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HIGH LEVELS OF DNA FRAGMENTATION OBSERVED AND IN NORMOSPERMIC MEN DUE TO EXPOSURE TO PESTICIDES

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ABSTRACT
Recent studies suggest that sperm DNA integrity may be altered by environmental exposure to some toxic chemicals (Aitken RJ, De Luiis GN.,2010). DNA fragmentation may be an excellent marker for exposure to potential reproductive toxicants and a diagnostic tool for male infertility (Ozmen B, et al., 2007). The aim of this study was to investigate the association between sperm DNA fragmentation in the population that lives in an environment of air pollution and exposure to pesticides. **Patients and selection.** Total 164 samples of semen from fertile men with respect to the parameters of semen and DNA fragmentation. This is a prospective study observation spent on Andrology Laboratory Polyclinic,, Biolab-Zafi,, in Klina in the Republic of Kosovo from April 2015 to Februare 2016. A portion of each semen sample was used to analyze the following parameters according to the World Health Organization (WHO) guidelines (2010). Halosperm is the SCD test based on the ability of intact DNA with deproteinized nuclei to create loops around nuclear matrix. Deproteinized nuclei create the halos of dispersed DNA that correspond to relaxed DNA loops attached to the residual nuclear structure (core) (Fernández et al., 2005). **Results.** The group of respondents farmers (exposed to pesticides) that have been tested in the period spring - summer, have a higher percentage index fragmented sperm (DFI = 36.2 ± 4.85), in contrast to the other two groups; Group 2 (air pollution), DFI = 27.8 ± 1.3, and the Group 1 (normal control), DFI = 18.5 ± 6.5 (** P <0.05 Groups 1 and 2). **Recommendations and conclusions.** Exposure to pesticides increases the percentage of DNA-fragmented sperm in normospermic men ejaculate. To reduce the percentage of DNA-fragmented sperm in the ejaculate, especially in infertile men, should be taken into account and avoid exposure to pesticides by taking antioxidant therapy.

KEYWORDS: Pesticides, DNA, Sperm DNA fragmentation, Sperm chromatin structure assay (SCSA).

INTRODUCTION
Spermatozoa are a highly specialized cell undergoing various biological changes necessary to achieve fertilization (Aitken & Henkel, 2011). Over the past few decades, several studies have been published indicating a longitudinal decline in sperm quality (Carlsen et al., 1992). In 2000, Swan et al. published an updated meta-analysis that further supported this finding, showing declines in sperm concentration over time (Swan et al., 2000). While genetic differences may play a role in these results, many studies suggest environmental factors as a potential cause of these declines. Geographical differences in semen quality support the idea that local factors present in some areas but not in other may be influencing declines in semen quality (Swan et al., 2003). Environmental pollutants, occupational exposures and lifestyle have been explored as possible contributors to those changes Chemicals could adversely affect male reproductive system by, either disrupting the gonadal endocrine axis or, the spermatogenesis process (Homan GF., 2007). The spermatogenic process is regulated by the male endocrine system (Dohle et al., 2003), and therefore, semen quality may be particularly sensitive to any pesticides or pesticide metabolites that may mimic male hormones or inflict tissue damage in the testes. Between 1991 and 2006, 20 studies were published in which the outcomes of interest were common semen quality measures (Perry, 2008). Recent studies suggest that sperm DNA integrity may be altered by environmental exposure to some toxic chemicals (Aitken RJ, De Luiis GN., 2010). DNA fragmentation may be an excellent marker for exposure to potential reproductive toxicants and a diagnostic tool for male infertility (Ozmen B, et al., 2007).

The aim of this study was to investigate the association between sperm DNA fragmentation in the population that lives in an environment of air pollution and exposure to pesticides. In addition, our retrospective study was aimed at clarifying whether seasonal effects on human semen parameters such as sperm count, motility,

morphology and vitality associated with the percentage of sperm DNA fragmentation.

Patients and selection

All participants in this study were volunteers, residents of the Republic of Kosovo. Age of participants from 39 to 45 years. Total 164 samples of semen from fertile men with respect to the parameters of semen and DNA fragmentation. This is a prospective study observation spent on Andrology Laboratory Polyclinic,, Biolab-Zafi,, in Klina in the Republic of Kosovo from April 2015 to Februare 2016. All respondents were asked to complete a questionnaire that included standard demographic data (age,BMI, etc.), as well as health status, lifestyle (smoking, alcohol consumption, diet, etc.) and working conditions (working hours per day, exposure, use protection, etc.). The main criterion for inclusion in the study was normospermic men, men who have more than one child. We excluded any individual who could not provide clear information on their job circumstances or exposure risks. Nevertheless, these men constitute a selected population, thus conclusions derived from such studies should be interpreted with caution.

Sample collection

Semen samples were collected in sterile containers by masturbation after a sexual abstinence period of 2–5 days. A portion of each semen sample was used to analyze the following parameters according to the World Health Organization (WHO) guidelines (2010): volume (ml), sperm concentration (x 10^6/ml), pH, percentage of spermatozoa with progressive motility (rapid + slow progression) and % normal morphology.

Sperm chromatin structure assay

The DNA integrity is the sperm functional parameter that together with conventional semen parameters gives more reliable and precise diagnosis of male reproductive potential (Agarwal & Allamaneni, 2004). Consequently, DNA integrity testing should be incorporated into standard semen analysis (Barratt et al., 2010). So far, there is no unique test to determine sperm DNA integrity, only numerous test measuring DNA fragmentation of which some have become part of laboratory practice: TUNEL (Terminal dUTP Nick-End Labeling) test, Comet test (Single Cell Gel Electrophoresis), SCSA (Sperm Chromatin Structure Assay) test and SCD (Sperm Chromatin Dispersion) test (Fernández et al., 2003).

Halosperm SCD test

Halosperm is the SCD test based on the ability of intact DNA with deproteinized nuclei to create loops around nuclear matrix. Deproteinized nuclei create the halos of dispersed DNA that correspond to relaxed DNA loops attached to the residual nuclear structure (core) (Fernández et al., 2005). Nuclei with fragmented DNA produce either small halos (sh) or no halos (wh) of dispersed DNA. DNA fragmentation is expressed as DNA fragmentation index (DFI). In contrast, the sperm nuclei without DNA fragmentation release DNA creating big halos (bh) or those slightly fragmented creating medium halos (mh). Halosperm test, actually measures undamaged spermatozoa rather than spermatozoa with DNA fragmentation (Lewis et al., 2013).

Determination of big halo and DFI

Halosperm test (Halotech DNA, Madrid, Spain) is a modified and improved SCD fragmentation test version (Fernández *et al*., 2005). Semen samples were diluted with Sperm washing medium (Sage, Trumbull, CT, USA) to the concentration of 5–10 × 10^6/mL. Agarose gel from kit was incubated for 5 min at 90–100 °C and 5 min at 37 °C, this was followed by adding and mixing of 25 µL of semen in marked eppendorf test tube. Twenty microlitres of mixture were placed on previously supercoated slide from kit and covered with 22 mm × 22 mm coverslip. Slides were kept for 5 min at 4 °C in refrigerator to create a microgel with an implanted spermatozoa. Coverslips were then carefully removed, a procedure followed by immersion of slides into the previously prepared acid solution (80 µL HCl in 10 mL of distilled water) for 7 min. Slides were then transferred to the tray with a lysing solution from the kit and incubated for 25 min. Rinsing with distilled water followed by dehydration for 2 min in increasing concentrations of ethanol (70, 90 and 100%). After drying, slides were stained with Giemsa dye (Merck KGaA), rinsed under tap water and allowed to dry at room temperature. Each slide was examined under the light microscope at ×100 magnification and 200 spermatozoa were scored. Non-fragmented spermatozoa immersed in agarose matrix and exposed to lysing solution to deproteinize nuclei create the halos of dispersed DNA. The halos correspond to relaxed DNA loops attached to the residual nuclear structure (Fernández *et al*., 2003). The spermatozoa without DNA fragmentation show halos of dispersed DNA which can be large (big halo, bh) or medium (medium halo, mh), whereas those sperm nuclei with fragmented DNA produce either small halos (small halo, sh) or no halos at all (wh). Fragmented spermatozoa can also be degraded (d). Big halo spermatozoa produce halos with thickness equal to or greater than the length of the minor diameter of the core, medium halo spermatozoa produce halos with thickness smaller than the length of the minor diameter of the core and greater than 1/3 of the minor diameter of the core, whereas small halo spermatozoa produce halos with thickness equal to or smaller than 1/3 diameter of the minor diameter of the core (Fernández *et al*., 2005).

Statistical analysis

Statistical analysis was To compare the semen parameters among the worker groups, one-way ANOVA and LSD post hoc test were used. The strength of the relationship between variables was analyzed using Pearson Correlation test. Both of the statistics that applied are programmed in SPSS version 18.. It was

considered a statistical significant difference when P<0.05.

RESULTS

The results were divided into three groups: Total 164 fertile men of three different geographical regions of Kosovo were included in this study. The first group of 53 people living in the villages of the mountainous region of Rugova in the municipality of Pec. The second group of 49 people who live and work in the company Feronikel, in the city of Drenas, which produces a chemical element nickel and iron,3rd group of 62 men, farmers from the region Podrimja the municipality Rahovec, that the cultivation of vegetables, and exposed to pesticides. Let frequent pesticides that are used by the farmer in our study are: alachlor, diazinon, acetochlor, malathion, atrazine, metolachlor, DEET (insect repellant), 2,4-D, aldicarb and cadusaphos, ethoprothos, isazophos, terbufos, pirimiphos-ethyl organophosphorus pesticides) from this catalog of Bayer (Bayer CropScience, Lyon, France). Analysis of human ejaculation is tested twice in the same people in two periods; for the first time from April to August 2015.(Table.1).The second time of the October 2015 to Februar 2016. The number of participants in the second study smaller because some of the current from different reasons not to participate in the research, but supports the main criteria that are the same people twice tested. (Table.3).

Table 1: Characteristics of semen samples collected and DNA fragmentation index comparison between the three groups evaluated. (April - August 2015).

	Group 1 (normal control) n =31	**Group 2 (polluted air) n = 28**	**Group 3 (exposed to pesticides) n = 37**
Age (mean ± SD)	43.2 ± 1.5	42.5 ± 2.8	43.8 ± 1.2
Body mass index	22.5 ± 4.4 (20–28.1)	21.7 ± 4.1 (19.9–22.8)	23.9 ± 4.4 (20.8–32.1)
Tobacco use	17.6%	17.8%	18.1%
The number of children born	4.4 ± 3.5 [b] (3–4)	4.1 ± 3.0 [a] (2–4)	3.1 ± 2.0 [a,b] (1–3)
Volume (mL)	3.5 ± 1.2	3.6 ± 1.8	3.1 ± 1. 7 [a,b]
pH	7.38 ± 0.33	7.41 ± 0.35	7.52 ± 0.36**
Sperm concentration (x 10^6/ml)	69.11±14.88	62.36±21.33	53.11±10.12 **
Motility (rapid + slow progression) %	67.81±6.42	55. 42±10.53	51.64±7.85 **
% Normal morphology	55.6 ± 1.4	52.5 ± 1.6	48.6 ± 1.8*** ***
DNA (%)Fragmentation	18.5 ± 6.5	27.8 ± 1.3	35.2 ± 4.85**

[a,b] p < 0.01

***P* <0.05 in relation to the group 1 and 2

The group of respondents farmers (exposed to pesticides) that have been tested in the period spring - summer, have a higher percentage index fragmented sperm (DFI = 36.2 ± 4.85), in contrast to the other two groups; Group 2 (air pollution), DFI = 27.8 ± 1.3, and the Group 1 (normal control), DFI = 18.5 ± 6.5 (** P <0.05 Groups 1 and 2).When all the seeds quality parameters correlate with DNA fragmentation spermatozoa using Pearson correlation test we get the correlation coefficient (r) as indicated in Table 2. According to the results of its analysis of variance and post-hoc test, only those parameters such as ejaculate, tobacco use, age and pH did not show any correlation with DNA fragmentation spermatozoa. However, five other parameters, ie the volume of semen, sperm concentration, motility, morphology, the number of children born are strongly and negatively correlated with DNA fragmentation of spermatozoa (p <0.01).

Table: 2. Correlation coefficient *(r)* of the relationship between the mean value of percentage index fragmented sperm and the semen parameters

Dependent variables	***r***	***P***
Volume (mL)	-.574	.000
The number of children born	-.412	.000
Sperm Concentration (x 10^6/ml)	-.615	.000
Motility (rapid + slow progression) %	-.608	.000
% Normal morphology	-.602	.000

Table: 3 The second time of the October 2015 to Februar 2016. Characteristics of semen samples collected and DNA fragmentation index comparison between the three groups

	Group 1 (normal control) n =22	Group 2 (polluted air) n = 21	Group 3 (exposed to pesticides) n = 25
Age (Mean ± SD)	43.5 ± 2.7	46.2 ± 4.5	44.8 ± 3.2
Volume (mL)	3.8 ± 2.2	3.7 ± 0.4	3.8± 1. 4
pH	7.41 ± 0.34	7.43 ± 0.37	7.57 ± 0.38
Sperm Concentration (x 10^6/ml)	65.45 ± 18.39	58 ± 12.15	52.55 ± 17.78 *
Motility (rapid + slow progression) %	59.11 ± 21. 7	53.18 ± 17.4	50.11± 16.0 *
% Normal morphology	55.45 ± 1.12	51.26 ± 2.14	50.72 ± 0.93*
DNA Fragmentation (%)	17.8 ± 2.1**	26.8 ± 1.4	30.2 ± 1.7**

*****P <0.05 in relation to the group 1

Shown in Table 3 the results show that there are significant differences between the investigated groups of parameters; sperm concentration, motility, morphology of spermatozoa. A significant difference exists between the three groups in sperm DNA fragmentation. The group of respondents farmers (exposed to pesticides) which have been tested in the autumn-winter period have reduced the index fragmented sperm (DFI = 30.2 ± 1.7), in contrast to the first inspection (DFI = 35.2 ± 2.6) (* p <0.05, and his relationship with the group 1). the other two groups; Group 2 (air pollution) of DFI = 27.8 ± 1.3, increased the percentage of DNA fragmentation of sperm DFI = 26.8 ± 1.4, but not any substantial difference, a Group 1 (normal control), no significant differences in the results of the analysis of DNA fragmentation spermaatozoida between two test period (DFI = 18.5 ± 6.5 vs DFI= 17.8± 2.1).

DISCUSSION

Male factors are responsible for at least 40% of cases of infertility. Male infertility is related to impaired semen quality and may be due to a variety of causes including genetic (Klinefelter's syndrome), congenital (cryptorchidism), endocrine (hypogonadism), obstructive (vasectomy), infective (chlamydia), vascular (varicocele), neoplastic (carcinoma of the testis), lifestyle, and environmental (heat, drugs, pesticides, irradiation) factors. Others causes include sexual dysfunction related to erection and ejaculation (Dohle et al., 2005). However, in many cases male infertility is regarded as idiopathic (40-75%), and no cause can be identified. Semen analysis is used to evaluate semen quality, which is taken as a surrogate measure of male infertility. The World Health Organization has provided reference values for human semen characteristics (Cooper et al., 2010). Normal sperm genetic material is required for successful fertilization, as well as for further embryo and fetal development that will result in a healthy offspring. In males, germ cells divide continuously. It has been estimated that 30 spermatogonial stem cell divisions take place before puberty, when they begin to undergo meiotic divisions. From then on, 23 mitotic divisions per year occur, resulting in 150 replications by the age of 20 years and 840 replications by the age of 50 years (Crow JF., 2000). Because of these numerous divisions of stem cells, men may have an increased risk of errors in DNA transcription. Furthermore, germ cell is also continuously under attack from endogenous and exogenous factors that can induce a wide range of DNA lesions that can be formed under normal processes such transcription, recombination, and replication (Baarends WM.,2001). Sperm DNA contributes half of the offspring's genomic material and abnormal DNA can lead to derangements in the reproductive process. Sperm DNA damage has been attributed to a variety of intra and extratesticular factors (Zini A, Libman J., 2006). The most important is the production of Reactive Oxygen Species (ROS). ROS can be enter the cell nucleus, bind to the DNA and cause its fragmentation (Baumber J., 2003). However, DNA fragmentation is also a feature of physiological processes like apoptosis and necrosis (Gandini L., et al.2000). During the past two decades a number of tests have been introduced for the analysis of sperm DNA fragmentation. In the present study, the presence or absence of DNA fragmentation was determined by examining the Halosperm by SCD test; a simple, highly reproducible and less expensive technique, yielding results highly correlated with those from other procedures like the DBD-FISH and the SCSA (Fernández JL.,et al.2005). Geographic variations in sperm quality must reflect different exposure endocrine disruptive, such as pesticides. Pesticides may have the ability to stop male fertility in several different locations in the reproductive time and by one or more mechanisms. So, they can affect the hypothalamic axis that regulates the production of gonadotropins FSH and LH, the function of Sertoli and Leydig cells, interfere with spermatogenesis and steroidogenesis (Swan, 2006). Pesticides fall into three major classes: insecticides, fungicides, and herbicides (Cox C, Surgan M., 2006). Another classification categorizes pesticides according their chemical structure. Insecticides include organochlorines organophosphates, and carbamates. Organophosphate and carbamates are less toxic and largely replaced organochlorines. Pesticides may differ according to their chemical

structure, their mechanism of action and the toxicity they exhibit, but typically each pesticide consists of one (or more) active ingredient, which exerts the pesticidal activity, and an inert ingredient, which is inactive and helps in handling the active ingredient (Surgan MH.,2005). Experimental evidence in the Laboratory has linked the chemicals present in pesticides to reduced sperm quality, testicular cancer and reproductive abnormalities. Humans have a great risk of exposure through several pathways in occupational, agricultural and household use. Inhalation, oral, dermal and ocular is four possible routes for pesticide exposure. Ingestion of food and water is thought to be the main routes of pesticide exposure in the general population, while dermal absorption is suspected to be the main source of occupational exposure (Toppari J., ET AL., 1996). In our study patients were from three different geographical areas in Kosovo. From the result of additional analysis parameters ejaculate significant differences in the number of sperm, motility and morphology between these three groups of patients across geographies in Kosovo. (Table 1 and 3). Especially significant differences in DNA fragmentation of sperm index ($p < 0.01$). We came to the conclusion that this is the reason for these results is the longer the explosion pesticides in a group of farmers ($n = 62$), in contrast to the first group who live in the other Regio Kosovo (mountain villages in the region Dugagjin in Kosovo). Pesticides can have the ability to stop male fertility in several different locations in the reproductive time and by one or more mechanisms. So, they can affect the hypothalamic axis that regulates the production of gonadotropins FSH and LH, function Sertoli and Leydig cells, interfere with spermatogenesis and steroidogenesis (Swan, 2006). Pesticides can be classified into three main groups (Cox C., Surganova M., 2006) insecticides, fungicides, herbicides. Another classification of pesticides classified according to their chemical structure. Insecticides are organophosphates and carbamates, organochlorines. Organophosphate and carbamates are less toxic and has been largely replaced organochlorines. Pesticides can be distinguished according to their chemical structure, their mechanism of action and toxicity indicate whether each pesticide is usually composed of one (or more) active ingredients, exhibiting pesticidal activity, and the inert ingredient, that is inactive and helps in handling active ingredient (Surganova MH., 2005). Experimental evidence in the Laboratory is related chemicals present in pesticides to reduced sperm quality, testicular cancer and reproductive disorders. People at high risk of exposure through several pathways to work, agriculture and domestic use. Inhalation, oral, dermal and ocular four possible routes of exposure to pesticides. A little food and water is thought to be the main route of exposure to pesticides in the general population, and absorption through the skin is suspected to be the main source of occupational exposure (Toppari J., et al.,1996). In our study, the patients were from three different geographical areas in Kosovo. From result of additional parameters analysis ejaculate significant differences in sperm count, motility and morphology between the three groups of patients from different geographical areas in Kosovo. (Table 1 and 3). Especially significant differences in sperm DNA fragmentation index ($p < 0.01$). From Table (1 and 3) results of the analysis of DNA fragmentation of sperm respondents farmer shows a significant difference in contrast to the other two groups. Results of the analysis DFI = 35.2 ± 4.85, higher than Normospermic ejaculate ($p < 0.01$), and significantly higher than the other two treatment groups ($p < 0.05$). Comparing the results of another analysis of the same respondents in the second seasonal period (autumn-winter), shows, the percentage of DNA fragmentation of sperm in DFI = 30.2 ± 1.7. The fall in the percentage of DNA fragmentation in this period justify the fact that these respondents were farmers working in their farms during this period (autumn-winter) and even greater truth is that in this seasonal period are not exposed pesticides because it is not used as in the spring and summer, so you have not been exposed to its effects in the reproductive system. Research results Ralf, H et al (2001), showing a seasonal dependence sperm count, sperm motility and sperm morphology. In our work by comparing the results of the statistical parameters of ejaculate between the two seasonal periods, are not significantly different in the sperm parameters within each group individually. Since the epididymal sperm maturation takes almost three months, seasonal regulation of human spermatogenesis with the largest number of spermatozoa in the spring seems likely. On the other hand, directly photoperiodic control spermatogenesis may also be possible. Melatonin, seasonal step of controlling reproduction of many species of mammals (Brzezinski A, Wurtman RJ., 1988), may be included in the link. Antigonadal effect of melatonin is known (Stetson MH., Et al., 1976). Analysis of DNA fragmentation of sperm in two different periods of the year (June to August 2015 and October 2015 to February 2016) was observed significant differences in the percentage of sperm DNA fragmentation. In the season of spring-summer, the percentage of DNA fragmentation is greater than the seasonal period in the autumn and winter months. From our results, the obvious is that there is a significant seasonal impact on sperm count and morphology. We can speculate significant seasonality in the condensation of chromatin as a functional parameter of sperm. Our hypothesis was confirmed the first report S'anchez al. (2000), who found a tendency of increasing the quality of human sperm chromatin condensation in South America late fall. The paper Veliu et al (2007) air pollution is a critical problem of environmental protection in urban areas in Kosovo, but not for the country as a whole. Air quality is particularly bad in some cities; Pristina, Obilic space Drenas area, and Mitrovica. The main sources of pollutants are sulfur dioxide (SO_2), nitrogen oxides NO and NO_2 (NO_x), ozone (O_3), lead (Pb), carbon dioxide (CO_2), particulate matter (PM or dust), and dioxin. The main sources of pollutants are industrial plants, such as nickel mining and production in Drenas (Ferronikeli). Nickel salts are

considered an industrial health hazard, because many nickel compounds reach the human environment (Venugopal and Luckey, 1978), and exert potent toxic effects on peripheral tissues as well as on the reproductive system. Reports suggested nickel exposure causes decrease in weights of testicular and accessory sex organs and decrease in testicular steroidogenic enzymes activities (Δ5 3β-HSD and 17β-HSD) (Das and Dasgupta, 2000). Some reports suggested high blood nickel levels have a significant positive correlation with morphologically abnormal sperm (Danadevi et al., 2003). Adamopoulos et al. (1996) reported decreasing sperm concentrations in Athenian men during a period when air pollution was increasing and hypothesized that air pollution might contribute to secular trends in declining sperm counts. Also consistent with the earlier study (Selevan et al., 2000), this study found a significant association between exposure to air pollution and the percentage of sperm with fragmented DNA (SCSA–%DFI) there by increasing the weight of evidence that exposure to high levels of air pollution may have damaging effects on sperm DNA. Another group of patients in our study of 49 people who live near and work in the company Feronikel, which produces a chemical element nickel and iron, (Drenas. The Republic of Kosovo). Analysis of DNA fragmentation spermatozida the second group of subjects was higher than normal for Normospermic samples of ejaculate (DFI <15%) ($p < 0.05$). Results from the first measurements (Table 1) DFI = 27.8 ± 1.3 and the second repeated measurements (Table 3) DFI = 26.8 ± 1.4 showed no significant differences. This fact can be justified that the air pollution in the environment is the same in the independence of the seasonal periods, and the truth is justified by the fact that these men live and work all the time in the vicinity of these companies that pollute the environment. We must point out that we did not order the concentration of any of the metals in the blood of patients we can only speculate that the reason for the increased percentage of DNA fragmentation of sperm more than the normal percentage of Normospermic the constant exposure to polluted air.

Recommendations and conclusions

Eternal (there are plenty) authors in their articles concluded that the percentage of DNA-fragmented sperm in the ejaculate can very effectively reduces the relatively short oral treatment with a combination of vitamins C and E (Ermanno G, et al., 2005). From our work we can recommend it with antioxidant and therapy vitamins treatment in order to reduce the percentage of DNA-fragmented sperm in the ejaculate should be taken into account and avoiding exposure to pesticides which should more increase the percentage of DNA-fragmented sperm in the ejaculate, it is confirmed in this work we at Normospermic samples of ejaculate. This conclusion can we gain and greater justification to ejaculate with Oligoasthenozoospermic that the majority should be introduced in the procedure of medically assisted fertilization (IVF / ICSI).

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