University of Szeged,

Albert Szent-Györgyi Medical and Pharmaceutical Centre

Programme co-ordinator: Prof. László Kovács MD, PhD, DSc

Tutor: Prof. János Szöllősi MD, PhD

Lifestyle factors affecting male fertility

Imre Zsolt Fejes MD

PhD thesis

2005

Publications related to the thesis

I. S. Koloszár, I. Fejes, Z. Závaczki, J. Daru, J. Szöllősi, A. Pál (2005) Effect of weight on sperm concentration in normozoospermic males. *Archives of Andrology* 51:299-304. **(IF: 0.654)**

II. I. Fejes, Z. Závaczki, J. Szöllősi, S. Koloszár, J. Daru, L. Kovács, A. Pál (2005) Is there a relationship between cell phone use and semen quality? *Archives of Andrology* 51: 385-393. **(IF: 0.654)**

III. I Fejes , S Koloszár, J Szöllősi, Z Závaczki, A Pál (2005) Is semen quality affected by male body fat distribution? *Andrologia* 37: 155-159. **(IF: 1.00)**

IV. I Fejes, S Koloszár, Z Závaczki, J Daru, J Szöllősi, A Pál: Effect of body weight on testosterone/estradiol ratio in oligozoospermic male. *Archives of Andrology* accepted for publication **(IF: 0.654)**

V. I. Fejes, Z. Závaczki, J. Szöllősi, S. Koloszár, L. Kovács, A. Pál: Relationship between regular cell phone use and human semen quality. European Society of Human Reproduction and Embryology, 20th. Annual Meeting. Berlin, Germany, 27-30 June 2004. *Human Reproduction* 2004 19. Suppl 1. i 57.

VI. Koloszár S, Fejes I, Daru J, Szöllősi J (2003) A testtömeg hatása a tesztoszteron/ösztrogén arány változására oligozoospermiában. *Magyar Andrológia* 8: 41-44

VII. Koloszár S, Fejes I, Závaczki Z, Daru J, Szöllősi J (2004) A testtömeg hatása a spermiumszámra normozoospermia esetében. *Magyar Andrológia* 9: 17-19

VIII. Fejes I, Koloszár S, Závaczki Z, Szöllősi J (2004) A test zsíreloszlása befolyásolja-e az ondó minőségét és a reproduktív hormonszinteket? *Magyar Andrológia* 10: 53-56.

IX. I. Fejes, S Koloszár, J Szöllősi, Z Závaczki, A Pál: Does fat distribution affect the semen quality? 4th Congress of European Society of Andrological Urology, Budapest, Hungary, 8-9 October 2005.

X. I. Fejes: Kann länger dauernder Gebrauch von Mobiltelefonen die männliche Fruchtbarkeit negativ beeinflussen? 3. Nationale Kongress Elektrosmog-Betroffener, Olten, Switzerland, 19 November 2005. (invited speaker)

Contents

List of abbreviations

6-OHMS:	6-hydroxymelatonin sulphate
BMI:	body mass index
DCS:	Digital Cellular System
DNA:	desoxyribonucleic acid
EMF:	electromagnetic field
FSH:	follicle-stimulating hormone
GnRH:	gonadotropin-releasing hormone
GSM:	General System for Mobile Communication
LH:	luteinizing hormone
NMT:	Nordic Mobile Telephone
OB-R:	obese receptor
OTA:	oligo-terato-asthenozoospermia
ROS:	reactive oxygen species
SAR:	specific absorption rate
SD:	standard deviation
SHBG:	sexual hormone-binding globulin
TMS:	total motile sperm count
TRPS:	rapid progressive motile sperm count
TSC:	total sperm count

1. Introduction

Humans are exposed to many environmental factors that may be hazardous to their reproductive health. The male reproductive function in the general population has recently attracted considerable attention due to reports suggesting that the occurrence of various biological problems affecting the male genital tract has increased during the last 50 years (Olive *et al.* 2001, Toppari *et al.* 1996). The prevalence of infertility among couples of reproductive age has been estimated as up to 15%. Infertility is defined as failed success to achieve pregnancy during a 1-year period of regular unprotected intercourse. In about half of these cases, the male partner exhibits some disturbance in the spermatogram (Nieschlag and Behre 2000, WHO Manual 2000). In more than half of the male infertility cases, a medical or surgical reason is detectable in the background, such as varicocele, hypogonadism, infections, etc. Treatment based on causal therapy mostly achieves success. However, in about 30% of the cases, in idiopathic infertility, the aetiology remains unclear.

It has been proven, that several illnesses, such as tumours, metabolic X syndrome or depression, are associated with modern lifestyle factors. Some of the aetiologic factors of these diseases are well known to impair male fertility too, e.g. smoking, chemicals or stress. The aim of my thesis was to investigate whether novel factors that play increasing roles in the present lifestyle may affect male fertility. The first part of the thesis focuses on the effects of the electromagnetic radiation of cell phones on male fertility. The second part examines the relationship of obesity and the spermatogram parameters.

1.1. Cell phones and male fertility

The use of cell phones has become widespread in the past ten years. There are currently over 1.6 billion cell phone users worldwide (Ahlbom *et al.* 2004). The analogue NMT (Nordic Mobile Telephone) system introduced in the 1980s operated at an electromagnetic resonance of 902.5 MHz. A decade later, the GSM (General System for Mobile Communication) succeeded it, with a radiofrequency of 902.4 MHz pulsing on 217 Hz. The recent DCS (Digital Cellular System), which uses a radiofrequency of 1,800 MHz, has spread rapidly

(Roelandts 2003). Cell phones operate at a typical power of 0.25 W, although the biological effect is related to their wide range of specific absorption rate (SAR): depending on the model used, this is approximately 0.1-1 W/kg. Cell phones with the earlier biological SAR limit (2 W/kg) are no longer available. There are a n number of factors that influence the adaptive power, therefore causing methodological problems in epidemiological studies, such as the distance from the base station, the frequency of handovers, and the radiofrequency traffic conditions. The emitted power is higher in rural areas or when the user is moving. The shielding effects of materials also influence it. The maximum of the absorption is on the side of the head where the phone is held, and the absorption is less than one-tenth on the opposite side of the head (Ahlbom *et al.* 2004).

Although the emission level of cell phones is below the defined safety thresholds, the effects of the electromagnetic resonance emitted by cell phones on living cells and organs are still unclear. There have been publications concerning effects on the central nervous system, such as alterations in the electroencephalogram pattern, the sleeping pattern or even the neuroendocrine functions (Burch *et al.* 2002, D'Costa *et al.* 2003, Huber *et al.* 2002, Kramarenko and Tan 2003). There have also been reports on the breakage of desoxyribonucleic acid as the cause of tumours (Hardell *et al.* 2001, Hardell *et al.* 2002, Hardell *et al.* 2003, Ivancsits *et al.* 2003, Mashevich *et al.* 2003), but the role of cell phones in engendering tumours is debated (Christensen 2004, Cook *et al.* 2003, Elwood 2003, Hansson Mild *et al.* 2003, Johansen 2002, Warren *et al.* 2003, Weisbrot *et al.* 2003).

Dasdag *et al.* (2003) recently reported that they had detected no effects of cell phone use on the testis of rats, whereas Davoudi *et al.* (2002) observed declining levels of rapid progressive spermatozoa among a small study group of cell phone users. Ahlbom *et al.* (2004) summarize literature on the effect of electromagnetic field (EMF) exposure on reproductive health: most of the authors found a reduction in sperm density and variable results for other semen parameters.

As far as we are aware, ours is the first human study of the possible relationship between cell phone use and semen quality; more than 300 males were examined.

1.2. Fat deposition and male fertility

It is well known that deviation from the ideal weight disturbs the endocrine system, and especially the gonadal hormones. In women, both pathologically low (anorexia nervosa) and pathologically high body weight (obesity) cause anovulation and abrogate the fertilizing capacity (Koloszar *et al.* 2002).

The body fat content is well characterized by the body mass index (BMI), calculated from the height and weight through the following formula:

$$BMI = \frac{\text{weight (kg)}}{\text{height}^2\ (\text{m}^2)}$$

In spite of its relative simplicity and easy use, this index does not describe the distribution of the fat. Measurement of the waist circumference and the hip circumference allows an evaluation of the upper body mass and the lower body mass, respectively.

The abdominal, android type of fat deposition is a risk factor for cardiovascular diseases, diabetes mellitus, insulin resistance, differences in serum lipid profile and acute pancreatitis (Herold 2001, Hollman *et al.* 1997, Mery *et al.* 2002). The fat deposition is classified as gynoid when the waist/hip ratio is less than 0.85, intermediate when it is between 0.85 and 1.0, and android when it is more than 1.0. Most of the women with polycystic ovary syndrome are also characterized by android fat distribution and by an inability to achieve pregnancy, together with other endocrinologic and metabolic changes, such as increased concentrations of free and total testosterone, androstenedione, oestradiol, insulin, light-density lipoprotein-cholesterol, triglyceride and blood glucose, but a decreased level of sexual hormone-binding globulin (SHBG).

A recent study revealed differences in fat distribution between infertile women and normal controls. The different fat patterns were accompanied by different prognoses of infertility (Kirchengast *et al.* 2004).

Wass *et al.* (1997) showed that the pregnancy rate after *in vitro* fertilisation and embryo transfer is lower in women with a waist/hip ratio over 0.8.

In the intercellular septa of fat tissue cytochrome p450 aromatases convert androstenedione to oestrone, and testosterone to 17ß-oestradiol, and there is therefore an oestrogen domination in fat women (Figure 1). The elevated oestrogen level inhibits follicle-stimulating hormone (FSH) secretion from the hypothalamo-pituitary system by negative feedback, and the luteinizing hormone/FSH (LH/FSH) ratio shifts towards LH (Carreau *et al.* 2002). In contrast, in pathologic anorexia a FSH predominance can be detected.

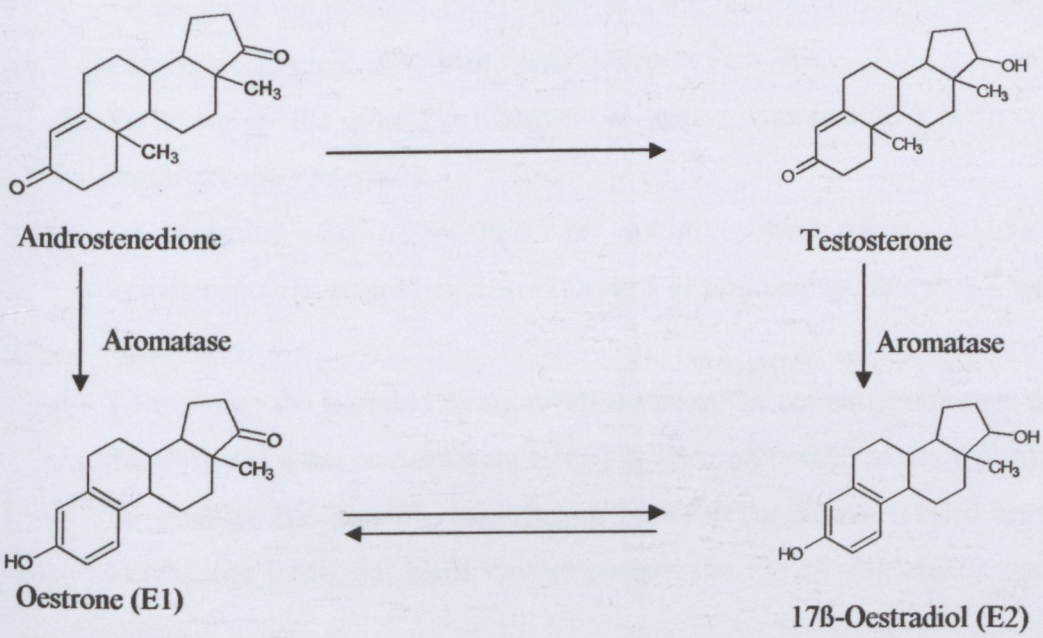

Figure 1. *Role of cytochrome p450 aromatase in the conversion of androstenedione and testosterone to oestrone and 17β-oestradiol.*

Among other factors, the peripheral production of oestrogen also plays a role in the regulation of the male hypothalamic-pituitary-gonadal system. Both pathologically low and pathologically high 17ß-oestradiol levels cause a disturbed regulation which can lead to impaired spermatogenesis. Just as in females, there is a feedback in males, which causes a shift in the gonadotropins and changes the LH/FSH ratio.

To investigate the effects of body weight on male fertility, we conducted a multi-level study. First, we examined the possible relationship between the sperm concentration and the male BMI to detect the effects of pathologically low and high body weights on the sperm concentration in cases of normozoospermia.

The next part of the study aimed at identifying how obesity affects the sperm concentration, the LH/FSH ratio and the testosterone/17ß-oestradiol ratio in oligozoospermic patients.
Finally, we conducted a prospective study to determine whether the waist/hip ratio, and thus the type of fat deposition correlate with the semen parameters and the sexual hormone levels (especially the testosterone/17ß-oestradiol ratio) in males who do not display other possible cofactors of deteriorated semen parameters.

2. Objectives

The objectives of the thesis were as follows:

1. To examine the possible relationships between the duration of cell phone possession, the duration of the cell phone being in the standby position near the examined males and the duration of cell phone use and the spermatogram parameters.
2. To examine the possible differences in sperm concentration of underweight, normal-weight, overweight and obese normozoospermic males.
3. To examine the possible changes in sperm concentration with aging in obese normozoospermic males.
4. To examine the differences in sperm concentration, LH/FSH ratio and testosterone/17ß-oestradiol ratio in groups of oligozoospermic males with a lower or a higher BMI.
5. To examine the possible relationship between the semen parameters and the weight, the BMI, the waist circumference, the hip circumference and the waist/hip ratio.
6. To examine the possible relationship between the sexual related hormones and the weight, the BMI, the waist circumference, the hip circumference and the waist/hip ratio.

3. Materials and methods

All of the studies were conducted at the Andrology Unit, Department of Obstetrics and Gynaecology, University of Szeged.

Besides a careful medical history taking and physical examination, the non-invasive methods of male fertility examination included semen analysis, measurement of reproductive hormone levels in the blood serum after venesection and ultrasound examination of the male genital organs. Semen analysis allows a direct evaluation of the male reproductive function and its changes, pointing to possible relationships between fertility and lifestyle factors. Investigation of semen samples from the general population is difficult as compared with other disciplines, as this is a highly intimate and sensitive area of the personality, and the participation rate in general population studies is therefore low (Bonde 1996). Studies on populations seeking medical help for their infertility are much more common, as semen analysis is an ultimate point in male fertility evaluation.

3.1.1. Semen analysis

In all of our studies, the semen analysis and evaluation procedures were the same, performed in accordance with the WHO 1999 standards criteria (WHO Manual 1999). Sperm samples were produced by masturbation into a sterile, wide-mouthed, calibrated glass container following a standardized 5 days of abstinence. After a 30-minute liquefaction period, the semen characteristics were quantified by using a Makler semen counting chamber (Sefi-Medical Instruments, Haifa, Israel) under the 200x magnification of an Olympus CH 2 phase contrast light microscope (Olympus Optical Co., Tokyo, Japan). The sperm concentration (10^6/ml) and the motile sperm ratio (%) were assessed. After a 3-week period, sample taking was repeated under the same conditions. The better findings were analysed.

3.1.2. Reproductive hormones

Peripheral venous blood samples were taken for hormone measurements between 8:00 and 9:00 a.m. Levels of spermatogenesis-related hormones were determined by using automated Immulite® chemiluminescent immunoassay (Diagnostic Products, Los Angeles, CA, USA.). Table 1 shows the normal hormone levels.

FSH	0.7-9.0 IU/l
LH	0.8-7.6 IU/l
prolactin	0.11-0.45 IU/l
testosterone	6.9-28 nmol/l
17ß-oestradiol	70-205 pmol/l
SHBG	7.2-33 nmol/l

Table 1. *Normal levels of reproductive hormones in our laboratory determined by Immulite® chemiluminescent immunoassay.*

3.1.3. Statistical analysis

Statistics were calculated with SPSS 11.0 for Windows statistical software (SPSS Inc. Chicago, IL, USA). The tests applied are detailed below.

3.2. Cell phones and male fertility

3.2.1. Patients

This study involved 611 consecutive male patients of reproductive age, who presented at our clinic because of infertility problems in their marriage.

We supplemented the history-taking with questions concerning cell phone use habits. The main aspects were: the duration of possession (in months), the duration of the cell phone being in the standby position closer than 50 cm to the patient (in hours) and the duration of daily transmission (in minutes).

As part of the semen analysis, the following categories of motility were assessed: the percentage of rapid progressive motile sperm (grade A): ≥ 20 µm/s at 20 °C, this being approximately equal to four head lengths or one half tail length; the percentage of slow

progressive motile sperm (grade B); the percentage of non-progressive motile sperm (grade C): < 5 μm/s; and the percentage of immotile sperm (grade D), according to the WHO standards (WHO Manual 1999). The total sperm count (TSC) (ejaculate volume * sperm concentration), the total number of motile sperm cells (TMS) (ejaculate volume * sperm concentration * motility/100) and the rapid progressive motile sperm count (TRPS) (ejaculate volume * sperm concentration * grade A motility/100) were calculated.

3.2.2. Exclusion criteria

Exclusion criteria were: (1) Some other potentially subfertility-causing factor in the patient's history, such as smoking (over 10 cigarettes/day), regular alcohol consumption (over 1 U/day) or drug abuse. (2) Any severe acute or chronic systemic non-gonadal illness (especially febrile illnesses) or trauma in the previous 6 weeks. (3) Some detectable organic alteration in the reproductive organs on physical examination, such as varicocele, obstruction or absence of the deferent duct, the absence of testes or a testis volume below 12 ml, or any abnormal localization of the testes. (5) Signs and symptoms of genital tract infection. Cultures were made from every examined semen sample. In the event of the presence of pathogenic aerobic or anaerobic bacteria or fungi in the semen, the patient was excluded from the study. (6) Certain alterations in the levels of the spermatogenesis-related hormones.

3.2.3. Formation of case and control groups

Control group 1 was subdivided into those who used a cell phone for less than 15 minutes/day (*low-transmitters*) to those who used it for over 60 minutes/day (*high-transmitters*). *Control group 2* was subdivided into those patients who kept their cell phone in the standby position within a distance of 50 cm for less than 1 hour daily (*short-standby group*) compared to those who kept their cell phone in the standby position within a distance of 50 cm for more than 20 hours daily (*long-standby group*).

3.2.4. Applied statistical methods

The parametric t-test and the Pearson correlation test were applied. Results are given as correlation coefficients or means ± standard deviation (SD). p values less than 0.05 were considered significant.

3.3. Effects of fat deposition on spermatogram

3.3.1. Normozoospermic males

3.3.1.1. Patients: The study involved 274 normozoospermic male patients of reproductive age, who presented at our clinic in consequence of infertility problems in their marriage. The characteristics of the study population are shown in Table 2.

Patient number	274
Mean age ± SD	26.3 ± 5.8 years
Mean BMI ± SD	27.6 ± 4.9 kg/m^2
Mean sperm concentration ± SD	38.9 ± 14.8 *10^6/ml

Table 2. *Characteristics of examined population of normozoospermic males.*

3.3.1.2. Measures: The weight and height were measured and expressed in kg and cm, respectively (Seca 880 Weight Scale, Leicester Height Measure, Seca Ltd, Vogel and Halke, Hamburg, Germany). BMI was calculated as weight/height^2 and expressed in kg/m^2.
Depending on the BMI, we formed four groups of patients as follows: *Group 1*: ≤ 20 kg/m^2, were underweight; *Group 2*: 20.1 to 25 kg/m^2, were of normal weight; *Group 3*: 25.1 to 30 kg/m^2, were overweight; and *Group 4*: >30 kg/m^2, were obese.

3.3.1.3. Applied statistical methods: Results are given as means ± SD. p values less than 0.05 were considered significant.

3.3.2. Oligozoospermic males

3.3.2.1. Patients: The study involved 42 oligozoospermic male patients of reproductive age. The characteristics of the study population are shown in Table 3. Hypogonadal male were excluded from the study. All basal testosterone, FSH and LH levels were in the normal ranges.

Patient number	42
Mean age ± SD	28.3 ± 5.3 years
Mean BMI ± SD	27.6 ± 4.6 kg/m^2
Mean volume ± SD	4.3 ± 0.7 ml
Mean sperm concentration ± SD	9.8 ± 2.9 * 10^6/ml
Motility mean ± SD	58.2 ± 10.1%
Morphology mean ± SD	42.8 ± 6.7%

Table 3. *Characteristics of examined population of oligozoospermic males.*

3.3.2.2. Measures: The weight and height were measured as described above. Depending on the BMI, two groups were formed: *Group 1*: ≤ 25 kg/m^2: underweight or normal-weight patients; and *Group 2:* BMI > 25 kg/m^2: overweight or obese patients.

3.3.2.3. Applied statistical methods: The sperm concentration, the LH/FSH ratio and the testosterone/17ß-oestradiol ratio were compared in the study groups. Student-tests were performed. Results are given as means ± SD. p values less than 0.05 were considered significant.

3.3.3. Male body fat distribution

3.3.3.1. Patients: This study involved 81 male patients of reproductive age selected according to the exclusion criteria, who presented at our clinic in consequence of infertility problems in their marriage.
In a one-year period of study, the variances in the seasons were examined.

3.3.3.2. The exclusion criteria were (1) smoking, (2) regular alcohol consumption, (3) drug abuse, prescription-only medication, (4) any acute disease in the previous 3 weeks, or any chronic disease such as hypertension or diabetes mellitus, (5) physical examination of the participants revealed organic alterations of the reproductive organs, such as varicocele, obstruction or absence of the deferent duct, absence of the testes or a testis volume below 12

ml, or any abnormal localization of the testes. (6) Cultures were made from every examined semen sample. In the event of the presence of pathogenic aerobic or anaerobic bacteria or fungi in the semen, the patient was excluded from the study. (7) We also excluded those who had azoospermia or severe oligozoospermia due to obstructive azoospermia, Y-chromosome microdeletion, Sertoli-cell-only syndrome, etc.

3.3.3.3. Anthropometric measures

The weight and height were measured and expressed in kg and cm as described previously. The circumference of the waist was measured halfway between the iliac crest and the bottom of the 12th costal bone, at the end of normal expiration, using a Waist watcher measure tape (meterex Karl Kuntze, Langenfeld, Germany). The hip circumference was determined with the same tape at the level of the tuberculi majoris. These reference points are well defined, as opposed to measurement of the abdominal circumference at the level of the umbilicus, for example, which can differ in the case of a pendulous abdomen. In studies to measure waist circumference, the minimum measurement between the xyphoid process and umbilicus has also been used (Wass et al. 1997), but for the above-mentioned reason this is not appropriate.

3.3.3.4. Applied statistical methods

Parametric or non-parametric tests such as the t-test, the Mann-Whitney test and the Pearson or Spearman correlation tests were applied where appropriate. Results are given as correlation coefficient, mean ± SD or median (range). p values less than 0.05 were considered statistically significant.

4. Results

4.1. Effects of cell phones on male fertility

A total of 611 consecutive Caucasian male patients were examined during the study period between 1 November 2002 and 31 March 2004. Thirty-nine percent of them (n = 240) did not meet the study criteria, and therefore the results on 371 of them were analysed. The mean age was 30.8 ± 4.4 years (range 17-41). The subjects were from every social class.
The semen parameters of the study population are presented in Table 4 and the regression results in Table 5.

	Total population *n = 371*	*Control group 1* *n = 195*	*High-transmitters* *n = 59*	*Control group 2* *n = 106*	*Long standby* *n = 88*
Sperm concentration (10^6/ml)	68.38 ± 48.86	67.6 ± 44.56	68.47 ± 46.43	69.32 ± 45.97	63.77 ± 42.3
Proportion of rapid progressive motile sperm (%)	47.17 ± 21.21	**48.75 ± 20.62**	**40.47 ± 21.62**	47.85 ± 21.4	46.17 ± 20.65
Proportion of slow progressive motile sperm (%)	12.38 ± 9.23	11.4 ± 7.13	16.98 ± 15.53	11.72 ± 6.79	13.58 ± 9.98
Proportion of non-progressive motile sperm (%)	7.99 ± 5.4	8.34 ± 5.76	7.73 ± 4.37	9.12 ± 5.67	7.59 ± 4.94
Motility (%)	59.56 ± 18.85	60.15 ± 18.8	57.46 ± 17.26	59.58 ± 19.13	59.75 ± 16.58

Table 4. *Main semen characteristics of the population in the study of cell phone effects on male fertility. All data are expressed as mean ± SD. Bold numbers indicate significant differences between control group 1 and the high-transmitters, with p = 0.01.*

	Volume (ml)	*Sperm conc. (10^6/ml)*	*Proportion of rapid progressive motile sperm (%)*	*Proportion of slow progressive motile sperm (%)*	*Proportion of non-progressive motile sperm (%)*	*Proportion of immotile sperm (%)*	*Total motility (%)*	*TSC (10^6/ejaculate)*	*TMS (10^6/ejaculate)*	*TRPS (10^6/ejaculate)*
Duration of possession (months)	r = -0.02; p = 0.64	r = -0.01; p = 0.91	**r = -0.12; p = 0.02**	**r = 0.12; p = 0.02**	r = 0.07; p = 0.15	r = 0.06; p = 0.28	r = -0.08; p = 0.14	r = -0.01; p = 0.81	r = -0.03; p = 0.53	r = -0.06; p = 0.26
Duration of daily standby (hours)	r = 0.05; p = 0.42	r = -0.01; p = 0.39	r = -0.05; p = 0.41	r = 0.05; p = 0.37	r = -0.05; p = 0.37	r = 0.04; p = 0.47	r = -0.03; p = 0.64	r = -0.05; p = 0.41	r = -0.07; p = 0.22	r = -0.08; p = 0.15
Duration of daily transmission (min)	r = -0.01; p = 84	r = 0.04; p = 0.84	**r = -0.19; p < 0.01**	**r = 0.28; p < 0.01**	r = -0.03; p = 0.56	r = 0.08; p = 0.12	r = -0.07; p = 0.16	r = 0.03; p = 0.58	r = 0.00; p = 0.54	r = -0.08; p = 0.14

Table 5. *Correlations between parameters of cell phone use and semen characteristics with Pearson correlation. (n = 371). Bold numbers denote a significant correlation ($p < 0.05$).*

The duration of possession correlated negatively with the proportion of rapid progressive motile sperm, and positively with the proportion of slow progressive motile sperm ($r = -0.12$; $p = 0.023$, and $r = 0.12$; $p = 0.024$, respectively) (Figures 2 and 3).

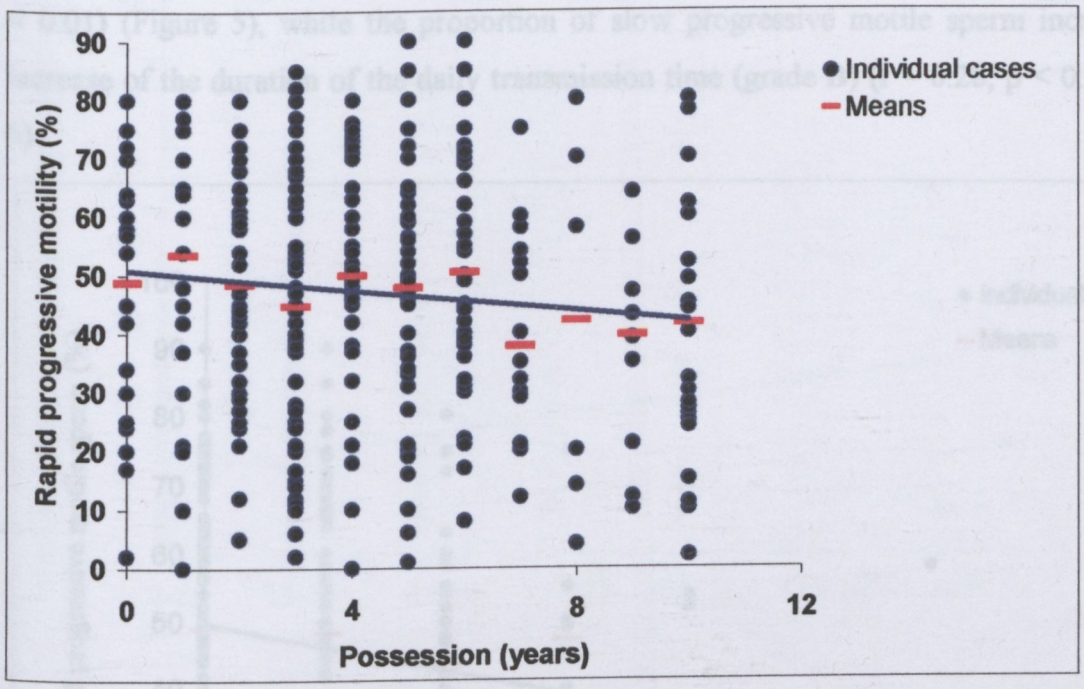

Figure 2. Correlation between duration of cell phone possession and rapid progressive motility. ($r = -0.12$; $p = 0.02$; $n = 372$)

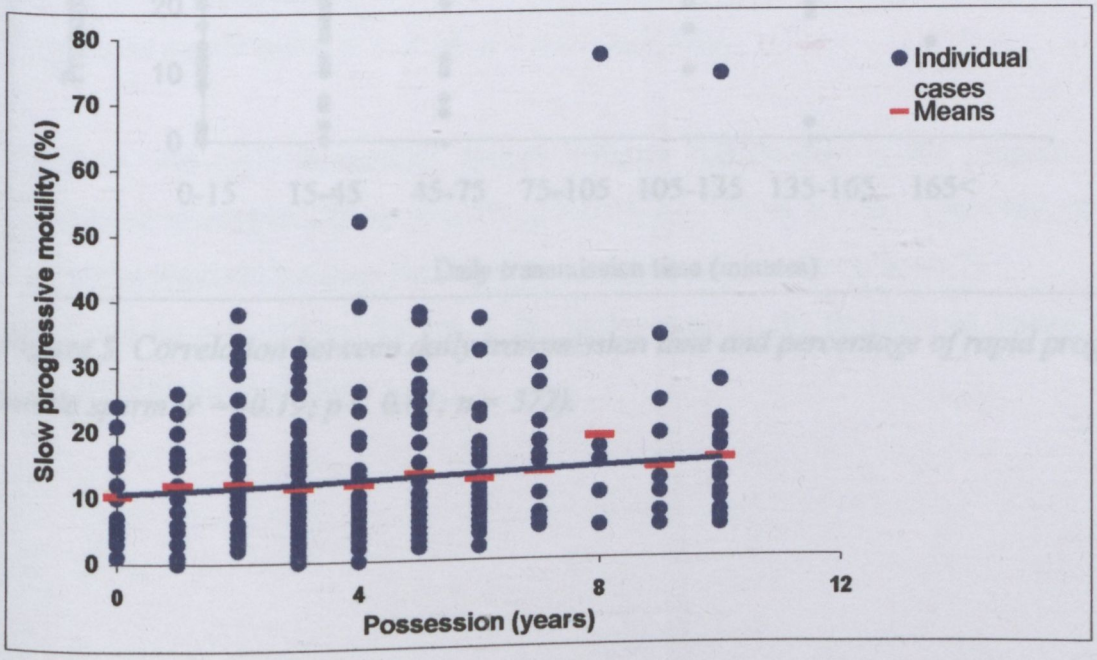

Figure 3. Correlation between duration of cell phone possession and slow progressive motility. ($r = 0.12$; $p = 0.02$; $n = 372$).

Although we found no changes in the total motility, the characteristics of the motile sperm had changed markedly. The results revealed a significant decrease in the proportion of rapid progressive motile sperm (grade A) with increase of the daily transmission time ($r = -0.19$; $p < 0.01$) (Figure 5), while the proportion of slow progressive motile sperm increased with increase of the duration of the daily transmission time (grade B) ($r = 0.28$; $p < 0.01$) (Figure 6).

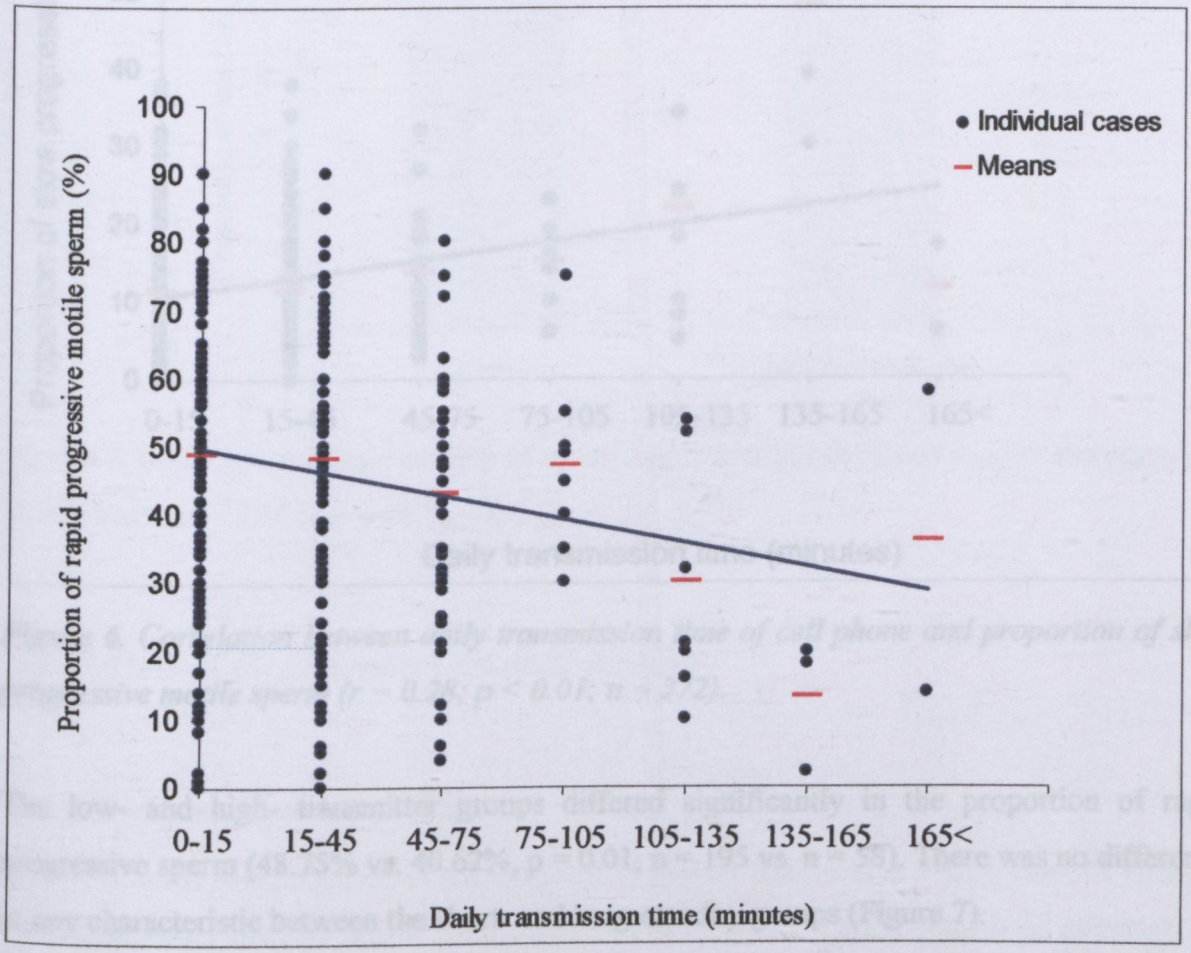

Figure 5. *Correlation between daily transmission time and percentage of rapid progressive motile sperm ($r = -0.19$; $p < 0.01$; $n = 372$).*

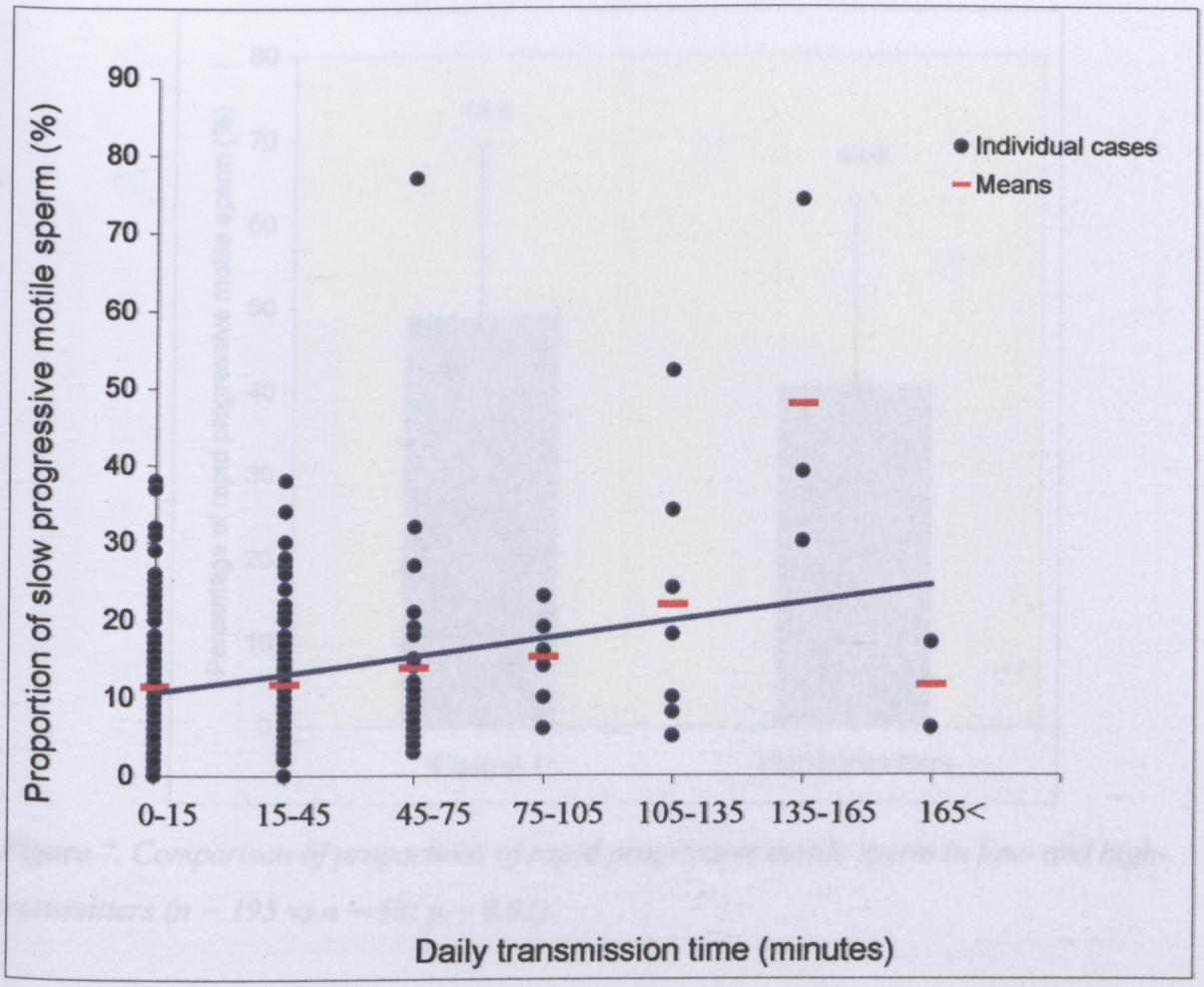

Figure 6. *Correlation between daily transmission time of cell phone and proportion of slow progressive motile sperm ($r = 0.28$; $p < 0.01$; $n = 372$).*

The low- and high- transmitter groups differed significantly in the proportion of rapid progressive sperm (48.75% vs. 40.62%, p = 0.01, n = 195 vs. n = 58). There was no difference in any characteristic between the short- and long-standby groups (Figure 7).

We found no occupational hazard in the background of the deteriorated semen parameters among the high-transmitters.

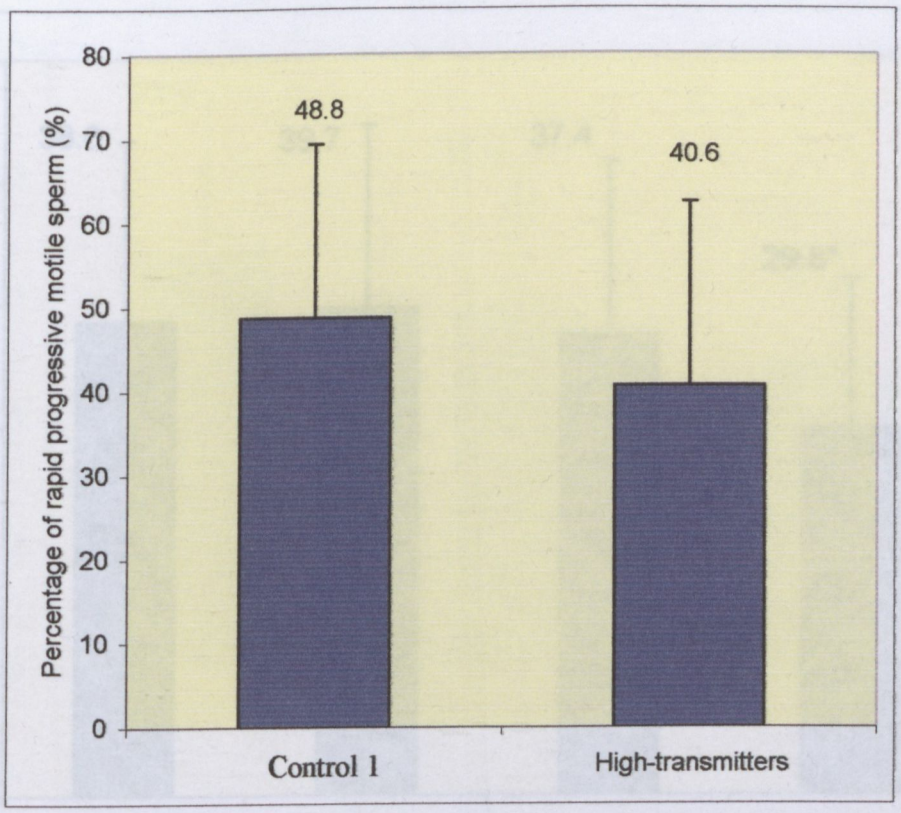

Figure 7. *Comparison of proportions of rapid progressive motile sperm in low- and high-transmitters (n = 195 vs n = 58; p = 0.01).*

4.2. Effects of fat deposition on male fertility

4.2.1. Normozoospermic males

The BMI in the study population was between 17 and 39 (mean: 27.6 ± 4.9). The 274 patients were grouped by BMI (Group 1: 29; Group 2: 96; Group 3: 91; and Group 4: 58 patients). The highest mean sperm concentration was found in Group 2 (39.7 ± 14.9 * 10^6/ml). In the groups of underweight and overweight patients, the sperm concentration was mildly lower (38.6 ± 14.9 * 10^6/ml and 37.4 ± 14.1 * 10^6/ml, respectively), but only in the group of obese patients was the decrease in sperm concentration significant (29.8 ± 12.3 * 10^6/ml; $p < 0.05$) (Figure 8).

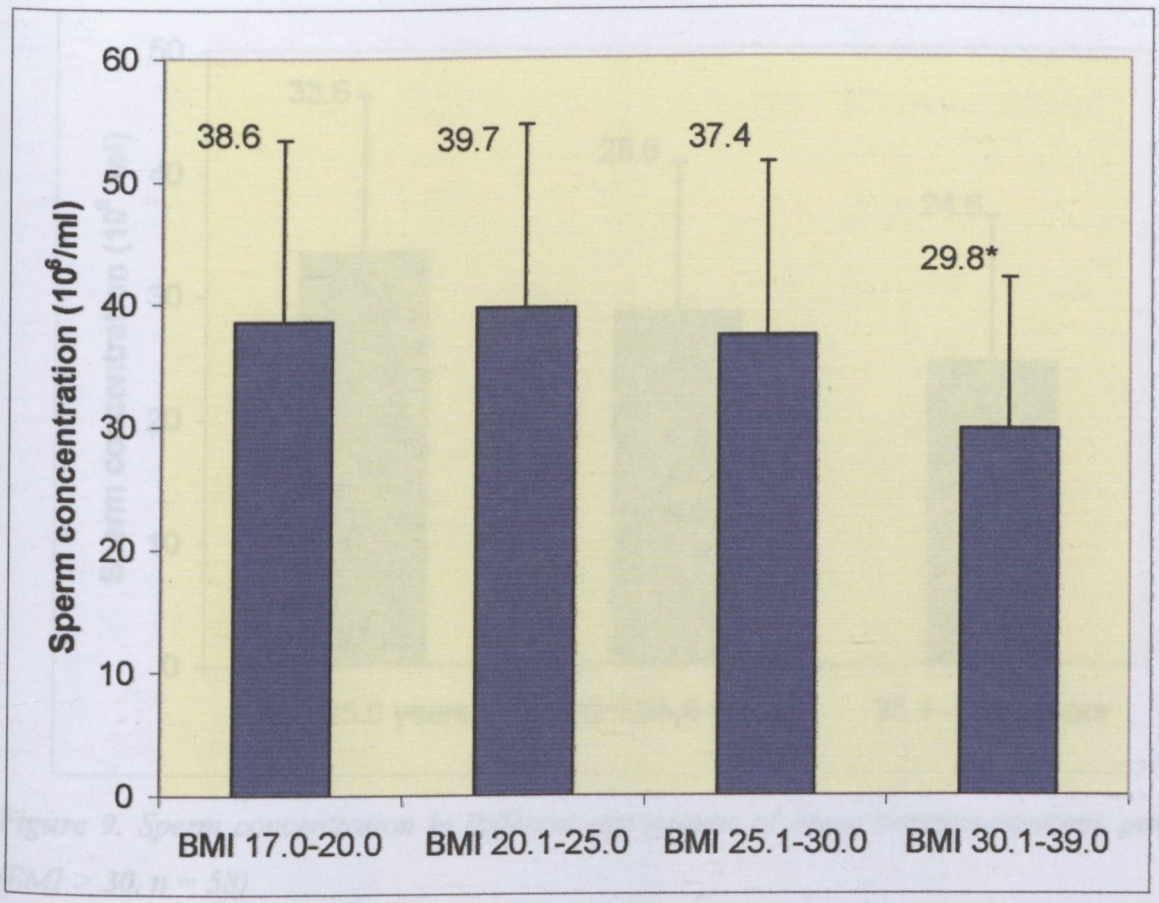

Figure 8. *Sperm concentration in groups of normozoospermic males with different BMI (n = 274). *Significant difference (p < 0.05).*

The analysis of Group 4 showed that the extent of spermatogenesis decreased with advancing age (Figure 9). Relative to the youngest (18-25 years) age group (n = 17), we found a decrease of 14.9% in the group aged 25.1-35 years (n = 26) and of 26.8% in the oldest (35.1-45 years, n = 15) age group.

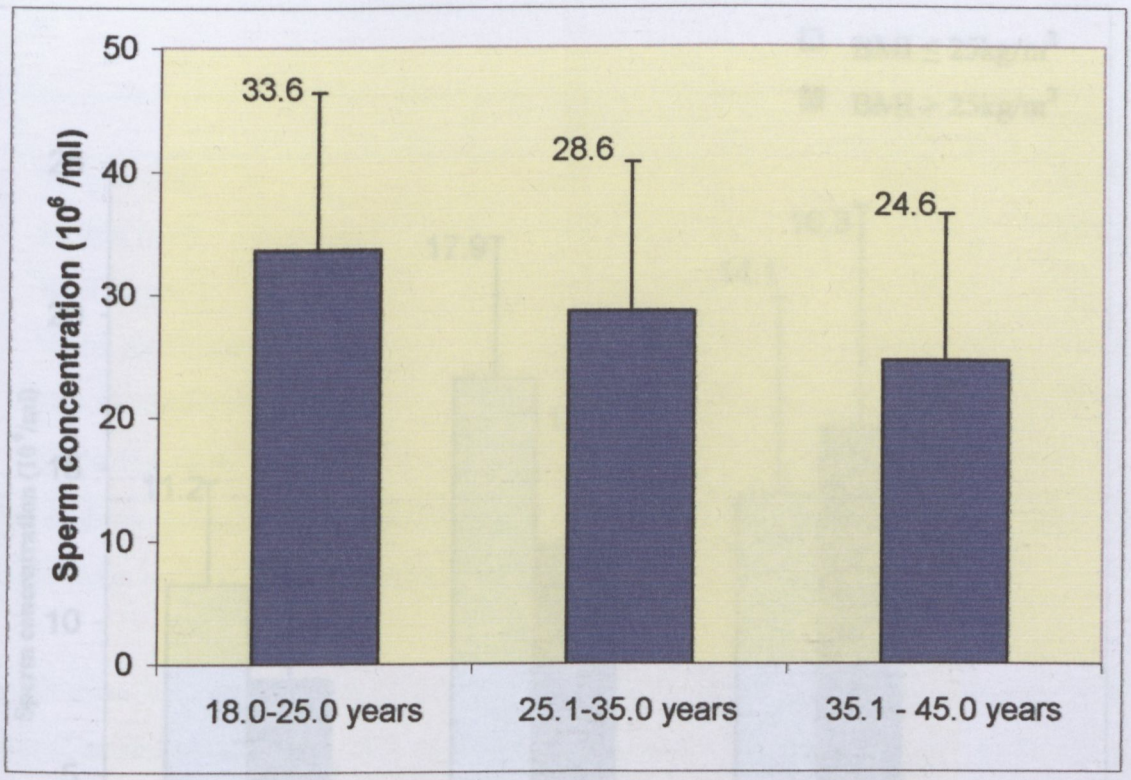

Figure 9. *Sperm concentration in different age groups of obese normozoospermic patients (BMI > 30, n = 58)*

4.2.2. Oligozoospermic males

The BMI of the overall study population ranged between 18 and 37 kg/m^2 (mean: 27.6 ± 4.6). The 42 patients were subdivided into two groups on the basis of the BMI. As shown in Figure 10, the mean sperm concentration was significantly lower in the group of overweight or obese patients (8.1 * 10^6/ml; n = 25) than in the group of underweight or normal-weight patients (11.2 * 10^6/ml; n = 17) ($p < 0.05$). There was no difference in semen volume, motility and morphology between the two groups. Similarly, the testosterone/17ß-oestradiol ratio was significantly reduced in the group of subfertile men with BMI > 25 kg/m^2 (17.9 ± 4.6 vs. 12.5 ± 2.3, $p < 0.05$). In contrast, the LH/FSH ratio was slightly, but not significantly increased in the latter group of patients (1.41 vs. 1.63).

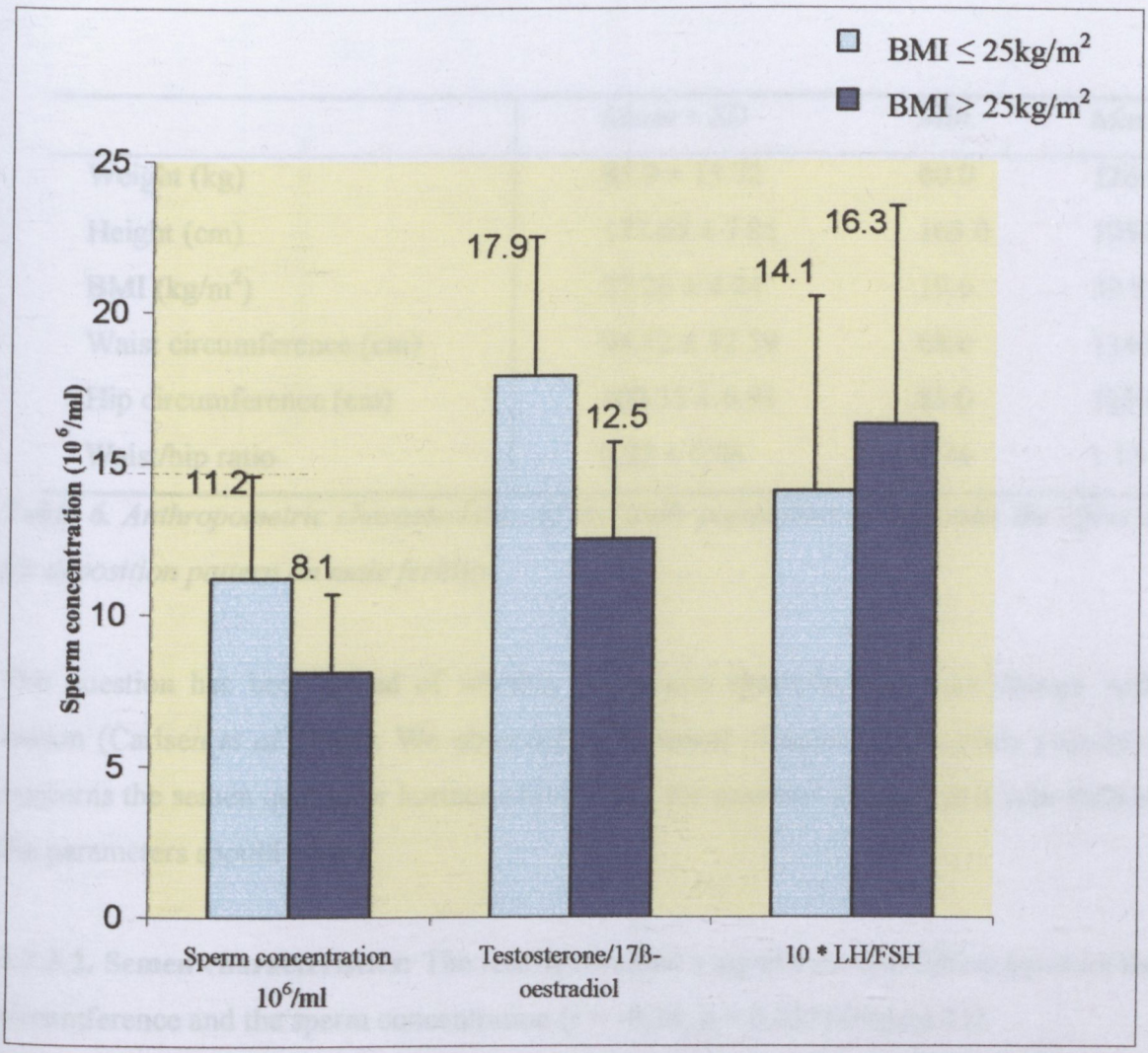

Figure 10. *Mean sperm concentration in the different BMI groups of oligozoospermic patients. The difference was significant between the sperm concentration ($p < 0.05$) and the testosterone/17β-oestradiol ratios ($p < 0.05$), but non-significant between the LH/FSH ratios. The results are given as mean ± SD ($n = 17$ vs. 25).*

4.2.3. Male body fat distribution

4.2.3.1. Characteristics of the study population: A total of 81 males were involved in the study during the one-year study period from 1 June, 2003 to 31 May, 2004. The mean age of the study population was 32.2 ± 5.6 years (range 23.7-52.2). The measured anthropometric characteristics are shown in Table 6.

	Mean ± SD	*Min.*	*Max.*
Weight (kg)	85.9 ± 13.72	60.0	126.0
Height (cm)	177.65 ± 7.85	163.0	195.0
BMI (kg/m^2)	27.26 ± 4.24	19.6	39.8
Waist circumference (cm)	94.52 ± 12.59	68.0	134.0
Hip circumference (cm)	102.35 ± 6.91	85.0	125.0
Waist/hip ratio	0.92 ± 0.08	0.74	1.15

Table 6. *Anthropometric characteristics of the study population as concerns the effect of the fat deposition pattern on male fertility.*

The question has been raised of whether the semen characteristics may change with the season (Carlsen *et al.* 2004). We observed no seasonal changes in this study population as concerns the semen quality or hormone levels. For the seasonal changes in a year-long study, the parameters should adjust.

4.2.3.2. Semen characteristics: The results revealed a significant correlation between the hip circumference and the sperm concentration ($r = -0.24$; $p = 0.033$) (Figure 11).

Both the waist circumference and the hip circumference correlated with the TSC, the TMS and the TRPS, but none of these sperm parameters correlated significantly with the waist/hip ratio (Figures 12 and 13).

The semen volume correlated significantly with the waist circumference and the waist/hip ratio, but not with the hip circumference (Table 7).

In this study population the oligozoospermic ($< 20 * 10^6$/ml; $n = 15$) patients did not differ from the normozoospermic ($\geq 20 * 10^6$/ml; $n = 66$) patients, and the asthenozoospermic ($n= 49$) patients did not differ from those with sperm with normal motility (>50%) ($n = 32$) as concerns the anthropometric parameters.

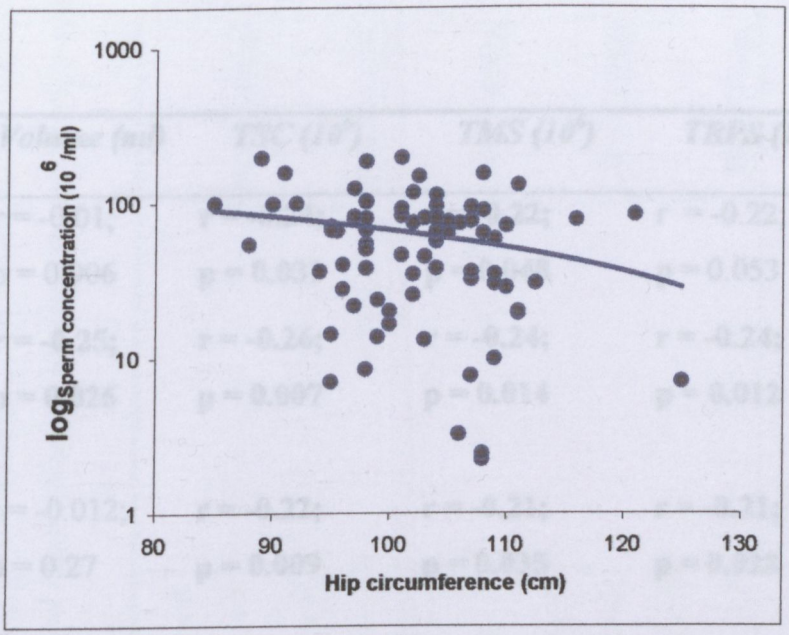

Figure 11. *Correlation between hip circumference and sperm concentration. (r = -0.24; p = 0.03; n = 81).*

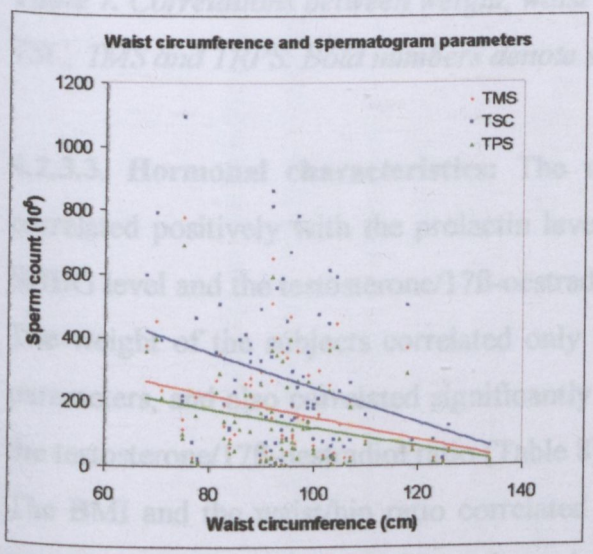

Figure 12. *Correlations between waist circumference and TSC, TMS and TRPS. r_{TSC} = -0.26; p = 0.007; r_{TMS} = -0.24; p = 0.014; r_{TRPS} = -0.24; p = 0.012).*

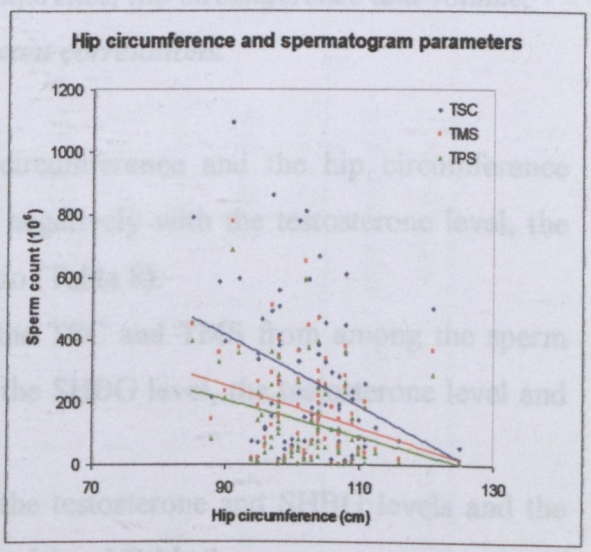

Figure 13. *Correlations between waist circumference and TSC, TMS and TRPS. r_{TSC} = -0.22; p=0.009; r_{TMS} = -0.21; p = 0.035; r_{TRPS} = -0.2; p = 0.028).*

	Volume (ml)	*TSC (10^6)*	*TMS (10^6)*	*TRPS (10^6)*
Weight (kg)	r = -0.01; p = 0.906	**r = -0.24; p = 0.031**	**r = -0.22; p = 0.048**	r = -0.22; p = 0.053
Waist circumference (cm)	**r = -0.25; p = 0.026**	**r = -0.26; p = 0.007**	**r = -0.24; p = 0.014**	**r = -0.24; p = 0.012**
Hip circumference (cm)	r = -0.012; p = 0.27	**r = -0.22; p = 0.009**	**r = -0.21; p = 0.035**	**r = -0.21; p = 0.028**
Waist/hip ratio	**r = -0.3; p = 0.007**	r = -0.2; p = 0.08	r = -0.2; p = 0.081	r = -0.18; p = 0.101

Table 7. *Correlations between weight, waist circumference, hip circumference and volume, TSC, TMS and TRPS. Bold numbers denote significant correlations.*

4.2.3.3. Hormonal characteristics: The waist circumference and the hip circumference correlated positively with the prolactin level and negatively with the testosterone level, the SHBG level and the testosterone/17ß-oestradiol ratio (Table 8).

The weight of the subjects correlated only with the TSC and TMS from among the sperm parameters, and also correlated significantly with the SHBG level, the testosterone level and the testosterone/17ß-oestradiol ratio (Table 8).

The BMI and the waist/hip ratio correlated with the testosterone and SHBG levels and the testosterone/17ß-oestradiol ratio, as shown in Figure 14 and Table 8.

The SHBG level was mostly affected by the body fat content: higher values of weight, BMI, waist and hip circumferences and waist/hip ratio were associated with lower SHBG levels (Table 8).

	*Testosterone level** *(nmol/l)*	*Testosterone/ 17β-oestradiol ratio**	*Prolactin level** *(IU/l)*	*SHBG level*** *(nmol/l)*
Weight (kg)	**r = -0.45; p < 0.001**	**r = -0.31; p = 0.005**	r = 0.19; p = 0.088	**r = -0.46; p < 0.001**
BMI (kg/m^2)	**r = -0.48; p < 0.001**	**r = -0.37; p < 0.001**	r = 0.18; p = 0.116	**r = -0.40; p < 0.001**
Waist circumference (cm)	**r = -0.42; p < 0.001**	**r = -0.38; p < 0.001**	**r = 0.28; p = 0.012**	**r = -0.46; p < 0.001**
Hip circumference (cm)	**r = -0.4; p < 0.001**	**r = -0.34; p = 0.002**	**r = 0.29; p = 0.09**	**r = -0.50; p < 0.001**
Waist/hip ratio	**r = -0.34; p = 0.001**	**r = -0.33; p = 0.002**	r = 0.19; p = 0.093	**r = -0.35; p = 0.001**

Table 8. *Correlations between anthropometric parameters and testosterone levels, testosterone/17β-oestradiol ratio, and prolactin and SHBG levels (n = 81). *Pearson test. **Spearman test. Bold numbers denote significant correlations.*

We found no correlation between the FSH, LH or 17ß-oestradiol levels and any of the anthropometric parameters.

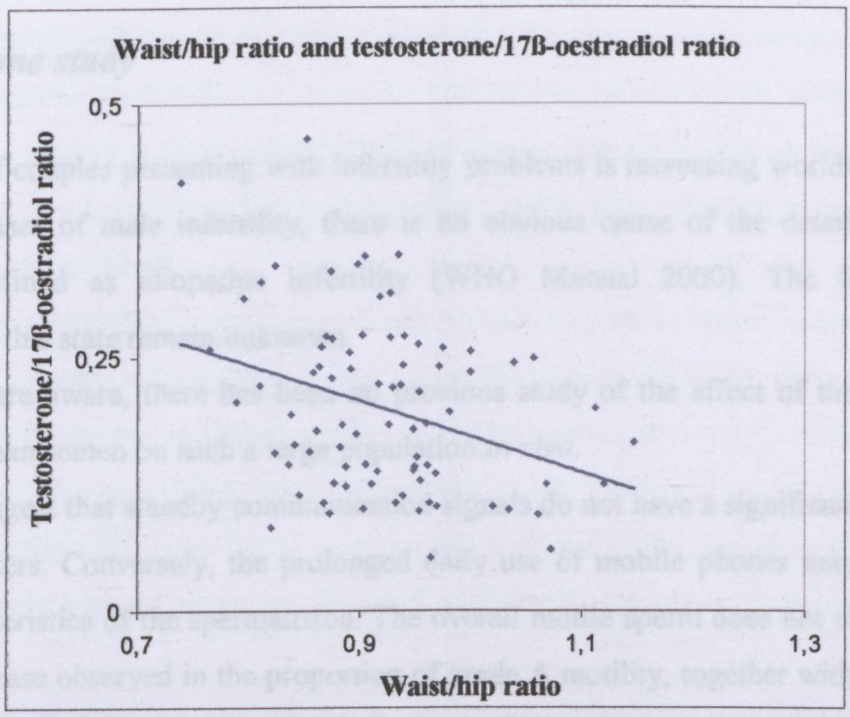

Figure 14. *Correlation between waist/hip ratio and testosterone/17β oestradiol ratio: r = -0.33, p = 0.002. The correlation reflects a relative testosterone deficiency in males with abdominal obesity.*

Android vs. intermediate fat deposition: Android (n = 10) and intermediate (n = 71) fat deposition were associated with differences in levels of serum 17ß-oestradiol (median: 73.4 (range 73.0-185.0) vs. 122 (range 73.0-228.0) pmol/l; p = 0.044) and SHBG (median 32.1 (range 12.7-87.4) vs. 29.25 (range 17.7-35.9) nmol/l; p = 0.022).

5. Discussion

5.1. Cell phone study

The number of couples presenting with infertility problems is increasing worldwide. In about 30% of the cases of male infertility, there is no obvious cause of the deteriorated semen parameters defined as idiopathic infertility (WHO Manual 2000). The factors in the background of this state remain unknown.

As far as we are aware, there has been no previous study of the effect of the EMF of cell phones on human semen on such a large population *in vivo*.

Our results suggest that standby communication signals do not have a significant effect on the sperm parameters. Conversely, the prolonged daily use of mobile phones may abrogate the motion characteristics of the spermatozoa. The overall motile sperm does not change, but the moderate decrease observed in the proportion of grade A motility, together with the moderate increase observed in the proportion of grade B motile sperm, may be a consequence of the electromagnetic radiation emitted from cell phones. These findings are similar to those of Davoudi *et al.*, who observed a reduction in the proportion of rapid progressive sperm from 32.3% to 26.1% after 1 month of cell phone use for 6 hours daily (Davoudi *et al.* 2002). Makler *et al.* examined the effects of 27 MHz electromagnetic resonance on human sperm velocity and survival *in vitro*, and found a decreased percentage motility and velocity, but no effect of UV light or X-rays (Makler *et al.* 1980).

The correlation between the duration of cell phone possession and the changes in the motility parameters suggests that these effects accumulate.

Electromagnetic radiation has both thermal and non-thermal effects on living cells. There is no consensus among authors as to which effect predominates (Dasdag *et al.* 2003, Weisbrot 2003). We believe that the thermal effects are possibly low at such low SAR levels as the cell phone emits.

We have formulated two hypotheses with which to interpret our results; however, both are in need of evidence.

First, as the brain region is close to the transmitting cell phone, the admittance is obvious: electromagnetic radiation affects the testis by changing the levels of hormones excreted

intracranially. de Seze *et al.* found no alterations in the levels of pituitary hormones in association with prolonged cell phone use (de Seze *et al.* 1998). Accordingly, we excluded those subjects who exhibited such an alteration. Burch *et al.* demonstrated a reduced 6-hydroxymelatonin sulphate (6-OHMS) level in the urine among those using a cell phone for over 25 minutes/day; 6-OHMS is the urinary metabolite reflecting the serum level of the pineal hormone melatonin (Burch *et al.* 2002). Melatonin is known to be an antioxidant agent that protects against lipid peroxidation in the retina, brain, liver cells and even human sperm (Gavella and Lipovac 2000).

Secondly, the electromagnetic radiation of cell phones may cause DNA breakage in cells in the male genital tract, which can occur in a low-frequency EMF. *In vitro* studies appear justified to investigate the increased numbers of chromosome aberrations of the micronuclei in human leucocytes and DNA breaks (Ivancsits *et al.* 2002, Lai *et al.* 1995, 1996). A moderate correlation has been found between the sperm motility and the sperm chromatin structure probably brought about by distorted epididymal protamination (Giwercman *et al.* 2003).

A possible connection of the two theories is reactive oxygen species (ROS) production. ROS cause DNA fragmentation in somatic cells, reducing protamination (Sun *et al.* 1997). Melatonin inhibits ROS production.

Additionally, a recent pilot study has shown that proteomics might be an effective tool in the search for of cell responses to weak stimuli, including cell phone radiation. It found several tens of protein targets of cell phone radiation that participate in basic physiological cell functions such as cell energy production, protein translation and cytoskeleton-dependent processes. The study was performed on an endothelial cell line. The most important from the aspect of spermatozoa movement might be the declined expression of isocitrate-dehydrogenase 3 (NAD+)α, a subunit of the mitochondrial enzyme, which catalyses the conversion of isocitrate to 2-oxoglutarate in the citric acid cycle (Nylund and Leszcynski 2004). The down-regulation might affect spermatozoa energy production, resulting in decreased movement characteristics.

A sedentary lifestyle and other occupational factors can lead to deteriorated semen parameters (Nieschlag and Behre 2001, Stoy *et al.* 2004). These effects are mostly occupation-related. We found no specific profession among the high-transmitters in our population to suggest that

some particular occupation might be responsible for the deteriorated semen parameters rather than excessive cell phone use.

Our study has some limits: the effects of the non-ionizing radiation emitted by cell phones depend on a number of factors besides the duration of transmission, e.g. the type of cell phone and the distance from the cell phone tower (ICNIRP 1998). We examined only the duration of use not specified to other variables, as this covered a fairly wide cross-section of males from the whole population.

Further, prospective, controlled studies on a larger population are required to prove whether the electromagnetic emission from cell phones affects the male fertilizing capacity, and to establish the mode of action of such a possible deteriorating effect.

5.2. Fat deposition study

There is an ever-growing number of reports concerning increasing proportions of overweight women and men in the developed countries. In Central Europe, an Austrian study reported that the proportion of moderately overweight men rose from 10.9% to 15.5% between 1985 and 2000, while that of overweight men rose from 1.8% to 4.9% (Rami *et al.* 2004).

Several illnesses, such as metabolic X syndrome, coronary disease, thrombosis, cholecystolithiasis, EPH gestosis, some types of carcinoma, arthrosis or even depression, have been proved to occur as complications of obesity. In women, both abnormally low (anorexia nervosa) and abnormally high body weights result in fertility problems (Herold 2001).

The glucose homeostasis also changes remarkably when the quality of abdominal fat grows, and the disturbed glucose metabolism causes decreased fertility in both women and men (Bujis *et al.* 2004).

On this basis, we studied the less documented field of body fat content relative to male fertility. We carried out a three-step study.

First, the aim of our study was to examine the sperm concentration of normozoospermic patients in different BMI groups.

Our results showed that the sperm concentration of obese patients is significantly lower than that of those who are underweight, of normal weight or overweight.

In the obese group we found a decrease in sperm concentration with aging; however, it was not significant, probably because of the low number of patient. This suggests that obesity and

the consequent oestrogen production have long-term effects on sperm production. Previous studies are contradictory as regards changes in sperm concentration with advancing age (Eskenazi *et al.* 2003, Kidd *et al.* 2001, Ng *et al.* 2004). Most of them reported changes in motility and volume, but not in sperm concentration. Some of the papers described an increase in sperm concentration with aging ranging, from 0.03 to 3.3% per year, whereas others showed no alteration and yet others reported a decrease in sperm concentration. The results most similar to ours were reported by Auger *et al.*, who observed a 3.3% decrease per year in 1351 healthy fertile men (Auger *et al.* 1995).

In aging males, oestrogen plays important roles in the regulation of gonadotropin production, the emotional state, bone mass and lipid synthesis (Vermeulen *et al.* 2002).

Secondly, we found a significant difference in the sperm concentration of patients with a normal or low BMI and in overweight or obese patients. The testosterone/17ß-oestradiol ratio was in harmony with this difference, and inversely the LH/FSH ratio was higher in the overweight group, but this was not significant. In this part of the study, however, the case number was relatively low.

As the testis has two important roles in male adults, sperm production and testosterone synthesis, these results correspond to the following idea: The testicular function is regulated by gonadotropins of the pituitary gland and testicular hormones, testosterone, 17ß-oestradiol and inhibin B. The LH stimulates Leydig cells to produce testosterone, and FSH promotes spermatogenesis (Amory and Bremner 2002). The FSH is regulated by the balance of hypothalamic gonadotropins-releasing hormone (GnRH) and the negative feedback of testosterone, 17ß-oestradiol and inhibin B (Hayes *et al.* 2001a). Testosterone exerts direct and indirect effects on LH secretion, whereas its effects on FSH are mediated by aromatization to oestradiol (Hayes *et al.* 2001 b). 17ß-oestradiol has dual sites of negative feedback: the decrease in GnRH pulse frequency at the hypothalamus and the decrease in pituitary responsiveness to GnRH (Hayes *et al.* 2000). Plant and Marshall (2001) emphazise the importance of inhibin B in the regulation of FSH secretion, rather than testosterone and 17ß-oestradiol.

The exact modes of action of LH, FSH and testosterone are not yet clear, but all of them are necessary for the normal development of spermatogenesis in puberty. Clinical evidence has given rise to the suggestion that a synergic effect of FSH, LH and testosterone is necessary for

the maintenance of appropriate spermatogenesis (Nieschlag and Behre 2000). In the event of their imbalance, the mechanism becomes disturbed.

If testosterone transforms to 17ß-oestradiol, it cannot exert its effect in spermatogenesis and its negative feedback on LH is less then the negative feedback of 17ß-oestradiol on FSH.

Our data accord to the results of Luboshitzky *et al.* (2002), who reported a decreased testosterone level and testosterone/17ß-oestradiol ratio in asthenozoospermic, oligozoospermic and oligo-terato-asthenozoospermia (OTA) syndrome patients, and an elevated 17ß-oestradiol level in oligozoospermic and OTA syndrome patients as compared to normal controls.

In the last part of this study, we found no correlation between the waist/hip ratio and any of the sperm characteristics but the semen volume. Although the significance of this last correlation is $p = 0.007$, the semen volume is a sensitive part of semen analysis, most influenced by the conditions of sample production. On the other hand, the semen volume influences the TSC, TMS and TRPS, which is important in the preparation of semen samples for artificial insemination. These parameters deteriorated with increasing weight and increasing waist and hip circumference. The findings suggest that it is not the type of fat deposition that plays an important role in sperm production, but merely the amount of fat.

The further results support our previous findings that the testosterone level and the testosterone/17ß-oestradiol ratio decline with increasing amount of fat. Further, the SHBG level showed a strong correlation with anthropometric parameters that further suggest a testosterone deficiency in obese men.

In spite of the fact that the spermatogram does not change with the waist/hip ratio, the sexual hormones and the SHBG level do.

In earlier work, a correlation was found between the weight and the semen concentration (Koloszar *et al.* 2002). It has been demonstrated that not only the BMI, but also the body fat distribution is a risk factor for several diseases (Hollman *et al.* 1997, Mery *et al.* 2002).

There are more precise methods for determination of the fat distribution in men, e.g. with the help of computer tomography or MRI, but these methods are not suitable in the daily routine because of the time and cost requirements. Hence, we chose the simple measurement of the waist and hip circumferences, and determination of their ratio.

In the background of the effect of an increased quantity fat on the human spermatogram, two mechanisms and their imbalance may be suspected: via increased leptin levels and changed sexual hormone levels on the action of aromatase.

Leptin is a 167-amino acid hormone secreted from the white adipose tissue that actively participates in the regulation of energy homeostasis. It reduces weight, body fat and food intake; it regulates neuroendocrine systems, energy expenditure, haematopoiesis, angiogenesis, puberty and reproduction (El-Hefnawy *et al.* 2000, Jope *et al.* 2003). It acts through binding to a specific obese receptor (OB-R), several isoforms of which exist. The OB-R is expressed in the ovary, where it regulates granulosa cell steroidogenesis and oocyte maturation. The expression of the messenger ribonucleic acid of the leptin receptor and functional OB-R is also localized in the rat testis, possibly playing a role in the proliferation and differentiation of germ cells (El-Hefnawy *et al.* 2000). The plasma leptin level is in strict relation with the amount of adipose tissue. It is known that leptin is detectable in the seminal plasma and in the human spermatozoa and it has direct and indirect effects on the gonadal functions, though the exact role is not yet known (Camina *et al.* 2002, Jope *et al.* 2003). Leptin influences the reproductive functions by stimulating GnRH. The serum leptin concentration was higher in non-obstructive azoospermia independently of the gonadotropins (Steinman *et al.* 2001), and the seminal plasma leptin levels were significantly lower in patients with normal spermatogram as compared with pathological semen samples and correlated negatively with the motility of the human spermatozoa (Glander *et al.* 2002). These findings suggest that serum leptin plays a role in the regulation of the gonadal functions. It passes through the blood-testis barrier and exists in the tubuli seminiferi and in the seminal plasma (Camina *et al.* 2002, Glander *et al.* 2002). Leptin suppresses testosterone secretion in the Leydig cells of rodents by interaction with its peripheral receptor (Caprio *et al.* 1999). These results indicate that leptin acts on the gonadal functions through two pathways: indirectly, via the central neuroendocine system, and directly, via peripheral tissue membrane receptors (Jope *et al.* 2003). In our studies, the leptin level was not measured, though it may be important from the aspect of the effect of obesitiy on male fertility, which is as yet an unclarified question.

Cytochrome p450 aromatase, the terminal enzyme that converts androgens into oestrogens, is present in different testicular cell types, such as the Leydig cells, Sertoli cells and germ cells, and aromatases and oestrogens play important roles in the function of the reproductive organs

(Carreau *et al.* 2002). Only 20% of the biologically active oestrogen is produced in the testis. The remainder is converted by aromatization from androstenedione in the adrenal gland. The 17ß-oestradiol level in males correlates with the amount of fat tissue. Aromatization occurs mostly in the subcutaneous abdominal tissue (Cohen *et al.* 2001, Vermeulen *et al.* 2002). Overweight patients have an increased level of cytochrome p450 aromatase, which therefore produces more oestrogen (Holbrook *et al.* 2003).

Oestrogen excretion can be decreased by administration of the recently introduced aromatase inhibitors. Their first success was achieved in the treatment of oestrogen-dependent breast cancers (Bray *et al.* 2002). Later, they were effectively used for the treatment of male infertility, where aromatase inhibitors markedly decreased the oestrogen level, with a resulting favourable effect on the testosterone/17ß-oestradiol ratio and consequently the sperm concentration rose too (Haidl 2002, Nieschlag and Behre 2000, Raman and Schegel 2002).

If a shift toward oestrogens occurs due to increased aromatization, a weight loss and the administration of pure FSH are suggested as treatment (Kamischke *et al.* 1998), if (as in Hungary) aromatase inhibitors are not available for the treatment of male infertility.

The weight of the patient and the amount of abdominal subcutaneous fat tissue may be important in oligozoospermia, especially in cases where no other reasonable explanation can be detected in the background of a deteriorated sperm concentration. In these cases, determination of the testosterone/17ß-oestradiol ratio can be instructive.

In the future, more attention should be paid to the weight of the patients, and especially the mass of abdominal subcutaneous fat, by measuring the waist/hip ratio not only in cases with oligozoospermia or asthenozoospermia, but also in those with normozoospermia, as an abnormal weight gain can result in deteriorated sperm motility characteristics. Further studies are required to examine the possible positive effects of a weight loss on the semen characteristics in obese patients.

6. Summary and conclusions

In this thesis, two major themes have been examined. In the first part the possible effects of electromagnetic radiation on human semen characteristics were studied. The results were as follows:

1.1. A relationship was found between the duration of possession of a cell phone and the spermatozoa motility characteristics.

1.2. There was no correlation between the daily duration of the cell phone being in the standby position near the males and any of the semen parameters.

1.3. A relationship was found between the duration of daily transmission and the spermatozoa motility characteristics.

The correlations were weak. Longer possession and longer daily use result in a decreased proportion of rapid progressive sperm and an increased proportion of slow progressive motile sperm. Previously, there had been no study of the effect of cell phone radiation on human semen on a relatively large population. The background mechanism is unknown, though the roles of melatonin, DNA breakage and the changed expression of proteins are suspected.

In the second part of the thesis, the effects of the body fat content on the spermatogram were examined. The findings were as follows:

2. In normozoospermic males, the sperm concentration was significantly lower in obese than in underweight, normal-weight or overweight males.

3. In obese normozoospermic males, the sperm concentration decreased with advancing age.

4. The sperm concentration and the LH/FSH ratio were lower, while the testosterone/17ß-oestradiol ratio was higher in those oligozoospermic males whose BMI was above the normal.

5. Relationships were found between the following parameters:

5.1. Hip circumference and sperm concentration.

5.2 Waist circumference and hip circumference vs. sperm count, TSC, TMS and TRPS.

5.3 Weight vs. TSC and TMS.

5.4 Waist circumference and waist/hip ratio vs. semen volume.

There was no other significant relationship between the examined anthropometric parameters and the semen parameters.

6. Relationships were found between the following parameters:

6.1 Weight, BMI, waist circumference and hip circumference vs. testosterone level, SHBG level and testosterone/17ß-oestradiol ratio.

6.2 Waist circumference and hip circumference vs. prolactin level.

No other significant relationship was found between the examined anthropometric parameters and the sexual related hormone levels.

An increased body fat content leads to deteriorated semen characteristics. This effect increases with advancing age. The type of fat deposition pattern did not affect a spermatogram, although a larger hip circumference was associated with a decreased sperm concentration. Hormonal changes were also detected. The testosterone level and the testosterone/17ß-oestradiol ratio were lower in males with increased body fat. Furthermore, the SHBG level was lower in these patients. This reflects deficiencies of both free and total testosterone. Similar hormonal changes were observed on changes in the waist/hip ratio. The duration of the fat status at the time of the study was not examined. The roles of aromatase p450 and leptin are suspected in the background.

Acknowledgements

I would like to express my grateful thanks to my tutor, Professor János Szöllősi, to my PhD programme coordinator, Professor László Kovács, to the Head of the Department, Professor Attila Pál, and to my principal investigator in the EMAS study group, Professor György Bártfai, for their invaluable advice, support and help in my scientific work and also in my private life.

Many thanks are due to all of those colleagues and friends who provided outstanding support and circumstances for my work: Gabriella Tari, Éva Lukácsné Gábor, Dr. Sándor Koloszár, Dr. Zoltán Závaczki, Dr. József Daru, Dr. Imre Földesi and Angéla Herédi.

I would like to express my thanks to Professor Gábor Huszár, Lynne Vigue, Leyla Sati and Dave Bennett for their help in my work and life at Yale University.

The studies were partially supported by the Hungarian OTKA programme. Grant number: T 038235.

Last, but not least, I owe many thanks to my family and all of my friends for helping me through the difficulties of life during the past years.

References

Ahlbom A, Green A, Kheifets L, Savitz D, Swerdlow A (2004) Epidemiology of health effects of radiofrequency exposure. *Environmental Medicine* 112: 1741-1754.

Amory JK, Bremner W (2001) Endocrine regulation of testicular function in men: implications for contraceptive development. *Molecular and Cellular Endocrinology* 182: 175-179.

Auger J, Kunstmann JM, Czyglik F, Jouannet P (1995) Decline in semen quality among fertile men in Paris during the past 20 years. *New England Journal of Medicine* 332: 281-285.

Bray GA (2002) The underlying basis for obesity: relationship to cancer. *Journal of Nutrition* 132: 3451S-3455S.

Buijs MM, Romijn JA, Burggraaf J, De Kam ML, Ackermans MT, Sauerwein HP, Cohen AF, Meinders AE, Pijl H (2004) Glucose homeostasis in abdominal obesity: hepatic hyperresponsiveness to growth hormone action. *American Journal of Physiology-Endocrinology and Metabolism* 287:63-68.

Burch JB, Reif JS, Noonan CW, Ichinose T, Bachand AM, Koleber TL, Yost MG (2002) Melatonin metabolite excretion among cellular telephone users. *International Journal of Radiation Biology* 78:1029-1036.

Camina JP, Lage M, Menendez C, Grana M, Garcia-Devesa J, Dieguez C, Casanueva FF (2002) Evidence of free leptin in human seminal plasma. *Endocrine* 17: 169-174.

Caprio M, Isidori AM, Carta AR, Moretti C, Dufau ML, Fabbri A (1999) Expression of functional leptin receptors in rodent Leydig cells. *Endocrinology* 140: 4939-4947.

Carlsen E, Petersen JH, Andersson A-M, Skakkebaek NE (2004) Effects of ejaculatory frequency and season on variations in semen quality. *Fertility and Sterility* 82: 358-366.

Carreau S, Bourguiba S, Lambard S, Galeraud-Denis I, Genissel C, Levallet J (2002) Reproductive system: aromatase and estrogens. *Molecular and Cellular Endocrinology* 193: 137-143.

Christensen HC, Schuz J, Kosteljanetz M, Poulsen HS, Thomsen J, Johansen C (2004) Cellular telephone use and risk of acoustic neuroma. *American Journal of Epidemiology* 159: 277-283.

Cohen PG (2001) Aromatase, adiposity, aging and disease. The hypogonadal metabolic-atherogenic- disease and aging connection. *Medical Hypotheses* 56: 702-708.

Cook A, Woodward A, Pearce N, Marshall C (2003) Cellular telephone use and time trends for brain, head and neck tumours. *New Zealand Medical Journal* 116: 457.

Dasdag S, Zulkuf Akdag M, Aksen F, Yilmaz F, Bashan M, Mutlu Dasdag M, Salih Celik M (2003) Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes. *Bioelectromagnetics* 24:182-188.

Davoudi M, Brössner C, Kuber W (2002) Der Einfluss elektromagnetischer Wellen auf die Spermienmotilität. *Journal für Urologie und Urogynäkologie* 9:18-22.

D'Costa H, Trueman G, Tang L, Abdel-rahman U, Abdel-rahman W, Ong K, Cosic I (2003) Human brain wave activity during exposure to radiofrequency field emissions from mobile phones. *Australasian Physical & Engineering Sciences in Medicine* 26: 162-167.

de Seze R, Fabbro-Peray P, Miro L (1998) GSM radiocellular telephones do not disturb the secretion of antepituitary hormones in humans. *Bioelectromagnetics* 19: 271-278.

El-Hefnawy T, Ioffe S, Dym M (2000) Expression of the leptin receptor during germ cell development in mouse testis. *Endocrinology* 141: 2624-2630.

Elwood JM (2003) Epidemiological studies of radio frequency exposures and human cancer. *Bioelectromagnetics* Suppl 6: 63-73.

Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, Young S, Moore D (2003) The association of age and semen quality in healthy men. *Human Reproduction* 18: 447-454.

Gavella M, Lipovac V (2000) Antioxidative effect of melatonin on human spermatozoa. *Archives of Andrology* 44: 23-27.

Giwercman A, Richthoff J, Hjollund H, Bonde JP, Jepson K, Frohm B, Spano M (2003) Correlation between sperm motility and sperm chromatin structure assay parameters. *Fertility and Sterility* 80: 1404-1412.

Glander HJ, Lammert A, Paasch U, Glasow A, Kratzsch J (2002) Leptin exists in tubuli seminiferi and in seminal plasma. *Andrologia* 34: 227-233.

Haidl G (2002) Management strategies for male factor infertility. *Drugs* 62: 1741-1753.

Hansson Mild K, Hardell L, Kundi M, Mattsson MO (2003) Mobile telephones and cancer: is there really no evidence of an association? (review). *International Journal of Molecular Medicine* 12: 67-72.

Hardell L, Mild KH, Pahlson A, Hallquist A (2001) Ionizing radiation, cellular telephones and the risk for brain tumours. *European Journal of Cancer Prevention* 10: 523-529.

Hardell L, Mild KH, Carlberg M (2002) Case-control study on the use of cellular and cordless phones and the risk for malignant brain tumours. *International Journal of Radiation Biology* 78: 931-936.

Hardell L, Mild KH, Johansson B (2003) Cellular and cordless telephones and basal cell carcinoma: a case report. *Archives of Environmental Health* 58: 380-382.

Hayes FJ, Seminara SB, DeCruz S, Boepple PA, Crowley WF (2000) Aromatase inhibition in the human male reveals a hypothalamic site of estrogen feedback. *Journal of Clinical Endocrinology and Metabolism* 85: 3027-3035.

Hayes FJ, Pitteloud N, DeCruz S, Crowley WF, Boepple PA (2001 a) Importance of inhibin B in the regulation of FSH secretion in the human male. *Journal of Clinical Endocrinology and Metabolism* 86: 5541-5546.

Hayes FJ, DeCruz S, Seminara SB, Boepple PA, Crowley WF (2001 b) Differential regulation of gonadotropin secretion by testosterone in the human male: absence of a negative feedback effect of testosterone on follicle-stimulating hormone secretion. *Journal of Clinical Endocrinology and Metabolism* 86: 53-58.

Herold G (2000) Innere Medizin-eine vorlesungsorientierte Darstellung-2000, Köln, Author's edition. Hungarian Translation, Budapest, B+V Medical and Technical Lap- és Könyvkiadó Kft.

Holbrook JM, Cohen PG (2003) Aromatase inhibition for the treatment of idiopathic hypogonadotropic hypogonadism in men with premature ejaculation. *Southern Medical Journal* 96: 544-547.

Hollmann M, Runnebaum B, Gerhard I (1997) Impact of waist-hip-ratio and body-mass-index on hormonal and metabolic parameters in young, obese women. *International Journal of Obesity Related Metabolic Disorders* 21: 476-483.

Huber R, Treyer V, Borbely AA, Schuderer J, Gottselig JM, Landolt HP, Werth E, Berthold T, Kuster N, Buck A, Achermann P (2002) Electromagnetic fields, such as those from mobile phones, alter regional cerebral blood flow and sleep and waking EEG. *Journal of Sleep Research* 11: 289-295.

International Commission on Non-Ionizing Radiation Protection (ICNIRP) (1998) Guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields (up to 300 GHz). *Health Physics* 74: 494-522.

Ivancsits S, Diem E, Pilger A, Rudiger HW, Jahn O (2002) Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. *Mutation Research* 519: 1-13.

Ivancsits S, Diem E, Jahn O, Rudiger HW (2003) Intermittent extremely low frequency electromagnetic fields cause DNA damage in a dose-dependent way. *International Archives of Occupational and Environmental Health* 76: 431-436.

Johansen C, Boice JD Jr, McLaughlin JK, Christensen HC, Olsen JH (2002) Mobile phones and malignant melanoma of the eye. *British Journal of Cancer* 86: 348-349.

Jope T, Lammert A, Kratzsch J, Paasch U, Glander HJ (2003) Leptin and leptin receptor in human seminal plasma and in human spermatozoa. *International Journal of Andrology* 26: 335-41.

Kamischke A, Behre HM, Bergmann M, Simoni M, Schafer T, Nieschlag E (1998) Recombinant human follicle stimulating hormone treatment of male idiopathic infertility: a randomized, double blind, placebo-controlled, clinical trial. *Human Reproduction* 13: 596-603.

Kidd SA, Eskenazi B, Wyrobek AJ (2001) Effects of male age on semen quality and fertility: a review of the literature. *Fertility and Sterility* 75: 137-248.

Kirchengast S, Huber J (2004) Body composition characteristics and fat distribution patterns in young infertile women. *Fertility and Sterility* 81: 539-544.

Koloszár S, Daru J, Keresztúri A, Závaczki Z, Szöllősi J, Pál A (2002) Effect of female body weight on efficiency of donor AI. *Archives of Andrology* 48: 323-327.

Kramarenko AV, Tan U (2003) Effects of high-frequency electromagnetic fields on human EEG: a brain mapping study. *International Journal of Neuroscience* 113: 1007-1019.

Lai H, Singh NP (1995) Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. *Bioelectromagnetics* 16: 207-210.

Lai H, Singh NP (1996) Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. *International Journal of Radiation Biology* 69:513-521.

Luboshitzky R, Kaplan-Zverling M, Shen-Orr Z, Nave R, Herer P (2002) Seminal plasma androgen/oestrogen balance in infertile men. *International Journal of Andrology* 25: 345-351.

Makler A, Tatcher M, Vilensky A, Brandes JM (1980) Factors affecting sperm motility. III. Influence of visible light and other electromagnetic radiations on human sperm velocity and survival. *Fertility and Sterility* 33: 439-444.

Mashevich M, Folkman D, Kesar A, Barbul A, Korenstein R, Jerby E, Avivi L (2003) Exposure of human peripheral blood lymphocytes to electromagnetic fields associated with cellular phones leads to chromosomal instability. *Bioelectromagnetics* 24: 82-90.

Mery CM., Rubio V, Duarte-Rojo A, Suazo-Barahona J, Pelaez-Luna M, Milke P, Robles-Diaz G (2002) Android fat distribution as predictor of severity in acute pancreatitis. *Pancreatology* 2: 543-549.

Ng KK, Donat R, Chan L, Lalak A, Di Pierro I, Handelsman DJ (2004) Sperm output of older men. *Human Reproduction* 19: 1811-1815.

Nieschlag E, Behre HM (2000) Andrology: male reproductive health and dysfunction. Berlin, Heidelberg, New York, Barcelona, Hong Kong, London, Milan, Paris, Singapore, Tokyo: Springer-Verlag.

Nylund R, Leszczynski D (2004) Proteomics analysis of human endothelial cell line EA.hy926 after exposure to GSM 900 radiation. *Proteomics* 4: 1359-1365.

Olive A, Spira A, Multigner L (2001) Contribution of environmental factors to the risk of male infertility. *Human Reproduction* 16: 1768-1776.

Plant TM, Marshall GR (2001) The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. *Endocrine Reviews* 22: 764-786.

Raman JD, Schlegel PN (2002) Aromatase inhibitors for male infertility. *Journal of Urology* 167: 624-629.

Rami B, Schober E, Kirchengast S, Waldhor T, Sefranek R (2004) Prevalence of overweight and obesity in male adolescents in Austria between 1985 and 2000. A population based study. *Journal of Pediatric Endocrinology and Metabolism* 17: 67-72.

Roelandts R (2003) Cellular phones and the skin. *Dermatology* 207: 3-5.

Steinman N, Gamzu R, Yogev L, Botchan A, Schreiber L, Yavetz H (2001) Serum leptin concentrations are higher in azoospermic than in normozoospermic men. *Fertility and Sterility* 75: 821-822.

Stoy J, Hjollund NH, Mortensen JT, Burr H, Bonde JP (2004) Semen quality and sedentary work position. *International Journal of Andrology* 27: 5-11.

Sun JG, Jurisicova A, Casper RF (1997) Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. *Biology of Reproduction* 56: 602-607.

Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ Jr, Jegou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Muller J, Rajpert-De Meyts E, Scheike T, Sharpe R, Sumpter J, Skakkebaek NE (1996) Male reproductive health and environmental xenoestrogens. *Environmental Health Perspectives* 104 Suppl 4: 741-803.

Vermeulen A, Kaufman JM, Goemaere S, van Pottelberg I (2002) Estradiol in elderly men. *Aging Male* 5: 98-102.

Warren HG, Prevatt AA, Daly KA, Antonelli PJ (2003) Cellular telephone use and risk of intratemporal facial nerve tumor. *Laryngoscope* 113: 663-667.

Wass P, Walderström U, Rössner S, Hellberg D (1997) An android body fat distribution in female impairs the pregnancy rate of in-vitro fertilization-embryo transfer. *Human Reproduction* 12: 2057-2060.

Weisbrot D, Lin H, Ye L, Blank M, Goodman R (2003) Effects of mobile phone radiation on reproduction and development in Drosophila melanogaster. *Journal of Cellular Biochemistry* 89: 48-55.

World Health Organization (1999) WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge, UK: Cambridge University Press.

World Health Organisation (2000) WHO manual for the standardised investigation, diagnosis and management of the infertile male. Cambridge, UK: Cambridge University Press.