



Experimental mild increase in testicular temperature has drastic, but reversible, effect on sperm aneuploidy in men: A pilot study.

Mohamed Hadi Mohamed Abdelhamid, Camille Esquerre-Lamare, Marie Walschaerts, Gulfam Ahmad, Roger Mieusset, Safouane Hamdi, Louis Bujan

► To cite this version:

Mohamed Hadi Mohamed Abdelhamid, Camille Esquerre-Lamare, Marie Walschaerts, Gulfam Ahmad, Roger Mieusset, et al.. Experimental mild increase in testicular temperature has drastic, but reversible, effect on sperm aneuploidy in men: A pilot study.. *Reproductive biology*, 2019, 19 (2), pp.189-194. 10.1016/j.repbio.2019.06.001 . hal-03018190

HAL Id: hal-03018190

<https://hal.science/hal-03018190>

Submitted on 25 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

Mohamed Hadi Mohamed Abdelhamid^a, Camille Esquerre-Lamare^a, Marie Walschaerts^{a,b},
Gulfam Ahmad^c, Roger Mieusset^{a,d}, Safouane Hamdi^{a,e} and Louis Bujan^{a,f}

^a Université de Toulouse; UPS; Groupe de Recherche en Fertilité Humaine (EA 3694, Human
Fertility Research Group), 330 avenue de Grande Bretagne, 31059 Toulouse, France

email:

Mohamed Hadi Abdelhamid: moh9363@gmail.com

Camille Esquerre-Lamare: camille_lamare@hotmail.com

Marie Walschaerts: walschaertsm@gmail.com

Roger Mieusset: mieusset.r@chu-toulouse.fr

Safouane Hamdi: hamdi.s@chu-toulouse.fr

^b Germethèque, Groupe d'activité de médecine de la reproduction, Hôpital Paule de Viguer,
CHU Toulouse, 330 avenue de Grande Bretagne, 31059 Toulouse, France

^c School of Medical Sciences, Sydney Medical School, Discipline of Pathology, Sydney
University, Australia

Gulfam Ahmad: gulfam.ahmed@sydney.edu.au

^d Unité d'Andrologie, Groupe d'activité de médecine de la reproduction, Hôpital Paule de
Viguer, CHU Toulouse, 330 avenue de Grande Bretagne, 31059 Toulouse, France

23 ^e Laboratoire de biochimie, Institut Fédératif de Biologie, CHU Toulouse, 330 avenue de Grande
24 Bretagne, 31059 Toulouse, France

25 ^f CECOS, Groupe d'activité de médecine de la reproduction, Hôpital Paule de Viguier, CHU
26 Toulouse, 330 avenue de Grande Bretagne, 31059 Toulouse, France

27

28 **Corresponding author:** Louis Bujan, Groupe de Recherche en Fertilité Humaine (EA 3694,
29 Human Fertility Research Group), UPS, Université de Toulouse and CECOS, CHU Paule de
30 Viguier, 31059 Toulouse Cedex 09, France; e-mail: bujan.l@chu-toulouse.fr; phone: 00 33
31 567771046; fax: 00 33 567771049

32

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

34

35 **Acknowledgments:**

36 This study was supported by a grant from CHU de Toulouse N° 09 161 02.

37 We would like to thank Nina Crowte for text editing.

38

39

40

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 ± 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).

1. Introduction

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

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

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

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

It has been suggested that a modification of testicular temperature could be used in two ways: increasing testes temperature to inhibit spermatogenesis or decreasing it to improve spermatogenesis in infertile men who have abnormally elevated testes temperatures. Experimental studies [29-31] and reviews [32-34] have reported that a mild testis temperature increase could be used as a male contraceptive approach. On the other hand, reducing the testicular temperature 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.

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

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

2. Materials and methods

2.1. Study Design

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

2.2. Study Population

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

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

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

2.3. Technique for Increasing Testicular Temperature

Increased testicular and epididymal temperature was induced by maintaining the 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, 10⁶ spermatozoa/ml) and total
180 sperm count (volume × SC = TSC, 10⁶ 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

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

At each time, a mean of 5149 ± 218 and 5232 ± 190 cells, per subject, were read respectively in experimental group and control group.

2.4. Statistical Analysis

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

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

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

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

3. Results

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

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

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

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

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

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

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

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

4. Discussion

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

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

At day 34 of heating, the percentage of chromosomal abnormalities were not affected by the mild temperature increase. In accordance with the physiology of the spermatogenesis process and epididymal transit timing, spermatozoa 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 ± 218 and 5232 ± 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 ± 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

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

REFERENCES

- [1] Setchell BP. The parkes lecture heat and the testis. *J Reprod Fertil.* 1998;114:179-94.
- [2] Liu Y. Temperature control of spermatogenesis and prospect of male contraception. *Front Biosci (Scholar edition).* 2010;2:730-55.
- [3] Mieusset R, Bujan L. Testicular heating and its possible contributions to male infertility: a review. *Int J Androl.* 1995;18:169-84.
- [4] Mieusset R, Fouda PJ, Vaysse P, Guitard J, Moscovici J, Juskiewenski S. Increase in testicular temperature in case of cryptorchidism in boys. *Fertil Steril.* 1993;59:1319-21.
- [5] Agger P. Scrotal and testicular temperature: its relation to sperm count before and after operation for varicocele. *Fertil Steril.* 1971;22:286-97.
- [6] Lund L, Nielsen K. Varicocele testis and testicular temperature. *Br J Urol.* 1996;78:113-5.
- [7] Wright EJ, Young GP, Goldstein M. Reduction in testicular temperature after varicocelectomy in infertile men. *Urology.* 1997;50:257-9.
- [8] Evenson DP, Jost LK, Corzett M, Balhorn R. Characteristics of human sperm chromatin structure following an episode of influenza and high fever: a case study. *J Androl.* 2000;21:739-46.
- [9] Sergerie M, Mieusset R, Croute Fo, Daudin M, Bujan L. High risk of temporary alteration of semen parameters after recent acute febrile illness. *Fertil Steril.* 2007;88:970. e1-. e7.
- [10] Bonde JP. Semen quality in welders exposed to radiant heat. *Br J Ind Med.* 1992;49:5-10.
- [11] Hjollund NHI, Storgaard L, Ernst E, Bonde JP, Olsen Jr. Impact of diurnal scrotal temperature on semen quality. *Reprod Toxicol.* 2002;16:215-21.
- [12] Bujan L, Daudin M, Charlet J-P, Thonneau P, Mieusset R. Increase in scrotal temperature in car drivers. *Hum Reprod.* 2000;15:1355-7.
- [13] Garolla A, Torino M, Sartini B, Cosci I, Patassini C, Carraro U, et al. Seminal and molecular evidence that sauna exposure affects human spermatogenesis. *Hum Reprod.* 2013;28:877-85.
- [14] Jockenhovel F, Grawe A, Nieschlag E. A portable digital data recorder for long-term monitoring of scrotal temperatures. *Fertil Steril.* 1990;54:694-700.
- [15] Saikhun J, Kitiyanant Y, Vanadurongwan V, Pavasuthipaisit K. Effects of sauna on sperm movement characteristics of normal men measured by computer-assisted sperm analysis. *Int J Androl.* 1998;21:358-62.
- [16] Rock J, Robinson D. Effect of induced intrascrotal hyperthermia on testicular function in man. *Amer J Obstet Gynecol.* 1965;93:793-801.
- [17] Hjollund NHI, Bonde JPE, Jensen TK, Olsen Jr, Team DFPPS. Diurnal scrotal skin temperature and semen quality. *Int J Androl.* 2000;23:309-18.
- [18] Mieusset R, Bengoudifa B, Bujan L. Effect of posture and clothing on scrotal temperature in fertile men. *J Androl.* 2007;28:170-5.

- [19] Jurewicz J, Radwan M, Sobala W, Polanska K, Radwan P, Jakubowski L, et al. The relationship between exposure to air pollution and sperm disomy. *Environ Mol Mutagen*. 2015;56:50-9.
- [20] Robbins W, Elashoff D, Xun L, Jia J, Li N, Wu G, et al. Effect of lifestyle exposures on sperm aneuploidy. *Cytogen Genome Res*. 2005;111:371-7.
- [21] Shi Q, Ko E, Barclay L, Hoang T, Rademaker A, Martin R. Cigarette smoking and aneuploidy in human sperm. *Mol Reprod Dev*. 2001;59:417-21.
- [22] Young HA, Meeker JD, Martenies SE, Figueroa ZI, Barr DB, Perry MJ. Environmental exposure to pyrethroids and sperm sex chromosome disomy: a cross-sectional study. *Environ Health*. 2013;12:111.
- [23] Jurewicz J, Radwan M, Sobala W, Radwan P, Jakubowski L, Hawula W, et al. Lifestyle factors and sperm aneuploidy. *Reprod Bio*. 2014;14:190-9.
- [24] Ahmad G, Moinard N, Esquerre-Lamare C, Mieusset R, Bujan L. Mild induced testicular and epididymal hyperthermia alters sperm chromatin integrity in men. *Fertil Steril*. 2012;97:546-53.
- [25] Mieusset R, Bujan L, Mansat A, Pontonnier F, Grandjean H. Hyperthermia and human spermatogenesis: enhancement of the inhibitory effect obtained by artificial cryptorchidism. *Int J Androl*. 1987;10:571-80.
- [26] Mieusset R, Grandjean H, Mansat A, Pontonnier F. Inhibiting effect of artificial cryptorchidism on spermatogenesis. *Fertil Steril*. 1985;43:589-94.
- [27] Rao M, Zhao X-L, Yang J, Hu S-F, Lei H, Xia W, et al. Effect of transient scrotal hyperthermia on sperm parameters, seminal plasma biochemical markers, and oxidative stress in men. *Asian J Androl*. 2015;17:668.
- [28] Robinson D, Rock J. Intrascrotal hyperthermia induced by scrotal insulation: Effect on spermatogenesis. *Obstet Gynecol*. 1967;29:217-23.
- [29] Mieusset R, Bujan L. The potential of mild testicular heating as a safe, effective and reversible contraceptive method for men. *Int J Androl*. 1994;17:186-91.
- [30] Shafik A. Testicular suspension as a method of male contraception: technique and results. *Advances in contraceptive delivery systems: CDS*. 1991;7:269.
- [31] Shafik A. Contraceptive efficacy of polyester-induced azoospermia in normal men. *Contraception*. 1992;45:439-51.
- [32] Anderson RA, Baird D. Male contraception. *Endocr Rev*. 2002;23:735-62.
- [33] Setchell B. Contraception: Possible physiological bases for contraceptive techniques in the male. *Hum Reprod*. 1994;9:1081-7.
- [34] Soufir J-C. Hormonal, chemical and thermal inhibition of spermatogenesis: contribution of French teams to international data with the aim of developing male contraception in France. *Basic Clin Androl*. 2017;27:3.
- [35] Robinson D, Rock J, Menkin MF. Control of human spermatogenesis intrascrotal temperature. *Jama*. 1968;204:290-7.
- [36] Jung A, Eberl M, Schill W. Improvement of semen quality by nocturnal scrotal cooling and moderate behavioural change to reduce genital heat stress in men with oligoasthenoteratozoospermia. *Reproduction*. 2001;121:595-603.
- [37] Zorngiotti A, Sealfon A, Toth A. Further clinical experience with testis hypothermia for infertility due to poor semen. *Urology*. 1982;19:636-40.
- [38] Zorngiotti AW, Sealfon AI. Scrotal Hypothermia: New therapy for poor semen. *Urology*. 1984;23:439-41.
- [39] Hendry W, Sommerville I, Hall R, Pugh R. Investigation and treatment of the subfertile male. *Br J Urol*. 1973;45:684-92.
- [40] Jung A, Leonhardt F, Schill W-B, Schuppe H-C. Influence of the type of undertrousers and physical activity on scrotal temperature. *Hum Reprod*. 2005;20:1022-7.
- [41] Lynch R, Lewis-Jones DI, Machin DG, Desmond AD. Improved seminal characteristics in infertile men after a conservative treatment regimen based on the avoidance of testicular hyperthermia. *Fertil Steril*. 1986;46:476-9.

460 [42] Waldbieser G, Chrisman C. X Y chromosome univalency in the testes of hyperthermic mice: I.
 461 Concomitant formation of multinucleated giant cells. *Gamete Res.* 1986;15:153-60.

462 [43] Zhang MH, Zhai LP, Fang ZY, Li AN, Qiu Y, Liu YX. Impact of a mild scrotal heating on sperm
 463 chromosomal abnormality, acrosin activity and seminal alpha glucosidase in human fertile males.
 464 *Andrologia.* 2018;50:e12985.

465 [44] Templado C, Uroz L, Estop A. New insights on the origin and relevance of aneuploidy in human
 466 spermatozoa. *Mol Hum Reprod.* 2013;19:634-43.

467 [45] Templado C, Vidal F, Estop A. Aneuploidy in human spermatozoa. *Cytogenet Genome Res.*
 468 2011;133:91-9.

469 [46] Eskenazi B, Wyrobek A, Kidd S, Lowe X, Moore D, 2nd, Weisiger K, et al. Sperm aneuploidy in fathers
 470 of children with paternally and maternally inherited Klinefelter syndrome. *Hum Reprod.* 2002;17:576-83.

471 [47] Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet.*
 472 2001;2:280.

473 [48] Kitayama T. Study on testicular temperature in men. *Hinyokika Kiyo Acta Urologica Japonica.*
 474 1965;11:435.

475 [49] WHO. WHO laboratory manual for the examination and processing of human semen. Geneva:
 476 World Health Organisation; 2010.

477 [50] Esquerré-Lamare C, Walschaerts M, Debordeaux LC, Moreau J, Bretelle F, Isus F, et al. Sperm
 478 aneuploidy and DNA fragmentation in unexplained recurrent pregnancy loss: a multicenter case-control
 479 study. *Basic Clin Androl.* 2018;28:4.

480 [51] Griffiths A, Miller J, Suzuki D, Lewontin R, Gelbart W. Chromosome mutation II: Changes in
 481 chromosome number. In: Griffiths AJF MJ, Suzuki DT, editor. *An Introduction to genetic analysis.* New
 482 York: WH Freeman; 2000.

483 [52] Uroz L, Templado C. Meiotic non-disjunction mechanisms in human fertile males. *Hum Reprod.*
 484 2012;27:1518-24.

485 [53] Jurewicz J, Radwan M, Sobala W, Radwan P, Jakubowski L, Hawula W, et al. Lifestyle factors and
 486 sperm aneuploidy. *Reprod Biol.* 2014;14:190-9.

487 [54] Van Zelst S, Zupp J, Hayman D, Setchell B. XY chromosome dissociation in mice and rats exposed to
 488 increased testicular or environmental temperatures. *Reprod Fertil Dev.* 1995;7:1117-21.

489 [55] Garriott M, Chrisman C. Hyperthermia induced dissociation of the X Y bivalent in mice. *Environ*
 490 *Mutagen.* 1980;2:465-71.

491 [56] Bomblies K, Higgins JD, Yant L. Meiosis evolves: adaptation to external and internal environments.
 492 *New Phytol.* 2015;208:306-23.

493 [57] Widlak W, Vydra N. The role of heat shock factors in mammalian spermatogenesis. The role of heat
 494 shock proteins in reproductive system development and function: Springer; 2017. p. 45-65.

495 [58] Eddy EM. Role of heat shock protein HSP70-2 in spermatogenesis. *Rev Reprod.* 1999;4:23-30.

496 [59] North M, Lellei I, Rives N, Erdei E, Dittmar A, Barbet J, et al. Reversible meiotic abnormalities in
 497 azoospermic men with bilateral varicocele after microsurgical correction. *Cell Mol Biol (Noisy-le-Grand,*
 498 *France).* 2004;50:281-9.

499 [60] Yadav SK, Pandey A, Kumar L, Devi A, Kushwaha B, Vishvkarma R, et al. The thermo-sensitive gene
 500 expression signatures of spermatogenesis. *Reprod Biol Endo.* 2018;16:56.

501 [61] Acar H, Kilinc M, Guven S, Yurdakul T, Celik R. Comparison of semen profile and frequency of
 502 chromosome aneuploidies in sperm nuclei of patients with varicocele before and after varicocelectomy.
 503 *Andrologia.* 2009;41:157-62.

504 [62] Hornak M, Vozdova M, Musilova P, Prinosilova P, Oracova E, Linkova V, et al. Comprehensive
 505 meiotic segregation analysis of a 4-breakpoint t (1; 3; 6) complex chromosome rearrangement using
 506 single sperm array comparative genomic hybridization and FISH. *Reprod Biomed Online.* 2014;29:499-
 507 508.

508 [63] Patassini C, Garolla A, Bottacin A, Menegazzo M, Speltra E, Foresta C, et al. Molecular karyotyping
 509 of human single sperm by array-comparative genomic hybridization. *PLoS One.* 2013;8:e60922.

510 [64] Frias S, Van Hummelen P, Meistrich ML, Lowe XR, Hagemeister FB, Shelby MD, et al. NOVP
511 chemotherapy for Hodgkins disease transiently induces sperm aneuploidies associated with the major
512 clinical aneuploidy syndromes involving chromosomes X, Y, 18, and 21. Cancer Res. 2003;63:44-51.

513

514

515

516

517

518

519

520

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). * $p < 0.016$.

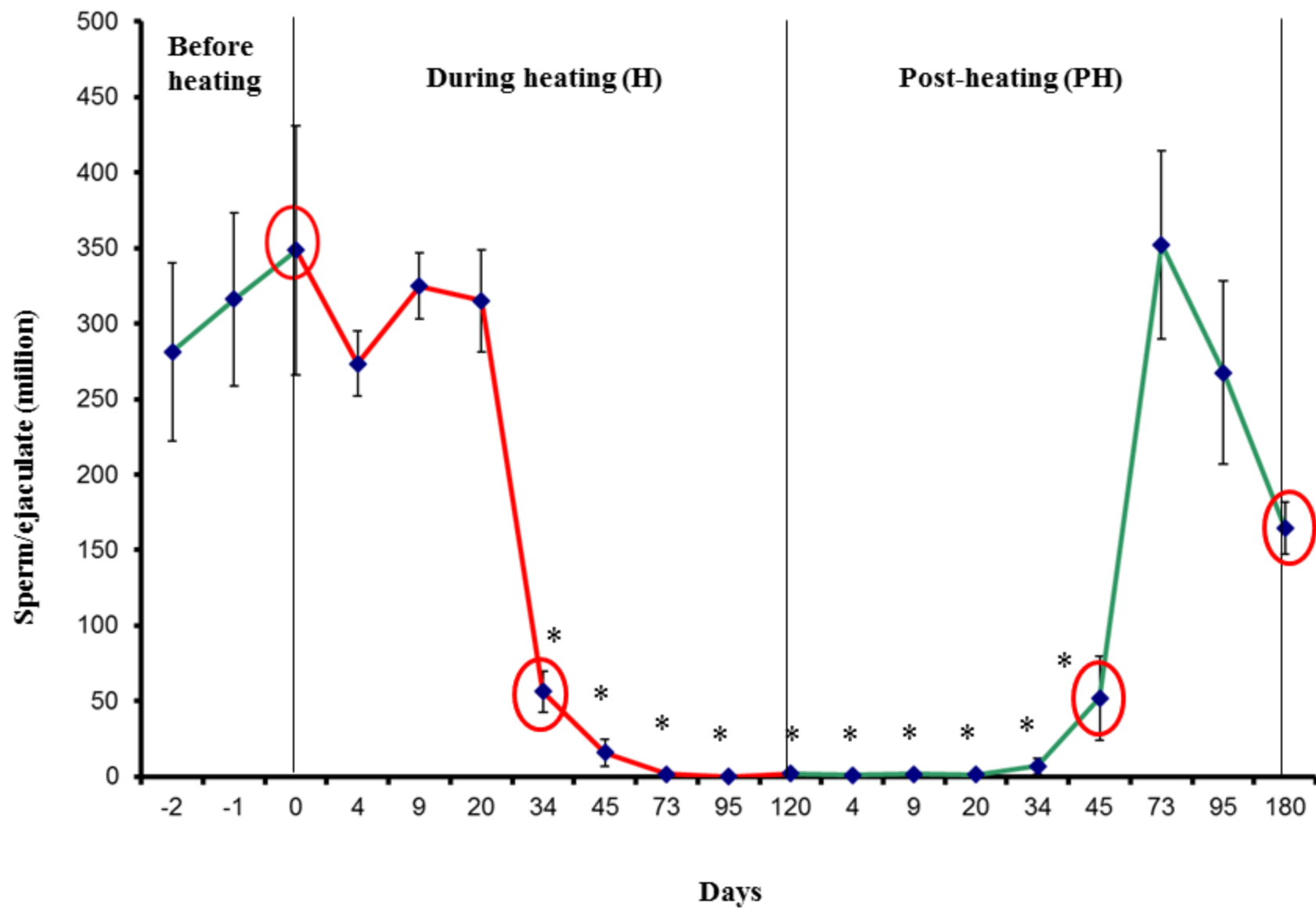
527 **Figure 2**

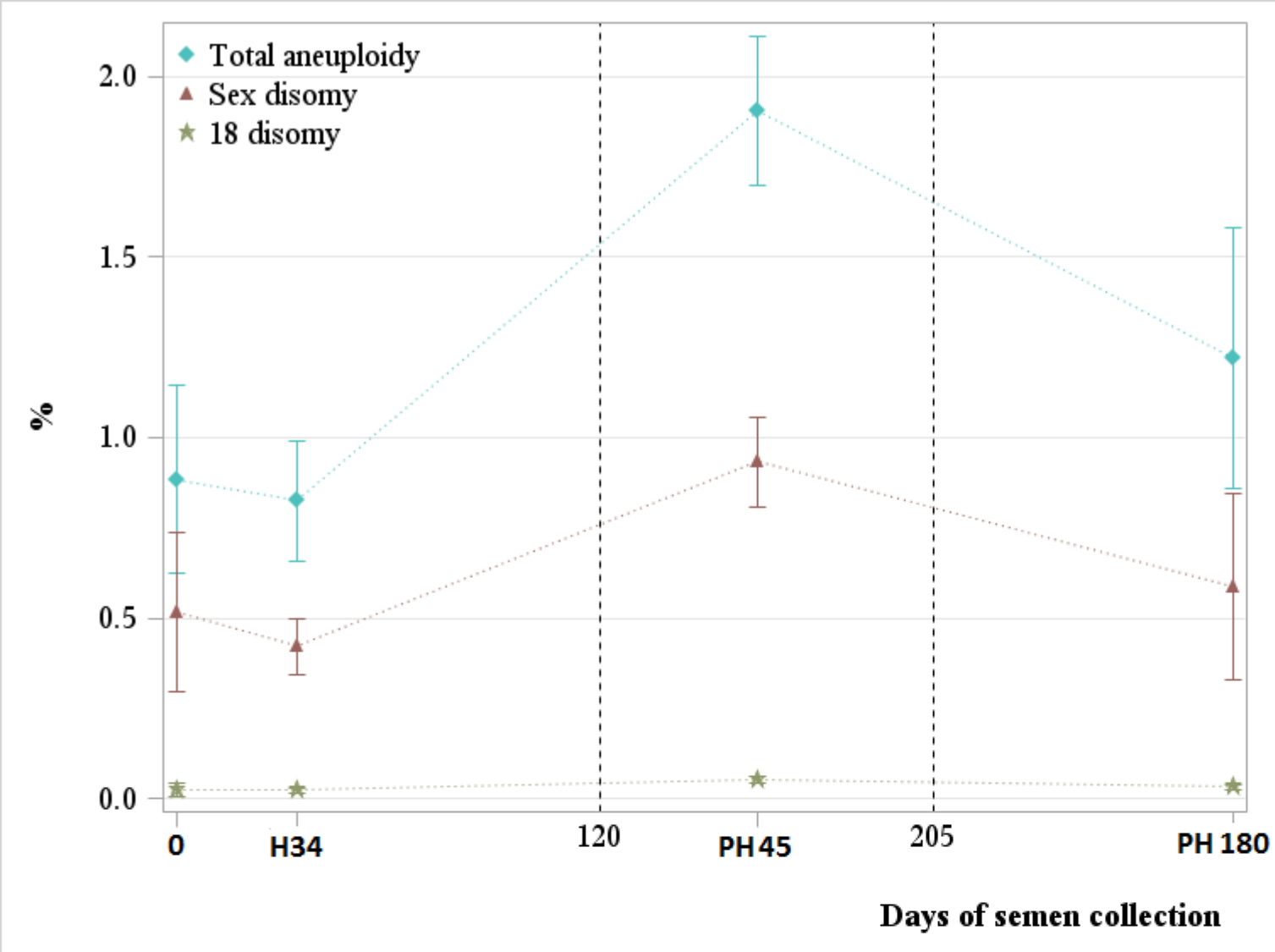
528 Sperm aneuploidies (%) before, during and after a mild increase in testicular and epididymal
529 temperature in men. Sperm chromosomal abnormalities (%) measured at the four time points:
530 before heating (D0), during heating (H34), and after heating (PH45 and PH180). Means \pm SEM
531 are represented.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

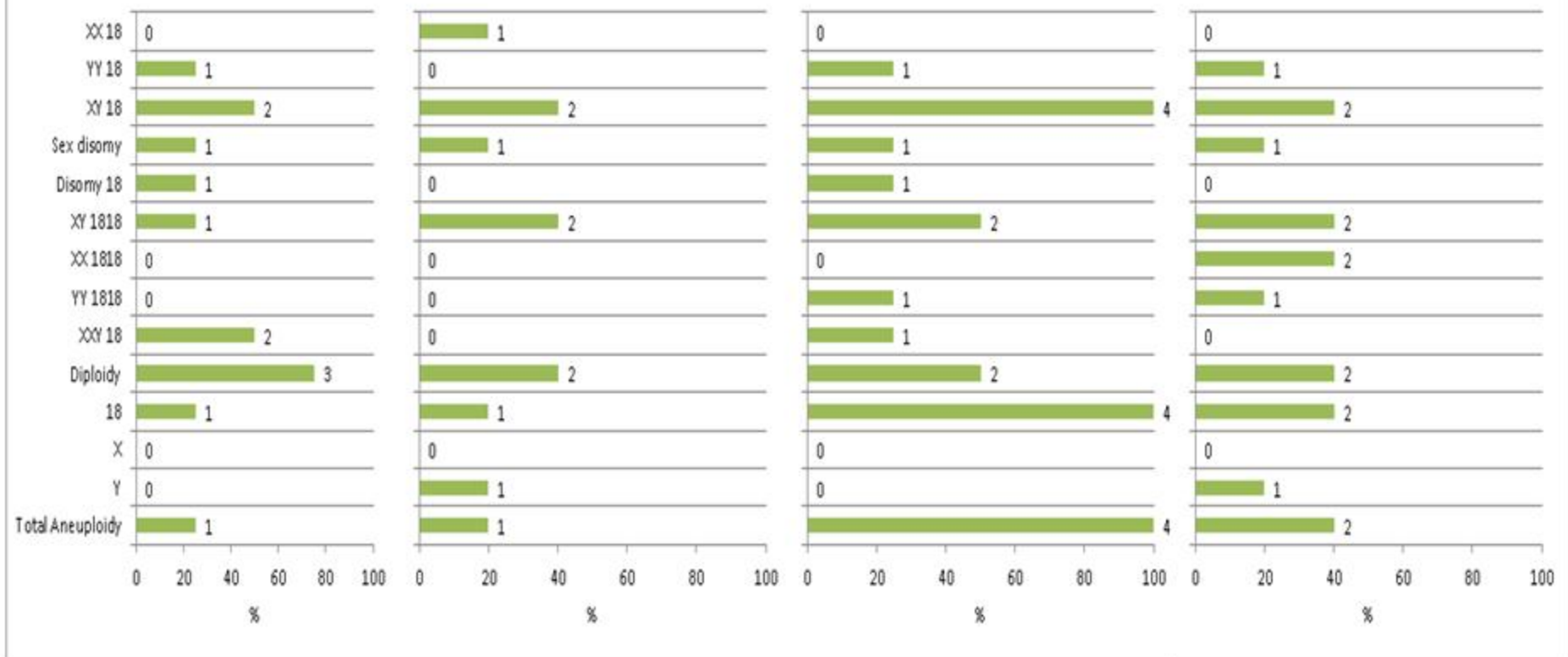
532 **Figure 3**

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.

536







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]
Number of spermatozoa counted per subject	5231 [5092-5335]	5082.5 [5048.5-5191.5]	5085 [5083-5143]	5076 [5047-5089.5]	5117 [5047-5120]
Y 18	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]
X 18	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]
XX 18	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]
YY 18	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]
XY 18	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]
Sex disomy	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]
X 1818	0 [0-0.02]	0 [0-0.02]	0 [0-0.02]	0.01 [0-0.02]	0.02 [0-0.04]
Y 1818	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]
Disomy 18	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]
XX 1818	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0.02] (p=0.001)*
YY 1818	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0.01]	0 [0-0]
XY 1818	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)*
Diploidy	0 [0-0]	0 [0-0.03]	0 [0-0.02]	0.02 [0-0.04]	0 [0-0.08]
XXY 18	0 [0-0]	0.01 [0-0.02] (p=0.003)*	0 [0-0]	0 [0-0.01]	0 [0-0]
YYX 18	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0.01]	0 [0-0]
18	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]
X	0.06 [0-0.23]	0.01 [0-0.03]	0 [0-0]	0 [0-0.03]	0 [0-0.04]
Y	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]
Nullisomy 18	0.06 [0-0.23]	0.01 [0-0.03]	0 [0-0]	0 [0-0.03]	0 [0-0.06]
Total aneuploidy	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]
Meiosis I	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]
Meiosis II	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.