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1 **Experimental mild increase in testicular temperature has drastic, but reversible,**  
2 **effect on sperm aneuploidy in men : a pilot study**

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32

33 **Short title:** Testis heating and sperm aneuploidy in men

34

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41

42 **Abstract**

43 In mammals testicular and epididymal temperature increase impairs  
44 spermatogenesis. This experimental study investigates the effects of a mild testis  
45 temperature increase (i.e. testis temperature remains below core body  
46 temperature) on sperm aneuploidy in men. In 5 fertile volunteers a testicular  
47 temperature increase was induced by maintaining the testes at suprascrotal  
48 position using specially designed underwear for  $15 \pm 1$  hours daily for 120  
49 consecutive days. After heating men were followed for next 180 days. A control  
50 group (27 men) was recruited. Semen samples were collected before, during and  
51 after heating period and analyzed for chromosomes X, Y and 18 for aneuploidy  
52 using FISH. A total of 234,038 spermatozoa were studied by FISH. At day 34 of  
53 heating, mean sperm aneuploidy values were not modified. From day 34 of  
54 heating until day 45 post heating, FISH evaluation was not possible due to the  
55 drastic fall of sperm count. At day 45 post-heating total sperm aneuploidy  
56 percentage was twice higher than before heating whereas. Sex disomy (sperm  
57 XY18), sex chromosome nullisomy (sperm 18) were significantly higher than  
58 controls. These effects were completely reversed at 180 days post heat exposure.  
59 Conclusion: A mild rise in testicular temperature significantly increases sperm  
60 aneuploidies, reflecting an effect on the meiosis stage of spermatogenesis. The  
61 effect of heating was reversible and suggests that recovery of aneuploidy to

62 normal values requires at least two cycles of spermatogenesis. Nonetheless, the  
63 low number of volunteers was a limitation of this pilot study and warrants further  
64 research on larger population.

65

66 **Keywords**

67 Sperm aneuploidy; spermatogenesis alteration; testis temperature increase; male  
68 infertility; Fluorescence In Situ Hybridization (FISH).

69        **1. Introduction**

70        The adverse effects of increased testicular temperature on spermatogenesis in  
71 mammals have been investigated and established for many years. In most  
72 mammals, testes are located in the scrotum outside the body, which provides  
73 anatomical location that offers lower testicular temperature than core body. In a  
74 thermoneutral environment, the scrotal testes are appreciably cooler than the  
75 body, but this does not mean that they are kept at a constantly lower  
76 temperature [1]. In man as in most other mammals, optimal spermatogenesis  
77 requires 2-6°C lower testicular temperature than body and any rise above the  
78 physiological temperature of the testes has adverse impact on spermatogenesis  
79 [2, 3].

80        Several pathological conditions are associated with an increase in testicular  
81 temperature which include cryptorchidism [4], varicocele [5-7] and febrile  
82 diseases [8, 9]. Exogenous factors such as occupational exposure to high  
83 temperatures [10, 11], car driving [12] or sauna exposure [13-15] also increase  
84 the testicular temperature. Notably, some living conditions such as wearing tight  
85 clothing [16] or sitting and sleeping postures [17] are also associated with an  
86 increase in testicular temperature which remains in the physiological range [18].  
87        The relation between lifestyle factors or exposure to environmental risk factors

88 and sperm aneuploidy was investigated in several studies [19-22], but to our  
89 knowledge, only one epidemiological study has reported the possible link  
90 between circumstances known to improve testicular temperature, such as sauna  
91 use or type of underwear, and sperm aneuploidy and have shown decrease in  
92 sperm aneuploidy in men wearing boxer shorts compared to those wearing tight  
93 underwear [23].

94 In man, different experimentally methods increasing testicular temperature  
95 resulted in a drastic, but reversible, inhibition of spermatogenesis with decrease  
96 in sperm count [13, 24-28] sperm motility [13, 24-27] and sperm normal  
97 morphology [13, 25, 26]. In addition to conventional sperm parameters (count,  
98 motility, morphology) the adverse effects of testicular hyperthermia on sperm  
99 DNA damage have also been reported [13, 24].

100 It has been suggested that a modification of testicular temperature could be used  
101 in two ways: increasing testes temperature to inhibit spermatogenesis or  
102 decreasing it to improve spermatogenesis in infertile men who have abnormally  
103 elevated testes temperatures. Experimental studies [29-31] and reviews [32-34]  
104 have reported that a mild testis temperature increase could be used as a male  
105 contraceptive approach. On the other hand, reducing the testicular temperature  
106 using application of an ice bag for 30 min per day for 14 days improves

107 spermatogenesis outcome [35]. Various devices have been used to decrease  
108 testicular temperature and improve spermatogenesis, either in varicocele or in  
109 oligozoospermic patients having higher scrotal temperatures [36-38] . In addition,  
110 avoidance of exposure to heat has been reported to prevent infertility [39-41].

111 We have previously demonstrated that a mild increase in testicular and  
112 epididymal temperature (+2°C, i.e. a testicular temperature below core body  
113 temperature), induced by maintaining the testes in a suprascrotal position, had  
114 drastic effects not only on sperm output and quality but also on sperm DNA  
115 fragmentation in humans [24].

116 While a study in mice [42] reported X-Y dissociation increase in spermatocytes  
117 from animals that were exposed to an environmental temperature of 35°C for 2-5  
118 days (whole body exposure), to date no study has investigated the effect of a mild  
119 testicular temperature increase (testicular temperature below core temperature)  
120 on sperm aneuploidy in men. A recent study in man reported increased sperm  
121 aneuploidy after testicular exposure to 43°C for two successive days per week for  
122 3 months. [43]. Noteworthy, 43°C is very high temperature compared to  
123 physiological temperature of testes ( $\approx 34.5^\circ\text{C}$ ) and sperm aneuploidy is more likely  
124 to occur at this higher temperature threshold.



125 Sperm aneuploidy can be a risk to the embryonic and fetal development and the  
126 offspring [44, 45]. The majority of the aneuploidy embryos are not viable resulting  
127 into miscarriages but several chromosomal abnormalities, such as autosomal  
128 trisomy or sex chromosome aneuploidy, could be non-lethal and compatible with  
129 survival resulting in birth and developmental defects [44-47].

130 In this context, the objective of this study was to evaluate the effects of a mild  
131 testicular temperature increase (+2°C) on sperm aneuploidy in fertile men and to  
132 examine its potential reversibility.

133

## 134 **2. Materials and methods**

### 135 **2.1. Study Design**

136 The present study is the second part of a study of the effects of mild induced  
137 testes temperature increase on gamete quality [24]. We used the biological  
138 specimens that had been frozen during the first part of the study.

### 139 **2.2. Study Population**

140 The study was approved by the Ethics Committee (Comité de Protection des  
141 Personnes Sud-Ouest et Outre Mer I) and the protocol has been previously  
142 described [24]. The volunteers were recruited through advertisement in the press

143 and local hospital communication portal. A total of 34 men volunteered for the  
144 study. After exclusion criteria and financial limitations 6 volunteers (aged 25-35  
145 years) were selected. Only the men who had fathered at least one child, had a  
146 normal clinical andrological examination, no current pathology, medical or  
147 surgical history, had either no, moderate or occasional alcohol intake were  
148 included. None of the participating volunteer was exposed to toxic agents and  
149 had no particular professional risk to fertility. One volunteer smoke 20 cigarettes  
150 a day in experimental group and 7 men smoke more than 10 cigarettes a day in  
151 control group.

152 One volunteer dropped the study at day 73 of heating due to personal reasons  
153 hence the data of this volunteer were excluded. Remaining five volunteers  
154 followed the instructions and the protocol and continued until the completion of  
155 study. None of the volunteer reported any discomfort during the entire period of  
156 study. The aneuploidy results of 27 fertile men were used as control values to  
157 compare with aneuploidy values of experimental volunteers (n=5).

158

### 159 **2.3. Technique for Increasing Testicular Temperature**

160 Increased testicular and epididymal temperature was induced by maintaining the  
161 testes in a supascrotal position by means of specially designed underwear worn

162 15 ± 1 hours daily [3, 24, 29] for 120 consecutive days. The method was  
163 developed by our group and has been tested safe, reversible and reproducible.  
164 Before, commencement of heating participating volunteers of experimental group  
165 were trained on how to put the underwear by lifting the testes up at the root of  
166 the penis during waking hours. The support to the testes keeping them at  
167 suprascrotal position was ensured with the underwear provided with an orifice  
168 allowing the scrotum and penis to be exteriorized specifically designed for each  
169 volunteer as we previously described [25]. The suprascrotal position of the testes  
170 results in 1.5 -2°C increase in testicular temperature. Notably, the testicular  
171 temperature remains below core human body temperature in this heating  
172 method [30, 48].

### 173 **Semen analysis**

174 Semen samples were collected, after mean sexual abstinence of 4.1 (±1.0) days,  
175 at specific times according to spermatogenesis chronology as we previously  
176 described [19]. After liquefaction at 37°C for minimum of 30 minutes, semen  
177 analysis was done according to WHO guidelines [49] on an aliquot (200 µL) by a  
178 trained technician. The characteristics considered were sexual abstinence (days),  
179 ejaculate volume (ml), sperm concentration (SC, 10<sup>6</sup> spermatozoa/ml) and total  
180 sperm count (volume × SC = TSC, 10<sup>6</sup> spermatozoa/ejaculate). Total sperm count

181 was the only sperm parameter presented, as it is the true reflect of sperm  
182 production.

183 After initial analysis a part of each sample was frozen in liquid nitrogen in the  
184 Germethèque Biobank (BB-0033-00081, France) using routine freezing method  
185 currently practiced in our laboratory [50] and stored until FISH analysis.

### 186 **Sperm Fluorescence in situ Hybridization (FISH)**

187 FISH analysis was performed at four time points i.e. before heating at D0, D34,  
188 post heating at PH45 and PH180 (Fig. 1). Between day 34 of heating and day 45  
189 post heating total sperm count was drastically reduced due to heating method  
190 and did not recover sufficiently until day 45 post heating which rendered the FISH  
191 analysis impossible (Figure 1). Frozen cells were thawed and washed twice with 5  
192 ml phosphate buffer saline (PBS) and centrifugation performed at 630g for 10  
193 minutes. Samples were then fixed with fixation solution (acetic acid and methyl  
194 alcohol) for 30 min at 4°C. After centrifugation at 1500g for 10 minutes, the  
195 supernatant was discarded and the pellet re-suspended. A volume of 10 µl was  
196 dropped on a slide and cell density was verified by microscopy and adjusted  
197 accordingly. Slides were incubated for a minimum of 2h at -20°C. DNA was  
198 decondensed by incubating slides in 1M NaOH for 1 min, washed twice in saline  
199 sodium citrate (SSC) buffer, then dehydrated in 70%, 90% and 100% ethyl alcohol

200 baths (2 min each). Each slide was then incubated overnight with the different  
201 probes at 37°C (Vysis probes (Abbott), CEP X spectrum green, CEP Y spectrum  
202 orange and CEP 18 spectrum aqua). After a 2 min wash in 2SSC 0.4% NP40 at 73°C  
203 followed by a 1 min wash in 2SSC 0.3% NP40, slides were incubated with 1/2000e  
204 Hoechst for 3 min and washed for 3 min in PBS. Slides were finally mounted with  
205 Antifade mounting medium (Promega, Germany) and stocked at -20°C until  
206 reading. Slides were analyzed under a Leica DM 6000 B microscope system.

207 At each time, a mean of  $5149 \pm 218$  and  $5232 \pm 190$  cells, per subject, were read  
208 respectively in experimental group and control group.

209

#### 210 **2.4. Statistical Analysis**

211 Data are presented in the Tables as median and interquartile range Q1–Q3 due to  
212 the number of patients and as mean and standard deviation in graphic  
213 representations.

214 Sperm aneuploidy data were compared before, during and after mild induced  
215 increase in testicular and epididymal temperature by the Wilcoxon signed rank-  
216 sum test. Due to the small number of men included in the experimental protocol,  
217 we also carried out statistical comparison with a control group of 27 fertile men (a  
218 single evaluation of sperm aneuploidy for each man) using the non-parametric

219 Mann-Whitney test. As there were multiple comparisons, a Bonferroni correction  
220 was used. P-values of 1.66% were considered significant for the Wilcoxon test and  
221 of 1.25% for the Mann-Whitney test.

222 Statistical analyses were performed using SAS software (9.3, SAS Institute). A p-  
223 value of 5% was considered significant without Bonferroni correction.

224

### 225 **3. Results**

226 The number of volunteers (n=5) was constant during the three study periods  
227 (before, during and after heating). Due to technical problems, at D0 and PH45  
228 four volunteers underwent FISH analyses instead of five.

229 Total sperm count decreased significantly as early as day 34 during heat exposure  
230 (Fig. 1) and remained persistently low (azoospermia or severe oligospermia)  
231 throughout the heating period (days 0-120). FISH analysis was not possible until  
232 day 45 after cessation of heating (PH45).

233 A total of 234,038 spermatozoa were analyzed by FISH. Before commencement of  
234 experimental heating (Day 0), no significant differences were found for  
235 aneuploidy percentages between the control group (n = 27) and the five

236 volunteers except for median frequencies of XXY18. However, the range of  
237 frequencies difference was very small, ranging from 0.00 to 0.02 (Table 1).

238 Table 1 represents sperm aneuploidy percentages at the different time points of  
239 the study. The major significant results were an increased total aneuploidy, sex  
240 disomy (sperm XY18) and sex chromosome nullisomy (sperm 18) at day 45 post  
241 heating (PH45). The percentages of sperm with sex disomy and sex nullisomy  
242 were two and three times more elevated, respectively, than before heating  
243 period (D0). Percentage of total aneuploidy was twofold higher at PH45 than  
244 before heating (D0) (Table 1, Fig. 2).

245 At day 180 post heating (PH180), sperm aneuploidy percentages did not differ  
246 from the values before heating (Fig. 2), which suggests that the effects of heating  
247 were reversible.

248 In this study, we defined an abnormal aneuploidy rate as a rate above the 90<sup>th</sup>  
249 percentile of control group values. This enabled us to calculate the number of  
250 volunteers who had an abnormal aneuploidy rate at different time points.

251 The results indicate that at 45 post heating, all volunteers had a higher number of  
252 abnormal sperm with sex disomy XY18, sex nullisomy and total sperm aneuploidy  
253 (Fig. 3) which did not recover to values before heating period until day 180 post  
254 heating suggesting that minimum of two cycles of spermatogenesis and

255 epididymal transit after discontinuation of heating are required in order to  
256 restore normal spermatogenesis with no risk of aneuploidy (Fig.3).



#### 257        **4. Discussion**

258        The negative impact on spermatogenesis after testicular exposure to a mild or  
259        high temperatures increase has been demonstrated by numerous experimental  
260        human and animal studies. A recent study reported an increases sperm  
261        aneuploidy rate when testes were exposed to 43°C [43]. However, these findings  
262        were taken at one-point time and reversibility was not evaluated. Moreover, 43°C  
263        is a pathological temperature which is much higher than testes and core body  
264        temperature and sperm aneuploidies are more likely to occur at this high  
265        threshold. No study has investigated the effect of a mild increase in testicular  
266        temperature (<37°C) on the frequency of sperm chromosomal abnormalities.

267        In present study by inducing a mild increase in testes and epididymal temperature  
268        for  $15 \pm 1$  hours daily for 120 consecutive days, we show that this temperature  
269        threshold is sufficient to increase sperm aneuploidy in men and that this effect is  
270        reversible after cessation of heating which suggests that testicular heating is the  
271        cause of sperm aneuploidy.

272        At day 34 of heating, the percentage of chromosomal abnormalities were not  
273        affected by the mild temperature increase. In accordance with the physiology of  
274        the spermatogenesis process and epididymal transit timing, spermatozoa  
275        collected at D34 were at the late spermatocyte stage (end of meiosis) or early

276 round spermatids in the testes when heat was induced: i.e. they were subjected  
277 to heat but were released from progenitor germ cells that were not exposed to  
278 heating. This is in agreement with previous reports indicating that most  
279 chromosomal abnormalities in sperm result from meiotic errors during the  
280 spermatogenesis process [51, 52].

281 At day 45 post heating (PH45) we found a significant increase of disomic sperm  
282 (XY18 sperm: non-disjunction of the sexual chromosome at meiosis I) or  
283 nullisomic sperm (18) and a slight increase of diploidy sperm (XY1818),  
284 consequently total aneuploidy increased at this time. It is noteworthy that  
285 spermatozoa collected at day 45 after heating were more probably affected by  
286 heating at the meiosis stage, according to spermatogenesis and epididymis  
287 physiology as previously published [24].

288 The effects of a mild increase in testes temperature are reversible, as indicated by  
289 recovery of aneuploidy values to pre-heating values at PH180. The time require to  
290 recover the aneuploidy to control values suggest that reversibility requires more  
291 than two cycles of spermatogenesis.

292 A recently published study examined sperm aneuploidy in 10 men before and  
293 during scrotal heating using an electric warming bag to increase the testes  
294 temperature to 43°C for 30-40 min two days per week for 3 months [43].

295 Compared to pre-heating values, a significant increase in the sum rate of sperm  
296 chromosomal 13, 18, 21, and sum of X and Y sperm was reported at the end of  
297 the 3 months of heating. In this last study, the increase of sum rate of aneuploidy  
298 for X and Y at the end of the 3-month period of heating was 14.2-fold higher than  
299 before heating values. This is much higher than the 2 to 3-fold increase we  
300 observed in the present study where temperature increase was very mild.  
301 However, the aneuploidy values after cessation of heating were not reported in  
302 the study by Zhang et al [43] which lacks information about the reversibility.  
303 Further, only 200 sperm cells were analyzed by Zhang et al [43] compared to  
304 present study which report analysis of  $5149 \pm 218$  and  $5232 \pm 190$  cells in  
305 experimental volunteers and controls respectively.

306 Relation of lifestyle factors, such clothing, with sperm aneuploidy has also been  
307 reported. Jurewicz et al [53] reported that use of boxer short decreases sperm  
308 aneuploidy rate compared to tight underwear.

309 In animal models, only three studies have reported the effect of induced  
310 increased temperatures on germ cell chromosomal abnormalities and abnormal  
311 chromosome configurations [42]. Exposing mice to an environment of  
312 approximately  $35 \pm 1^\circ\text{C}$  and 65% relative humidity for 2-5 days led to significant  
313 peaks of X-Y dissociation 5 days after the end of heat stress which recovered to

314 control levels 50-70 days after cessation of heat stress ( $\approx$ 1.5 to 2 cycles of  
315 spermatogenesis in mice).

316 In another study [54], testes of mice and rats under anesthesia were heated in a  
317 water bath at 42°C for 20 min which resulted in an increase in the percentage of  
318 X-Y univalents after 6 days of heat exposure in mice and 12 days in rats. These  
319 results suggest an action of heating in pre-meiotic or early prophase I stages of  
320 spermatogenesis, which was also suggested by Garriott and Chrisman [55].

321 The precise mechanism of aneuploidy induction in man following mild testis  
322 temperature increase is not known. However, meiosis is a structural dynamic  
323 process, broadly conserved across eukaryotes, and temperature appears to be a  
324 major challenge for chromosome segregation during the meiosis stage.

325 Temperature increase was associated with failure of synapsis and reduced  
326 chiasma frequency in plant and animals at both high and low temperatures [56].

327 Heat shock proteins (HSPs) play a protective role in response to cell stress and  
328 several HSPs were induced mainly in spermatocytes and round spermatids when  
329 testis temperature was increased [57]. The HSP70 gene is expressed during the  
330 meiosis prophase, and HSP70 protein is present in the synaptonemal complex and  
331 in attachment plaques at the ends of chromosomes. Desynapsis is disrupted in  
332 male mice lacking this protein [58, 59]. Moreover, heat stress following

333 experimental cryptorchidism in rats resulted in modifications in the expression of  
334 594 genes in pachytene spermatocytes [60].

335 Some cases of varicoceles are associated with abnormally elevated testes  
336 temperature. It is noteworthy that in patients with varicocele, the frequency of  
337 meiotic abnormalities such as partial asynapsis, chromosome breaks and a high  
338 rate of sex vesicle-autosome association were higher than in a control group [59].  
339 Further analysis after varicocele surgery showed no abnormalities suggesting that  
340 the meiotic alterations were due to increased temperature and were reversible.  
341 Likewise, a comparison of sperm aneuploidy frequency before and after  
342 varicocelectomy showed decrease, though not significant, in the sperm  
343 aneuploidy frequency [61].

344 One of the limitations of our study is the small number of volunteers included in  
345 this experimental protocol. The other limitation is the small number of  
346 chromosomes studied by FISH (X, Y, 18). However, the inter-chromosomal effect  
347 has been reported using CGH array [62, 63] and it has been suggested that the  
348 results obtained by studying one set of chromosomes could be extended to  
349 others [64]. The absence of longitudinal follow-up of the control group could be  
350 another limitation of our study. Testicular temperature was not measured in this

351 study owing to invasive approach and was based on the existing literature [30,  
352 48].

353 While further studies are needed to clarify the relationship between sperm  
354 aneuploidy and various circumstances resulting in testes temperature increase,  
355 findings of current study may have clinical implications. In infertile men, it seems  
356 reasonable to avoid or treat situations (fever, varicocele, ascending testis or  
357 mispositioning of testis, and occupational or professional exposure) which can  
358 result in increased scrotal or testicular temperature. If such circumstances are  
359 unavoidable, FISH analysis of sperm may be advised to identify aneuploidy which  
360 can pose possible risk to the future embryo.

361 In conclusion, this study shows that a mild induced increase ( $\approx 2^{\circ}\text{C}$ ) in testicular  
362 and epididymal temperature (15  $\pm$  1 hour daily for 120 consecutive days) results  
363 in increased sperm aneuploidy in men, which reflects an effect of temperature on  
364 the meiosis stage of spermatogenesis. This effect was reversible and suggests  
365 waiting period of minimum 2 cycles of spermatogenesis if a couple is planning to  
366 conceive allowing cells sufficient time to recover from heat stress. Nevertheless,  
367 the current results are based on a smaller population which does warrant  
368 validation of these findings on larger cohort of men.

369

370 **Conflicts of Interest:** The authors declare that there is no conflict of interest that  
371 could be perceived as prejudicing the impartiality of the research reported

372

373

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521 **Figure legends**

522 **Figure 1**

523 Mean (SEM) total sperm count (millions per ejaculate) before, during and after a mild induced  
524 testicular and epididymal temperature increase in healthy men. Red circles: semen probes  
525 used for sperm aneuploidy study: before heating (day 0), during heating (day 34) and during  
526 post heating period (day 45 and day 180). \* $p < 0.016$ .

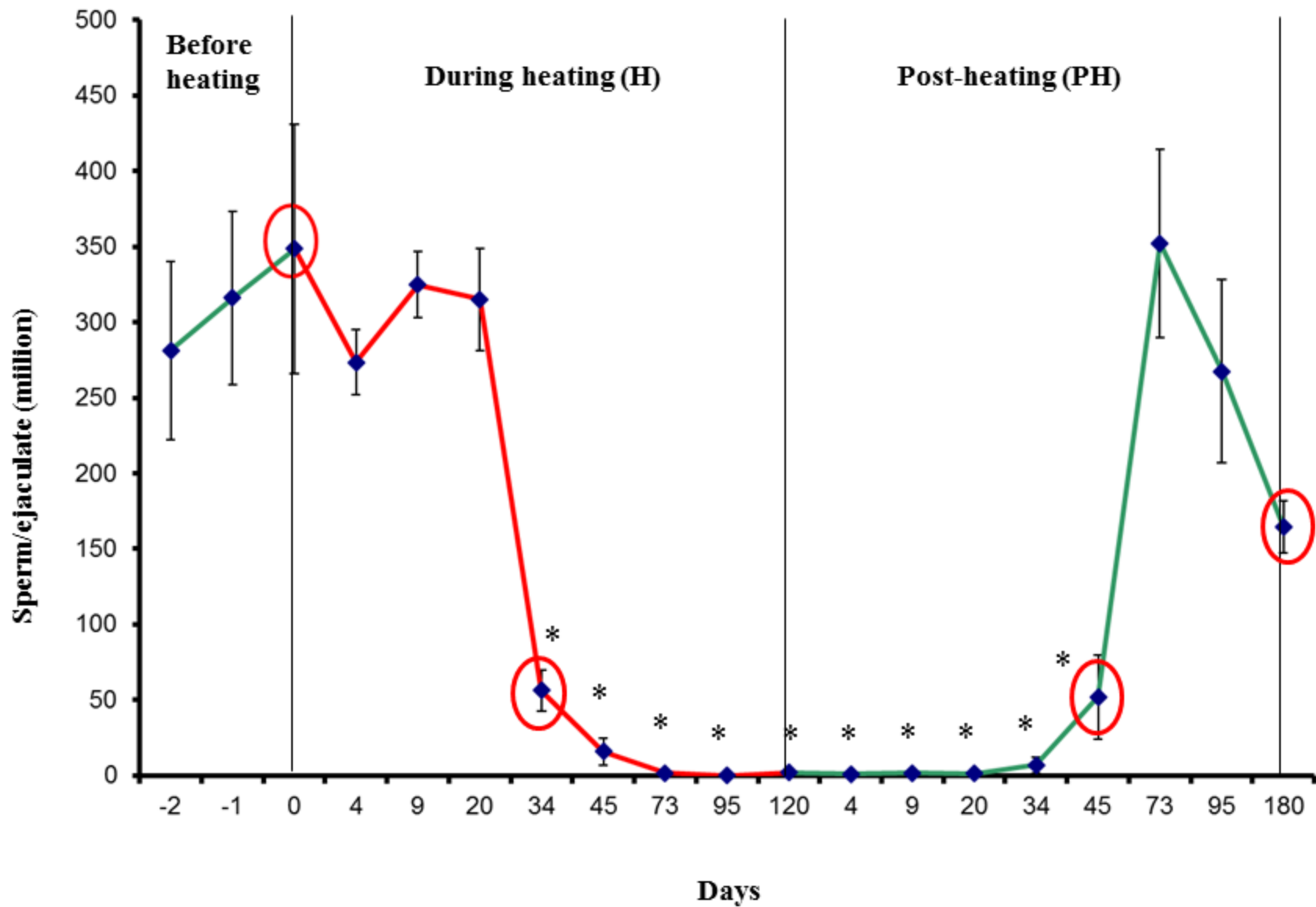
527 **Figure 2**

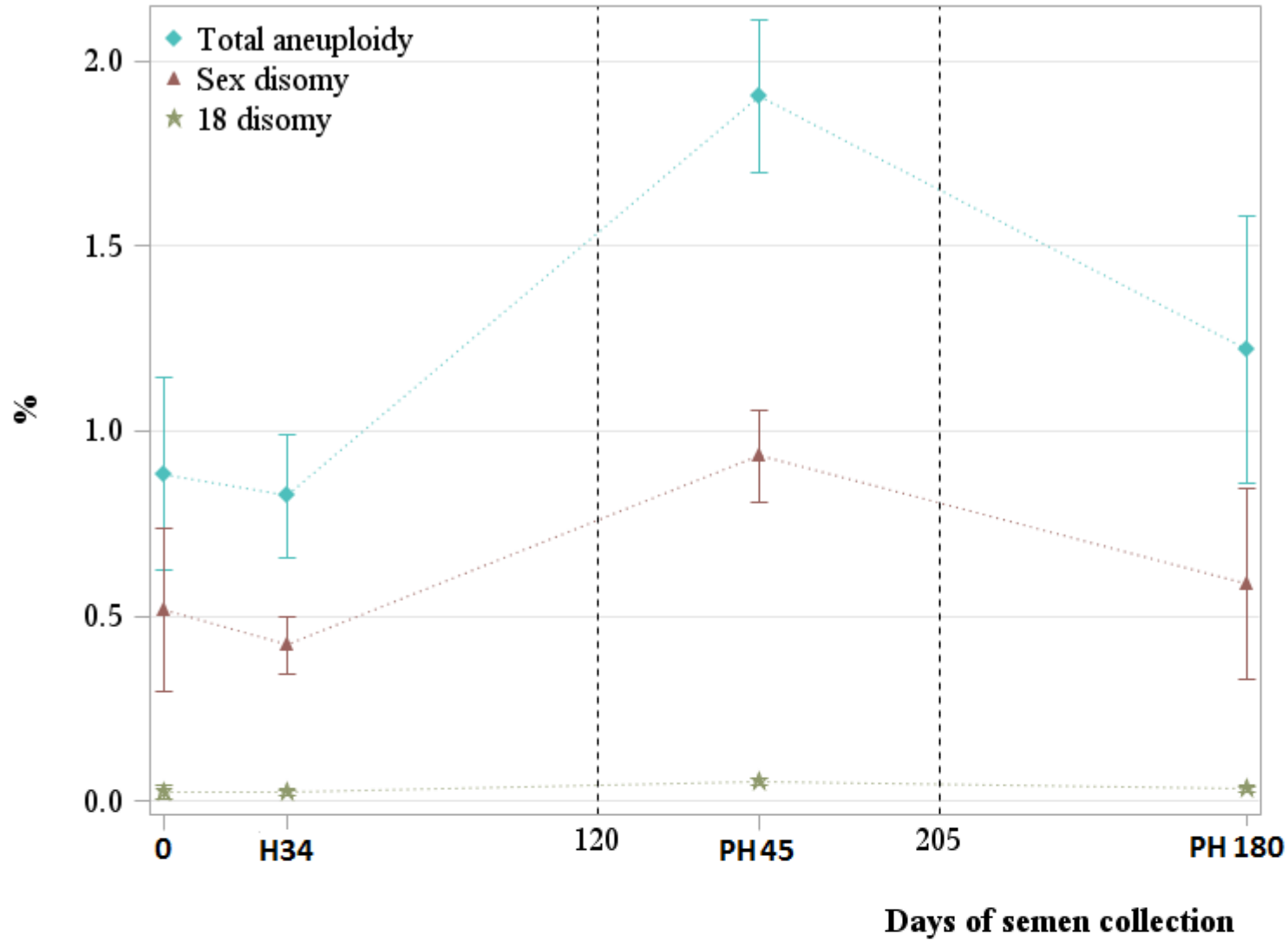
528 Sperm aneuploidies (%) before, during and after a mild increase in testicular and epididymal  
529 temperature in men. Sperm chromosomal abnormalities (%) measured at the four time points:  
530 before heating (D0), during heating (H34), and after heating (PH45 and PH180). Means  $\pm$  SEM  
531 are represented. Mild induced testicular and epididymal heating was discontinued at D120.

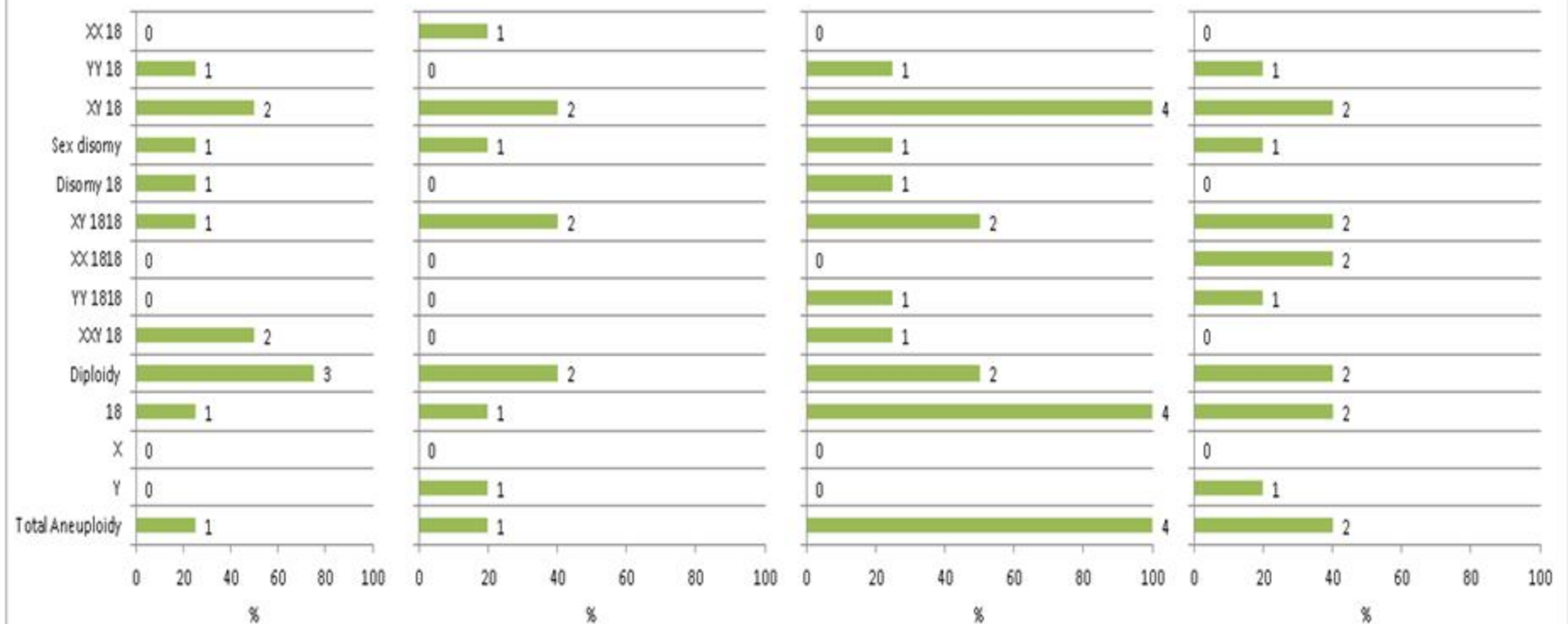
532 **Figure 3**

533 Number of volunteers whose percentage of sperm aneuploidies before, during and after a mild  
534 induced increase in testicular and epididymal temperature was greater than the 90e percentile  
535 of the control group.

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O  
(n=4)

H34  
(n=5)

PH45  
(n=4)

PH180  
(n=5)

**Table 1. Sperm aneuploidies (%) in controls and before (Day 0), during (H34) and after (PH45, 180) testicular and epididymidal heating in healthy men.**

	Controls (n=27)	Day 0 (n=4)	H 34 (n=5)	PH 45 (n=4)	PH 180 (n=5)
	median [Q1-Q3]	median [Q1-Q3]	median [Q1-Q3]	median [Q1-Q3]	median [Q1-Q3]
<b>Number of spermatozoa counted per subject</b>	5231 [5092-5335]	5082.5 [5048.5-5191.5]	5085 [5083-5143]	5076 [5047-5089.5]	5117 [5047-5120]
<b>Y 18</b>	49.43 [47.67-50.6]	49.96 [49.16-50.55]	50.39 [49.78-51.34]	49.15 [48.47-50.32]	48.89 [48.21-49.64]
<b>X 18</b>	49.82 [48.85-51.3]	49.44 [48.72-49.8]	48.98 [47.92-49.09]	48.97 [47.93-49.47]	49.66 [49.28-51.23]
<b>XX 18</b>	0.06 [0.04-0.06]	0.04 [0.01-0.06]	0.02 [0.02-0.04]	0.03 [0.02-0.04]	0.02 [0.02-0.02]
<b>YY 18</b>	0.04 [0-0.06]	0 [0-0.04]	0.02 [0-0.04]	0.04 [0.03-0.06]	0.02 [0-0.02]
<b>XY 18</b>	0.21 [0.13-0.31]	0.35 [0.21-0.72]	0.34 [0.32-0.49]	0.85 [0.68-1.03] (p=0.001)*	0.18 [0.16-0.8]
<b>Sex disomy</b>	0.30 [0.19-0.38]	0.35 [0.24-0.80]	0.45 [0.35-0.53]	0.93 [0.76-1.10] (p=0.001)*	0.22 [0.18-1.04]
<b>X 1818</b>	0 [0-0.02]	0 [0-0.02]	0 [0-0.02]	0.01 [0-0.02]	0.02 [0-0.04]
<b>Y 1818</b>	0.02 [0-0.04]	0.01 [0-0.03]	0.02 [0-0.02]	0.04 [0.04-0.05]	0.02 [0-0.02]
<b>Disomy 18</b>	0.04 [0-0.06]	0.01 [0-0.05]	0.02 [0.02-0.03]	0.05 [0.04-0.07]	0.02 [0.02-0.04]
<b>XX 1818</b>	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0.02] (p=0.001)*
<b>YY 1818</b>	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0.01]	0 [0-0]
<b>XY 1818</b>	0 [0-0]	0 [0-0.03]	0 [0-0.02] (p=0.010)*	0.01 [0-0.03] (p=0.003)*	0 [0-0.02] (p=0.010)*
<b>Diploidy</b>	0 [0-0]	0 [0-0.03]	0 [0-0.02]	0.02 [0-0.04]	0 [0-0.08]
<b>XXY 18</b>	0 [0-0]	0.01 [0-0.02] (p=0.003)*	0 [0-0]	0 [0-0.01]	0 [0-0]
<b>YYX 18</b>	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0.01]	0 [0-0]
<b>18</b>	0.24 [0.17-0.33]	0.29 [0.23-0.37]	0.26 [0.25-0.31]	0.91 [0.76-0.99] (p=0.001)*	0.41 [0.34-0.46]
<b>X</b>	0.06 [0-0.23]	0.01 [0-0.03]	0 [0-0]	0 [0-0.03]	0 [0-0.04]
<b>Y</b>	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]
<b>Nullisomy 18</b>	0.06 [0-0.23]	0.01 [0-0.03]	0 [0-0]	0 [0-0.03]	0 [0-0.06]
<b>Total aneuploidy</b>	0.65 [0.46-0.81]	0.73 [0.58-1.19]	0.73 [0.64-0.87]	1.93 [1.62-2.19] (p=0.002)*	0.7 [0.67-1.84]
<b>Meiosis I</b>	0.28 [0.17-0.32]	0.35 [0.21-0.79]	0.37 [0.34-0.51]	0.91 [0.74-1.11] (p=0.001)*	0.20 [0.20-1.19]
<b>Meiosis II</b>	0.08[0.06-0.11]	0.04 [0.01-0.10]	0.039 [0.038-0.039]	0.08 [0.07-0.09]	0.04 [0.02-0.08]

\* p <0.0125 between controls and volunteers at time 0, H34, PH 45 and PH180. Values are expressed as median with interquartile range [q1-q3]. Day 0:

before heating, H: heating period, PH: post heating period. Sex disomy: sum of XX18, YY18, XY18. Disomy 18: sum of X1818 and Y1818. Diploidy: sum of

XX1818, YY1818 and XY1818. Nullisomy 18: sum of X and Y. Meiosis I: sum of frequencies of XY18 and XY1818. Meiosis II: sum of XX18, YY18, XX1818 and YY18.