Evaluation of semen quality among men from the southeastern Poland in 2003–2013

Agnieszka Leszcz¹ (ABCDEF), Marzena Rzeszowska¹ (BE), Lechosław Putowski^{1,2} (E), Joanna Tkaczuk-Włach^{1,2} (DE), Grzegorz Polak^{1,3} (ADE)

- Ab ovo Sp. Z o.o., Family Health Center, Lublin, Poland
- ² Department of Gynecology and Gynecological Endocrinology, Medical University of Lublin, Poland ³ ¹⁵ Department of Gynecological Operational Gynecology, Medical University of Lublin,
- ³ 1st Department of Gynecological Oncology and Gynecology, Medical University of Lublin (SPSK No. 1), Poland

AUTHORS' CONTRIBUTION: (A) Study Design \cdot (B) Data Collection \cdot (C) Statistical Analysis \cdot (D) Data Interpretation \cdot (E) Manuscript Preparation \cdot (F) Literature Search \cdot (G) Funds Collection

SUMMARY

Introduction. The problem of infertility in Poland affects about 1.5 million pairs. It is estimated that the male factor is responsible for 40–60% of reproductive disorders. Over recent years, there has been a decline in sperm quality. This phenomenon may be the result of external factors, such as stress, poor testicular thermoregulation, exposure to heavy metals and xenoestrogens, alcohol consumption, smoking and drug use.

Aim. Analysis of sperm parameters of patients Ab ovo in the Family Health Center in Lublin in 2003–2013.

Material and methods. The study included 4,235 men diagnosed for infertility in the years 2003–2013. Semen parameters were evaluated according to the guidelines of the World Health Organization.

Results. The analysis showed a statistically significant decreasing trend for concentration and normal sperm morphology. The average concentration of sperm in the age group 18–29 years decreased from 42.31 million per ml in 2003 to 23.80 million per ml in 2013. In the age group 30–40 years, the average concentration decreased from 47.05 million per ml in 2003 to 24.17 million per ml in 2013. The average percentage of valid forms of sperm decreased annually by 2.2665% and 2.3505% for age groups 18–29 years and 30–40 years, respectively.

Conclusions. Our results are consistent with the generally observed global trend for a decline in sperm quality. **Key words:** infertility; semen; sperm concentration; sperm morphology

Address for correspondence: Grzegorz Polak Ab ovo Sp. z o.o., NZOZ Centrum Zdrowia Rodziny ul. Bociania 6, 20-542 Lublin Tel.: +48 081 527 71 36; e-mail: g.polak@ab-ovo.pl

Word count: 1565 Tables: 2 Figures: 2 References: 42

Received:29.09.2016Accepted:07.10.2016Published:12.12.2016

INTRODUCTION

Infertility is defined as the failure to achieve a pregnancy after a year of regular unprotected sexual intercourse. It is estimated that this problem affects approximately 15% of the population, i.e. about 1 million pairs in Poland [1]. The male factor is believed to be responsible for 40-60% of infertility cases [2]. At the end of the previous century, numerous reports on the global decline in semen quality appeared. It was demonstrated that the average concentration of sperm decreased from 113 to 66 million per ml in 1938-1990, which accounts for a 1% decline annually. At the same time, the average ejaculate volume decreased from 3.4 to 2.8 ml [3]. Also, comparative studies encompassing years 1934–1996, conducted by Swan et al. [4], revealed significant deterioration of semen quality. These results have been confirmed by studies conducted in other countries (Tab. 1).

External factors are believed to be the major cause of deteriorating fertility in men. Various forms of stress, including psychological stress, can affect male fertility. Both mild and severe stress decreases blood testosterone levels and impairs spermatogenesis [5]. Analyses of a relationship between the quality of life, measured with the Campbell questionnaire, and semen parameters have shown a significant positive correlation. Susceptibility to stress and deterioration of the quality of life associated with mood, which might lead to depression, can cause secondary infertility [6]. Also, occupational stress has been found to affect ejaculate volume and the number of progressively motile spermatozoa [7]. The phenomenon of infertility more frequently concerns men with improper testicular thermoregulation. Work conditions, sedentary lifestyle, using sauna and hot baths, and placing laptops near the scrotum lead to the overheating of the testicles, which is conductive to a decline in sperm concentration

w of the literature on the decline in sperm quality	Conclusions	 Average sperm concentration decreased by 2.1% annually; Sperm motility decreased by 0.6% annually; Number of morphologically normal spermatozoa decreased by 0.5% annually. 	 Median sperm concentration decreased from 98 million per ml in 1959 to 78 millic per ml in 1970; concentration decreased by 2.1% annually; Total number of motile spermatozoa in the ejaculate fell from 169 to 129 million. 	 Average sperm concentration was 51.07 million per ml in 1977 and fell to 39.32 millio per ml in 1993. 	 Sperm count decreased by 5.2 million annually; Motility decreased by 0.5% annually. 	 Average ejaculate volume declined from 3.7 to 3.3 million over 20 years; Average sperm concentration decreased from 110 million per ml in 1987 to 50 millio per ml in 2007. 	 Average semen volume declined from 3.5 ml in 1996 to 3.2 ml in 2007; Average sperm concentration decreased from 102.9 million per ml in 1996 to 96. million per ml in 2007; Average number of morphologically normal spermatozoa decreased by 26.1%. 	 Median sperm volume declined from 2.7 ml in 1980 to 2.5 ml in 2000; Median sperm concentration decreased from 80 to 77 million per ml ; Median sperm motility decreased by 5% from 1980 to 2000. 	 Median ejaculate volume declined from 3.4 ml (men born between 1979–81) to 3.3 ml (men born in 1987); Median sperm concentration was 67, 60 and 48 million per ml in men born in 1975 81, 1982–83 and 1987, respectively; Median percentage of morphologically normal spermatozoa decreased from 9.8 to 8. million per ml. 	 Sperm concentration for a 35-year-old man in 1989 was 73.6 million per ml ar decreased to 49.9 million per ml in 2005; concentration decreased by 1.9% annual! Average number of morphologically normal spermatozoa decreased by 21.7%. 	 Average semen volume declined from 3.4 ml in 2000–2002 to 3.3 ml in 2010–201. Average concentration declined from 67.1 to 26.7 million per ml ; Average number of morphologically normal spermatozoa decreased from 4.6% to 2.7⁶
	Country	France	Scotland	Greece	Israel	New Zealand	Tunisia	India	Finland	France	Brazil
	Time period	1973-1992	1984-1995	1977-1993	1990-2000	1987-2007	1996-2007	1981-1985 2000-2006	1998-2006	1989-2005	2000-2002 2010-2012
	Number of patients	1351	577	23850	2638	975	2940	3729	858	26609	2300
	Authors	Auger et al. [29]	Irvine et al. [31]	Adamopoulos et al. [32]	Almagor et al. [33]	Shine et al. [34]	Feki et al. [36]	Mukhopadhyay et al. [39]	Jørgensen et al. [35]	Rolland et al. [30]	Borges et al. [41]
Tab. 1. Revie	Year of publication	1995	1996	1996	2003	2008	2009	2010	2011	2013	2013

and motility [8–10]. The exposure of men to xenoestrogens, such as diethylstilbestrol (DES), industrial products and phenols (onylphenol, bisphenol A or octylphenol), pesticides and herbicides as well as phytoestrogens, disrupts the natural estrogen-androgen balance. Estrogen-like compounds have a negative influence on the development of male fetuses and spermatogenesis in adult men [11,12]. Pesticide exposure lowers the concentration, viability and count of normal spermatozoa [13]. Moreover, sperm concentration, motility and morphology can be also affected by smoking. Smokers present 23% lower sperm counts, 13% lower motility and a higher grade of sperm DNA damage compared with non-smokers [14,15]. Smoking is associated with a 48% increase in leukocyte concentrations in semen and 107% increase in ROS levels [16]. Moreover, alcohol consumption also contributes to disorders in male reproductive health. Alcohol lowers testosterone production, leading to a decline in libido and decrease in sperm count. Alcohol consumption greater than 15.4 g daily results in an increase in the number of abnormal spermatozoa [17,18]. It has also been proven that drug use (marijuana, opiates, cocaine or methamphetamine) can cause male infertility. Cannabinoids contained in marijuana have a negative impact on spermatogenesis and decrease testosterone concentration, thereby restricting sperm motility and negatively affecting the acrosome reaction [19]. Men using opiates present lower libido and symptoms of hypogonadism due to lower testosterone and luteinizing hormone concentrations in blood [20].

AIM

The aim of the study was to evaluate semen parameters in men diagnosed for infertility in the years 2003–2013.

MATERIAL AND METHODS

The analysis involved sperm specimens collected from patients examined in the Family Health Center in Lublin in the years 2003– 2013. The study is retrospective and concerns semen analysis results of 4,235 men diagnosed for infertility. The male population came from, lived and worked in both rural and urban areas of the southeastern Poland. The men were divided into two groups based on age: group M (young patients aged 18–29, n=1,638) and group S (older patients aged 30–40 years, n=2,597). Cancer patients and those with azoospermia were excluded from the study.

Ejaculate was collected to a sterile container by masturbation in a room next to the laboratory. Sexual abstinence lasted from 2 to 7 days. All semen parameters (volume, concentration, motility and morphology) were assessed in accordance with the guidelines of the World Health Organization (up to 2010, the methods from 1999 were used [21], whereas after 2010, the 2010 guidelines were followed [22]). The semen samples were put aside for liquefaction at 37°C. Ejaculate was examined within 30 minutes from liquefaction for no longer than 60 minutes. Sample volume was specified using sterile serological pipettes. Concentration and motility were determined at 37°C using a hemocytometer (Helber Counting Chamber Hawksley 0.02 mm, for sperm counting). Spermatozoa were characterized based on progressive motility "A," progressive motility "B," nonprogressive motility "C" and immotility "D"; motility was expressed in percentage. The samples were examined by three identically trained lab workers. An internal semen quality control was conducted in the laboratory.

The results were analyzed using Statistica 10 and Microsoft Office Excel. The significance of individual indices of the linear trend was tested with the Student's t-test. The significance level was p = 0.05.

RESULTS

Table 2 presents the results of the analysis for each of the tested parameters (Tab. 2). The average sperm concentration in the age group 18-29 years decreased from 42.31 million per ml in 2003 to 23.80 million per ml in 2013. In the age group 30-40 years, the average concentration decreased from 47.05 million per ml in 2003 to 24.17 million per ml in 2013. According to the linear trend diagram, a decreasing trend for sperm concentration in the tested period was observed in both age groups (Fig. 1 A, B) and was statistically significant (p < 0.05). Concentration decreased each year by 1.7073 million in group M, and by 2.1305 million in group S. The Student t-test, used to compare the concentration values in both groups in the consecutive years, showed that the mean values were statistically significantly higher in group S only in 2007, 2011 and 2012. Sperm motility (A+B%) increased annually in patients aged 18-29 by 0.4203%, and in men aged 30-40 by 0.4119%. The linear trend for

sperm motility was not statistically significant (p>0.05). The mean percentage of valid sperm forms decreased from 24.40% and 26.09% in 2003 to 3.68% and 3.93% in 2013 for group

M and S, respectively. The decreasing trend for sperm morphology was statistically significant in both groups (p<0.0001). Among patients aged 18-29, sperm morphology decreased an-

Tab.	ab. 2. Semen parameters (mean \pm SD) of men in the years 2003 to 2013									
Age group	Year	Number of	Concentration (million per ml)	Motility (A+B%)	Morphology (%)	Volume (ml)				
	patients		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD				
18-29 years	2003	156	42,31 ± 53,81	43,22 ± 21,01	24,40 ± 14,41	3,63 ± 1,92				
	2004	115	38,97 ± 37,39	37,67 ± 20,77	20,78 ± 12,35	3,93 ± 1,67				
	2005	138	30,80 ± 34,96	38,16 ± 17,60	18,47 ± 12,05	4,15 ± 1,82				
	2006	154	32,72 ± 56,23	40,38 ± 17,63	15,76 ±10,31	$4,03 \pm 1,75$				
	2007	98	$23,53 \pm 25,09$	38,22 ± 18,16	15,92 ± 12,66	4,15 ± 1,99				
	2008	153	26,80 ± 28,75	41,55 ± 17,06	9,03 ± 7,44	4,08 ± 1,65				
	2009	191	20,59 ±22,83	41,55 ± 17,89	6,42 ± 5,29	3,91 ± 1,92				
	2010	142	27,85 ± 31,01	$44,06 \pm 14,62$	4,77 ± 3,96	$4,08 \pm 1,89$				
	2011	159	23,49 ±26,73	42,26 ± 15,28	3,53 ± 3,25	4,11 ± 2,01				
	2012	178	23,81 ±19,74	41,93 ± 15,93	3,44 ± 3,67	3,85 ± 1,82				
	2013	154	$23,80 \pm 24,26$	44,46 ± 15,76	3,68 ± 2,89	4,30 ± 2,02				
30-40 years	2003	195	47,05 ± 54,67	45,65 ± 19,89	26,09 ± 12,89	3,89 ± 1,91				
	2004	264	47,61 ± 62,30	36,22 ± 19,18	$21,40 \pm 12,45$	3,70 ± 1,96				
	2005	234	32,27 ± 35,14	38,44 ± 18,63	19,32 ± 12,71	$3,84 \pm 1,69$				
	2006	193	$35,99 \pm 41,94$	38,82 ± 17,73	$16,19 \pm 10,44$	$3,82 \pm 1,74$				
	2007	247	$34,61 \pm 46,76$	39,98 ± 17,48	16,24 ± 11,71	$3,98 \pm 1,92$				
	2008	196	26,34 ± 32,14	42,52 ± 15,81	8,64 ± 6,45	3,80 ± 1,81				
	2009	310	$22,81 \pm 21,24$	43,93 ± 14,96	6,95 ± 5,80	4,17 ± 1,88				
	2010	283	$25,10 \pm 23,73$	$41,25 \pm 16,01$	$4,38 \pm 4,00$	3,85 ± 1,85				
	2011	195	$29,36 \pm 26,24$	$44,65 \pm 14,41$	$4,56 \pm 3,84$	$3,95 \pm 1,68$				
	2012	287	$28,20 \pm 24,98$	41,70 ± 15,19	$3,76 \pm 2,79$	$3,65 \pm 1,70$				
	2013	193	24,17 ± 21,17	44,84 ± 16,19	3,93 ± 2,93	3,94 ± 1,80				





18–29 years



nually by 2.2665%, whereas among patients aged 30-40, the average percentage of valid forms of spermatozoa decreased by 2.3505%. The Student t-test used to compare the percentage values between both groups in the consecutive years has shown that sperm morphology was statistically significantly higher in group S only in 2011 (p=0.0066). In the remaining years, the mean values did not differ significantly (with the significance level of 0.05) between the groups.

DISCUSSION

Recent years have shown an increased need for assisted reproductive technology in pairs wanting to have children. This is largely associated with semen quality decline. Sperm parameters, such as concentration, motility and morphology, are strictly connected with one another. Factors responsible for deterioration of one of them tend to affect the remaining two [23]. It is suggested that stress, exposure to harmful substances, estrogen-like compounds, stimulants, drugs, bacterial infections and sexually transmitted diseases can contribute to semen parameter deterioration [6,14,19,20,24]. The World Health Organization has changed referential values for individual sperm parameters over the years. In 1980, the range for normal concentration was 20-200 million per ml, and normal morphology - 80.5%. In 1992, the norms were changed: for concentration to ≥ 20 million per ml, and for morphology to $\geq 30\%$. According to the latest guidelines, sperm concentration should be \geq 15 million per ml, and morphology >4% [22,25]. The data presented above indicate that semen parameters do deteriorate over time thus supporting previous reports about the global decline in sperm quality [3,4]. However, Cocuzza M and Esteves SC [26] argue that there is no sufficient evidence to support the global decline in semen parameters. In some studies, it has been noted that sperm quality has neither decreased nor stayed the same, but even slightly increased in recent years [27,28]. However, the author's own studies demonstrate a statistically significant decreasing trend concerning sperm concentration. Similar results were obtained in France [29,30], Scotland [31], Greece [32], Israel [33], New Zealand [34], Finland [35] and Tunisia [36]. Nevertheless, other authors present different outcomes: an increased sperm concentration over time [37]. Our study, conducted from 2003–2013, showed a slight increase in sperm motility (progressive motility A and B). This trend occurred not to be statistically significant. Andolz et al. [38] also observed increased sperm motility. However, opposite findings were noted in certain countries [29,33,39], and other analyses present no tendencies concerning this parameter [36,40]. Our data also show a decrease in the number of valid forms of spermatozoa over time. The number of spermatozoa with normal morphology decreased by 2.2665% in men aged 18-29 and by 2.3505% in men aged 30-40 annually. Other authors have noted similar tendencies [29,36,38,41]. However, one study reports an increase in the number of spermatozoa with normal morphology [27]. It is suggested that sperm morphology could change over time due to different classification criteria and experience of lab workers evaluating samples [38].

CONCLUSIONS

Despite the fact that the existence of global semen quality deterioration is still a matter of debate [42], our results seem to be in line with this hypothesis and the generally observed trend concerning the decline in concentration and valid sperm forms. Further studies should focus on the cause of these trends in order to reverse them.

- Bakunowicz A, Brenk A, Olejek A. Analiza czynników społecznych, demograficznych i psychicznych u kobiet leczonych z powodu niepłodności. *Gin Pol Med Project* 2015;1(35):67-71.
- Aitken RJ. The human spermatozoon a cell in crisis? J Reprod Fertil 1999;115:1-7.
- Carlsen E, Giwercman A, Keiding N et al. Evidence for decreasing quality of semen during past 50 years. BMJ 1992;305:609-13.
- Swan S, Elkin E, Fenster L. The question of declining sperm density revisited: An analysis of 101 studies published 1934-1996. Environ Health Perspect 2000;108: 961-6
- Hall E, Burt VK. Male fertility: psychiatric considerations. Fertil Steril 2012;97(2):434-9.
- Depa-Martynow M, Walczyk-Matyja K, Szyfter J et al. Quality of life versus semen parameters. *Ginekol Pol* 2008;79:115-9.
- Jurewicz J, Hanke W, Sobala W et al. The effect of stress on the semen quality. *Medycyna Pracy* 2010;61(6):607-13.
- Figa-Talamanca I, Cini C, Varricchio GC et al. Effects of prolonged auto vehicle driving on male reproductive function: a study among taxi drivers. *Am J Ind Med* 1996; 30:750-8.
- Brown-Woodman PDC, Post EJ, Gass GC et al. The effect of a single sauna exposure on spermatozoa. Arch Androl 1984;12:9-15.
- Sheynkin Y, Jung M, Yoo P. Increase in scrotal temperature in laptop computer users. *Hum Reprod* 2005;2:452-7.

FERENCES

- 11. McLachlan JA. Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals. *Endocr Rev* 2001;22:319-41.
- 12. Mitchell JH, Cawood E, Kinniburgh D et al. Effect of a phytoestrogen food supplement on reproductive health in normal males. *Clin Sci* 2001;100:613-8.
- Hanke W, Jurewicz J. The risk of adverse reproductive and developmental disorders due to occupational pesticide exposure: an overview of current epidemiological evidence. Int J Occup Med Environ Health 2004;17(2):223-43.
- Taha EA, Ezz-Aldin AM, Sayed SK et al. Smoking influence on sperm vitality, DNA fragmentation, reactive oxygen species and zinc in oligoasthenoteratozoospermic men with varicocele. *Andrologia* 2013;19. doi: 10.1111/ and.12136
- Davar R, Sekhavat L, Naserzadeh N. Semen parameters of non-infertile smoker and non-smoker men. J Med Life 2012;5(4):465-8.
- Saleh RA, Agarwal A, Sharma RK et al. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. *Fertil Steril* 2002; 78(3):491-9.
- Emanuele MA, Emanuele N. Alcohol and the Male Reproductive System. Alcohol Res Health 2001;25(4):282-87.
- Joo KJ, Kwon YW, Myung SC et al. The Effects of Smoking and Alcohol Intake on Sperm Quality: Light and Transmission Electron Microscopy Findings. J International Medical Res 2012;40:2327-35.
- Rossato M, Ion Popa F, Ferigo M et al. Human sperm express cannabinoid receptor Cb1, the activation of which inhibits motility, acrosome reaction, and mitochondrial function. J Clin Endocrinol Metab 2005;90:984-91.
- Abs R, Verhelst J, Maeyaert J et al. Endocrine consequences of long-term intrathecal administration of opioids. J Clin Endocrinol Metab 2000;8: 2215-22.
- Laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 4th ed. WHO 1999.
- WHO laboratory manual for the examination and processing of human semen. 5th ed. WHO 2010.
- Bonde JP, Giwercman A, Ernst E. Identifying environmental risk to male reproductive function by occupational sperm studies: logistics and design options. Occup Environ Med 1996;53:511- 9.
- Forti G, Serio M. Male infertility: is its rising incidence due to better methods of detection or an increasing frequency? *Hum Reprod* 1993;8:1153-4.
- Esteves SC. Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. Int Braz J Urol 2014;40:443-53.
- Cocuzza M, Esteves SC. Shedding light on the controversy surrounding the temporal decline in human sperm counts: a systematic review. *Scientific World Journal 2014*; 2014:365691.
- 27. Berling S, Wölner-Hanssen P. No evidence of deteriorating semen quality among men in infertile relationships

during the last decade: a study of males from Southern Sweden. *Hum Reprod* 1997;12:1002-5.

- Fisch H, Goluboff ET, Olson JH et al. Semen analyses in 1,283 men from the United States over a 25-year period: no decline in quality. *Fertil Steril* 1996;65:1009-14.
- 29. Auger J, Kunstmann JM, Czyglik F et al. Decline in semen quality among fertile men in Paris during the past 20 years. N Engl J Med 1995;332(5):281-5.
- 30. Rolland M, Le Moal J, Wagner V et al. Decline in semen concentration and morphology in a sample of 26,609 men close to general population between 1989 and 2005 in France. *Hum Reprod* 2013;28(2):462-70.
- Irvine S, Cawood E, Richardson D et al. Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *BMJ* 1996;312(7029):467-71.
- Adamopoulos DA, Pappa A, Nicopoulou S et al. Seminal volume and total sperm number trends in men attending subfertility clinics in the greater Athens area during the period 1977-1993. *Hum Reprod* 1996; 11(9): 1936-41.
- Almagor M, Ivnitzki I, Yaffe H et al. Changes in semen quality in Jerusalem between 1990 and 2000: a crosssectional and longitudinal study. *Arch Androl* 2003; 49(2);139-44.
- Shine R, Peek J, Birdsal M. Declining sperm quality in New Zealand over 20 years. N Z Med J 2008;121(1287): 50-6.
- Jørgensen N, Vierula M, Jacobsen R et al. Recent adverse trends in semen quality and testis cancer incidence among Finnish men. Int J Androl 2011;34:37-48.
- Feki NC, Abid N, Rebai A et al. Semen Quality Decline Among Men in Infertile Relationships: Experience Over 12 Years in the South of Tunisia. J Androl 2009;30(5):541-7.
- Jørgensen N, Joensen UN, Jensen TK et al. Human semen quality in the new millennium: a prospective crosssectional population-based study of 4867 men. BMJ 2012;2:e000990.
- Andolz P, Bielsa MA, Vila J. Evolution of semen quality in North-eastern Spain: a study in 22 759 infertile men over a 36 year period. *Hum Reprod* 1999;3:731-5.
- Mukhopadhyay D, Varghese AC, Pal M et al. Semen quality and age-specific changes: a study between two decades on 3,729 male partners of couples with normal sperm count and attending an andrology laboratory for infertility-related problems in an Indian city. *Fertil Steril* 2010;93(7):2247-54.
- Fisch H. Declining worldwide sperm counts: disproving a myth. Urol Clin North Am 2008;2:137–146, vii.
- 41. Borges EJ, Setti AS, Vingris L et al. Decreasing sperm quality: findings from a 10 year gap longitudinal analysis of 2300 sperm samples from Brazil. *JBRA Assist Reprod* 2013;17(2):89-92.
- 42. Wilcox AJ. On sperm counts and data responsibility. *Epidemiol* 2011;22(5):615-6.