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1 **ADVANCED AGE INCREASES CHROMOSOME STRUCTURAL ABNORMALITIES**
2 **IN HUMAN SPERMATOZOA**

3

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16

17 **Running title:** Age and sperm chromosome structural abnormalities

18

19 **Key words:** paternal age, spermatozoa, chromosome duplications, FISH, structural
20 chromosome abnormalities

21

22 **ABSTRACT**

23 This study explores the relationship between sperm structural aberrations and age by utilizing a
24 multi-color-multi-chromosome FISH strategy that provides information on the incidence of
25 duplications and deletions on all the autosomes. TelVysion kit (Vysis) with telomere specific
26 probes was used. We investigated the sperm of 10 male donors aged from 23 to 74 years old.
27 The donors were divided into two groups according to age: a cohort of 5 individuals younger
28 than 40 and a cohort of 5 individuals older than 60. The goal of this study was to determine: 1)
29 the relationship between donor age and frequency and type of chromosome structural
30 abnormalities and 2) chromosomes more frequently involved in sperm structural aberrations.
31 We found that the older patients had a higher rate of structural abnormalities (6.6%) compared
32 to the younger cohort (4.9%). Although both duplications and deletions were seen more
33 frequently in older men, our findings demonstrate the presence of an excess of duplications
34 versus deletions in both groups at a ratio of 2 to 1. We demonstrate that the distribution of
35 duplications and deletions was not linear along the chromosomes, although a trend towards a
36 higher rate of abnormalities in larger chromosomes was observed. This work is the first study
37 addressing the frequencies of sperm chromosome structural aberrations of all autosomes in a
38 single assay thus making a contribution to the clarification of the amount and origin of damage
39 present in human spermatozoa and in relation to age.

40

41

42 INTRODUCTION

43 The causes of negative reproductive outcomes are poorly understood but chromosome
44 abnormalities are known to be a very significant factor. In humans, it has been estimated that at
45 least 8.1% of all clinically recognized pregnancies have a numerical (~7%) or structural (~1%)
46 chromosome abnormality. Chromosome abnormalities are seen in 50% of spontaneous
47 abortions, 6% still births, and 1% live births.¹ At birth, the incidence of aneuploidy (0.33%) is
48 only slightly higher than structural abnormalities 0.25%.² Most chromosomally abnormal
49 conceptions are the result of an error in gametogenesis and, less frequently, the result of a post
50 fertilization event.

51 In a study of the origin of *de novo* structural chromosome rearrangements using chromosome
52 heteromorphisms, Olson and Magenis³ found that 80% of *de novo* reciprocal translocations and
53 deletions and 100% of duplication and ring chromosomes were of paternal origin. The majority
54 of 115 *de novo* unbalanced structural chromosome abnormalities detectable by light
55 microscopy are of paternal origin⁴ varying from 84% of interstitial deletions to 58% of
56 duplications and rings. Deletions of the long arm of chromosome 18 are disproportionately
57 male⁵ as well as the deletions that cause Wolf-Hirschhorn syndrome⁶ and Cri-du-chat
58 syndrome.⁷ More recently, the parental origin of *de novo* microdeletions associated with *de*
59 *novo* reciprocal translocations and cases of complex chromosomes rearrangements was
60 determined to be paternal in all cases.⁸ Likewise all *de novo* deletions described by aCGH in
61 carriers of balanced translocations and abnormal phenotype turned out to be of paternal origin.⁹
62 Moreover, studies on the origin of the recurrent *de novo* t(11;22) found that it was paternal in
63 100% of cases^{10,11} and Thomas et al¹² confirmed a paternal origin of non recurrent *de novo*
64 balanced reciprocal translocations in 96% of cases.

65 Despite the high paternal contribution of *de novo* rearrangements compared to the low

66 frequency of paternally derived numerical abnormalities, the available information on
67 structural aberrations in human sperm is more limited. In sperm karyotypes, obtained after *in*
68 *vitro* penetration of hamster oocytes, structural chromosome abnormalities have been
69 observed far more frequently than numerical aberrations.¹³⁻¹⁵ In a review, Templado et al¹⁶
70 reported a median percentage of 6.6% of structural aberrations and 1.8% of numerical
71 abnormalities. Several authors have explored the relationship between age of the donor and
72 sperm structural aberrations (reviewed by Buwe et al¹⁷). Martin and Rademaker¹⁸ found
73 positive correlations with chromosomal breaks, and Sartorelli et al¹⁹ with acentric fragments
74 and complex radial figures. Other authors noticed a trend towards elevated frequencies of
75 breaks and /or acentric fragments with age but without reaching statistical significance.^{15,20,21}
76 There have been further reports using multicolor FISH in decondensed human sperm heads but
77 each study has been limited to a particular chromosome. Using this approach, a positive age
78 effect on the frequencies of duplications and deletions for the centromeric and subtelomeric
79 regions of chromosome 9 was reported by Bosch et al²² and, similarly, Slotter et al²³ found that
80 sperm of older men carried more breaks and segmental duplications and deletions of
81 chromosome 1.

82 In Western societies, women's access to contraception and to the work force coupled with the
83 availability of new reproductive technologies has resulted in couples delaying childbearing.
84 Both the age of the father and that of the mother are thought to influence reproductive outcome.
85 It has long been known that the age of the female increases the chances for meiotic errors in
86 oogenesis resulting in offspring with aneuploid chromosome abnormalities. There seems to be
87 a lesser impact for paternal than maternal age and the means by which advanced paternal age
88 affects human reproduction are poorly understood.

89 The purpose of this study is to explore the relationship between sperm structural aberrations
90 and age by utilizing a multi-color-multi-chromosome FISH strategy (*To TelVysion multi-color*
91 *FISH*, Vysis, Abbot) that provides information on the incidence of duplications and deletions
92 on all the autosomes. We investigated the sperm of 10 male donors aged from 23 to 74 years
93 old. The donors were divided into two groups according to age: a cohort of 5 individuals
94 younger than 40 (between 23 and 37 years old) and a cohort of 5 individuals older than 60
95 years of age (ages 60 to 74). We analyzed 1,000 spermatozoa per chromosome with a total of
96 15,000 spermatozoa per donor for a total of 150,000 cells. The multi-color-multi-chromosome
97 assay was used to explore: 1) the nature of the relationship between donor age and frequency
98 and type of chromosome structural abnormalities and 2) Chromosomes more frequently
99 involved in sperm structural aberrations.

100

101

102 **MATERIALS AND METHODS**

103 *Sperm Donors*

104 We worked with spermatozoa from 10 healthy male donors ages from 23 to 74 years old from
105 the Andrology Service of the Fundació Puigvert (Barcelona, Spain). Prior to the start of the
106 research, all of the sperm donors signed their consent to the study. The study was approved by
107 the Institutional Ethics Committee of the Universitat Autònoma of Barcelona, and the Ethics
108 Committee of the Fundació Puigvert.

109 Our donors had no history of exposure to any known mutagens, clastogens, radiation or
110 recreational drugs. Six of our donors, namely C1, C2, C6, C7, C9 and C10, had children. The
111 same donors had been used in previous studies of the frequency of sperm structural
112 abnormalities of chromosomes 6, 9, 21, X and Y.^{22,24} Sperm samples were obtained by
113 masturbation after an abstinence period of at least 3 days.

114 ***FISH with subtelomeric DNA probes***

115 Slide preparation and pretreatments prior to hybridization were performed following protocols
116 described elsewhere.²⁴ Similarly, the supplier's protocol with slight modifications was followed
117 for the hybridization procedure.²⁴

118 We utilized the panel of DNA probes *To TelVysion multi-color FISH* (Vysis, Abbot). Figure 1
119 depicts the chromosome location, color and aliquot distribution of the panel of probes used in
120 this study. These include subtelomeric probes specific for the short and long arms of all
121 chromosomes except the p arm of the acrocentrics (13, 14, 15, 21 and 22) and a number of
122 centromeric (CEP) probes (CEP X, CEP 17, CEP 18) and locus specific probes (LSI's): LSI 13
123 (13q14), LSI PML, (15q22), LSI AML, (21q22), LSI BCR, (22q11). For the sex chromosomes,
124 the probe mix used in this study (Figure 1) does not allow the discrimination between
125 numerical and structural aberrations nor the determination of structural aberrations present in
126 each of the sex chromosome, thus the sex chromosomes results have not been included in this
127 study.

128 Figure 1

129 This FISH protocol will measure duplications and deletions that include the following
130 chromosomal regions a) telomeres q of all autosomes, b) telomeres p of all autosomes except
131 acrocentric ones, and c) centromere and LSI specific for 6 out of 22 autosomes. The
132 duplications and deletions detected are not necessarily limited to subtelomeric ones but could
133 be whole arm or partial. They would correspond to chromosome structural aberrations such as:
134 unbalanced reciprocal translocations, unbalanced Robertsonian translocations, unbalanced peri
135 and paracentric inversions, isochromosomes, dicentric chromosomes, terminal deletions and
136 duplications, segmental deletions and duplications, whole arm deletions and duplications,
137 acentric fragments, and ring chromosomes. Interstitial deletions and /or duplications not

138 including the targeted probes, most microdeletions and microduplications, and balanced
139 structural aberrations would not be detected.

140

141 ***Data collection and scoring criteria***

142 Only slides with a hybridization efficiency of at least 99% were scored. Only intact and non-
143 overlapped decondensed sperm, identified due to the presence of a tail, were evaluated. For
144 each probe, two signals were scored as such only if they had the same intensity, size and color
145 and were separated by a distance equal to or higher than the probe signal diameter. If two
146 signals were recorded for a subtelomeric, centromeric or LSI probe it was considered a
147 duplication (partial or whole arm). When we observed the absence of one of these signals for a
148 chromosome, it was recorded as deletion (partial or whole arm). The presence of two signals
149 for subtelomere p and q regions and two signals for the corresponding centromeric or LSI
150 probe was considered disomy and it was not scored as a structural duplication. In mixtures with
151 only two subtelomeric probes, the presence of two signals for both subtelomeric probes was
152 considered disomy. Slides were coded prior to microscope scoring.

153

154 Figure 2

155

156 ***Statistical analysis***

157 The propensity to duplications versus the propensity to deletions for each individual was
158 assessed by both the paired Student's t-test and the non-parametric Wilcoxon signed-rank test.
159 The chi-square test was used to analyze the propensity of structural abnormalities for each
160 chromosome relative to a uniform distribution after including a correction for chromosome
161 length. A generalized estimating equation (GEE)²⁵ was used for assessing the differences

162 between the two age groups for the different types of chromosomal instability. The GEE
163 approach is an extension of generalized linear models designed to account for repeated within-
164 individual measurements. The GEE statistical model has been proved instrumental for the
165 comparison of chromosomal instability between two groups (see De la Chica et al²⁶ for further
166 statistical details). Statistical significance was set at $P < .05$. Statistical analyses were carried out
167 with SAS/STAT release 9.1 (SAS Institute Inc, Cary, NC).

168

169

170 **RESULTS**

171 We analyzed 1,000 spermatozoa per chromosome (15 aliquots) with a total of 15,000 sperm for
172 each donor for a total of 150,000 cells. The donors were divided into two groups according to
173 age: a cohort of 5 individuals younger than 40 (between 23 and 37 years old) and a cohort of 5
174 individuals older than 60 years of age (ages 60 to 74).

175

176 *Frequency of structural abnormalities*

177 Table 1 shows the percentages of structural chromosome abnormalities (duplications and
178 deletions) in each individual, and the mean and standard error of the mean (SEM) of response
179 variables for each group age

180 Table 1

181 The frequency of structural abnormalities ranged from 4.4% (C3 and C5) to 6.7% (C9) in the
182 younger group and from 4.5% (C1) to 7.7% (C6) in the older group. The standard error of the
183 mean (SEM) for the total of structural abnormalities was higher (0.60) in the older group than
184 in the younger group (0.44) meaning that there was more heterogeneity or interindividual
185 variability within the older group. Two outliers contribute significantly to the increase in
186 variability within each group. Indeed, the frequency of deletions (2.5%), duplications (4.2%)

187 and total structural abnormalities (6.7%) of donor C9 from the younger group was at or above
188 the mean for older donors. Likewise, within the older group there was C1 whose percentages of
189 deletions (1.0%), duplications (3.5%) and total structural (4.5%) were close to the mean of
190 younger donors.

191 The mean frequency of duplications was statistically higher than that of deletions in both the
192 older (4.5%) vs. (2.1%) (P=0.0053) and the younger group (3.3%) vs. (1.62%) (P=0.0012)
193 when using the paired Student's t-test. However under a more conservative approach (the non
194 parametric Wilcoxon signed-rank test) the p values switch to P=0.0625 for both older and
195 younger groups resulting in a lack of statistical relevance. All men from both groups had higher
196 frequencies of duplications than deletions, regardless of age (see Table 1). The ratio of
197 duplications to deletions is maintained throughout the age groups: 2.1 in the younger cohort
198 and 2.2 in the older, thus, in each group of age, for each deletion observed we scored
199 approximately 2 duplications. Table 2 shows the frequencies of duplications and deletions per
200 chromosome arm and the total of structural abnormalities per chromosome in all donors
201 studied. The higher frequency of duplications is observed across the range of all chromosome
202 arms with some exceptions, such as 2q, 3q, 4p, 7p, 8p, and 19q.

203

204 Table 2

205

206 ***Structural abnormalities in the older and younger groups:***

207 We scored a total of 330 structural abnormalities in the older group and 247 in the younger
208 group. The older cohort had a higher incidence of structural abnormalities across the board,
209 including deletions, duplications and total structural anomalies.

210 The statistical analysis showed a significant difference in the incidence of total structural
211 abnormalities between the older (6.6%) and younger groups (4.9%) (P=0.0499, GEE analysis).
212 If donor C9 from the younger group who has outlier values for the group, was removed from
213 the analysis, the P value shifted to P=0.0190, increasing the significance between older and
214 younger. A similar trend was obtained when removing instead the outlier in the older group
215 (donor C1), the P-value shifted to P=0.0154.). The frequency of duplications was statistically
216 different when comparing both groups of age (4.5% vs. 3.3%) (P=0.0400 GEE analysis). As for
217 deletions, although more were found in the older group (2.1%) than in the younger (1.6%), the
218 difference was not statistically relevant.

219

220 *Age effect on the distribution of structural abnormalities per chromosome*

221 Pooling all chromosomes together, the mean frequency of structural abnormalities per
222 chromosome was 0.31% in the older group and was higher (P=0,004) than that of the younger
223 group (0.23%).

224 The percentage of structural abnormalities for each chromosome (Figure 3) follows the same
225 trajectory in both groups with the exception of chromosome 12 where there is a statistical
226 divergence between older and younger (0.34% and 0.12% respectively) (P=0.01, GEE
227 analysis). For chromosome 16 the frequency of structural abnormalities also shows a divergent
228 trajectory but it is not statistically significant (Figure 3a). The percentage of duplications is
229 statistically different for chromosome 12 (0.28 older vs. 0.1 younger) (P=0.0286), and for
230 chromosome 19 (0.26 older vs. 0.16 younger) where there is a clear divergence between the
231 curves, (P=0.0239) (Figure 3b). The incidence of deletions follows a similar trajectory for both
232 groups with absence of statistical significance in any of the chromosomes (Figure 3c).

233 Figure 3

234 When we pooled all donors together (Table 2) we observed a percentage of structural
235 abnormalities ranging from 0.12 for chromosome 22 to 0.43 for chromosome 1. The second
236 chromosome in number of structural abnormalities was chromosome 5 (0.37%) followed by
237 chromosome 9 (0.36%), which was third. A tendency to a higher rate of abnormalities in larger
238 chromosomes was observed, however, a direct proportionality between the number of
239 abnormalities and chromosome length was not maintained. Indeed, the rate of abnormalities in
240 chromosomes 19 and 20 (0.32 and 0.35 respectively) were on the same order as larger
241 chromosomes, namely chromosome 2 (0.34), 5 (0.37), 8 (0.35) and 9 (0.36). In those donors
242 younger than 40 chromosome 3 had a lower (0.14%) than expected (0.34%) percentage of
243 duplications and deletions whereas chromosomes 19 (0.28%), 20 (0.34%) and 21 (0.18%) had
244 higher than expected percentages (0.13%, 0.13%, 0.09%, respectively) ($P < 0.05$). In those 60 or
245 older, chromosomes 19 and 20 demonstrated a higher percentage of structural abnormalities
246 than expected (0.36% vs. 0.18%, respectively) and (0.36% vs. 0.17%, respectively) ($P < 0.05$).

247

248

249 **DISCUSSION**

250 The multi-color-multi-chromosome assay provides a measure of the number of duplications
251 and deletions (terminal, partial including the subtelomere or other targeted areas, or whole arm)
252 present in the spermatozoa of an ejaculate. These duplications and deletions result from
253 presumably *de novo* structural chromosome rearrangements that may arise during the
254 spermatogonial divisions or in meiosis. The specific type of structural aberration that lead to
255 the duplications and deletions observed by our assay could not be determined because the
256 multicolor-multi-chromosome FISH assay does not discriminate among these aberrations.

257 The overall mean of structural abnormalities was 5.8 % which is in line with what has been
258 reported with the hamster oocyte assay for normal donors (6.6%)¹⁵ and reports of FISH studies
259 of structural abnormalities of chromosome 1 extrapolated to the whole genome (4.4-6.1%).²⁷
260 Although these numbers are comparatively similar it is important to keep in mind that our
261 study includes only unbalanced rearrangements and direct chromosome studies with the
262 hamster test included both balanced and unbalanced aberrations.

263 The older patients had a higher rate of structural abnormalities (6.6%) compared to the younger
264 cohort (4.9%). Along these lines, a positive age effect on the frequencies of duplications and
265 deletions for chromosome 9²² and for chromosome 1²³ was reported in FISH studies of
266 spermatozoa. We demonstrate that distribution of duplications and deletions was not linear
267 along the chromosomes. A tendency towards a higher rate of abnormalities in larger
268 chromosomes was observed, however, direct proportionality between the number of
269 abnormalities and chromosome length was not detected. In both younger and older patients,
270 chromosomes 19 and 20 had an excess of abnormalities whereas chromosomes 3 and 6 had less
271 than expected. Non-random distribution of breaks on chromosome 9 has been reported in
272 sperm karyotypes¹⁴ and in FISH studies an age dependent linear trend on chromosome 9 breaks
273 was reported.²² Our observations show a propensity of chromosome 9 to break higher than
274 expected yet without statistical significance. Moreover, previous reports of chromosome 4
275 having a lower than expected number of breaks in sperm karyotypes¹⁵ were confirmed in the
276 present study but without reaching statistical significance.

277 Both duplications and deletions were seen more frequently in the older men. The increase in
278 duplications with age was statistically significant as opposed to the increase in the frequency of
279 deletions, which, although higher in older men, was not significant. The ratio of duplications to
280 deletions was maintained around 2 in both groups, meaning that for each deletion observed we

281 scored 2 duplications (2.1 in the younger cohort and 2.2 in the older). The higher frequency of
282 duplications was observed in all donors, younger and older, and across the range of all
283 chromosome arms with a few exceptions (2q, 3q, 4p, 7p, 8p and 19q). Slotter et al²³ observed a
284 slight trend towards higher frequencies of sperm with segmental duplications vs. deletions in
285 each man, regardless of age. Likewise, several authors²⁸⁻³⁰ showed that duplications of 1p were
286 more frequent than deletions in human spermatozoa. The excess of duplications observed in
287 sperm nuclei could be a reflection of the excess in acentric fragments described in sperm
288 karyotypes.^{15, 18-21} and metaphase I spermatocytes.³¹ Acentric fragments can either acquire a
289 neocentromere and be rescued, or be lost.³² This could explain the excess of duplications
290 detected in spermatozoa with respect to the rate of subtelomeric duplications in subjects with a
291 clinical phenotype

292 The higher rate of duplications could be explained by a mitotic origin rather than a meiotic
293 origin for some of these *de novo* abnormalities. Translocations, inversions, insertions,
294 isochromosomes and small deletions and duplications are considered stable rearrangements and
295 it has been postulated that they would originate in spermatogenic mitotic divisions or during
296 meiosis.¹⁵

297 The number of spermatogonial divisions accumulates with increasing age. In males, germ cells
298 divide continuously. At the spermatogonial phase a man of 50 years could accumulate 840
299 replications each increasing the chances for DNA damage and errors in transcription. Thomas
300 et al¹² report a paternal origin in 96% of *de novo* non-recurrent balanced reciprocal
301 translocations. They all appeared to be unique events not mediated by sequence homology
302 (unlike meiotic rearrangements) and showed a strong paternal age effect suggesting a mitotic
303 spermatogonial origin. On the contrary, the frequency of recurrent *de novo* t(11;22) in sperm

304 has been found to be independent of the age of the donors³³ and it might be homology mediated
305 and of meiotic origin.

306

307 ***Environmental insults and age***

308 Environmental insults and exposures over a lifetime may increase DNA damage in sperm of
309 older men and contribute to the incidence of childhood diseases that increase with paternal
310 age.³⁴ Men over 50 have a higher pregnancy loss and decreased live birth rate.³⁵ There is
311 increased DNA fragmentation with age, a consequence of defective apoptosis, oxidative stress,
312 meiotic and spermiogenic abnormalities.³⁶ Singh et al³⁷ report that the amount of DNA damage
313 in sperm of men aged 35-57 is three times that of men <35 years. The accumulation of
314 unrepaired DNA damage in male germ cells, plus a progressively deteriorating testicular
315 environment –host factors- could manifest as an age-related increase in chromosomal structural
316 abnormalities that could be passed onto the zygote in the form of *de novo* structural
317 rearrangements.

318

319 ***Clinical significance***

320 The sperm carrying *de novo* structural rearrangements may father offspring with clinically
321 significant *de novo* chromosome aberrations (microscopic and submicroscopic) or *de novo*
322 submicroscopic clinical variants.

323 Contrary to that observed in sperm nuclei, studies carried out in selected populations of patients,
324 have found that *de novo* deletions are more frequent than duplications. Ravnan et al³⁸ in a FISH
325 study limited to subtelomeric regions of a highly selected population of patients with
326 developmental disabilities and normal karyotypes, detected more *de novo* clinically significant
327 subtelomeric deletions than subtelomeric duplications, and more subtelomeric deletion variants

328 than subtelomeric duplication variants. A contributing factor to explain the differences between
329 spermatozoa and clinical studies could be that duplications are associated with milder
330 phenotypes and thus individuals with duplications are less likely to be included in highly
331 selected populations such as patients with developmental disabilities. Moreover, the FISH
332 subtelomeric probes used in this study can potentially detect benign polymorphic variants^{39, 40}
333 that do not result in pathologic phenotype. This could be another contributing factor to explain
334 the differences between spermatozoa and clinical studies.

335

336

337 **CONCLUSION**

338 Our work shows an increase in structural abnormalities (duplications and deletions) in the
339 spermatozoa of older men. Our findings demonstrate the presence of an excess of duplications
340 versus deletions in both groups at a ratio of 2 to 1 which can only be explained by mechanisms
341 at play other than meiotic recombination as their source or by a negative selection of germinal
342 cells carrying deletions during spermatogenesis. Partial or whole arm duplications exceeded
343 deletions across the board for most of the chromosome arms. Chromosome 12 rates of
344 structural abnormalities differ significantly in the two groups of age. This work is the first
345 study addressing the frequencies sperm chromosome structural aberrations of all autosomes in
346 a single assay thus contributing to clarify the amount of damage present in human spermatozoa
347 and in relation to age. The next challenge resides in the elucidation of the specific types of
348 chromosome abnormalities that are produced at each stage of germ cell production, the relative
349 contributions of each mechanism involved in the production of sperm with structural
350 chromosome abnormalities, and how both processes are affected by age.

351

352

353

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357

358 **CONFLICT OF INTEREST**

359 The authors declare no conflict of interest

360

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454

455 39 **Table 1.** Percentages of structural chromosome abnormalities in spermatozoa from 10
 456 healthy donors in each group of age

457

Donor	Age	% Duplications	% Deletions	% Total Structural
C3	24	2.9	1.5	4.4
C4	25	3.5	1.1	4.6
C5	37	3.1	1.3	4.4
C8	23	2.9	1.7	4.6
C9	30	4.2	2.5	6.7
Mean (SEM) ¹	28	3.3 (0.25)	1.6 (0.2)	4.9 (0.4)
C1	74	3.5	1.0	4.5
C2	64	4.4	2.7	7.1
C6	72	5.3	2.4	7.7
C7	60	3.7	2.4	6.1
C10	62	5.7	1.9	7.6
Mean (SEM) ¹	66	4.5 (0.4)	2.1 (0.3)	6.6 (0.6)

458

459 ¹Standard error of the mean (SEM)

460

461

462 **Table 2.** Percentages of structural abnormalities in each chromosome arm in spermatozoa from
 463 all of the 10 individuals studied

464

Chromosome ¹	p arm			q arm			% Total structural
	% dup	% del	% Total	% dup	% del	% Total	
1	0.15	0.10	0.25	0.12	0.06	0.18	0.43
2	0.09	0.02	0.11	0.07*	0.16*	0.23	0.47
3	0.11	0.01	0.12	0.04*	0.05*	0.09	0.21
4	0.08*	0.08*	0.16	0.07	0.06	0.13	0.29
5	0.14	0.02	0.16	0.12	0.09	0.21	0.37
6	0.10	0.02	0.12	0.06	0.04	0.10	0.22
7	0.02*	0.08*	0.10	0.10	0.06	0.16	0.40
8	0.04*	0.09*	0.13	0.12	0.10	0.22	0.35
9	0.08	0.06	0.14	0.15	0.07	0.22	0.36
10	0.11	0.04	0.15	0.05	0.03	0.08	0.23
11	0.05	0.04	0.09	0.18	0.03	0.21	0.30
12	0.12	0.04	0.16	0.07	0.00	0.07	0.23
13	ND	ND	ND	0.22	0.04	0.26	0.26
14	ND	ND	ND	0.14	0.07	0.21	0.21
15	ND	ND	ND	0.15	0.03	0.18	0.18
16	0.08	0.05	0.13	0.06	0.03	0.09	0.22
17	0.01	0.00	0.01	0.16	0.0	0.16	0.17
18	0.09	0.00	0.09	0.09	0.0	0.09	0.18
19	0.18	0.04	0.22	0.03*	0.07*	0.10	0.32
20	0.12	0.09	0.21	0.11	0.03	0.14	0.28
21	ND	ND	ND	0.13	0.04	0.17	0.17
22	ND	ND	ND	0.11	0.01	0.12	0.12

480

481 ¹ 10,000 spermatozoa analyzed/chromosome

482 *Chromosome arms for which the frequency of duplications is NOT higher than that of
 483 deletions

484 ND = Not determined

485

486 **TITLES AND LEGENDS TO FIGURES**

487

488 **Figure 1.** The 15 aliquots of the FISH probe panel used showing both the chromosome location
489 and the color of the probes (Spectrum Orange, Spectrum Green and SpectrumAqua) from each
490 aliquot.

491

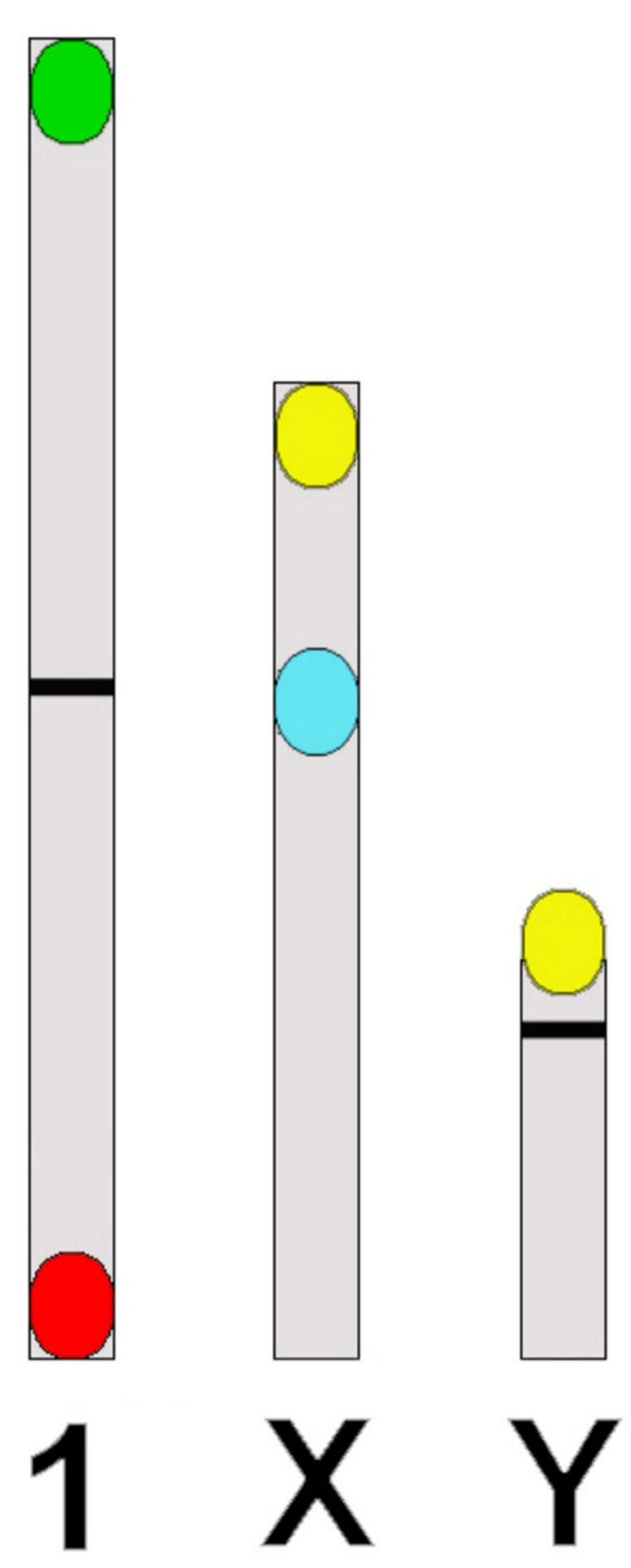
492 **Figure 2.** FISH in human decondensed sperm nuclei using one of the 15 mixtures of the FISH
493 panel (mixture 12) showing structural aberrations for chromosome 12: deletions for
494 subtelomere 12p and duplications for subtelomeres 12p and 12q.

495

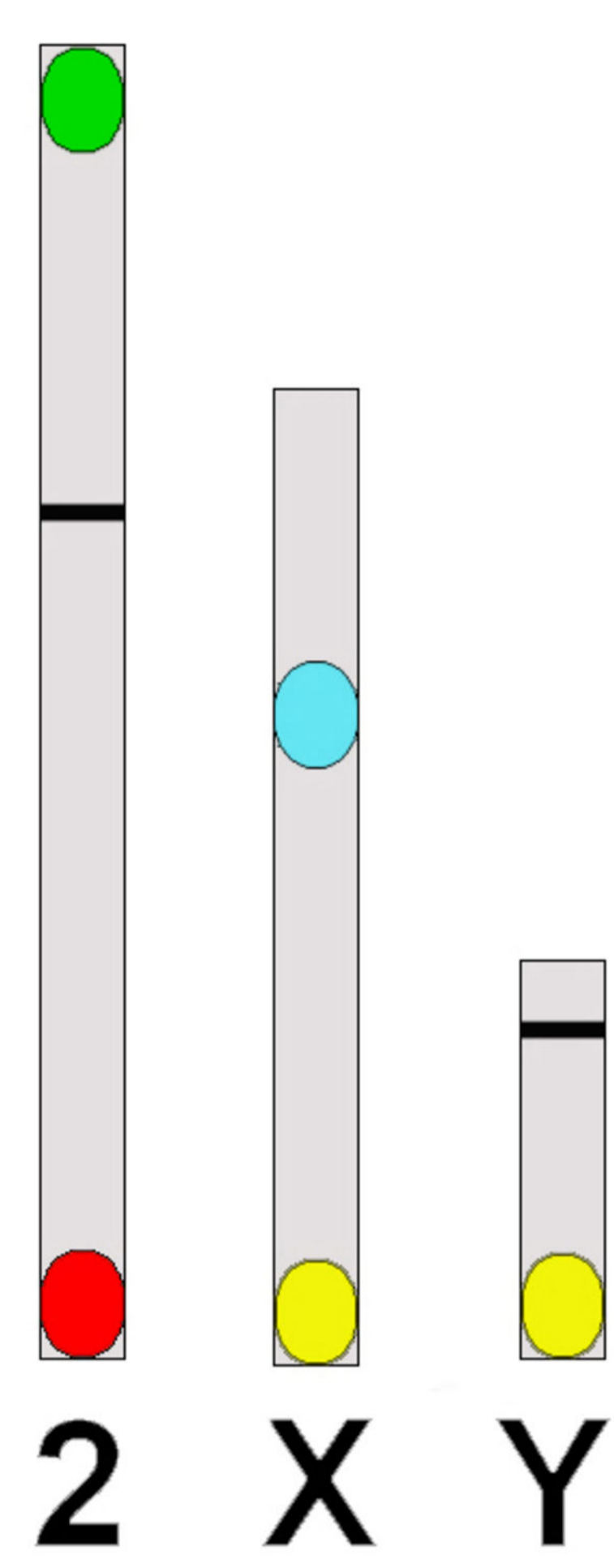
496 **Figure 3.** a) Percentage of chromosome structural abnormalities per chromosome in each
497 group of age. b) Percentage of duplications per chromosome. c) Percentage of deletions per
498 chromosome.

499

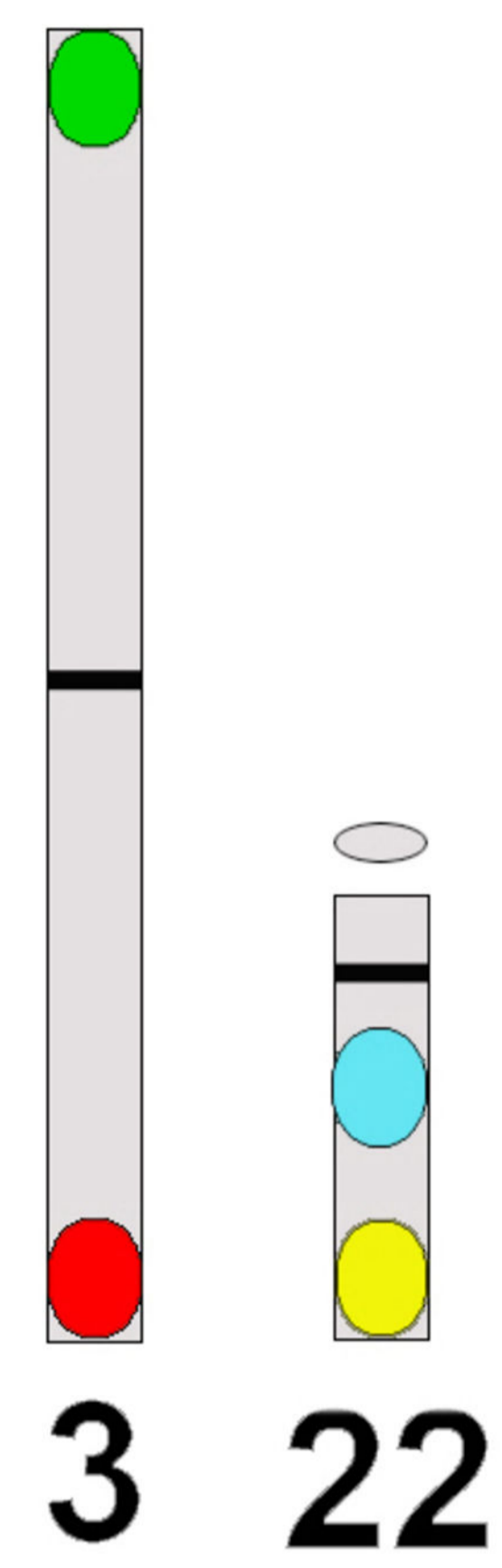
500



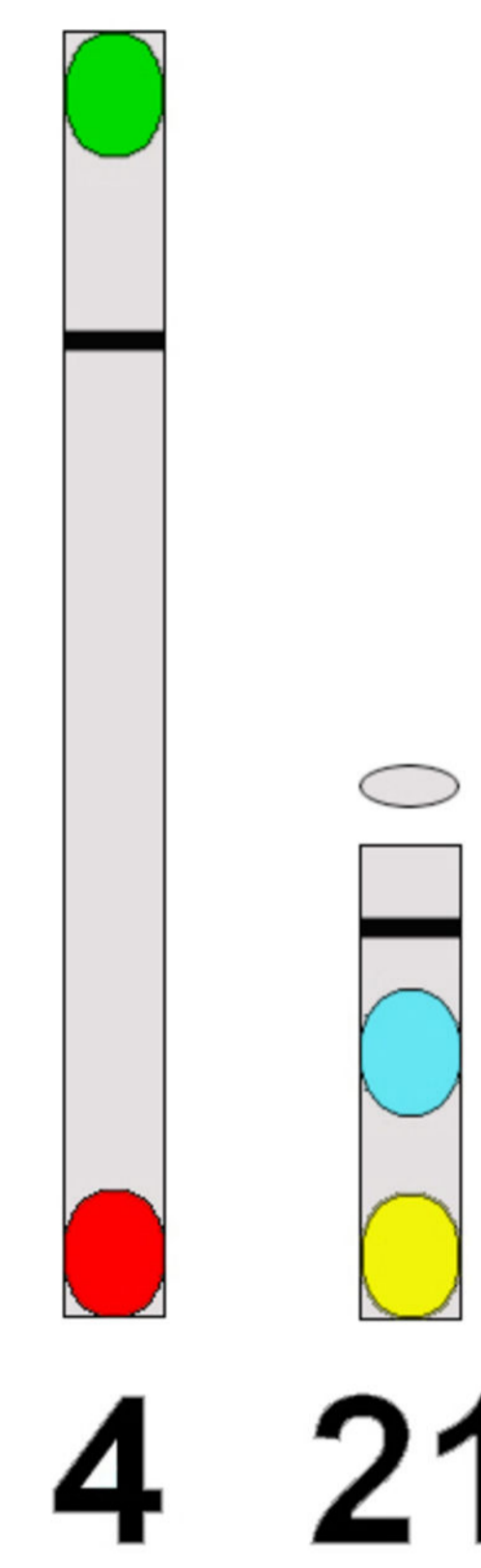
1 **X** **Y**
Aliquot 1
1p Green
1q Orange
Xp/Yp Orange&Green
CEP X Aqua



2 **X** **Y**
Aliquot 2
2p Green
2q Orange
Xq/Yq Orange&Green
CEP X Aqua



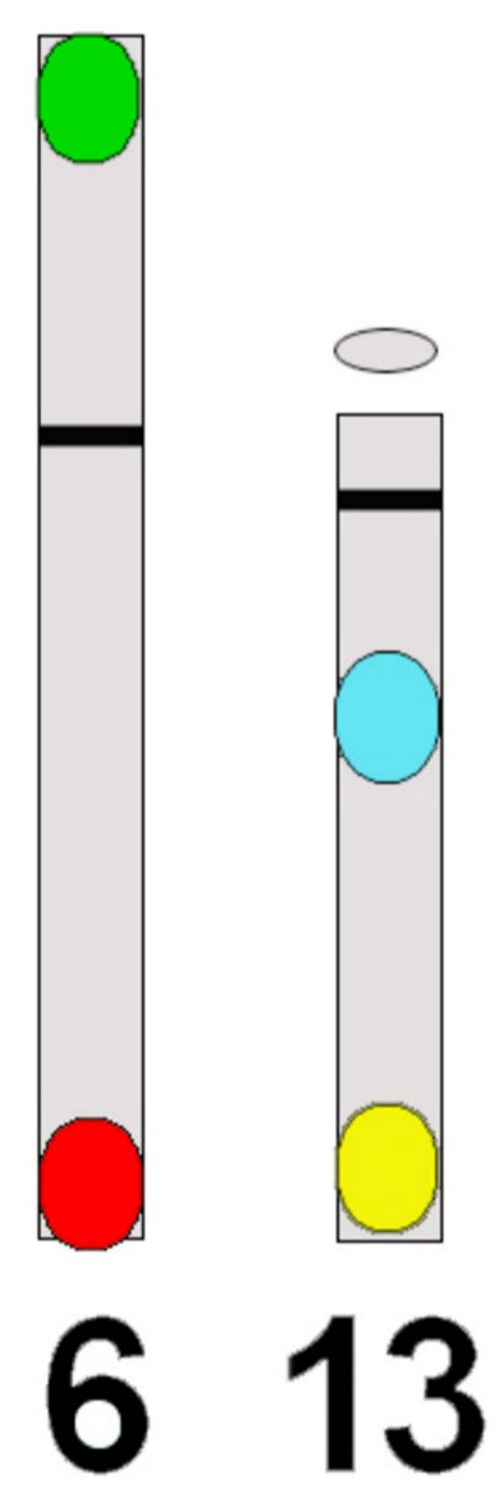
3 **22**
Aliquot 3
3p Green
3q Orange
22q Orange&Green
LSI BCR Aqua



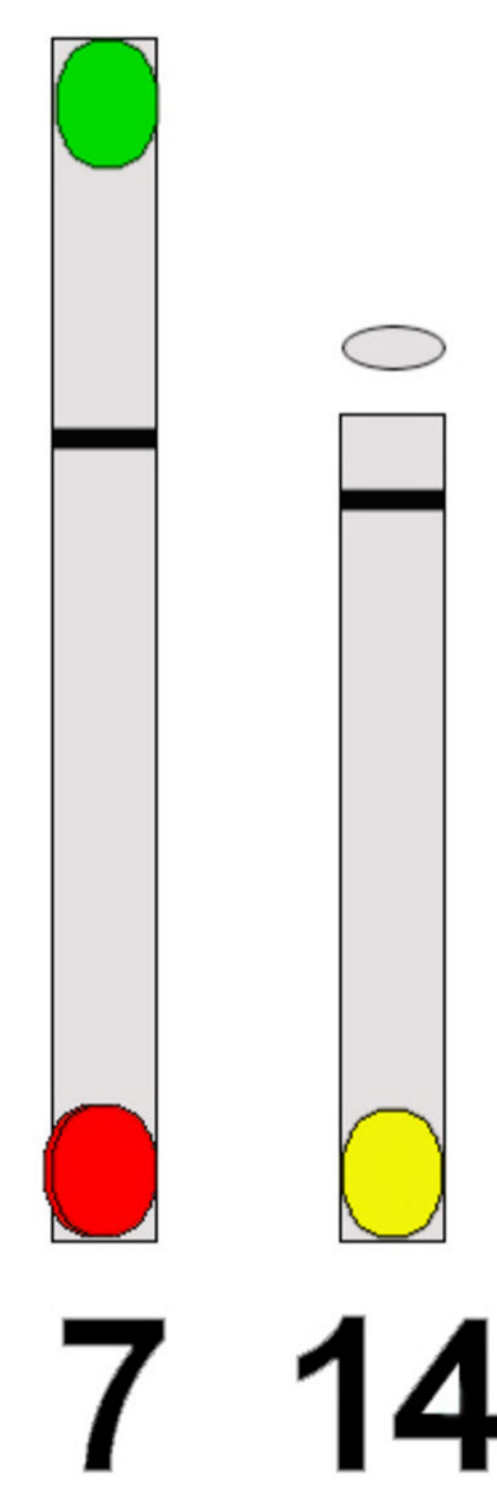
4 **21**
Aliquot 4
4p Green
4q Orange
21q Orange&Green
LSI AML1 Aqua



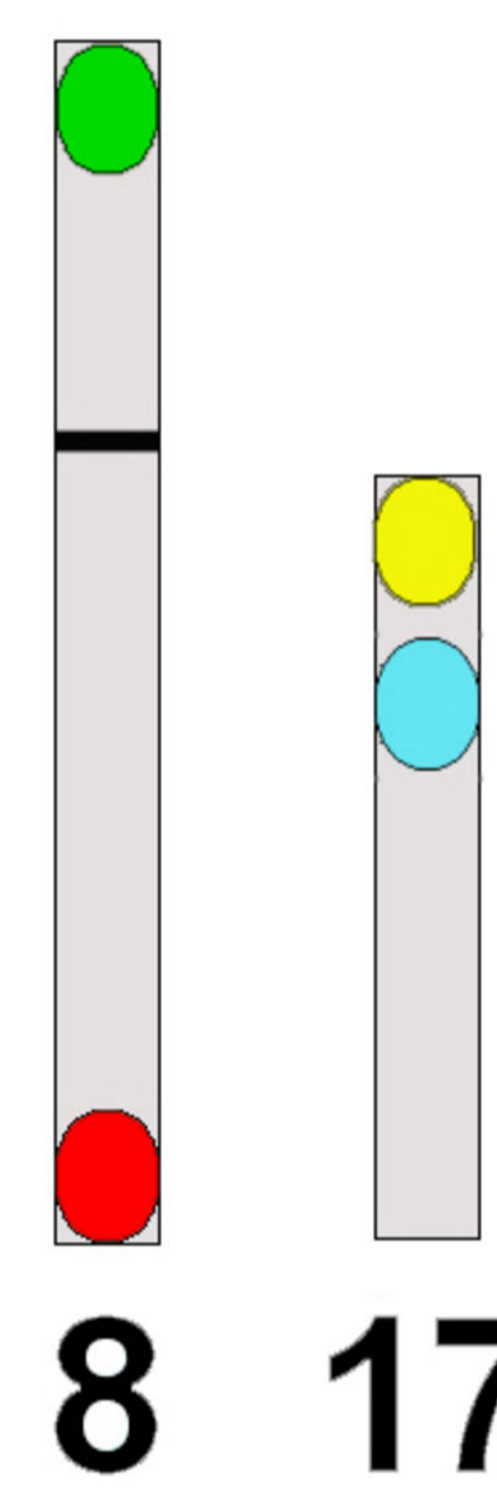
5
Aliquot 5
5p Green
5q Orange



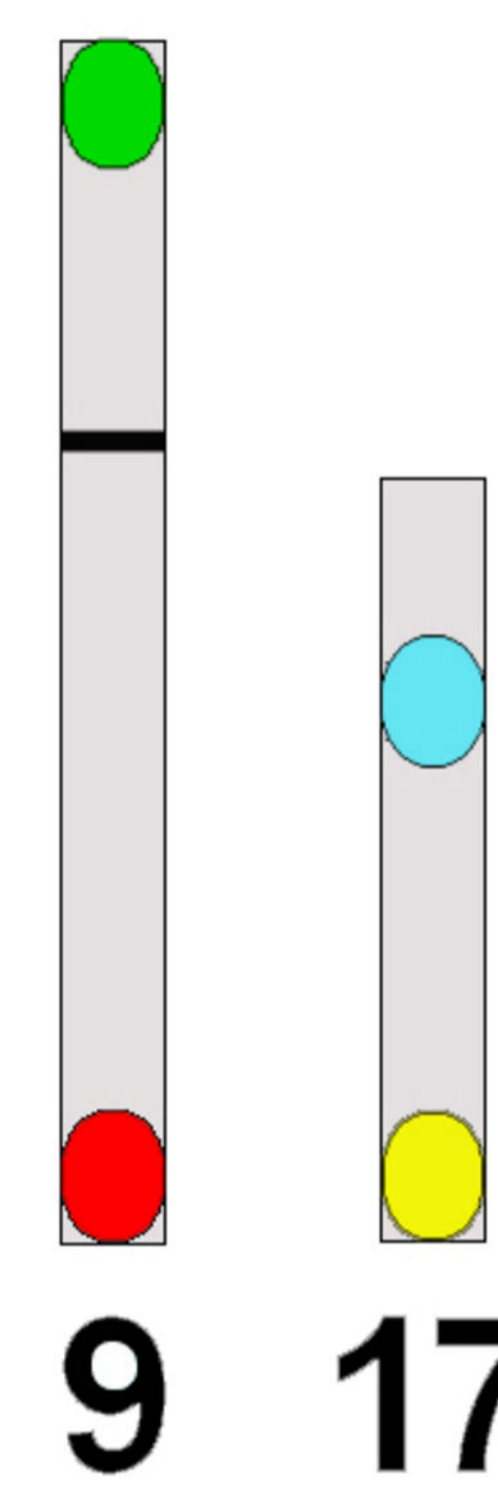
6 **13**
Aliquot 6
6p Green
6q Orange
13q Orange&Green
LSI 13 Aqua



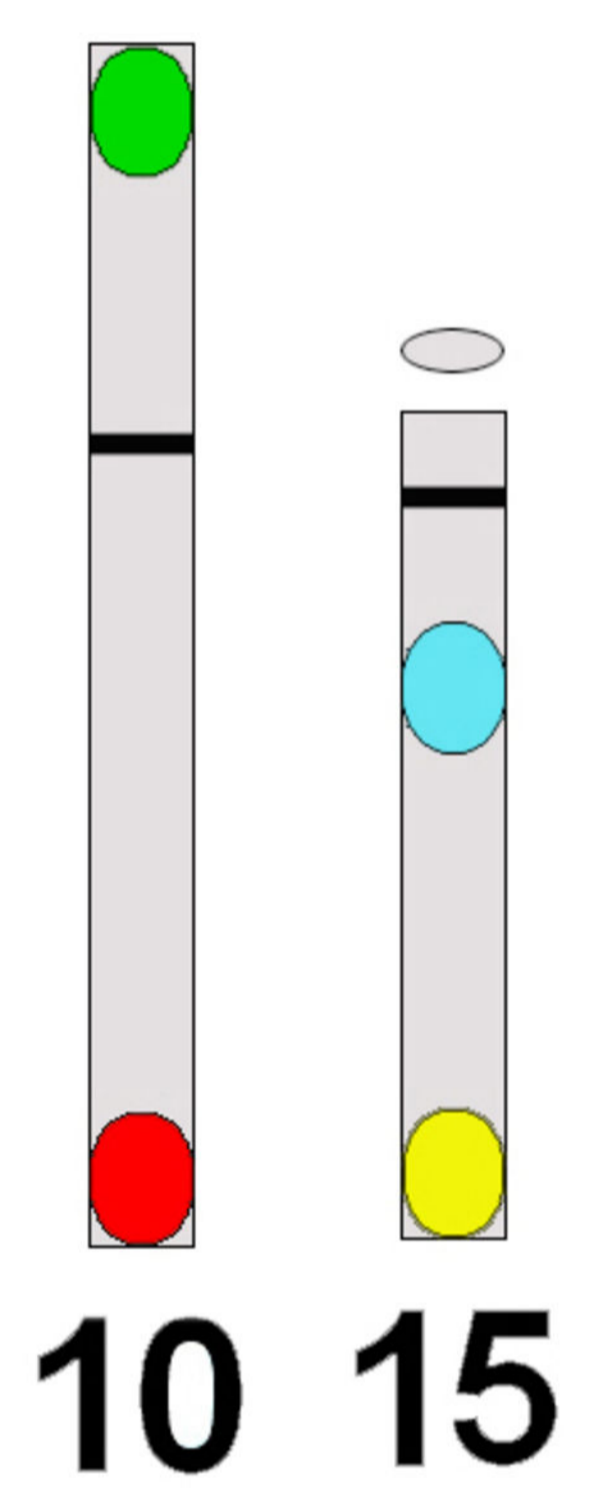
7 **14**
Aliquot 7
7p Green
7q Orange
14q Orange&Green



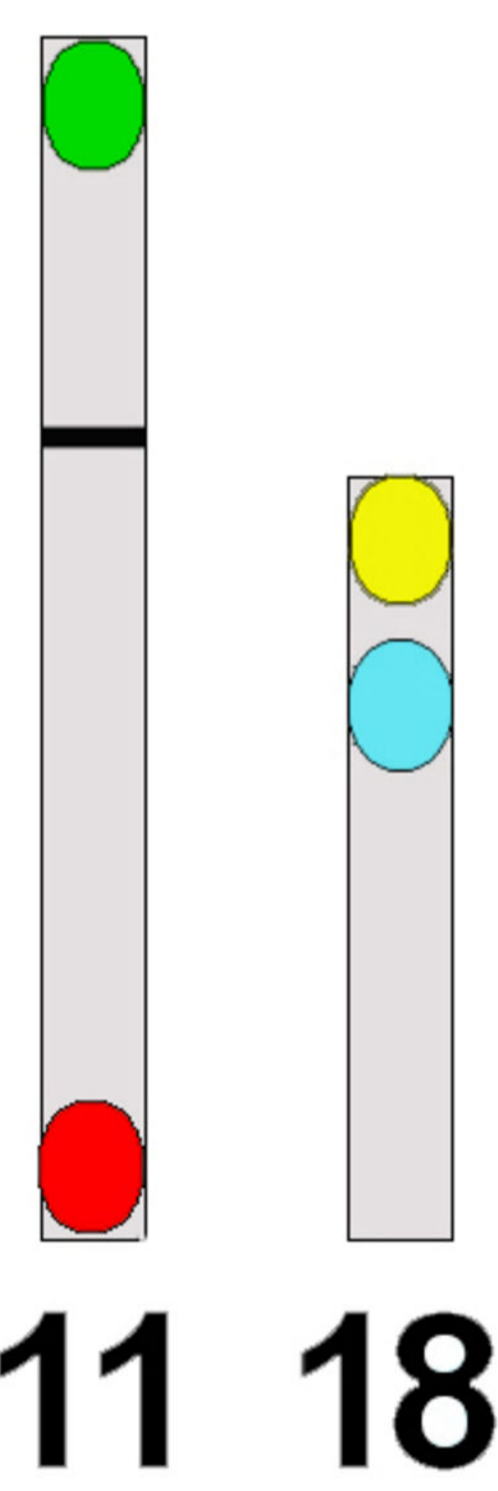
8 **17**
Aliquot 8
8p Green
8q Orange
17p Orange&Green
CEP 17 Aqua



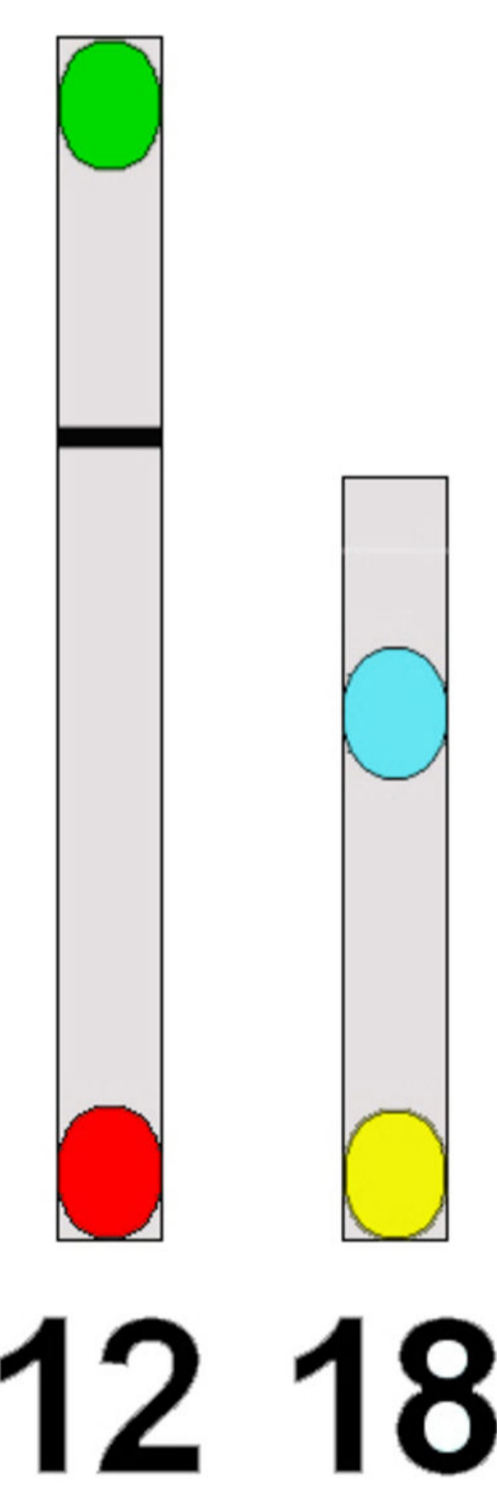
9 **17**
Aliquot 9
9p Green
9q Orange
17q Orange&Green
CEP 17 Aqua



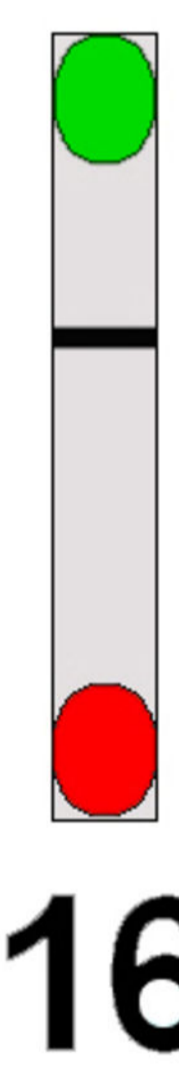
10 **15**
Aliquot 10
10p Green
10q Orange
15q Orange&Green
LSI PML Aqua



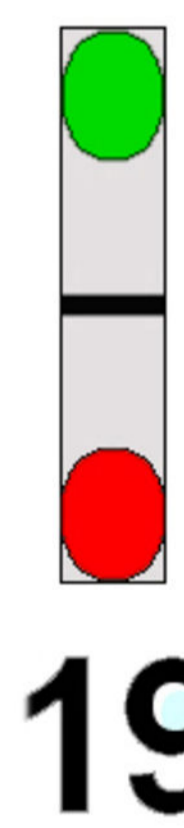
11 **18**
Aliquot 11
11p Green
11q Orange
18p Orange&Green
CEP 18 Aqua



12 **18**
Aliquot 12
12p Green
12q Orange
18q Orange&Green
CEP 18 Aqua



16
Aliquot 13
16p Green
16q Orange



19
Aliquot 14
19p Green
19q Orange



20
Aliquot 15
20p Green
20q Orange

normal

dup(12)(p)

dup(12)(q)

del(12)(p)

Mixture 12

subtel 12p

subtel 12q

subtel 18q

CEP 18

