Mild induced testicular and epididymal hyperthermia alters sperm chromatin integrity in men

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Objective: To investigate the effects of a mild induced testicular and epididymal hyperthermia (+2°C) on sperm chromatin integrity in men.
Design: Experimental prospective study.
Setting: University hospital.
Patient(s): Five healthy fertile volunteers.
Intervention(s): Testicular and epididymal hyperthermia was induced by maintaining the testes at inguinal position with the support of specially designed underwear 15 ± 1 hours daily for 120 consecutive days.
Main Outcome Measure(s): Classic semen characteristics. Sperm DNA fragmentation index (DFI) and high DNA stainability (HDS) were analyzed by sperm chromatin structure assay.
Result(s): Compared with baseline values, sperm DFI and HDS were significantly increased as early as day (D) 20 and D34, respectively, and remained elevated during the entire period of hyperthermia. Percentages of motile and viable spermatozoa decreased as early as D20 and D34, respectively, and total sperm count decreased at D34 during hyperthermia and remained low during the entire hyperthermia period. All studied parameters returned to respective baseline values at D73 after cessation of hyperthermia.
Conclusion(s): Mild induced testicular and epididymal hyperthermia largely impaired sperm chromatin integrity, which appeared before any changes in sperm output. These findings may have clinical implications in male contraception, infertility, and assisted reproductive technology. (Fertil Steril® 2012;97:546–53. ©2012 by American Society for Reproductive Medicine.)
Key Words: Testis, sperm chromatin, infertility, temperature, humans

Heat stress has deleterious effects on testicular functioning and is a well known cause of impaired spermatogenesis (1). Several studies in different animal species, e.g., mice (2, 3), rats (4, 5), and sheep (6), have shown harmful effects of induced testicular hyperthermia on sperm characteristics. Testicular and epididymal hyperthermia also has undesirable effects on epididymal function, causing rapid epididymal transit of sperm and resulting in a decreased number of motile and mature ejaculated spermatozoa (7, 8).

Several endogenous factors can modify human testicular temperature, such as cryptorchidism in children (9) and a history of cryptorchidism in infertile men (10, 11). Varicocele can also disturb testicular thermoregulation, causing scrotal hyperthermia and alterations in sperm parameters (1, 12, 13). An episode of fever in a man with body temperature 39°C–40°C for 2 days caused reduced sperm output, motility, and viability (14). Exogenous factors also have been reported to increase scrotal temperature, such as sitting or sleeping postures (15–19), clothing (20), sauna baths (21), driving for long periods (22, 23), and occupational exposure to high environmental temperatures (24).

In view of the potential effects of heat stress on spermatogenesis, some studies have been conducted on male contraceptive methods using different approaches to increase testicular temperature. In a previous study, we developed a diurnal artificial cryptorchidism (DAC) method to induce mild testicular and epididymal hyperthermia (36.5°C, inferior to body temperature), which resulted in a decrease in total sperm output and sperm motility (25) and an increase in morphologically abnormal spermatozoa (26). This type of method was later used as a contraceptive method in men (27–30).

Moreover, testicular heat stress has shown damaging effects on sperm DNA quality, causing arrest in early embryo development and high miscarriage rates, as reported in animal studies.

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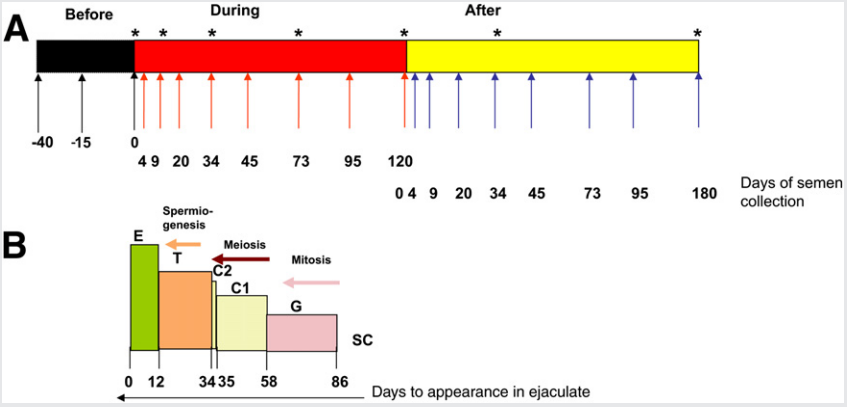
FIGURE 1



Chronology of semen sampling. (A) Schematic representation of semen sampling timing during the three study periods: before, during, and after mild induced testicular and epididymal hyperthermia in men. *Asterisks* indicate days when volunteers underwent complete clinical evaluations during the three study periods. (B) Location and evolutionary stages of sperm during the spermatogenic process at induction of hyperthermia (D0) and their expected appearance in ejaculates. E = epididymal sperm; T = spermatids; C2 = spermatocytes II; C1 = spermatocytes I; G = spermatogonia; SC = stem cells (modified from May et al. [36]).

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Mieusset et al. (31) observed no changes in sperm viability or motility in rams after 4 and 15 days of diurnal scrotal insulation, but there was an increase in embryo loss that may suggest possible harmful effects of heat stress on sperm genetic and/or epigenetic information. In mice, a paternal effect on embryo development has been demonstrated after 24 hours' exposure to an ambient temperature of 36°C, resulting in impaired embryo development (32). In addition, local scrotal heating of male mice to a high temperature (40–42°C for 30 minutes) caused sperm DNA breakdown, reduced pregnancy rates, low placental weight and litter size, and arrested embryo development at blastocyst stage (33, 34).

In men, few studies have examined the effect of testicular heat stress on sperm DNA quality. Evenson et al. (35) described the case of a patient whose sperm showed a high DNA fragmentation index (DFI) and altered composition of nuclear proteins after an episode of influenza and high fever (39.9°C). Likewise, our group reported another case of high fever (39–40°C) in a man resulting in a postfever increase in DFI which returned to baseline values 58 days after the fever (14).

Although studies on animal models have provided solid evidence on sperm DNA damage after heat exposure, no study in men has yet documented such damage during mild induced testicular and epididymal hyperthermia. This is the first report in men of the deleterious effects of diurnal mild testicular and epididymal hyperthermia (at a temperature inferior to core body temperature) on sperm DNA quality.

MATERIALS AND METHODS

Study Population

The study was approved by the Toulouse Ethics Committee (Comité de Protection des Personnes Sud-Ouest et Outre Mer I). Five healthy fertile volunteers (age 25–35 years, having fathered at least one child) with no andrologic, medical, or surgical history were recruited after their written informed consents.

Study Design

The study was divided into three periods: before (baseline/control), during, and after hyperthermia. The days of semen collection were established in accordance with the physiologic chronology of spermatogenesis and epididymal transit (36–38) (Fig. 1).

Testicular and Epididymal Hyperthermia Induction

We used the DAC method developed by our group (25), which is well tolerated and reversible. Each participant was provided with specially designed underwear with an orifice allowing the penis and the scrotum to be exteriorized. A comprehensive demonstration was given to the volunteers explaining how to push up and maintain the testicles at the upper part of the root of the penis. All volunteers were able to push and maintain the testicles in this position during waking hours (15 ± 1 hours daily) for 120 consecutive days during hyperthermia. Regular clinical examinations were performed on different days (Ds) during and after hyperthermia (Fig. 1) by two andrologists.

Semen Sampling

Three semen samples were collected before, eight during, and eight after hyperthermia. At all data points, samples from five volunteers were collected, except at D95 during hyperthermia when one volunteer did not attend for family reasons. Samples were collected by masturbation in our laboratory after a mean sexual abstinence duration of 4.1 ± 0.1 days. After liquefaction (37°C for 30 minutes), semen analysis was performed as previously published (14, 39). Briefly, semen volume was measured by a graduated pipette and the pH measured with reaction paper. Sperm motility was observed

TABLE 1

Semen parameters before, during and after mild induced testicular and epididymal hyperthermia in men.

	Day of semen collection								
	Before			During					
	40	15	0	4	9	20	34	45	73
AD (d)	3.8 ± 0.2	4.4 ± 0.6	4.2 ± 0.2	3.8 ± 0.2	4.2 ± 0.2	4.4 ± 0.2	4 ± 0.3	3.8 ± 0.3	3.8 ± 0.2
Volume (mL)	5.1 ± 1.2	4.1 ± 0.7	5.1 ± 1.4	5.6 ± 1	4.4 ± 0.6	5.0 ± 0.8	4.0 ± 1.1	4.9 ± 1.3	4.2 ± 0.7
Sperm count ($\times 10^6$/mL)	62 ± 14.2	83 ± 19.3	88 ± 28.6	68 ± 15.2	75 ± 12.8	68 ± 12.9	15 ± 3.1*	2.8 ± 1.0*	0.4 ± 0.2*
Round cell count ($\times 10^6$/mL)	1.1 ± 0.4	2.0 ± 1.1	1.2 ± 0.6	1 ± 0.5	2 ± 0.8	6.3 ± 1.7	6.0 ± 1.9*	2.1 ± 0.8	0.5 ± 0.8*
Sperm viability (%)	70.4 ± 7	72.8 ± 4.6	76.4 ± 2.3	71 ± 3.8	76 ± 3.4	70 ± 3.7	49 ± 9.2*	36 ± 10.4*	23 ± 14.3*
Progressive motility (%)	49 ± 5.7	46 ± 2.9	46 ± 3.6	50 ± 5.4	45 ± 4.1	31 ± 2.9*	21 ± 4.8*	7.4 ± 3.5[a]*	8.6 ± 7.8[b]*

Note: Values shown are mean ± SEM of five volunteers at all time points except: [a] n = 4; [b] n = 2; [c] n = 1 and [d] n = 3. One man became azoospermic at D95 and another at D120 during hyperthermia, and the remaining 3 men had rare motile or nonmotile spermatozoa in their ejaculates. The means ± SEM of the three means before hyperthermia, which were compared with the means during and after hyperthermia, were AD 4.1 ± 0.1 (d), volume 4.8 ± 0.3 (mL), sperm concentration 78.1 ± 8.1 ($\times 10^6$/mL), round cell count 1.5 ± 0.26 ($\times 10^6$/mL), sperm viability 73.2 ± 1.7 (%), and sperm motility 47 ± 1.0 (%). AD = abstinence duration.
*$P<.05$.

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under optical microscope and expressed as percentage progressive motility (sperm crossing the microscopic field). Sperm viability was assessed by nigrosin and eosin staining (Sigma Aldrich) and expressed as percentage viability. Sperm and round cell counts ($\times 10^6$) were assessed with the use of a Malassez cell (Rogo Sanlab) as published previously (25). Total sperm and round cell counts were calculated per ejaculate (i.e., sperm/round cell count × semen volume). Semen analyses were performed blindly, and all readings were taken in duplicate within 1 hour of sample collection.

Sperm Chromatin Structure Assay

Sperm DFI and high DNA stainability (HDS) were measured by conventional sperm chromatin structure assay (SCSA) techniques (35, 40) routinely used in our laboratory (14, 39). Briefly, a fraction of semen sample containing a minimum of 4×10^6 spermatozoa was separated from the original sample. For sperm fixation, 5 mL Dulbecco phosphate-buffered saline solution (DPBS; pH 7.4, 1×; Gibco) was added drop by drop to 1 mL, the remaining 4 mL were then added more rapidly, and centrifugation was performed at 630 *g* for 10 minutes. The supernatant was removed and the precipitate was mixed in 1 mL of DPBS. Then, drop by drop, 4 mL paraformaldehyde (PFA; 1%; Merck; >95%, NaOH, PBS, pH 7.4) was added to make a final volume of 5 mL and incubated at room temperature for 30 minutes. A second centrifugation was carried out at 1,500 *g* for 10 minutes, the supernatant was discarded, and the precipitate was resuspended in the required volume of DPBS according to sperm count. A volume containing a minimum of 2×10^6 spermatozoa was drawn from the sample already fixed in PFA and centrifuged at 1,500 *g* for 10 minutes. The precipitate was resuspended in 200 μL DPBS and transfered into a cytometric tube with identity of the sample. Samples were run in duplicates at all data points except where sperm count dropped lower than the required sperm number for SCSA analysis. The flow cytometer was preequilibrated by passing a tube containing 1.2 mL acridine orange and 0.4 mL acid detergent (pH 1.2) for 5 minutes. After vortexing, 0.4 mL acid detergent was added to the sample, and mixing was continued by gently shaking the tube for 30 seconds. Then 1.2 mL acridine orange was added and incubated in ice for 3 minutes. After incubation the sample was passed through the cytometer and at least 5,000 sperm cells counted.

Statistical Analysis

Means (±SEM) of the three means of the three data points (each data point: n = 5) before hyperthermia were compared with the means of each data point (n = 5) during and after hyperthermia for all semen parameters (significance level at $P<.05$). Mean values of sperm viability, motility, total sperm and round cell counts, and sperm DFI and HDS were compared by applying Wilcoxon test using Stata software version 8.

RESULTS

No volunteer withdrew from the study because of discomfort, pain, or any other medical reasons. No change was observed in mean ± SEM semen pH (data not shown), semen volume, or duration of abstinence (Table 1) during the three periods of the study.

During Hyperthermia

Sperm motility, viability and count. Percentages of motile and viable sperm decreased significantly ($P<.05$) compared with baseline values as early as D20 and D34, respectively, and the difference remained significant until D120 (Table 1). Sperm concentration decreased significantly as early as D34 and remained low during the entire period of hyperthermia (Table 1). Baseline total sperm count was 315.2 ± 19.4 $\times 10^6$, which significantly decreased to $56.0 \pm 13 \times 10^6$ at D34 and to $16.0 \pm 8 \times 10^6$ at D45 (95% decrease in sperm output). One of the five volunteers presented with

TABLE 1

Continued.

Day of semen collection									
After									
95	120	4	9	20	34	45	73	95	180
4.4 ± 0.4[a]	3.8 ± 0.2	3.8 ± 0.2	4.4 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	3.8 ± 0.3	4 ± 0.0	4.2 ± 0.2	3.8 ± 0.2
4.7 ± 0.7[a]	4.0 ± 0.5	4.1 ± 0.5	5.1 ± 0.9	4.1 ± 0.6	4.1 ± 0.8	4.3 ± 1.0	5.3 ± 1.0	4.5 ± 0.8	4.1 ± 0.6
0.04 ± 0.03[a]*	0.4 ± 0.4*	0.2 ± 0.2*	0.2 ± 0.1*	0.2 ± 0.2*	2.2 ± 1.2*	19 ± 11.2*	86 ± 23.1	70 ± 17.1	59.2 ± 19.2
0.8 ± 0.5[a]	3.2 ± 2.9	1.6 ± 1.1	1.5 ± 0.6	1.4 ± 1.1	1.7 ± 1.0	2 ± 0.6	3 ± 1.6*	1.9 ± 0.4	0.9 ± 0.3
20 ± 15[b]*	12.5[c]	13.8[c]	16[c]	26 ± 16[b]	46 ± 19.2[d]	56 ± 14.3[a]	75 ± 4.2	77 ± 4.8	72 ± 5.2
7.6 ± 5.8[b]*	5.0[c]	5.8[c]	4[c]	11 ± 5[d]*	22.2 ± 8[a]*	34 ± 5.3*	41 ± 4.8	49 ± 7.8	45 ± 5

azoospermia at D95, a second at D120, and the remainder had rare spermatozoa in their ejaculates at D120 (Fig. 2B).

Round cells. Round cell count and total count started to increase at D20 ($P=.07$) and to be significantly higher than baseline values at D34, then decreased to baseline values until the end of hyperthermia (D120) with the exception of a low significant value at D73 (Table 1; Fig. 2A).

SCSA. Mean ± SEM sperm DFI (%) was significantly increased at D20 (16.7 ± 3.9), D34 (23.8 ± 2.9), and D45 (31.3 ± 5.4) compared with baseline (11.9 ± 1.5; ~200% increase; Fig. 3B). Mean sperm HDS started to increase as early as D20 (7.4 ± 1.5; $P=.07$) and was significantly higher than baseline value (5.9 ± 0.3) at D34 (10.9 ± 1.0) and D45 (13.0 ± 1.1; Fig. 3A). The percentage values of sperm DFI (27.8 ± 4.3) and HDS (14.9 ± 2.3, $P=.06$) remained higher at D73 but appeared statistically nonsignificant, most probably owing to the smaller number of volunteers (n = 4) at this time point (Fig. 3).

Because total sperm count was drastically decreased from D73 until D120 during hyperthermia, the number of spermatozoa was insufficient for SCSA, which requires a minimum of 2 million sperm (35). For this reason, both sperm DFI and HDS were analyzed in only one volunteer at D95 and two at D120 having sufficient number of spermatozoa (Fig. 3).

After Hyperthermia

Sperm motility, viability, and count. Percentages of motile and viable spermatozoa, sperm count (Table 1), and total count (Fig. 2B) reached respective baseline values at D73 after cessation of hyperthermia.

Round cells. Round cell count and total count did not differ from baseline values, except at D73 with a significantly higher value (Table 1; Fig. 2A).

SCSA. Percentage values of sperm DFI and HDS were higher, but statistically nonsignificant, than baseline values at D45 (n = 3) and recovered baseline values from D73 to D180 (Fig. 3).

DISCUSSION

This is the first study in men that reports the drastic but reversible effects of mild induced increase in testicular and epididymal temperature on sperm DNA integrity. To date, an increase in sperm DNA fragmentation in men has been observed in only two case reports (14, 41) of high fever (39–40C°) which correspond to the complex conditions of whole body heating. To focus on the testes and epididymides alone, we investigated the effects of a mild increase (~2C°) in testicular and epididymal temperature in men (15 h/d for 120 consecutive days) on sperm parameters and sperm DNA quality.

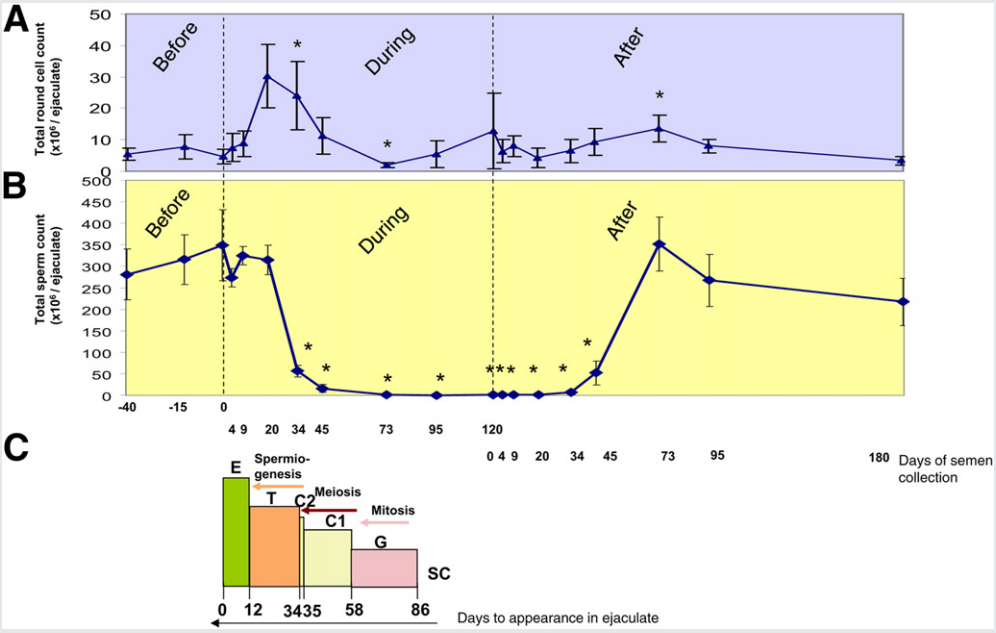
Based on widely accepted concepts of duration of human spermatogenesis, almost 74 days are required for one complete spermatogenic cycle (i.e., spermatogonia to spermatozoa) in men (37). It takes a further ~12 days (1–22) for the spermatozoa to pass through the epididymis and vas deferens and to reach the ejaculate as measured in the only in vivo physiologic study after incorporation of H_3-thymidine in men (42). Therefore, we followed those authors and the reports on the investigations of the effects of repeated exposures to chemotherapy (38) or radiotherapy (36) on spermatogenesis in men.

Sperm Parameters

Effects on epididymal spermatozoa. According to chronology of spermatogenesis and epididymal transit, spermatozoa collected at D4 and D9 during hyperthermia most probably were stored in the epididymis when hyperthermia was induced. At these time points (D4 and D9), we found no change in total sperm count or percentage of motile and viable spermatozoa. This finding is in accordance with previously published animal studies (31, 43).

Effects on testicular germ cells. The spermatozoa collected at D20 were at the elongated spermatid stage (late

FIGURE 2



Total sperm and round cell counts ($\times 10^6$/ejaculate) measured during the three study periods: before, during, and after mild induced testicular and epididymal hyperthermia in men. (A) Total round cell count; (B) total sperm count. Means ± SEM (6 ± 0.9 and 315.24 ± 19.24 $\times 10^6$/ejaculate, respectively) of the three means ± SEM before hyperthermia were compared with the means ± SEM of each data point during and after hyperthermia. However, for easier interpretation of the data, all three means before hyperthermia are plotted in the figure. *$P<.05$. (C) Location and evolutionary stages of sperm during the spermatogenic process at induction of hyperthermia (D0) and their expected appearance in ejaculates. Abbreviations as in Figure 1.

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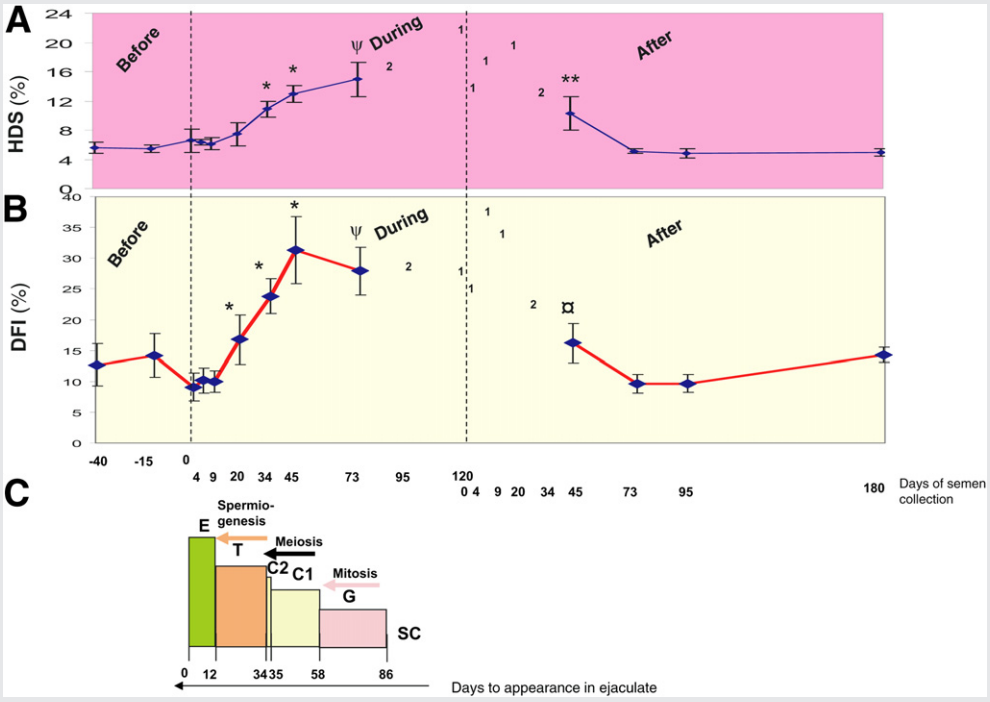
spermiogenesis) in the testes, and those collected at D34 were at the late spermatocyte stage (end of meiosis) or early round spermatid stage (beginning of spermiogenesis) when hyperthermia was induced. A significant drop in the percentage of motile spermatozoa occurred as early as D20 during hyperthermia. This fall may be the consequence of two cumulative effects of heating. First, elongated spermatids may be altered during spermiogenesis, as observed 48 hours after induced cryptorchidism in rats (44). Second, when passing through the epididymis, spermatozoa acquire the capacity of motility, which can be impaired due to inappropriate ion and protein exchange during epididymal transit (45–47). Testicular and epididymal heat stress alters the normal functioning of the epididymis, which may lead to faster sperm epididymal transit, thus reducing the time required for spermatozoa maturation and resulting in a large number of immotile spermatozoa in the ejaculate. This has been reviewed elsewhere in various animal species (7, 8).

The major decrease in sperm output at D34 (~15% of baseline value) during hyperthermia suggests that a 2°C increase in testicular temperature has an active impact mainly on the meiosis stage. This fall may result from: 1) an early germ cell release from germinal epithelium that was reflected in an increased total round cell count as early as D20 during hyperthermia and that corresponded to degeneration of germ cells (spermatocytes) resulting from either a direct effect of heat on germ cells, Sertoli-Sertoli and Sertoli-germ cell junctions, and/or on permeability of the blood-testes barrier (48); or 2) a possible reduction in cell proliferation and cell survival together with a simultaneous increase in germ cell apoptosis (49), as shown in men after local heating (43°C in a water bath for 30 minutes for 6 consecutive days). Damage to the seminiferous epithelium 2 weeks after the end of this heat treatment and impaired expression of several proteins mainly expressed during germ cell proliferation, survival, as well as in apoptosis, was observed (49). Yet, ejaculates at D34 contained spermatozoa (~15% of control value), which suggests that some, but not all, germ cells were more specifically affected by heat; this was reflected in the increased sperm DFI at D20 during hyperthermia without a drop in sperm output. Moreover, some germ cells could have arrested at the spermatocytes stage. This possibility is supported by the total sperm count values, which started to increase as early as D45 after cessation of hyperthermia. An explanation could be that the sperm ejaculated at D45 were in fact at D33 toward the end of their spermatogenic cycle (45 − 12 days of epididymal transit = 33 days) and after cessation of hyperthermia spermatocytes restarted their evolutionary process from the spermatocyte stage onward. When heating was withdrawn, arrested spermatocytes were able to regain their differentiation into round spermatids and then into spermatozoa.

Furthermore, during heating, the first stage of spermatogenesis (mitosis and differentiation of spermatogonia) seems not to be affected, as has been reported in induced

FIGURE 3



Analysis of sperm (A) high DNA stainability (HDS) and (B) DNA fragmentation index (DFI) by sperm chromatin structure assay (SCSA) during the three study periods: before, during, and after mild induced testicular and epididymal hyperthermia in men. Means ± SEM of all five volunteers are presented, except n = 4 where indicated by *Psi* and n = 3 where indicated by **, and Arabic numbers (1 and 2) between *Psi* and ** correspond to the number of volunteers and their mean values who had the minimal required sperm count for SCSA to be performed on the corresponding days. Means ± SEM (5.9 ± 0.3 and 11.9 ± 1.5, respectively) of the three means ± SEM before hyperthermia were compared with the means ± SEM of each data point during and after hyperthermia. However, for easier interpretation of the data all three means before hyperthermia are plotted in the figure. *$P<.05$. (C) Location and evolutionary stage of sperm during the spermatogenic process at induction of hyperthermia (D0) and their expected appearance in ejaculates. Abbreviations as in Figure 1.

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cryptorchidism in rats (50) and after unilateral cryptorchidism in rabbits (51).

We hypothesize that at the spermatocyte stage some cells underwent apoptosis, some appeared as round cells, a few continued to develop into spermatozoa, and others became arrested in a "frozen state." As spermatogonia continued dividing and differentiating, several waves of germ cells accumulated as late spermatogonia B and spermatocytes in the "frozen state"; finally, when heating was stopped, all arrested germ cells restarted their evolutionary process together, giving a sperm output similar to baseline value at D73 after hyperthermia, and those germ cells which were unsuccessful in completing the rest of their spermatogenic cycle appeared as round cells, giving a higher total round cell count compared with the control value. This concept is further supported by the studies in rodents where scrotal hyperthermia (43°C for 15 minutes) (52) and vitamin A withdrawal (53) resulted in spermatogenesis arrest at spermatogonia in mice and at preleptotene spermatocytes in rats (54).

Sperm DNA Fragmentation

Effects on epididymal spermatozoa. No significant change was revealed in percentages of sperm DFI and HDS in spermatozoa collected at D4 and D9 during hyperthermia. Nevertheless, the effects of hyperthermia on the quality of epididymal spermatozoa can not be neglected. Indeed, the effects of hyperthermia on epididymal spermatozoa depend on the degree, as observed in mice (34), and duration, as observed in sheep (31), of heat exposure. In mice, no changes in sperm DNA were reported at 38°C compared with 40°C and 42°C (30 minutes' exposure), whereas higher embryo loss occurred in sheep as early as D4 (epididymal sperm) after a heat exposure of 16 h/d for 21 days achieving an intrascrotal temperature ~35°C.

Effects on testicular germ cells. A significant increase in percentage sperm DFI at D20, D34, and D45 and in HDS at D34 and D45 was observed and appeared to continue until the end of the hyperthermia period (D73–D120), but owing to the small number of volunteers at D73–D120 these data were not significant. This growing increase in sperm DFI/HDS during hyperthermia may be explained by the chronology of spermatogenesis: 1) At D20, hyperthermia affected the last phase of spermiogenesis (elongated spermatids), when nuclear compaction takes place and the majority of nuclear proteins (histones) are replaced with protamines; during transit through the caput and corpus epididymis when disulfide cross-linking process takes place, as previously shown (55) and reviewed (56, 57),

sperm were more sensitive to external stresses; 2) at D34, heat affected the cells that were at late spermatocyte and/or early spermatid stages; they traveled the whole spermiogenesis and epididymal transit period and therefore were exposed to the heat longer than the cells at D20; 3) at D45, the cells were at primary spermatocyte stages and traveled the rest of the meiotic stage, whole spermiogenesis, and epididymis and were exposed to heat longer than both D20 and D34. This marked increase in sperm DFI and HDS at D34 and D45 is most probably because at these stages (spermatocyte and/or early spermatid), DNA strands are not firmly bound and are less dense than in late spermatids (D20), which is in accordance with the study by Paul et al. (34) in mice where most affected cells were the spermatocytes and spermatids after scrotal heating. In conclusion, the longer the time of heat exposure, the more damaging effects on the DNA quality of ejaculated spermatozoa.

Moreover, increased percentage of DFI and HDS and ratio of histones to protamines after an episode of fever have been shown in human sperm (35). Protamines are critical for proper sperm DNA packaging (58) and may serve a protective function against DNA damage (59). In mice it has been established that protamine haploinsufficiency is a direct causative factor involved in DNA damage induction (60). Furthermore, DNA polymerase β, which is maximally involved in DNA repair and function during meiosis, lost its activity in rats after artificial cryptorchidism (61).

It is noteworthy that we observed alterations in sperm chromatin integrity when the sperm count was still compatible with natural conception. Damaged sperm DNA may have a negative impact on the fate of the embryo in both natural and assisted reproduction. Some authors have reported reduced blastulation rates after in vitro fertilization (62) and increased frequency of miscarriages (63–65) in women whose partners had high sperm DNA damage compared with women whose partners had low damage. Furthermore in sheep, embryo loss has been reported without any effect on fertilization after scrotal heating (~35°C) (31). Furthermore, impaired embryo development was observed in mice at an ambient temperature of 36°C (32) or scrotal heating at 40°C and 42°C (34). This shows that sperm with compromised DNA quality can fertilize the oocyte but a negative impact becomes evident at later stages of embryo development.

Taken together, our findings argue that in conditions of moderate induced testicular and epididymal heat stress (+2°C) sperm chromatin is largely impaired before the sperm count drops, and it recovers when the stress is withdrawn, as has been observed in infertile men with varicocele (66, 67). This leads us to suggest that when increased sperm DNA fragmentation is observed in couples seeking to conceive by assisted reproductive techniques (ART), underlying cause(s) such as varicocele or occupational habits must first be investigated and treated before moving on to assisted reproductive options. Additionally, it would be useful to evaluate sperm chromatin structure in couples where use of ART has resulted in reduced embryo development and high abortion rates without involvement of a female factor.

In conclusion, we have presented the first study in men that investigated the direct relationship between sperm DNA damage and mild induced testicular and epididymal hyperthermia. We suggest that sperm chromatin may be the earliest indicator of alterations in sperm characteristics in a stress situation. Although additional studies on a larger population are required, our findings may have clinical implications for male contraception specifically during the inhibition and/or recovery phases of spermatogenesis.

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