# Changes in the selected sperm parameters after 2010 in Lublin region

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## Abstract

Epidemiological data show that human reproductive disorders are a common problem worldwide because they concern one-sixth of all couples. Recently, a number of reports have been published concerning a considerable decrease observed in male and female fertility. Reports concerning changes in fertility in Poland are ambiguous. The aim of our study was to analyze the semen parameters and DNA fragmentation over the years 2010-2014 among patients treated at Ovum Infertility Treatment Center in Lublin, Poland. The years 2010-2013 showed a decrease in sperm density and total count of semen. Moreover, the research has shown that the number of semen of abnormal morphological structure increased. No changes in sperm motility were observed between 2010 and 2013. It has been shown that DNA fragmentation of sperm was increasing in the years 2011-2014, which was on the verge of statistical significance.

## Key words:

ifertility, semen, DNA fragmentation

Epidemiological data show that human reproductive disorders are a common problem worldwide because they concern one-sixth of all couples. Therefore, infertility has been considered by the World Health Organization as a social disease. The obtaining of pregnancy and the state of health of the baby depends on the quality of the genetic material of both the woman and man. Health behaviours and environmental factors directly affect the quality of sperm, as well as the human egg cell, and consequently on the reproductive capabilities, the course of pregnancy and the state of the newborn. Experiments conducted on animals indicate that hazardous environmental factors may contribute, among other things to the development of cancer in future generations, male infertility, and premature exhaustion of the ovarian reserve.

Recently, a number of reports have been published concerning a considerable decrease observed in male and female fertility [1]. Low sperm count in contemporary males, apart from deterioration of the quality of semen, result in the reduction of the chance to reproduce, constitutes a great problem from the aspect of public health, and may be among the causes of the negative birth rates in many European countries [2].

The causes for a decrease in male infertility have not been fully recognized. This is due to the fact that it is very difficult to select individual hazardous factors. The main causes for a decrease in male infertility which are taken into consideration are: exposure to plant protection products, heavy metals, increasingly more frequent occurrence of overweight and obesity among males, sedentary life style causing overheating of the testicles, tobacco smoking habit, alcohol consumption, and narcotics, as well as the effect of the surrounding sources of magnetic waves (TV, GSM, Wi-Fi, radio) [3-10]. It might be expected that these hazards also affect female reproductive cells; however, a direct assessment of the quality of the oocyte is difficult considering the invasive way in which it is obtained.

Reports concerning changes in fertility in Poland are ambiguous. The political transformation that occurred after Poland joined the EU has introduced changes in living conditions, work time and stress load. Furthermore, working conditions have changed, and new potential environmental threats have also come to life. One can thus deduce that it may have an influence on men's fertility as well.

The aim of our study was to analyze the semen parameters and DNA fragmentation over the years 2010-2014 among patients treated at Ovum Infertility Treatment Center in Lublin, Poland.

## Matherial and methods

The presented study was conducted in 2010-2014 in the Non-Public Health Care Unit "Ovum Reproduction and Andrology" in Lublin, Lublin, Poland, and covered 1636 men aged. The sperm was obtained by way of masturbation, and examined directly after liquidation according to the 2010 criteria of the World Health Organization [11]. Prior to the examination, the men maintained a 4-day abstinence from sex and alcohol. In order to determine the percentage of fragmented DNA in 500 sperm samples, the sperm chromatin dispersion test (SCD) was used, according to the instructions provided by the producers (DynHalosperm, ®Kit Halotech DNA SL, Madrid, Spain [12]). Sperm cells suspended in agarose gel were treated with an acidic solution and then lysing solution. It was noticed that sperm cells with fragmented DNA had very little or no "halo" of the decompressed DNA, whereas no DNA fragmentation of sperm had long loops forming a rich nucleic acid "halo". There were 300 sperm cells counted from each sample. As a result, the sperm DNA fragmentation index (DFI) was obtained – the percent of cells that have detectable sperm fragmentation. The studies were approved by the Ethics Committee.

The results of the study obtained were subjected to statistical analysis. Differences between groups were investigated using Mann-Whitney U test, Kruskal-Wallis test, Spearman test , and the relationships between variables were tested by means of r-Pearson correlation. The p level $p<0.05$ was considered statistically significant. The database and statistical analysis were performed using the software Statistica 9.1 (StatSoft, Poland).

## Results

The results of the research show that the mean density of sperm decreased between 2010 and 2013. The differences between 2010 and 2011 as well as changes between 2011 and 2012 were statistically significant (p=0.00). The differences between 2012 and 2013 were not statistically significant (p=0.66) (Fig. 1).

The mean total number of semen in sperm in the aforementioned years was decreasing, whereas in the last year of research – it increased. Between years 2010 and 2011 there were no statistically significant differences (p=0.29), whereas between 2011 and 2012 differences were statistically significant (p=0.00). No statistically significant changes have been shown in the mean density of sperm between 2012 and 2013 (p=0.60) (Fig. 2).

The statistical analysis has shown that differences in the mean count of semen in forward progression in the years 2010-2013 were not statistically significant (p=1.00). However, they kept reducing over the examined years (Fig. 3).

It has been shown that the number of abnormal semen in sperm was increasing in the mentioned years. The statistical analysis has shown that differences in the mean number of abnormal semen between 2010 and 2011, as well as in the years 2011-2012, were statistically significant (p=0.00). The analysis has also demonstrated that differences in the mean number of abnormal semen between 2012 and 2013 were not statistically significant (p=1.00) (Fig. 4).

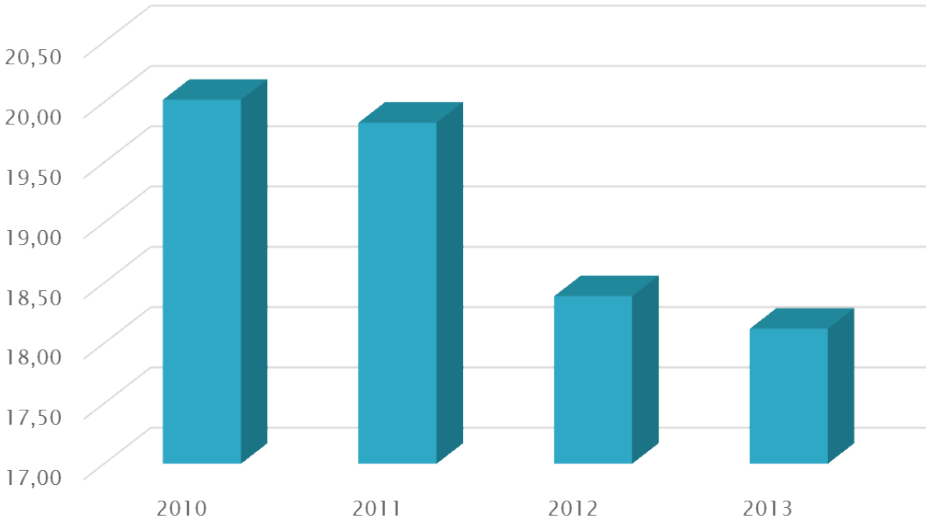


**Fig. 1.**
The mean density of sperm examined in the years 2010-2013

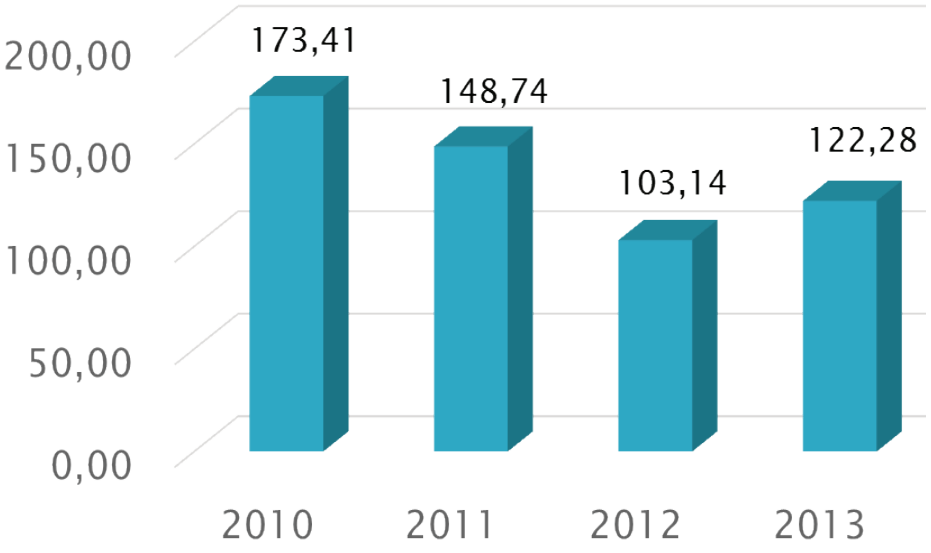


**Fig. 2.**
Changes in the mean density of sperm in the years 2010-2013


**Fig. 3.**
The mean values of forward progression in the examined sperm in the years 2010-2013

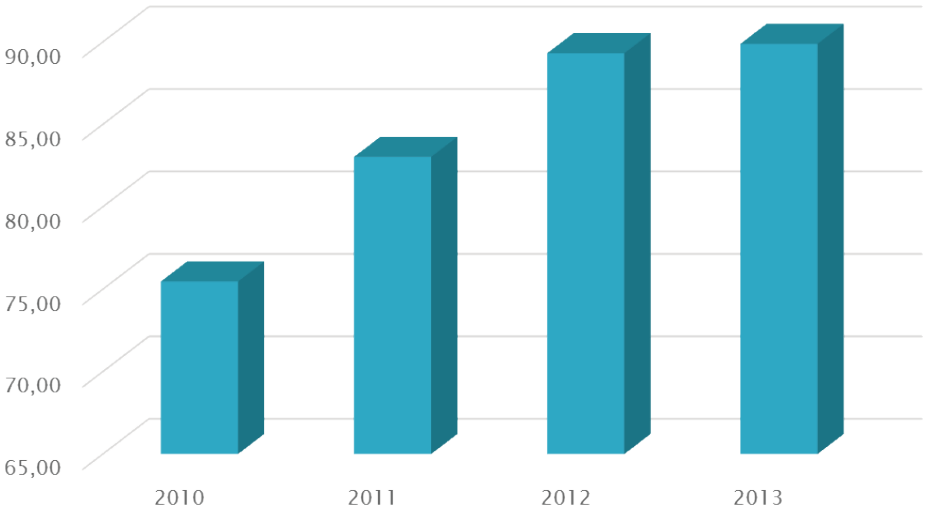


**Fig. 4.**
Changes in the count of semen of abnormal morphological structure between 2010 and 2013.

In 2011, mean DFI was 26.18, in 2012 – 29.51, in 2013 – 29.93 and in 2014 – 31.25. The conducted r-Pearson's correlation has shown that mean DFI was increasing over the years, but correlation was on the verge of statistical significance, whereas Spearman's rank order analysis did not yield a statistically significant result (Fig. 5).

## Discussion

The decrease of sperm parameters, which we have observed in our research, is a reflection of changes introduced by WHO, lowering the standards of semen evaluation over the years, which is presented in table no. 1.

Carlsen et al. [14] reviewed 61 heterogeneous observational studies on semen quality, which indicated that mean sperm density decreased significantly between 1940 and 1990. The study inspired many investigators to perform studies on semen quality from the general population. However, there was a controversy about the trend on semen quality. Swan et al. [15] carried out an updated and expanded meta-analysis and confirmed a trend towards lower sperm counts in Europe as well as inthe United States. In many other countries, many investigators also performed retrospective studies and reported that semen quality had been declining in the past several decades [16-18]. While several others reported that there were no significant changes in human semen quality [19]. Research conducted in China shows

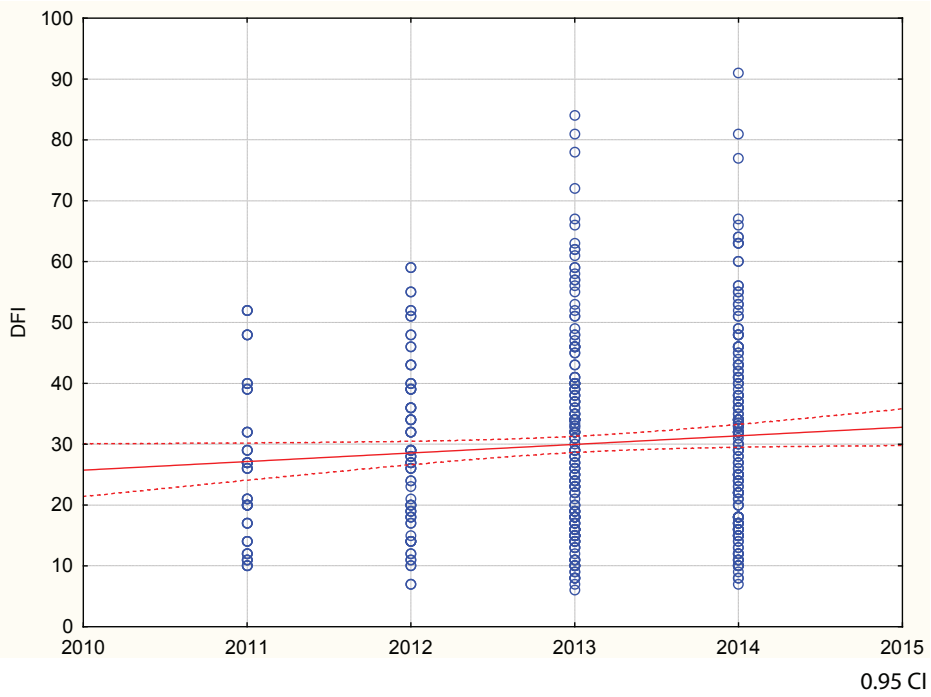


**Fig. 5.**
Changes mean DFI from 2011 until 2014

**Table 1.**
Changes in semen evaluation standards recommended by WHO over the years

| Semen parameters | WHO 1980 | WHO 1987 | WHO 1992 | WHO 1999 | WHO 2010 |
|---|---|---|---|---|---|
| **Volume ( ml )** | BD | ≥2 | ≥2 | ≥2 | **1.5** |
| **Semen concentration ($10^6$/ml)** | 20-200 | ≥20 | ≥20 | ≥20 | **≥15** |
| **Total count of semen ($10^6$/ejaculate)** | BD | ≥40 | ≥40 | ≥40 | **≥39** |
| **Forward progression (%)** | ≥2 | ≥25 | ≥25 | ≥25 | **≥38** |
| **Motile semen (%)** | BD | ≥50 | ≥75 | ≥75 | **≥58** |
| **Normal morphology (%)** | 80.5 | ≥50 | ≥30 | ≥14 | **≥4** |

that, the trends in semen quality are also being debated. Liang et al. [20] analysed semen parameters of 5834 Chinese fertile men from 14 provinces in 1980-2005, which indicated that semen concentration declined in the past 25 years, but there was no significant decline after 1996. Li et al. [21] found that sperm concentration and sperm viability rate of the college students in Chengdu area had a tendency to decrease. In contrast, Zhu et al. [22] reviewed 36 papers and analysed the semen parameters from 2318 healthy Chinese males and showed that there was no significant decline in sperm density and semen volume during 13 years (1985–1997).

Polish retrospective studies conducted by Olejek et al. [23] covered 2 116 men who had been not previously treated and who reported to the Infertility Treatment Outpatient Clinic in years 1982-1997. Authors analyzed the results of semen for resident patients. They showed no relationship between the place of residence of the patients and sperm parameters. They also found no reduction in the quality of sperm in the studied years.

As we can see, differences in the results of various researchers differ from one another, which may have been caused by diverse research models. Some of the authors qualified men with attested fertility, others – men treated due to infertility. Tendencies for the occurrences of changes may depend on the place of examination and the occurrence of factors affecting semen parameters in the given place. The years taken into consideration are also different.

One may suspect then that research carried out in various places of the world will differ. However, the general trend towards deterioration of sperm quality is disturbing. That ought to induce academic groups to search for factors responsible for this and to monitor the trends toward changes in sperm parameters in the future.

Our findings are cause for concern and suggests the existence of harmful causative factor in our region, the finding will require further research.

## Conclusions

1. The years 2010-2013 showed a decrease in sperm density and total count of semen. Moreover, the research has shown that the number of semen of abnormal morphological structure increased.
2. No changes in sperm motility were observed between 2010 and 2013.
3. It has been shown that DNA fragmentation of sperm was increasing in the years 2011--2014, which was on the verge of statistical significance.

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