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The impact of sedentary work on sperm nuclear DNA integrity

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Abstract

Introduction. Contemporary professional jobs that often enforce a sedentary lifestyle and are frequently associated with testicular overheat, deserve special attention with respect to male fertility potential. Interestingly, the harmful effect of testicular heat stress on sperm characteristics including nuclear DNA integrity was well characterized; however, the influence of sedentary work on sperm chromatin has not yet been documented. Therefore, our research was designed to examine the potential effects of sedentary work not only on conventional semen features but also on sperm nuclear DNA status.

Materials and methods. The study was carried out on ejaculated sperm cells obtained from men who spent ≥ 50% of their time at work (≥ 17.5 h per week) in a sedentary position (n = 152) and from men who spent < 50% of their time at work in a sedentary position (n = 102). Standard semen characteristics were assessed according to the WHO 2010 recommendations, while sperm nuclear DNA fragmentation (SDF) was evaluated using the Halosperm test.

Results. There were no significant differences in the standard semen parameters between the study groups. The groups differed only in SDF parameter. The men who spent at least 50% of their work time in a sedentary position had a higher proportion of SDF than the men who spent < 50% of their time at work in a sedentary position (median value 21.00% *vs.* 16.50%, respectively). The incidence of low SDF levels (related to 0–15% sperm cells with abnormal DNA dispersion) was significantly lower (27.63% *vs.* 45.10%), the percentage of men with high SDF levels (related to > 30%) was significantly higher (30.92% *vs.* 16.67%) in group of men who spent at least 50% of their work time in a sedentary positon. Furthermore, these men were more than twice as likely to have not a low SDF level (OR: 0.4648) and had more than twice the risk of having a high SDF level (OR: 2.2381) than the men in less sedentary occupations.

Conclusions. Despite lack of association between sedentary work and conventional semen characteristics our study revealed detrimental effect of seated work on sperm nuclear DNA integrity. A sedentary job doubled the risk of high levels of sperm DNA damage. The pathomechanism could be related to testicular heat stress resulting in sperm chromatin remodelling failure during spermiogenesis. Therefore, it seems reasonable to simultaneously carry out routine seminological analyses and tests assessing sperm chromatin status while diagnosing male infertility.*(Folia Histochemica et Cytobiologica 2019, Vol. 57, No. 1, 15–22)*

Key words: sperm parameters; DNA fragmentation; sedentary work; male reproductive health

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Introduction

Infertility has become a global disorder affecting up to 20% of couples trying to conceive. Infertility is not only a medical challenge but also a sociological and economic issue [1–3]. Couples affected by this problem struggle with stress, depression, and family crisis, even leading to divorce. Moreover, treatment is very expensive and not universally affordable [1–4]. It is estimated that male factors (coexisting with female factors) contribute to infertility in up to half or even 70% of infertility cases (20–43% in Africa, 37% in Asia, 40% in Oceania, 50% in North America, 50–56%, in Europe, 52% in South America, 70% in Middle-East). One-third of these cases can be caused by male factors alone [1–3, 5, 6]. Male factors (*e.g.*, congenital and acquired urogenital defects, urogenital infections, genetic, hormonal and immunological disorders, cancers, systematic diseases, age, lifestyle) can affect fertilization, embryo gene expression and development. They may also be responsible for idiopathic pregnancy loss as well as autosomal dominant diseases and neurobehavioural disorders in offspring, especially in cases of advanced paternal age [7–12].

It should be emphasized that unhealthy lifestyle of men (*e.g.*, drug use, smoking, alcohol consumption, obesity, psychological stress, environmental pollution) are very important and simultaneously modifiable factors affecting male reproductive ability [13–17]. Some reports revealed that contemporary professional jobs that are often associated with a sedentary lifestyle may contribute to declining semen characteristics (*e.g.*, number of sperm cells, sperm motility, morphology) due to testicular heat stress [18–21]. Importantly, a physiological temperature of human testes between 32°C and 35°C (the optimal temperature is 1–2°C below the body core temperature) is needed for the normal course of spermatogenesis associated with correct essential gene expression. Therefore, the differentiation and maturation of spermatocytes and spermatids, germ cells of seminiferous epithelium, are temperature-dependent processes. However, a seated position leads to testes overheating due to poor air circulation in the groin area and impaired blood flow. The increase in the scrotal temperature may trigger pathological changes in both seminiferous epithelium (Sertoli cells and germinal cells) and endocrine cells located in testicular interstitial tissue, *i.e.* Leydig cells. Therefore, spermatogenic arrest leading to a decrease in sperm concentration or even testicular atrophy may appear [22, 23]. Likewise, heat stress induces damage of mitochondria, dilatation of the smooth endoplasmic reticulum in germinal cells and increases the distance between spermatids and Sertoli cells. In the latter case, premature exfoliation of immature spermatids is often associated with their apoptosis [22–24]. Furthermore, DNA damage (defects in chromosomes' synapsis, DNA strand breaks, suppression of DNA repair) is observed in germinal cells, particularly in pachytene and diplotene spermatocytes. The DNA damage may be caused not only by the direct action of heat stress but also by the pathological heat-dependent generation of reactive oxygen species (ROS) [22–24].

It should be highlighted that the harmful effect of testicular heat stress on sperm nuclear DNA integrity was well elaborated but the influence of sedentary work on sperm chromatin has not yet been documented. Therefore, our study was designed to examine the potential effects of sedentary work not only on conventional semen features but also on sperm nuclear DNA status.

Materials and methods

Subjects. The study was carried out on ejaculated sperm cells obtained from general population of men (n = 254) attending the Andrology Laboratory of the Department of Histology and Developmental Biology (Pomeranian Medical University in Szczecin, Poland). We designed a questionnaire to record personal characteristics and medical history. Men also reported the average time *per* week spent in a sedentary position at work. Subjects who worked at least 35 h *per* week were considered for seminological analyses. Based on Støy *et al.* [25], the subjects were divided into two groups: group 1 comprised men who spent ≥ 50% of time at work in a sedentary position, *i.e.*, a minimum of 17.5 h per week (n = 152, median age = 31.50 years), whereas group 2 comprised men who spent < 50% of time at work in a sedentary position (n = 102, median age = 31.00 years). In both groups, the exclusion criteria included the following: working time less than 35 h *per* week, a clinical picture suggestive of obstructive azoospermia, a history of testicular torsion, varicocele, maldescent of testis, cryptorchidism, injury or cancer and co-existing systemic disease. The ethics committee of the Pomeranian Medical University, Szczecin, Poland approved the study protocol (ethical authorization number: KB-0012/21/18).

Conventional semen analyses. The semen samples were obtained by masturbation after 2–7 days of sexual abstinence. The standard semen characteristics were evaluated according to the WHO 2010 recommendations [26]. Sperm concentration was estimated in an improved Neubauer hemocytometer (Heinz Hernez Medizinalbedarf GmbH, Hamburg, Germany), whereas sperm motility (total and progressive motility), vitality (live sperm cells: eosin-negative or hypo-osmotic-reactive sperm cells – HOS test) and morphology in a bright light microscope (CX 31, Olympus Optical Co., Ltd., Tokyo, Japan). Papanicolaou-stained

spermatozoa was used for sperm morphology and teratozoospermia index (TZI) assessment. Concentration of leukocytes in the semen samples (peroxidase-positive cells) was calculated using the Endtz test (LeucoScreen kit, FertiPro N.V., Beernem, Belgium).

A normozoospermia (positive results of the basic seminological examination) was considered according to the following criteria: sperm concentration ≥ 15 mln/mL, total number of sperm cells ≥ 39 mln, sperm progressive motility ≥ 32% and morphology ≥ 4%. Furthermore, the teratozoospermia index, vitality and concentration of peroxidase-positive cells (leukocytes) were evaluated (Table 1). In the group of 152 men who spent ≥ 50% of time at work in a sedentary position, the following seminological categories were distinguished: normozoospermia (n = 54), asthenozoospermia (abnormal sperm motility, n = 2), oligozoospermia (abnormal number of sperm cells, n = 3), teratozoospermia (abnormal sperm morphology n = 40), asthenoteratozoospermia (abnormal sperm motility and morphology, n = 17), oligoteratozoospermia (abnormal sperm number and morphology, n = 15) and oligoasthenoteratozoospermia (abnormal sperm number, motility and morphology, n = 21). For the group of 102 men who spent < 50% of time at work in a sedentary position, the following categories were identified: normozoospermia (n = 42), asthenozoospermia (n = 1), oligozoospermia (n = 2), teratozoospermia (n = 28), asthenoteratozoospermia (n = 10), oligoteratozoospermia (n = 9) and oligoasthenoteratozoospermia (n = 10).

Sperm chromatin dispersion (SCD) test (Halosperm test). The SCD test was used to assess sperm nuclear DNA fragmentation (SDF). The evaluation of DNA fragment dispersion after denaturation was carried out using a Halosperm G2 kit (Halotech DNA, Madrid, Spain) following the manufacturer's guidelines. Semen samples were diluted with PBS (phosphate buffered saline, pH = 7.4) to adjust the concentration to no more than 20 mln/mL. Agarose gel from the kit was incubated for 5 min in hot water at 95°C to ensure complete melting and then was incubated at 37°C. Fifty microliters of warm agarose (37°C) was added and mixed with 25 μL of semen in an Eppendorf tube. The mixtures (10 μL) were placed on a super-coated slide and covered with a coverslip. The smears were kept for 5 minutes at 4°C in a fridge to solidify the agarose. Thereafter, the coverslips were carefully removed, and the reaction area was fully immersed in a denaturation solution for 7 min. The smears were drained by tilting the slides. Afterwards, the reaction area was fully immersed in the lysing solution for 20 min. Then, the smears were placed in distilled water for 5 min and dehydrated by flooding with 70% ethanol and then 100% ethanol (each for 2 min). After drying, the slides were stained with an eosin staining solution and thiazine staining solution (each for 7 min).

Sperm DNA fragmentation scoring. The smears were evaluated under a bright light microscope at 1000× magnification (CX 31 Olympus microscope). A minimum of 300 spermatozoa *per* sample were counted. Sperm cells without SDF can produce the characteristic halo of dispersed DNA loops (large halo: halo width similar to or higher than the diameter of the sperm head; medium halo: halo width > 1/3 the diameter of the sperm head), while spermatozoa with damaged DNA fail to form a halo of dispersed DNA loops (small halo: halo width ≤ 1/3 the diameter of the sperm head; sperm cells without a halo or with degraded DNA: spermatozoa with no halo or irregularly, weakly stained sperm head) (Fig. 1). The results are presented as the total number of spermatozoa with small or no halo, *i.e.*, degraded, divided by the total number of assessed sperm cells and multiplied by 100% [16]. The levels of sperm chromatin damage were estimated based on the following criteria: 0–15% SDF (low level of sperm cells with fragmented DNA, high fertility potential), 16–30% SDF (moderate level, moderate fertility potential) and > 30% SDF (high level, low fertility potential) [27–30].

Statistical analysis. The statistical analyses were performed using Statistica version 13.3 (StatSoft, Poland) and MedCalc version 15.2.2 (MedCalc Software, Ostend, Belgium) software with significance set at $p < 0.05$. The quantitative variables are expressed as the mean ± standard deviation (SD) and median (range), while qualitative data are reported as percentages. The conformity of numerical variables with the normal distribution was examined using the Shapiro-Wilk test. The nonparametric Mann-Whitney U test was used to compare data from two independent groups. A chi-square test was used to compare the categorical data. The odds ratios (ORs) for SDF levels (their 95% confidence intervals and p value) to define the relative risk in predicting the level of SDF in men who spent ≥ 50% of time at work in a sedentary position with respect to men who spent < 50% were calculated using the method of Altman [31].

Results

The compared groups did not significantly differ in body mass index (BMI) (the evaluation was carried out by medical staff), *i.e.*, kilograms of body weight divided by the square of the person's height in metres [32], age and standard semen parameters, but they did differ in SDF (Table 1). The men who spent at least 50% of their professional work time in a sedentary position (Group 1) had a higher proportion of SDF than the men who spent less than 50% of their work time in a sedentary position (Group 2); median: 21.00% *vs.* 16.50%, respectively. Moreover, the groups differed significantly in the incidence of low SDF levels (27.63% *vs.* 45.10% of subjects, respectively) and high SDF levels (30.92% *vs.* 16.67% of subjects,

Table 1. Descriptive statistics and comparison of body mass index (BMI), standard semen parameters and sperm DNA fragmentation (SDF) between groups of men who spent ≥ 50% of time at work in a sedentary position (Group 1) and men who spent < 50% of time at work in a sedentary position (Group 2)

Parameter	Reference = normal values	Group 1 (n, median with range and or mean ± SD)	Group 2 (n, median with range and mean ± SD)	*P*
BMI	18.50–24.99 kg/m^2*	150 25.75 (19.40–37.46) 26.34 ± 3.24	100 25.90 (18.83–47.77) 26.50 ± 4.42	0.574469
Age (y)	no data	152 31.50 (21.00–51.00) 32.9 6 ± 5.67	102 31.00 (22.00–48.00) 32.16 ± 5.98	0.202004
Semen volume (mL)	≥ 1.50 mL**	152 3.22 (1.10–11.50) 3.50 ± 1.55	102 3.00 (0.50–8.80) 3.46 ± 1.63	0.588980
Sperm concentration (× 10^6/mL)	≥ 15 mln/mL**	152 22.55 (0.25–166.00) 33.22 ± 34.01	102 27.69 (0.80–283.00) 37.74 ± 4 0.10	0.157160
Total number of spermatozoa (× 10^6)	≥ 39 mln**	152 73.10 (0.75–660.25) 104.33 ± 99.98	102 79.07 (1.60–566.00) 121.45 ± 118.45	0.307699
Morphologically normal spermatozoa (%)	≥ 4%**	152 2.00 (0.00–13.00) 2.98 ± 2.88	102 3.00 (0.00–12.00) 3.33 ±3.02	0.373186
TZI	≤ 1.8**	152 1.53 (1.20–2.14) 1.56 ± 0.17	102 1.50 (1.17–2.09) 1.52 ± 0.16	0.098516
Progressive motility (%)	≥ 32%**	152 49.00 (0.00–86.00) 46.15 ± 21.70	102 53.50 (0.00–87.00) 50.25 ± 21.86	0.082646
Non-progressive motility (%)	no data	152 5.00 (0.00–23.00) 6.39 ± 4.18	102 5.00 (0.00–26.00) 5.95 ± 4.03	0.540435
Total sperm motility (%)	≥ 40%**	152 57.00 (0.00–92.00) 52.55 ± 22.02	102 60.50 (0.00–91.00) 56.20 ± 22.04	0.167037
Eosin-negative spermatozoa — live cells (%)	≥ 58%**	152 75.50 (2.00–94.00) 71.54 ± 16.67	102 79.00 (0.00–95.00) 73.40 ± 18.99	0.060613
HOS test-positive spermatozoa — live cells (%)	≥ 58%**	145 73.00 (0.00–94.00) 69.68 ± 16.46	96 77.50 (0.00–93.00) 71.18 ± 19.47	0.066224
Peroxidase-positive cells (mln/mL)	< 1.00 mln/mL**	152 0.25 (0.00–10.00) 0.59 ± 1.35	102 0.25 (0.00–6.50) 0.50 ± 0.90	0.649891
SDF	< 30%***	152 21.00 (5.00–89.00) 24.76 ± 15.01	102 16.50 (3.00–89.00) 19.99 ± 13.73	0.001816

n — number of subjects, HOS test — hypo-osmotic swelling test; SD — standard deviation, TZI — teratozoospermia index, p — significance of differences between compared groups, Mann-Whitney U test. *reference values according to WHO 2000 recommendations [32] (2000); **reference values according to WHO 2010 recommendations [26]; *** reference values according to Evenson and Wixon [42].

respectively) (Table 2). Furthermore, men in the Group 1 had significantly lower ORs for a low SDF level (OR: 0.4648) and significantly higher ORs for a high SDF (OR: 2.2381) than the men in Group 2 (Table 3).

Discussion

In our study, we analysed relationships between seminological parameters and a sedentary work position. Therefore, considering the suggestions of

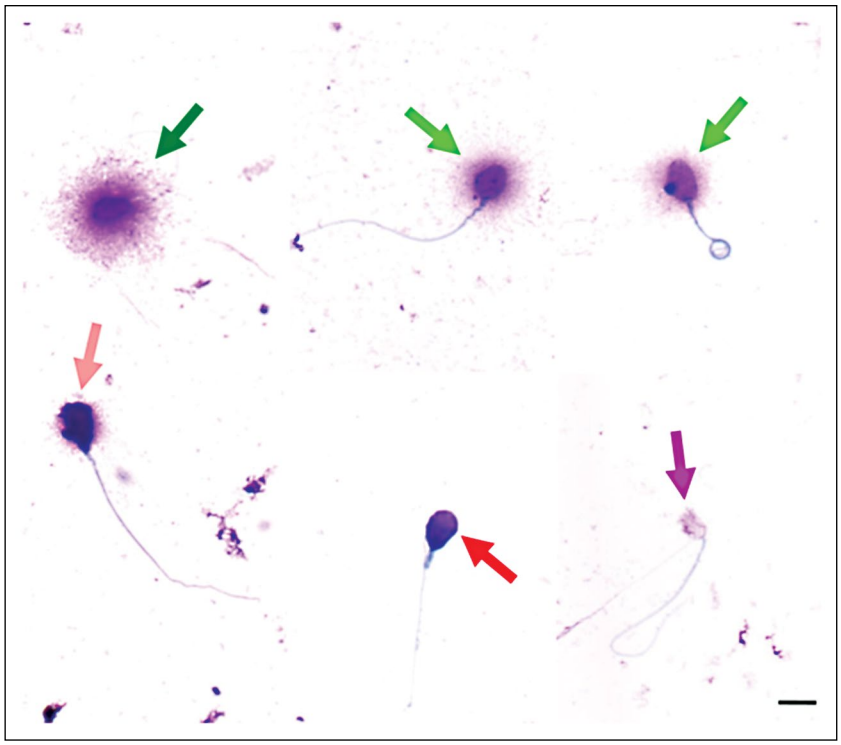

Figure 1. Micrographs presenting the results of the sperm chromatin dispersion test. Sperm cells without fragmented nuclear DNA: large halo (dark green arrow) and medium halo (bright green arrows). Sperm cells with fragmented nuclear DNA: small halo (rose arrow) and no halo (red arrow). Sperm cell with degraded DNA (purple arrow). Scale bar = 10 μm.

Table 2. Prevalence of sperm chromatin fragmentation (SDF) levels in groups of men who spent ≥ 50% of time at work in a sedentary position (Group 1) and who spent < 50% of time at work in a sedentary position (Group 2)

Group	SDF (%)		
	0–15% n (%)	**16–30% n (%)**	**> 30% n (%)**
Group 1 (n = 152)	42 (27.63)	63 (41.45)	47 (30.92)
Group 2 (n = 102)	**46 (45.10)****	39 (38.24)	**17 (16.67)***

n — number of subjects; *significant difference between compared groups at p = 0.0157; **significant difference between compared groups at p = = 0.0063 (bold); Chi^2 test.

Table 3. Odds ratio (OR) of forming sperm chromatin fragmentation (SDF) levels in the group of men who spent ≥ 50% of work time in a sedentary position (Group 1) and who spent < 50% of work time in a sedentary position (Group 2)

	Group 1 (n = 152)	**Group 2 (n = 102)**	**OR (95%CI)**
SDF 0–15%	42 (27.63)	46 (45.10)	**0.4648** (0.2742–0.7879)**
SDF 16–30%	63 (41.45)	39 (38.24)	1.1435 (0.6843–1.9108)
SDF > 30%	47 (30.92)	17 (16.67)	**2.2381* (1.1991–4.1775)**

n — number of subjects and percentage of the whole group in parentheses; *statistical significance at p = 0.0114, **statistical significance at p = = 0.0044. Test of significance, the P-value is calculated according to Sheskin [43], 95%CI — 95% confidential interval.

other authors [25], the study subjects were divided into two groups: men who spent ≥ 50% of working time in a sedentary position (≥ 17.5 h per week) and men who spent < 50% of their time at work in a sedentary position. The obtained data suggested no association between sedentary work and conventional semen characteristics. Our findings are in agreement with the data obtained by other researchers [18, 19, 25, 33, 34]. Støy *et al.* [25] suggested that sedentary work was not a risk factor for abnormal semen characteristics. Moreover, De Fleurian *et al.* [34] did not observe differences in the prevalence of normal and abnormal conventional semen parameters with respect to the number of hours spent in a sitting position. In turn, Figà-Talamanca *et al.* [19] published results that were partly consistent with our data. They com-

pared semen parameters of taxi drivers with those of healthy individuals. The authors showed a significant difference only in the proportion of normal sperm cell morphology (taxi drivers had fewer normal sperm forms). In another publication, Boggia *et al.* [18] described that men who worked in sitting positions and those who had free work positions differed only in total sperm motility. Furthermore, Magnusdottir *et al.* [33] reported that the prevalence of sedentary work was significantly lower among men with high normal sperm concentration compared to that for men with low normal sperm concentration.

It should be highlighted that we did not observe a significant difference in the standard semen parameters between men who spent ≥ 50% of their work time in a sedentary position (≥ 17.5 h per week) and men who spent < 50% of their time at work in a sedentary position. However, we found a difference in the percentage of spermatozoa with SDF, the prevalence of low and high SDF levels and the ORs for a low or high proportion of sperm cells with abnormal DNA structure. Moreover, the incidence of high DNA damage (> 30% sperm cells with SDF) was also significantly higher in this group and could be related to a low fertility potential [27–30] because a high proportion of sperm cells with DNA damage is associated with negative effects on fertilization, embryo and pregnancy rate [28, 35–37]. Therefore, we can conclude that sedentary work affects sperm DNA and doubles the risk of having a high SDF level without changing conventional semen characteristics and could lead to reduced male fertility.

It has to be noted that according to our best knowledge this is the first study about the influence of sedentary work on sperm chromatin status. It is interesting to consider which pathomechanism could be responsible for sperm DNA damage in 'sedentary' men. It has been shown that a sitting position may lead to testicular heat stress [20, 38, 39], which may provoke DNA damage [21–24, 40, 41]. As reported by Koskelo *et al.* [38], only 20 minutes of sitting in an office chair can increase the scrotal temperature by up to 3°C. Moreover, Bujan *et al.* [39] observed that the mean scrotal temperature increased in drivers after 2 hours of driving, reaching a value 1.7–2.2°C higher than the corresponding mean scrotal temperature during walking. Additionally, Hjollund *et al.* [20] showed that in periods of sedentary work, the median scrotal temperature was on average 0.7°C higher. It has been proven that a high body temperature reduces sperm DNA integrity [21, 40, 41]. Sergerie *et al.* [40] revealed that a 2-day fever of 39–40°C significantly affected sperm cell concentration, motility and vitality as well as sperm DNA integrity. The proportion of sperm with DNA fragmentation increased from 9% (before fever) to 24% and 36% (15 and 37 days after fever, respectively). In turn, in a prospective randomized clinical study, Rao *et al.* [21] showed that men undergoing warming in a 43°C bath had significantly affected sperm DNA integrity as well as reduced standard semen parameters. Moreover, a direct impact of temperature on sperm DNA fragmentation was reported by Santiso *et al.* [41], who incubated sperm cells at 37°C, 41°C and 45°C for 24 hours. The authors revealed that a higher incubation temperature was associated with a higher SDF. Based on the described data, we can speculate that an increase in the proportion of spermatozoa with abnormal DNA integrity in men who spent ≥ 50% of their work time in a sedentary position was most likely related to the testicular temperature stress.

Conclusions

Our study suggests that an influence of sedentary work on semen fertility potential is possible but not clearly verifiable. Although this and other studies revealed a lack of association between sedentary work and standard semen characteristics, we have demonstrated the detrimental effect of sedentary work on sperm nuclear DNA integrity since the sedentary job doubled the risk of high sperm DNA damage. We can speculate that the discovered DNA damage could be related to testicular heat stress resulting in sperm chromatin remodelling failure during spermiogenesis. Therefore, it seems reasonable to simultaneously carry out routine seminological analyses and tests assessing sperm chromatin status while diagnosing male infertility.

Conflicts of Interest

The authors declare there is no conflict of interest regarding the publication of this article.

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