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Synapsis and meiotic recombination analyses: MLH1 focus in the XY pair as an indicator

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BACKGROUND: Anomalies in meiotic prophase I have been related to partial or total meiotic arrest. These anomalies include an abnormal synaptic process, resulting in disorders in meiotic recombination. METHODS: In the present study, we analyse primary spermatocytes from 12 infertile men (four with non-obstructive azoospermia, six with oligoastenoteratozoospermia, one with astenoteratozoospermia and one normozoospermic) and five control fertile donors using immunocytological techniques for synaptonemal complex, meiotic recombination and centromeric proteins. RESULTS: Mean numbers of MLH1 foci per cell, frequencies of cells presenting an MLH1 focus in the XY pair and percentages of cells affected by abnormal synaptic patterns (gaps and splits) are reported for each of the infertile patients and control men. A positive correlation between the frequency of cells showing a recombination focus in the XY pair and the number of autosomal recombination foci per cell is found. CONCLUSIONS: Reduced recombination in the XY pair and an increased number of cells affected by gaps may explain some idiopathic male infertility cases. The results suggest that recombination in the XY pair could be an indicator for general recombination frequency and for a successful meiotic process.

Key words: male infertility/meiotic recombination/pachytene/synaptonemal complexes/XY pair

Introduction

Meiosis is the process by which the whole genome of a cell is reduced to half to produce genetically different haploid cells for sexual reproduction. During meiotic prophase I, homologous chromosomes pair, synapse and recombine. These three events are essential for a successful meiotic process; any alteration of the meiotic process results in a reduction of the quality and quantity of gametes and, in the most severe cases, it can cause a complete meiotic arrest. Several meiotic checkpoints have been proposed to control this process (Roeder and Bailis, 2000). When any of the meiotic sequential events does not occur properly, the checkpoint switches on and prevents the cell from proceeding.

The incidence of infertility in human males is ~10%. Synaptic anomalies in meiotic chromosomes have been described and associated with male infertility. Indeed, up to 8% of the general infertile population show meiotic defects, and 74% of them correspond to an abnormal synapsis (revised in Egozcue *et al.*, 2005). A correlation between a higher number of meiotic abnormalities and more severely affected semenograms has also been reported (Vendrell *et al.*, 1999).

When chromosomes synapse, a tri-axial proteinaceous core, the synaptonemal complex (SC), is built along the pairing axis. At pachytene, homologous chromosomes are fully synapsed. Some structural meiosis-specific proteins of the SC, SCP1, SCP2 and SCP3, have been identified in the last few years (Meuwissen *et al.*, 1992; Lammers *et al.*, 1994; Schalk *et al.*, 1998). Mutations of the *Scp3* gene in mice (Yuan *et al.*, 2000) and in human males (Miyamoto *et al.*, 2003) demonstrated that defects in synapsis of homologous chromosomes at pachytene arrests the meiotic process and leads to infertility. In the past, studies of the SC were performed using unspecific protein staining ($AgNO_3$). In a series of infertile men, abnormal synaptic processes were reported (Navarro *et al.*, 1986). Since the development of immunolabelling methods, the interest in SC analysis has been renewed. Recently, abnormal synaptic processes have been reported in two azoospermic men by immunocytogenetic analysis of their spermatocytes (Judis *et al.*, 2004; Sun *et al.*, 2004a).

Disorders in meiotic recombination have also been described as a possible cause of meiotic arrest (revised in Egozcue *et al.*, 2005). A DNA mismatch repair protein MLH1 (Baker *et al.*, 1996; Barlow and Hultén, 1998; Anderson *et al.*, 1999) and a cyclin-dependent kinase Cdk2 (Ashley *et al.*, 2001) co-localize in late recombination nodules. Both of them have been shown to be involved in reciprocal recombination. A reduction in the MLH1 foci

number per cell has also been reported in some azoospermic men (Gonsalves *et al.*, 2004; Sun *et al.*, 2004a).

The aim of this study was to analyse synapsis and meiotic recombination patterns in infertile and control men. Spermatocyte spreads were immunolabelled by using antibodies against synaptonemal complex proteins (SCP3 and SCP1), a late recombination nodule protein (MLH1) and centromeric proteins (CENP).

Materials and methods

Testicular biopsies from 12 infertile patients and from five control donors, of proven fertility, were obtained under local anaesthesia. Infertility patients were diagnosed after 2 years of pregnancy failure. Written consent was given by all patients, and the study was approved by the Institutional Ethics Committee.

Four infertile patients were azoospermic (AZO 1–4), six oligoasthenoteratozoospermic (OTA 1–6), one astenoteratozoospermic (AST) and another normozoospermic (NOR). Semenograms were classified according to the WHO parameters (World Health Organization, 1999). In the control group, the testicular biopsies of patients C1, C2 and C3 were obtained while they were undergoing vasectomy. Testicular biopsies of patients C4 and C5 were obtained while they were undergoing a vasectomy reversal.

Sample treatment

The testicular tissue was processed for meiotic chromosome analysis (Evans *et al.*, 1964) and for SC immunocytogenetic analysis (Codina-Pascual *et al.*, 2004). For the immunocytology of spermatocytes, the primary antibodies used were rabbit anti-SCP3 (Lammers *et al.*, 1994) and rabbit anti-SCP1 (Meuwissen *et al.*, 1992) (both gifts from Dr Christa Heyting, University of Wageningen, The Netherlands), anti-CENP (CREST serum given by Dr William Earnshaw, University of Edinburgh, UK) and mouse anti-MLH1 (Pharmingen, San Diego, CA). The secondary antibodies applied were tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit IgG antibody and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG antibody (both from Sigma; Madrid, Spain). The Pacific Blue-conjugated rabbit anti-human IgG (from Sigma; Madrid, Spain) labelled with Zenon Reaction (Molecular Probes, Spain) was applied in a third round. Finally, slides were counterstained with antifade (Vector lab Inc., Burlingame, CA). A fluorescent photomicroscope (Olympus B × 60) and Power Macintosh G3 with Smartcapture software (Digital Scientific, Cambridge, UK) were used for cell evaluation and image capture.

Cell analysis

Pachytene cells immunolabelled for SCP3, SCP1, MLH1 and CENP were captured and analysed. We considered as pachytene only the cells in which the XY pair was identifiable. Only pachytene nuclei with clear MLH1 labelling were included in the study. Cells were analysed according to three main variables: pachytene stage, meiotic recombination and synapsis.

According to the XY pair stages (Solari, 1980), nuclei were classified as early pachytene (stages 1 and 2) or late pachytene (stages 3, 4 and 5) (Figure 1a).

For meiotic recombination analysis, the number of MLH1 foci per cell and the presence of an MLH1 focus in the XY pair were evaluated.

To analyse synapsis, the existence of discontinuities in the SCs (gaps) and of splits in bivalents (unpaired lateral elements) was analysed. Based on the number of gaps present in the cell, nuclei were initially classified into four groups: NA, nuclei not affected by gaps; SA, nuclei slightly affected having from one to two SCs with gaps; MA, nuclei moderately affected with three or more SCs with gaps; and HA, nuclei highly affected with gaps in all the SCs. However, for data analysis the NA+SA and MA+HA groups were joined, respectively, into normal and gap-affected nuclei groups.

Data analysis

The χ^2 test and Fisher test were applied when needed for qualitative data analysis. The Student test and Mann–Whitney test were applied to quantitative data comparisons between two groups. For other quantitative comparisons, analysis of variance (ANOVA) was used. Pearson's correlation coefficient was calculated for correlation analysis.

Results

Classic meiotic chromosome analysis results are displayed in Table I.

For immunocytogenetic analysis, a total of 846 pachytene nuclei, 224 for the control group and 622 for the infertile men, were studied. Table I shows the number of cells analysed and the frequency of early pachytenes (complementary to that of late pachytenes) for the infertile cases, for each semenogram group and for controls. In the control group, 35.5% of pachytene cells were classified as early pachytene and the rest (64.5%) as late (Figure 1a). Similar results were found for the infertile group in which 34.4% were early and 65.6% were late pachytenes. Interindividual differences in the early and late pachytene percentages are detected in both control and infertile groups ($P < 0.0005$). Early pachytene ranged from 18.8 to 52.8% for the control group and from 17.9 to 61.7% for the infertile group.

MLH1 foci analysis

The number of MLH1 foci was scored per each cell. Table I also displays the mean number of MLH1 foci per pachytene and the frequency of cells presenting an MLH1 focus in the XY pair for the analysed cases. The mean number of MLH1 foci per cell observed in controls is 48.8 ± 2.3 and ranges from 36 to 63 foci per cell. In the infertile group, a mean of 47.3 ± 3.3 foci per cell is found, ranging from 34 to 66, which is not different from the control results. A significant interindividual variation in the average number of MLH1 foci per cell in all cases is detected ($P < 0.0005$), ranging from 42.9 ± 2.1 to 52.3 ± 4.2 MLH1 foci (Figure 2). However, two infertile cases showing univalents in metaphase I chromosome spreads (OTA3 and OTA4) and the NOR patient present a significant reduction in the number of MLH1 foci per cell when compared with the mean ($P < 0.0001$) and minimum ($P < 0.003$) control values (Table I). Pachytene nuclei were also classified regarding the presence or absence of an MLH1 focus in the XY pair. In the infertile group, the mean average of pachytene cells having an MLH1 focus in the XY pair (59.2%, range 40–76.2%) is not significantly lower than that seen in the control group (69.9%, range 59.7–80.5%). In both control and infertile groups, the average frequencies of cells showing an MLH1 focus in the XY pair in early pachytene cells (72.7

and 61.7%, respectively) are not significantly different from the average frequencies observed in late pachytenes (67.9 and 58.2%, respectively). Two cases, OTA6 and AZO4, displayed a significantly lower number of cells presenting an MLH1 focus in the XY pair (40 and 45%, respectively) when compared with the control mean value (69.9%) ($P < 0.006$). However, when individually comparing the above results with the lower control value (59.7%), the difference is not significant (Table I).

The mean number of autosomal MLH1 foci per cell was compared between the nuclei with an MLH1 focus in the XY pair and the nuclei without an MLH1 focus. Interestingly, the number of autosomal MLH1 foci per cell displayed a relationship with the presence or absence of the MLH1 focus

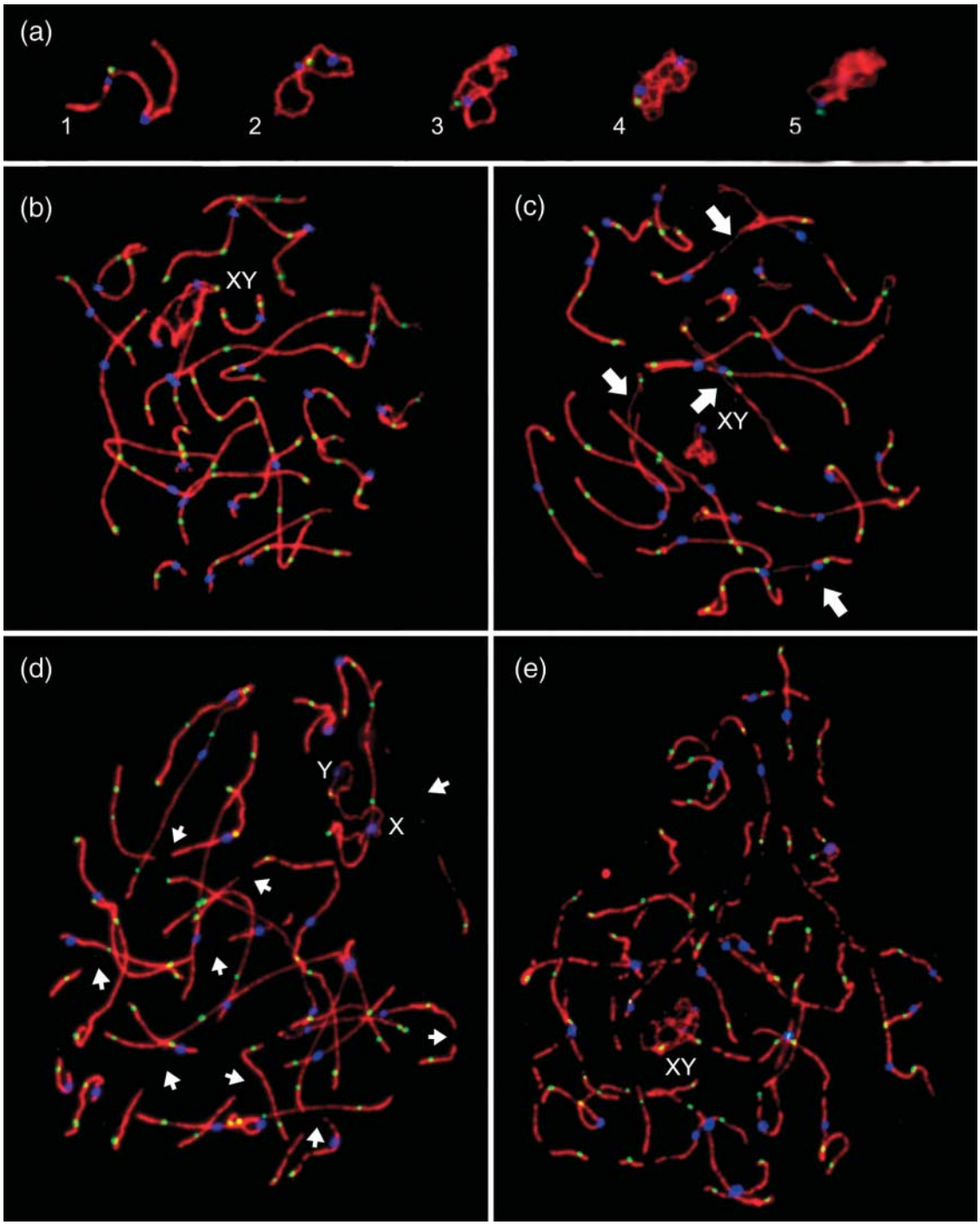

Figure 1. Immunolabelled pachytene cells with synaptonemal complexes (SCs) in red, MLH1 in green and the centromere in blue. XY is identified in each cell. (**a**) XY pair morphologies observed during pachytene. XY pair stages 1 and 2 correspond to early pachytene (EP) and stages 3, 4 and 5 to late pachytene (LP). (**b**) Normal pachytene cell of a control donor. (**c**) Pachytene cell of an infertile patient showing multiple splits (large arrows). (**d**) Pachytene cell of an infertile patient showing multiple gaps (small arrows). (**e**) Pachytene cell of an infertile patient showing a generalized fragmentation of the SCs.

Table I. MLH1 immunocytogenetic results in spermatocytes of infertile and control men

	Age (years)	Meiotic chromosome analysis	Immunocytogenetic analysis				
			n	Early pachytene (% cells)	MLH1 foci/cell		MLH1 in XY (% cells)
					Mean	SD	
Infertile men ($\Sigma n = 622$ cells)							
AZO1	31	Ma	47	61.7	49.5	4.2	57.4
AZO2	26	Ma	103	39.8	52.3	4.2	68
AZO3	41	NA	84	17.9	48.3	6.2	76.2
AZO4	30	Ma	40	30	49.3	5.2	45^{Dd}
Mean				37.4	49.9	1.7	61.7
OTA1	35	Ach	59	37.3	50.7	5.1	72.9
OTA2	33	Ma	45	35.6	49.9	5.3	64.4
OTA3	33	Ach	38	34.2	43.3^{Aa}	3.5	55.3
OTA4	39	Ach	39	33.3	42.9^{Bb}	2.1	53.8
OTA5	35	NPI, Ma	41	29.3	46.8	5	48.8
OTA6	36	Ma	25	32	44.2	4.1	40^{Ee}
Mean				33.6	46.3	3.4	55.9
AST	31	Ma	45	40	47.4	3.8	73.3
NOR	35	NM	56	21.4	43.1^{Cc}	4	55.4
Total mean				34.4	47.3	3.3	59.2
Control men ($\Sigma n = 224$ cells)							
C1	34	NM	48	18.8	45.8^{abc}	4.1	64.6
C2	35	NM	41	34.1	51.2	4.6	80.5
C3	37	NM	72	52.8	47	4.4	59.7^{de}
C4	43	NM	37	48.6	49.3	4.4	67.6
C5	38	NM	26	23.1	50.6	3.9	76.9
Mean				35.5	48.8^{ABC}	2.3	69.9^{DE}

Frequencies of early pachytene, means of MLH1 foci per cell and frequencies of XY pair with an MLH1 focus are indicated.
Upper case superscripts indicate comparisons with mean control values. Lower case superscripts indicate comparisons with the minimum control value. $^{abc}P < 0.003$; $^{ABC}P < 0.0001$; ^{de}NS; $^{DE}P < 0.006$.
Ma = partial or total meiotic arrest; Ach = achiasmatic bivalents; NPI = normal prophase I; NM = normal meiosis; NA = not available.

in the XY pair ($P = 0.001$). The mean value of autosomal MLH1 foci per cell was significantly higher in nuclei with an MLH1 focus in the XY pair. This increase was found for both control ($P = 0.022$) and infertile ($P = 0.004$) groups. A significant correlation between the frequency of cells with an MLH1 focus in the XY pair and the mean number of autosomal MLH1 foci per nuclei was observed for the 17 cases ($P = 0.013$, $r^2 = 0.34$) (Figure 3).

Synapsis analysis

Unsynapsed bivalent regions (splits) were seen in both control (11.7%) and infertile (11.2%) groups (Figure 1c). Table II shows the frequencies of pachytene nuclei showing a single split and multiple splits in SC regions in infertile patients and in control men. High interindividual variation in the presence of splits is detected in the control ($P = 0.002$) and in the infertile groups ($P < 0.0005$). Observed ranges are from 2.4 to 29.2% in control donors and from 0 to 26.2% in infertile patients. In general, the presence of a single small split is much more frequent in pachytene nuclei (80.4% of splits) than two or more splits. For almost all cases analysed, splits were seen more frequently in early nuclei than in late ones. No variation in the number of MLH1 foci per cell or in the frequency of MLH1 foci in the XY pair was detected in cells with splits.

Some pachytene cells had discontinuities (gaps) in their SCs. Table III shows frequencies of pachytene nuclei showing SCP3 and SCP1 gaps in infertile and control men. The results are indicated according to the degree to which the bivalent set is affected. A significantly higher frequency of nuclei affected by gaps is observed in infertile cases (31.3%) when compared with control donors (20.2%) ($P = 0.046$) (Figure 1d and e). Patients AST and AZO1 have more gap-affected cells (60 and 55.3%, respectively) than control donors ($P < 0.0001$).

About 3% of cells presented a general fragmentation of their SCs (Figure 1e). MLH1 foci have always been seen in

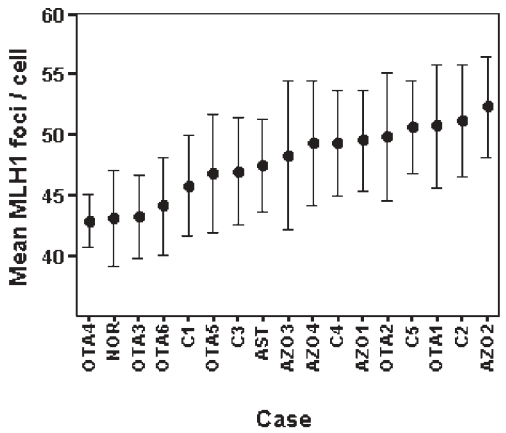

Figure 2. Mean (± SD) number of MLH1 foci per cell is drawn for the 17 individuals.

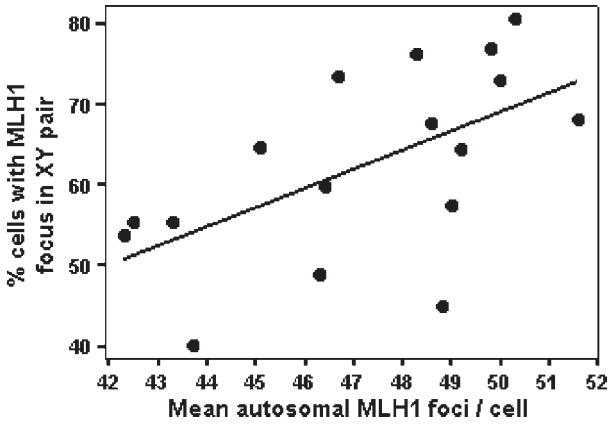

Figure 3. Relationship between the frequency of cells with an MLH1 focus in the XY pair and the mean number of MLH1 foci per cell in autosomal SCs ($y = 2.37 \times - 49.5$; $P = 0.013$, $r^2 = 0.34$).

Table II. Frequencies of pachytene nuclei showing single (1S) and multiple splits (≥2S) in infertile patients and in control men

	1S (% cells)	≥2S (% cells)	Total S (% cells)
Infertile men			
AZO1	10.6	8.5	19.1
AZO2	20.4	5.8	26.2
AZO3	8.3	4.8	13.1
AZO4	10	2.5	12.5
Mean	12.3	5.4	17.7
OTA1	3.4	1.7	5.1
OTA2	13.3	0	13.3
OTA3	0	0	0
OTA4	0	0	0
OTA5	0	2.4	2.4
OTA6	0	0	0
Mean	2.8	0.7	3.5
AST	24.4	0	24.4
NOR	17.9	0	17.9
Total mean	9	2.1	11.2
Control men			
C1	22.9	6.3	29.2
C2	2.4	0	2.4
C3	9.7	0	9.7
C4	8.1	5.4	13.5
C5	3.8	0	3.8
Mean	9.4	2.3	11.7

regions where the SC is structured. The number of MLH1 foci in the cell and the presence of MLH1 in the XY pair do not vary significantly whatever the degree to which cells were affected by gaps.

Discussion

This work reports the results of synapsis and meiotic recombination analysis by the immunocytogenetic labelling technique of pachytene spermatocytes of infertile and control men. In control donors, 35.5% of the pachytenes cells correspond to the early pachytene stage. In the infertile group, a close average frequency is found (34.4%). A previous study showed similar frequencies of early (37%) and late (63%) nuclei in 46 pachytene cells of a control donor (Barlow and Hultén, 1998).

Table III. Frequencies of pachytene nuclei having SCP3 and SCP1 gaps in infertile and control men

	Non-affected cells (NA) (% cells)	Normal cells (NA + SA)	Affected cells (MA + HA)
Infertile men			
AZO1	25.5	44.7	55.3^{Bb}
AZO2	48.5	87.4	12.6
AZO3	36.9	78.6	21.4
AZO4	47.5	67.5	32.5
Mean	39.6	69.5	30.5
OTA1	52.5	81.4	18.6
OTA2	51.1	62.2	37.7
OTA3	26.3	65.8	34.2
OTA4	35.9	74.4	25.6
OTA5	39	70.7	29.3
OTA6	48	64	36
Mean	42.1	69.8	30.2
AST	13.3	40	60^{Cc}
NOR	69.6	87.5	12.5
Total mean	41.2	68.7	31.3^{A}
Control men			
C1	47.9	66.7	33.3
C2	29.3	65.9	34.1^{bc}
C3	52.8	94.4	5.6
C4	45.9	83.8	16.2
C5	73.1	88.5	11.5
Mean	49.8	79.8	20.2^{ABC}

Results are indicated according to the degree to which the bivalent set is affected.
Upper case superscripts indicate comparisons with mean control values. Lower case superscripts indicate comparisons with the minimum control value. $^{a}P < 0.05$; $^{bc}P < 0.05$; $^{BC}P < 0.0001$.
NA = nuclei not affected by gaps; SA = nuclei slightly affected having from one to two SCs with gaps; MA = nuclei moderately affected with three or more SCs with gaps; HA = nuclei highly affected with gaps in all the SCs.

Recombination levels in spermatocytes

In this study, no significant difference in the number of MLH1 foci at early or at late pachytene has been detected, corroborating previous observations in which no relationship between MLH1 foci count and early–late pachytene stage was found (Barlow and Hultén, 1998; Lynn *et al.*, 2002). These results indicate that, once the cell has acquired the full MLH1 complement by early pachytene, the mean number of MLH1 foci per cell remains the same throughout the pachytene stages.

Significant interindividual variation in the mean number of MLH1 foci per cell was encountered, which ranged from 42.9 ± 2.1 to 52.3 ± 4.2 MLH1 foci. All reported studies of MLH1 foci count in men with normal spermatogenesis showed interindividual variations in the range described here (Barlow and Hultén, 1998; Lynn *et al.*, 2002; Gonsalves *et al.*, 2004; Hassold *et al.*, 2004; Sun *et al.*, 2004b,c). The presence of an interindividual variation in the MLH1 foci frequency suggests the idea that the number of MLH1 foci would be more related to an individual behaviour than to a specific fertility group.

A significant reduction in MLH1 foci number has been found in three patients when compared with our controls. However, this reduction would not be significant if compared with control donors of other reported studies (Gonsalves *et al.*, 2004; Sun *et al.*, 2004c). This discordance between

the results could be due to the interindividual variation in the number of MLH1 foci per cell. In cases presenting a strong reduction in the MLH1 foci counts, as reported in some azoospermic males (Gonsalves *et al.*, 2004; Sun *et al.*, 2004a), no discordances in the results could be found when compared with any control series.

In this study, we have obtained ranges of MLH1 foci per cell from 36 to 63 for the control group, and from 34 to 66 for the infertile group. Similar ranges are also observed in previous studies (Barlow and Hultén, 1998; Lynn *et al.*, 2002; Gonsalves *et al.*, 2004; Hassold *et al.*, 2004; Sun *et al.*, 2004b,c). It has been proposed recently that the minimum number of expected MLH1 foci is 39, one for each of the 39 autosomal arms (excluding the short arms of acrocentric chromosomes) (Lynn *et al.*, 2002). Recently, the MLH1 foci pattern of each single bivalent has been analysed (Sun *et al.*, 2004b). Taking into account ranges of MLH1 foci per bivalent, the theoretically expected range of recombination events in a single nucleus would be from 33 to 74. In this study, minimum values of meiotic recombination ranges in a cell are similar to the theoretical ones, but maximum values differ by $\sim$10 units. This indicates the existence of a positive interference in the number of MLH1 foci in the cell. This interference would ensure a minimum of crossing-overs, but would reduce the maximum potential of recombination events in human males. Sexual differences in this interference may exist. This interference would be stronger in human males, as spermatocytes show fewer recombination events than oocytes (Tease *et al.*, 2002). Differences between individuals and between sexes in epigenetic factors controlling this interference might explain interindividual and intersex variation in interference intensity.

Frequencies of cells with an MLH1 focus in the XY pair observed in the present study are similar to the 56.5 and 73% previously reported (Barlow and Hultén, 1998; Sun *et al.*, 2004b). Nevertheless, patients AZO4 and OTA6 have a frequency of cells with an MLH1 focus in the XY pair lower than 45%, indicating a low recombination frequency between sex chromosomes. A reduction in the recombination in the PAR1 region of the XY pair can lead to an abnormal disjunction of the sex chromosomes. Consequently, it may increase the sex chromosome aneuploidy (Shi *et al.*, 2001) or even cause a meiotic arrest (Hale, 1994). In patients AZO4 and OTA6, partial meiotic arrest and meiotic arrest, respectively, were detected by meiotic chromosomes analysis, which is in agreement with the XY recombination results.

The present study describes for the first time a correlation between the percentage of XY pairs showing an MLH1 focus and the mean number of autosomal MLH1 foci per cell. The presence of an MLH1 focus at the XY pair was strongly correlated with a higher autosomal recombination frequency. Similarly, levels of XY and autosomal pairing were described to correlate positively (Mittwoch and Mahadevaiah, 1992). Also, the rate of XY bivalents has been reported as an indicator for successful spermatogenesis in azoospermic men (Yogev *et al.*, 2002).

The results obtained in our study suggest that the frequency of an MLH1 focus in the XY pair could be a marker for general recombination frequency and for the meiotic process to proceed.

Synaptic behaviour: split and gap incidence

Pachytene cells of infertile men presented splits in a mean of 11.2% cells, not different from those observed in control patients. However, considerable differences among cases have been seen in both infertile and control groups. The fact that 80.4% of the splits seen are small and unique in the pachytene nuclei suggests that these splits correspond to the heterochromatic regions of bivalents 1 and 9. These splits have been observed more frequently in early than in late pachytene nuclei, indicating that they may result from a delay of the heterochromatin to synapse. Polymorphisms in these heterochromatic regions may explain the high interindividual variation observed in the frequency of splits. Discontinuities (gaps) in the SCs of a cell are seen in $\sim$50% of pachytene nuclei in control patients. According to a study of a fertile man in which SCs of chromosomes 1 and 9 frequently ($>$50%) showed gaps in their heterochromatic regions (Barlow and Hultén, 1996), pachytene nuclei presenting a gap in one or two SCs have been considered as normal nuclei.

The observed pachytene cells showing a general fragmentation of their SCs (Figure 1e) could correspond to nuclei of degenerative cells in apoptosis.

The AST and AZO1 infertile cases show a significant increase of nuclei ($>$55%) affected by gaps. If more nuclei are affected, more cells may fail to go through meiotic checkpoints, leading to a reduction in cell counts or to cells with an abnormal morphology and motility.

AZO1 has the highest frequency of early pachytene nuclei (61.7%), some of them with general SC fragmentation, and also shows $<$45% of normal pachytene cells. These results suggest an early pachytene partial arrest caused by synaptic defects, in agreement with the few post-pachytene meiotic stages observed in meiotic chromosome analysis.

In summary, we report the results of an immunocytogenetic analysis of synapsis and meiotic recombination in a series of infertile and control men. We have demonstrated that reduced recombination in the XY pair and increased number of cells affected by gaps may explain male infertility in certain cases. Therefore, this cytological analysis is a useful tool to better understand some idiopathic male infertility. However, the great interindividual variability in meiotic recombination and in the synaptic process, seen in this and in other studies, makes comparisons between groups and individuals still complicated. To better understand the effects of meiotic recombination and synaptic abnormalities on fertility and to mark out limits between pathological and normal incidence values, further analysis in infertile patients will be required. Also, the application of centromere or subtelomere multiplex-fluorescence *in situ* hybridization (FISH) methodologies for the identification of all SCs (Oliver-Bonet *et al.*, 2003; Codina-Pascual *et al.*, 2004) should provide information about the bivalents most affected by synaptic and recombination disorders. Finally, the relationship between frequency of MLH1 foci in the XY pair and the average number of MLH1 foci per cell found in the present study

suggests that the meiotic recombination frequency in the XY pair could be an indicator for general recombination frequency and for a successful meiotic process.

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References

Anderson LK, Reeves A, Webb LM and Ashley T (1999) Distribution of crossing over on mouse synaptonemal complexes using immunofluorescent localization of MLH1 protein. Genetics 151,1569–1579.

Ashley T, Walpita D and de Rooij DG (2001) Localization of two mammalian cyclin dependent kinases during mammalian meiosis. J Cell Sci 114,685–693.

Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie D-M, Monell C, Arnheim N, Bradley A, Ashley T and Liksay RM (1996) Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nature Genet 13,336–342.

Barlow AL and Hultén MA (1996) Combined immunocytogenetic and molecular cytogenetic analysis of meiosis I human spermatocytes. Chromosome Res 4,562–573.

Barlow AL and Hultén MA (1998) Crossing over analysis at pachytene in man. Eur J Hum Genet 6,350–358.

Codina-Pascual M, Kraus J, Speicher M, Oliver-Bonet M, Murcia V, Sarquella J, Egozcue J, Navarro J and Benet J (2004) Characterization of all human male synaptonemal complexes by subtelomere multiplex-FISH. Cytogenet Genome Res 107,18–21.

Egozcue J, Sarrate Z, Codina-Pascual M, Egozcue S, Oliver-Bonet M, Blanco J, Navarro J, Benet J and Vidal F (2005) Meiotic abnormalities in infertile males. Cytogenet Genome Res, (in press).

Evans EP, Breckon G and Ford CE (1964) An air-drying method for meiotic preparations from mammalian testes. Cytogenetics 3,289–294.

Gonsalves J, Sun F, Schlegel PN, Turek PJ, Hopps CV, Greene C, Martin RH and Reijo Pera RA (2004) Defective recombination in infertile men. Hum Mol Genet 13,2875–2883.

Hale DW (1994) Is X–Y recombination necessary for spermatocyte survival during mammalian spermatogenesis? Cytogenet Cell Genet 65,278–282.

Hassold TJ, Judis L, Chan ER, Schwartz S, Seftel A and Lynn A (2004) Cytological studies of meiotic recombination in human males. Cytogenet Cell Genet 107,249–255.

Judis L, Chan ER, Schwartz S, Seftel A and Hassold TJ (2004) Meiosis I arrest and azoospermia in an infertile male explained by failure of formation of a component of the synaptonemal complex. Fertil Steril 81,205–209.

Lammers JHM, Offenberg HH, van Aalderen M, Vink ACG, Dietrich AJJ and Heyting C (1994) The gene encoding a major component of the lateral elements of synaptonemal complexes of the rat is related to X-linked lymphocyte-regulated genes. Mol Cell Biol 14,1137–1146.

Lynn A, Koehler KE, Judis L, Chan ER, Cherry JP, Schwartz S, Seftel A, Hunt PA and Hassold TJ (2002) Covariation of synaptonemal complex length and mammalian meiotic exchange rates. Science 296,2222–2225.

Meuwissen RL, Offenberg HH, Dietrich AJ, Riesewijk A, van Iersel M and Heyting C (1992) A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. EMBO J 11,5091–5100.

Mittwoch U and Mahadevaiah SK (1992) Unpaired chromosomes at meiosis: cause or effect of gametogenic insufficiency? Cytogenet Cell Genet 59, 274–279.

Miyamoto T, Hasuike S, Yogev L, Maduro MR, Ishikawa M, Westphal H and Lamb DJ (2003) Azoospermia in patients heterozygous for a mutation in SYCP3. Lancet 362,1714–1719.

Navarro J, Vidal F, Templado C, Benet J, Marina S, Pomerol JM and Egozcue J (1986) Meiotic chromosome studies and synaptonemal complex analysis by light and electron microscopy in 47 infertile or sterile males. Hum Reprod 1,523–527.

Oliver-Bonet M, Liehr T, Nietzel A, Heller A, Starke H, Claussen U, Codina-Pascual M, Pujol A, Abad C, Egozcue J, Navarro J and Benet J (2003) Karyotyping of human synaptonemal complexes by cenM-FISH. Eur J Hum Genet 11,879–883.

Roeder GS and Bailis JM (2000) The pachytene checkpoint. Trends Genet 16,395–403.

Schalk JAC, Dietrich AJJ, Vink ACG, Offenberg HH, van Aalderen M and Heyting C (1998) Localization of SCP2 and SCP3 protein molecules within synaptonemal complexes of the rat. Chromosoma 107,540–548.

Shi Q, Spriggs E, Field L, Ko E, Barclay L and Martin RH (2001) Single sperm typing demonstrates that reduced recombination is associated with the production of aneuploid 24,XY human sperm. Am J Med Genet 99,34–38.

Solari AJ (1980) Synaptonemal complexes and associated structures in microspread human spermatocytes. Chromosoma 81,315–337.

Sun F, Kozak G, Scott S, Trpkov K, Ko E, Mikhaail-Philips M, Bestor TH, Moens PB and Martin RH (2004a) Meiotic defects in a man with non-obstructive azoospermia: case report. Hum Reprod 19,1770–1773.

Sun F, Oliver-Bonet M, Liehr T, Starke H, Ko E, Rademaker AW, Navarro J, Benet J and Martin RH (2004b) Human male recombination maps for individual chromosomes. Am J Hum Genet 74,521–531.

Sun F, Trpkov K, Rademaker AW, Ko E and Martin RH (2004c) Variation in meiotic recombination frequencies among human males. Hum Genet 116,172–178.

Tease C, Hartshorne GM and Hultén MA (2002) Patterns of meiotic recombination in human fetal oocytes. Am J Hum Genet 70,1469–1479.

Vendrell JM, García F, Veiga A, Calderon G, Egozcue S, Egozcue J and Barri PN (1999) Meiotic abnormalities and spermatogenic parameters in severe oligoasthenozoospermia. Hum Reprod 14,375–378.

World Health Organization (1999) WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction, 4th edn. Cambridge University Press, Cambridge.

Yogev L, Gamzu R, Paz G, Kleiman S, Botchan A, Hauser R and Yavetz H (2002) Rate of homologous chromosome bivalents in spermatocytes may predict completion of spermatogenesis in azoospermic men. Hum Genet 110,30–35.

Yuan L, Liu J-G, Zhao J, Brundell E, Daneholt B and Höög C (2000) The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. Mol Cell 5,73–83.

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