Neuroendocrinology Letters Volume 35 No. 1 2014
ISSN: 0172-780X; ISSN-L: 0172-780X; online ISSN: 2354-4716

Levels of FSH, LH and testosterone, and sperm DNA fragmentation

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Submitted: 2013-11-06 Accepted: 2013-12-05 Published online: 2014-02-27

Key words: **DNA fragmentation; FSH; LH; testosterone; semen; infertility**

Neuroendocrinol Lett 2014; **35**(1):73–79 **PMID:** 24625916 NEL350114A10

Abstract

OBJECTIVE: Having an offspring is the most important human biological goal, which is necessary for survival of the human species. Lack of offspring is a phenomenon concerning approximately 15% of married couples in Poland. In a half of the cases, a causative agent is the male factor infertility problem. There is evidence that certain male fertility problems are related with disorders of the process of spermatogenesis. The course of normal spermatogenesis depends on proper pituitary secretion of folliculostimulin (FSH), luteinizing hormone (LH), as well as testicular secretion of testosterone. It is considered that in approximately 20% of patients with idiopathic infertility an elevated level of sperm DNA fragmentation may be the cause of failure in reproduction. The objective of the present study was determination of the relationship between FSH, LH and testosterone levels, and the occurrence of sperm DNA fragmentation.

METHODS: The present study was conducted in the year 2012 in the Non-Public Health Care Unit 'Ovum Reproduction and Andrology' in Lublin, and covered 186 men treated for infertility. For inclusion into the study group we qualified males aged 25–35, who have been treated for infertility for more than 1 year, with no pathological features observed in the female partner. The structure of sperm chromatin was evaluated using the technique of flow cytometry – Sperm Chromatin Structure Assay (SCSA). The result of the examination was a sperm DNA Fragmentation Index (DFI), i.e., the percentage of sperm with DNA lesions (DNA fragmentation). A morning blood sample (5 mL volume) was obtained and sent to an authorized laboratory to assess serum levels of testosterone, LH and FSH.

REULTS: An intensified sperm DNA fragmentation co-occurred with both extremely low and extremely high levels of FSH and LH. Sperm DNA fragmentation was negatively correlated with testosterone level.

ORIGINAL ARTICLE

INTRODUCTION

Having an offspring is the most important human biological goal, which is necessary for survival of the human species. Lack of offspring is a phenomenon concerning approximately 15% of married couples in Poland. In a half of the cases, a causative agent is the male factor infertility problem. There is evidence that certain male fertility problems are related with disorders of the process of spermatogenesis. The course of normal spermatogenesis depends on proper pituitary secretion of folliculostimulin (follicle-stimulating hormone – FSH), luteinizing hormone (LH), as well as testicular secretion of testosterone. Hormonal testicular function is controlled by pituitary gonadotropins: LH, via Leydig cells, stimulates the production of sex steroids, while FSH, together with testosterone, act on seminiferous tubules via Sertoli cells in order to support and maintain spermatogenesis. Pituitary secretion of these two gonadotropins is controlled by decapeptide GnRH (Gonadotropin Releasing Hormone). This peptide is synthesized in hypothalamic neurons and released in a pulse pattern (every 15–30 minutes). Subsequently, it is secreted into the hypophyseal pituitary portal system and bound to its membrane receptors on gonadotropic cells of the anterior lobe of the pituitary gland. Secretion of the GnRH is modulated by sex hormones. FSH plays a major role in the hormonal control of spermatogenesis; however, secretion of androgens independent on FSH is also assumed. FSH stimulates cell division and differentiation, inhibits apoptosis during spermatogenesis, and stimulates meiosis, while testosterone controls the course of meiosis, the transformation of spermatids, especially their elongation during maturation phase, and adhesion of spermatids to Sertoli cells. Several types of Sertoli cells uncorrelated with individual stages of the cycle are distinguished in humans, which is probably associated with the patch-like distribution of the stages in the epithelium. In the vicinity of Sertoli cells surface, in the region of the zonula occludens, the presence of pinocytotic vesicles was confirmed, the number of which increases in conditions of stimulation by LH-like gonadotropin, called human chorionic gonadotropin. Hormones control spermatogenesis indirectly by maintaining the activity of somatic cells, especially Sertoli cells. Directly, spermatogenesis is controlled by means of function of the local regulatory system. Gametogenic cells lack FSH and androgen receptors. Sertoli cells, as the only receptor cells for these hormones in the epithelium, form the mechanism of transmission of signals required at individual stages of spermatogenesis. This mechanism functions on the principle of cross-talk cellular interaction. This is a complex mechanism, in which intercellular junctions participate. It has been found that the process of spermatogenesis can neither be hormonally accelerated nor slowed down, but only inhibited (Hampl *et al.* 2013; Fietz *et al.* 2013; Latronico *et al.* 2013; Rey *et al.* 2013). By the action of hormones it is possible to increase the number of gametogenic cells, which will manage to complete the process of spermatogenesis, due to, among others, reduction in number of cells undergoing apoptosis. FSH and LH deficiency occurs in males with hypogonadotropic hypogonadism, as well as in those who receive treatment with antidepressants, whereas the level of these hormones is elevated in the case of primary (including genetic causes) or secondary testicular failure.

It is considered that in approximately 20% of patients with idiopathic infertility, an elevated level of sperm DNA fragmentation may be the cause of failure in reproduction (Aktan *et al.* 2013; Bungum *et al.* 2011). Intensified sperm DNA fragmentation has also been associated with decreased oocyte fecundation, embryo quality and pregnancy rate (Aktan *et al.* 2013; Bungum *et al.* 2011). Intriguingly, an intracytoplasmatic sperm injection (ICSI) study of mouse oocytes, using spermatozoa with a high percentage of DNA Fragmentation Index (DFI), revealed that a relevant proportion of adult offspring produced by this procedure showed a significant increase in the incidence of abnormal behavior, aberrant growth, congenital malformations, tumor formation and premature aging (Fernández-González *et al.* 2008). DFI is also an informative biomarker in studies of reproductive toxicology, as exposure of testis to reproductive toxicants, such as thermal stress, ionizing radiation, chemotherapy, pesticides, air pollution and smoking, has resulted in a higher incidence of sperm DNA damage (Perrault *et al.* 2003; Evenson *et al.* 2005; García-Contreras *et al.* 2011). The quality of genetic material carried in male sperm becomes a prognostic factor in the area of both the effectiveness of treatment of an infertile couple and bearing a healthy child, as well as the risk of contracting cancer in the future generations (Erenpreiss *et al.* 2006).

The present study was aimed to investigate a relationship between levels of FSH, LH and testosterone (TTE), and the occurrence of sperm DNA fragmentation.

MATERIAL AND METHODS

The present study was conducted in the year 2012 in the Non-Public Health Care Unit 'Ovum Reproduction and Andrology' in Lublin.

The studied group covered 186 men aged 25–35, treated for infertility for more than 1 year, with no pathological features observed in the female partner. Exclusion criteria included: offspring in another relationship, symptoms of systemic diseases, clinically diagnosed features of inflammatory state of a reproductive organ, smoking, body weight disorders (BMI below 17 or over 30 kg/m^2), pre- and post-natal developmental defects within male reproductive organs, presence of clinically examinable varices of the spermatic cord, taking certain drugs (which may affect the quality and density of the semen) during the period up to three months prior to the study.

The sperm was obtained by the way of masturbation, and examined directly after liquidation, according to the WHO standards. The structure of sperm chromatin was evaluated using the technique of flow cytometry – Sperm Chromatin Structure Assay (SCSA). The result of the examination was the sperm DFI, i.e., the percentage of sperm with DNA lesions (DNA fragmentation). The semen samples with DFI value of less than or equal to 15% represent low levels, between 15% and 30% represent moderate levels, and more than or equal to 30% represent high levels of DNA fragmentation (Chonan *et al.* 2006).

A morning blood sample (5 mL volume) was obtained and sent to an authorized laboratory to assess serum levels of testosterone (nmol/l), luteinizing hormone (mIu/mL) and follicle-stimulating hormone (mIU/mL). The male normal level of testosterone ranges from 9.00 to 34.72 nmol/l, luteinizing hormone – from 0.8 to 9.9 mIU/mL and follicle-stimulating hormone – from 0.8 to 7.6 mIU/mL.

The studies were approved by the ethics committee of the Medical University of Lublin. All patients gave their informed consent to participate in the study.

The data obtained in the study were statistically analyzed using STATISTICA software. Characteristics of DFI, FSH, LH and TTE in the examined group were assessed using descriptive statistics, including: minimum and maximum, quartiles, mean and standard deviation. Kruskal-Wallis H and Mann-Whitney U tests were used to compare DFI by normal and beyond normal levels of FSH, LH and TTE. The point relationship between DFI and FSH, LH, TTE levels was investigated using multiple regression analysis with non-linear predictor variables transformed into linear ones. Regressions of DFI to FSH and LH levels were approximated by quadratic functions, while regression of DFI to TTE level was approximated by hyperbolic function. The *p*-value below or equal to 0.05 was considered significant.

RESULTS

FSH, LH and TTE levels in blood plasma of the examined men with infertility were presented in Table 1. The level of FSH in the examined patients ranged from 0.67 to 16.78 and the mean reached 6.43±3.74 mIU/mL. Normal FSH level was found in 2/3 of the patients, while about 1/3 of them had above normal FSH level and 1% – below normal. LH level in blood plasma of the patients ranged from 0.77 to 17.07 and the mean was 6.45±4.05 mIU/mL. The lowest testosterone value was 5 nmol/L and the highest 31 nmol/L, while the mean reached 14.5±7.32 nmol/L. About 2/3 of the examined men with infertility had normal LH and testosterone levels, while 21% of them had above normal LH levels, and 23% – below normal TTE levels. Additionally, a positive correlation between LH and FSH levels in the blood plasma of the examined

Table 1. FSH, LH and TTE levels in the infertile men.

Parameter		FSH (mlu/mL)	LH (mlu/mL)	TTE (nmol/L)
Minimum		0.67	0.77	5.00
Lower quartile		3.65	3.19	9.00
Median		6.13	5.95	12.50
Upper quartile		7.89	8.00	18.00
Maximum		16.78	17.07	31.00
Mean		6.43	6.45	14.51
Standard deviation		3.74	4.05	7.32
Below normal	n (%)	2 (1.08)	4 (2.15)	43 (23.12)
Normal	n (%)	123 (66.13)	143 (76.88)	143 (76.88)
Above normal	n (%)	61 (32.80)	39 (20.97)	0 (0.00)

DFI – DNA Fragmentation Index; FSH – follicle-stimulating hormone; LH – luteinizing hormone; TTE – total testosterone

Tab. 2. DFI (%) total and by FSH, LH and TTE norm in the infertile men.

DFI (%)		Total	FSH			LH			TTE	
			below	normal	above	below	normal	above	below	normal
Minimum		7	27	7	7	24	7	7	7	7
Lower quartile		10	27	10	13	25	10	21	26	10
Median		16	28	14	25	28	14	27	27	13
Upper quartile		26	29	22	29	29.5	21	29	30	20
Maximum		32	29	32	32	30	32	32	32	32
Mean		17.89	28.00	16.04	21.28	27.50	15.73	24.79	26.30	15.36
Standard deviation		8.11	1.41	7.30	8.51	2.65	7.32	6.59	5.73	6.94
Low	n (%)	90 (48.39)	0 (0.00)	71 (57.72)	9 (31.15)	0 (0.00)	85 (59.44)	5 (12.82)	2 (6.98)	87 (60.84)
Moderate	n (%)	76 (40.86)	2 (100.00)	43 (34.96)	31 (50.82)	3 (75.00)	48 (33.57)	25 (64.10)	28 (65.12)	48 (33.57)
High	n (%)	20 (10.75)	0 (0.00)	9 (7.32)	11 (18.03)	1 (25.00)	16 (6.99)	9 (23.08)	12 (27.91)	8 (5.59)

DFI – DNA Fragmentation Index; FSH – follicle-stimulating hormone; LH – luteinizing hormone; TTE – total testosterone

men with infertility was observed (r=0.260; $p<0.001$). However, their testosterone level did not negatively correlate with FSH (r=–0.135; $p=0.066$) and LH (r=–0.100; $p=0.185$) in a significant way (Table 1).

DFI in the total group of patients ranged from 7 to 32% and the mean reached 17.89±8.11% (Table 2). In 90 of the examined infertile men (48.39%) DFI was lower than or equal to 15%, which represents low levels of DNA fragmentation. In 76 (40.86%) of those men DFI was greater than 15% but lower than 30%, which represents moderate levels of DNA fragmentation, while in 20 of them (10.75%) DFI was higher than or equal to 30%, that represent high levels of DNA fragmentation.

Considering DFI by FSH, LH and TTE levels in the interfile men (Table 2 and Figure 1 a, b, c), it was shown that DFI was significantly higher in men with the studied hormones concentrations beyond normal range: FSH (H=17.543; $p<0.001$), LH (H=39.656; $p<0.001$) and TTE (U=7.307; $p<0.001$), in comparison with the men with normal levels of these hormones. The average DFI value in the examined men with normal FSH level reached 14% and was significantly lower than in men with FSH concentration both below and above the normal range (the average DFI – 28% and 25% respectively). The same relation was observed between DFI and LH level: the average DFI in the examined men with normal LH level reached 14% and was significantly lower than for men with LH level both below the normal range (the average DFI 28%) and above the normal range (27%). Similarly, the average DFI in the men with normal TTE level reached 13% and was significantly lower than in men with LH level below normal range (the average DFI 27%).

Most of the examined men with normal FSH level (57.72%) and – similarly – with normal LH level (59.44%) had low levels of DNA fragmentation, while most of the men with above normal FSH level (50.82%) and – similarly – with above normal LH level (64.10%) had moderate ones. Most of examined men with normal TTE level (60.84%) had low levels of DNA fragmentation, while most men with below normal FSH level (65.12%) had moderate ones.

Tab. 3. Results of regression DFI to FSH, LH and TTE in the infertile men.

Variable	Estimate	Standard error	t	*p*-value
Slope	29.536	2.046	14.433	<0.001
FSH	–2.723	0.431	–6.313	<0.001
FSH^2	0.183	0.028	6.564	<0.001
LH	–2.382	0.419	–5.681	<0.001
LH^2	0.166	0.027	6.231	<0.001
1/TTE	16.853	8.215	2.052	0.042

DFI – DNA Fragmentation Index; FSH – follicle-stimulating hormone; LH – luteinizing hormone; TTE – total testosterone

Scatter diagrams were used to investigate the character of relationships between DFI and levels of the three examined hormones in the studied group (Figure 1 d, e, f). The U-shaped graphs approximated relationships between DFI and FSH (d), as well as between DFI and LH (e). The decreasing upward curved graph shows relationship between DFI and TTE (f). The results of regression of DFI to FSH, LH and TTE in the studied group are presented in Table 3. The DFI values kept decreasing as FSH level was increasing up to the upper limit of normal FSH range, that was a critical point, and then DFI value started to increase. Similarly, the DFI value was decreasing as LH level was going up to about 7.5 mIU/mL, that was a critical point, and then the DFI value started to increase. The DFI value increased when FSH or LH levels reached abnormally low (below normal range) or abnormally high values (above normal level). However, the DFI value decreased with an increase of TTE in almost the whole range of its values. This decrease was rapid until the lower limit of normal TTE level, and much slower for greater TTE values. The estimated regression of DFI to FSH, LH and TTE levels in the blood plasma of the infertile men was well fitted to the empirical data (adjusted $R^2=0.525$; F=41.897; $p<0.001$), so explained 52.5% of DFI variability.

DISCUSSION

The results of many studies suggest that problems in male fertility are frequently associated with abnormalities in spermatogenesis, which may be caused by hormonal disorders, as well as oxidative stress. The results of the present study indicated an increase in the percentage of sperm with fragmented DNA in the case of decreased FSH or LH levels, as well as in the case of elevated levels of these hormones. Increased levels of FSH and LH accompany testicular failure, which may explain an intensification of DNA fragmentation in this case. Decreased FSH and LH levels are observed in the case of hypogonadotropic hypogonadism, and may also be the consequence of stress exerting an unfavourable effect on pulsatile secretion of GnRH. Several studies dealing with the effect of FSH supplementation on DNA fragmentation indirectly confirm the results of the present study because, according to the researchers, treatment with FSH reduces the percentage of chromatin damage. This may result from the fact that FSH inhibits sperm apoptosis.

Ruvolo *et al.* observed decreased DFI value after treatment with 150 IU FSH given three times a week for at least 3 months. After recombinant human FSH therapy, they did not find any differences in terms of sperm count, motility and morphology. The average DFI value was significantly reduced (21.15 vs. 15.2, $p<0.05$), but they found a significant reduction in the number of patients with high basal DFI values (>15%), while no significant variation occurred in patients

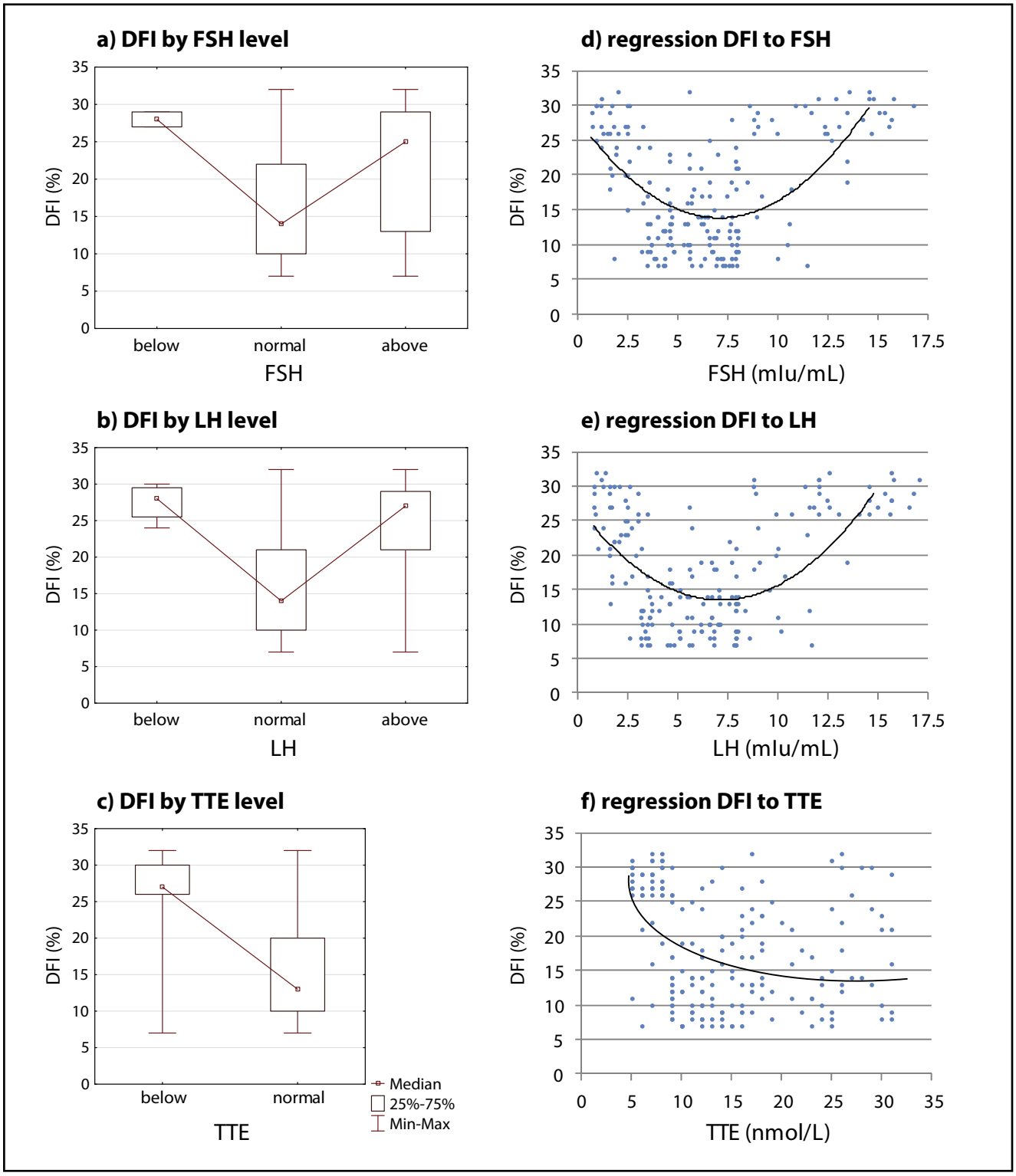

Fig. 1. Relationships between DFI and FSH, LH, TTE in the infertile men. DFI – DNA Fragmentation Index; FSH – follicle-stimulating hormone; LH – luteinizing hormone; TTE – total testosterone.

with DFI values ≤15% (Ruvolo *et al.* 2013). The results obtained in the present study are consistent with the results obtained by Palomba, who observed the effect of highly purified FSH (hpFSH) administration on standard semen parameters, sperm oxidative stress, and sperm chromatin structure and DNA fragmentation. In the prospective baseline-controlled study covering 36 males with idiopathic subfertility, a significant improvement in standard seminal parameters was observed after FSH therapy. A significant reduction in DNA fragmentation was also proven. Statistically significant correlations between variation in reactive

oxygen species levels and both seminal parameters and DNA fragmentation variations were detected (Palomba *et al.* 2011). Similar results were obtained by Abbasihormozi *et al.* in experimental study on rats. The researchers confirmed the present observations of an increase in DNA fragmentation in conditions of FSH deficiency (Abbasihormozi *et al.* 2013). Similar findings were also obtained by Colacurci et al., who compared the effects of 90-day FSH therapy and 90-day supplementation with vitamin preparations on DFI in men with idiopathic oligoasthenoteratozoospermia (iOAT). The DFI value was similar in the 2 groups at the time of the enrollment but reduced significantly after rFSH therapy, whereas no significant variation occurred in the control group. In the subgroup of patients with high basal DFI values (>15%), rFSH treatment significantly decreased DFI, whereas no significant variation occurred after 90 days of vitamin supplements administration. They concluded that rFSH administration improves sperm DNA integrity in iOAT men with increased DFI values (Colacurci *et al.* 2012).

The present study showed a relationship between a quality of spermatogenesis and sperm DNA fragmentation. The results of our study are consistent with those obtained by Smit et al., who observed that in all studied patient categories, except for those including men with hypogonadotrophic hypogonadism, sperm antibodies, or normospermia, DFI was significantly higher compared with proven fertile controls. After classification of the quality of spermatogenesis based on mean testicular volume (<10 ml vs. >15 ml), as well as on levels of follicle stimulating hormone (FSH >10 U/L vs. <5 U/L) and inhibin-B (<100 nmol/L vs. >150 nmol/L), the DFI value was showed to be significantly higher in patients with poor spermatogenesis (35.9%) than in patients with normal spermatogenesis (25.9%). In a multiple regression analysis, teratozoospermia index, sperm vitality, and FSH level were significant determinants of the DFI value (Smit *et al.* 2010).

Appasamy *et al.* investigated the relationship between male reproductive hormones and sperm DNA damage and markers of oxidative stress in men undergoing infertility evaluation for male factor and non-male factor infertility. Semen samples were analyzed for DFI, while serum samples were tested for FSH, inhibin B, anti-Müllerian hormone (AMH) and testosterone levels, and for total antioxidant capacity (TAC). There was no significant relationship between hormone concentrations, sperm DNA damage and total antioxidant capacity, suggesting other mechanisms for sperm dysfunction, which is not consistent with our results (Appasamy *et al.* 2007).

In studies performed by Richthoff *et al.* DFI was negatively correlated with levels of free testosterone, which is consistent with our results (Richthoff *et al.* 2002).

Manish *et al.* investigated status of maturation and function of Sertoli cells in 37 patients with Sertoli cell only syndrome (SCOS). They found low level of inhibin B and high level of FSH in 29 cases (78.37%). Among 37 cases of SCOS, there were 6 cases of chromosomal abnormality (4 cases of Klinefelter syndrome and 2 cases of mosaic Klinefelter syndrome) and 4 cases of Yq microdeletions (2 cases of AZFbc and 1 each of AZFa and AZFc). These studies attract attention to the genetic background of gonadal failure in men, which may be related with sperm chromatin abnormalities (Manish *et al.* 2012).

In studies performed by Dobrzynska *et al.* a correlation between low sperm concentration in ejaculate and enhanced level of DNA damage was observed. The highest DNA damage was noted in samples with low sperm concentration. In gametes from this group, the lowest percent of DNA in comet head, the highest mean tail length, and the highest tail moment were observed (Dobrzynska *et al.* 2010), which may indirectly explain the results of the present study. A study conducted by Norambuena *et al.* also suggests a relationship between DNA damage and low sperm concentration, which may be related to the level of FSH, LH and testosterone (Norambuena *et al.* 2012).

In studies performed by Wasilewska-Dziubińska et al., the relationship between male reproductive hormones, spermatogenesis and neurological disorders was proven (Wasilewska-Dziubińska *et al.* 2011).

The results obtained by various researchers do not unequivocally explain the relationship between the levels of FSH, LH and testosterone on sperm chromatin damage, which may suggest that the scope of problems concerning the causes of sperm DNA fragmentation will require further studies.

CONCLUSIONS

1. An intensification of sperm DNA fragmentation accompanied both abnormally low and abnormally high levels of FSH and LH.
2. Sperm DNA fragmentation kept increasing together with a decrease in the level of testosterone.

ACKNOWLEDGEMENTS

This study was financially supported by the Institute of Rural Health, Lublin, Poland.

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