of resolution between the light and electron microscopes appears to severely impair the ability to recognise this feature.

The nucleus shown in Plate 5*b* contains aberrant SCs with protruding rods and loops (Plate 5*b*, arrows). Very similar features were observed by Garcia *et al.* (1987) in all stages of prophase I from a single fetus during both light and electron microscopic studies of silver-stained microspread oocytes. Garcia *et al.* (1987) speculated that these projections might provide support for models of SCs as multi-stranded features (e.g. Heyting *et al.*, 1985; del Mazo & Gil Alberdi, 1986; Dietrich *et al.*, 1992). Clearly the immunocytogenetic techniques employed during this study do not hinder the ability to identify such features.

A clear univalent was detected in the nucleus shown in Plate 5*c*. Univalents have been detected in previous studies of microspread oocytes from normal females. Speed (1985) detected univalents at a rate of 2 in 154 during light microscopic studies and 17 in 162 during electron microscopic studies of silver-stained microspread oocytes. Speed's (1985) data clearly indicate that electron microscopic studies are

far more effective than light microscopic studies in the detection of univalents. However, univalents that have been stained with non-specific heavy metal stains such as silver-nitrate or uranyl acetate develop a thickened appearance, making them difficult to distinguish from bivalents in the light microscope (e.g. Wallace & Hultén, 1983; Speed, 1985, 1988). Univalents labelled with anti-lateral element antibodies remain thin in appearance, making them easier to spot with fluorescence microscopy and indicating that the proteins that accumulate to produce the thickening are not lateral element proteins.

An apparently late diplotene nucleus was observed with angular, nodular lateral elements. The observation of few diplotene nuclei during this study (Table 1) make it unclear whether this single nucleus is representative of human diplotene oocytes; however, data from previous studies of microspread oocytes indicate that it is not. Studies by Wallace & Hultén (1985), Speed (1985) and Garcia *et al.* (1987) all suggest that the lateral elements of diplotene oocytes are not angular or nodular. It therefore seems likely that this nucleus is either aberrant or degenerative, especially as diplotene nuclei have been regarded as a significant source of atresia, with these cells characterised by shrinkage and wrinkling of membranes (Baker, 1963; Kurilo, 1981).

The success of attempts to use immunocytogenetically labelled microspread oocytes as substrates for FISH experiments proved to be highly dependent upon the type of probe used, with repetitive probes being universally successful (Plate 6*d*,*f*) whilst single-copy probes and whole-chromosome paints (Plate 6*b*) proved much more problematic. The reasons for this discrepancy are unclear, but may be related to degenerative changes that occur to SC-associated chromatin between fetal demise and fixation with formaldehyde. Human female oocytes have been used highly successfully as substrates for chromosome painting in studies of normal and trisomy 18 fetuses (Cheng & Gartler, 1994; Cheng *et al.*, 1995) and of a chromosomally rearranged fetus (Barlow & Hultén, 1997), so there is no fundamental reason why paints should not be successfully utilised against microspread oocytes. Cheng & Gartler (1994) and Cheng *et al.* (1995) did stress that freshness of the specimens was critical to hybridisation efficiency; success with repetitive but not single-copy probes therefore suggests that the specimens used for this study were 'borderline' with respect to freshness. Every attempt was made to process specimens in the minimum time possible, but nothing could be done to decrease the delay between termination and removal of ovaries.

Numerous examples of FISH against SC-associated chromatin now exist in the literature and the patterns obtained have become relatively predictable. The tight association of telomeric sequences to the SC is well established (Moens & Pearlman, 1990): single-copy sequences have been shown to produce signals in the form of spots (Heng *et al.*, 1994), with other sequences forming loops that associate with SCs only at their bases (Barlow & Hultén, 1996). Heng *et al.* (1994, 1996) have presented evidence that chromatin packaging mechanisms (loop size) are dependent upon both DNA sequence and chromosomal position. Terminal sequences form small loops and remain tightly associated with SCs whilst interstitial sequences form large loops (Heng *et al.*, 1996). Our data from human females appear to conform with the model of Heng *et al.* (1996).

Comparison of the meiotic stages present in all three fetuses reveals some striking differences between the three, such as the total absence of leptotene nuclei with partially polymerised axial elements from the 16 wks B fetus, and the very large number of nuclei containing condensed, speckled chromatin in the same fetus. This may suggest that the nuclei in the earliest stages of prophase in this fetus have degenerated into the condensed speckled nuclei. If this is the case, it raises the possibility that the structures regarded by other authors as representing prochromosomes (e.g. Stahl & Luciani, 1972; Kurilo, 1981) may represent degenerative structures and not a genuine phase at all. Further comparison of the stages present in all three fetuses (Table 1) reveals that each had nuclei in all stages from leptotene to diplotene, although the numbers of nuclei at each stage varied greatly despite the similar ages of the three fetuses. Comparison with other authors reveals little consensus, with enormous discrepancies existing. For example, Speed (1985) found no diplotene nuclei in a 16 weeks gestation fetus (Speed only reported diplotene at 23 weeks), whilst Garcia *et al.* (1987) found this to be by far the most common stage in four fetuses of the same age. This study is somewhere between these two extremes, with diplotene nuclei found from all three fetuses in low numbers. Some have taken such huge discrepancies as a symptom of differing interpretations of material that has been studied with various techniques (Cheng & Gartler, 1994). Whilst this conclusion is plausible, it could equally be the case that human female meiosis is a highly variable process, with the published discrepancies reflecting genuine differences. The numbers of individuals studied is still far too low to discriminate between these two possibilities, and electron microscopic studies are too time-consuming to generate large quantities of data without the investment of much time and money. Immunocytogenetic studies, on the other hand, have the potential to generate large quantities of data of adequate resolution in a relatively short time.

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