Repositorio Institucional de la Universidad Autónoma de Madrid

https://repositorio.uam.es

Esta es la **versión de autor** del artículo publicado en:
This is an **author produced version** of a paper published in:

Andrologia 50.10 (2018): e13115

DOI: http://dx.doi.org/10.1111/and.13115

Copyright: © 2018 Blackwell Verlag GmbH

Access to the published version may require subscription
El acceso a la versión del editor puede requerir la suscripción del recurso

Impact of polymorphism in DNA repair genes *OGG1* and *XRCC1* on seminal parameters and human male infertility

Running Title: *OGG1* and *XRCC1* SNPs and male infertility

Authors: Garcia-Rodriguez A[1], de la Casa M[2], Serrano M[3], Gosálvez J[1], Roy R[1*]

[1]Biology Department, University Autónoma of Madrid, Madrid.

[2]GINEFIV, Assisted Reproduction Centre, Madrid.

[3]Animal Genetics Department, National Institute for Agricultural Research (INIA), Madrid

* Corresponding author:

Rosa Roy Barcelona

Biology Department, Unit of Genetics
University Autónoma of Madrid

Cantoblanco Campus

28049

Madrid

rosa.roy@uam.es

Keywords: infertility, polymorphism, BER route, OGG1, XRCC1.

Abstract

The DNA repair capacity in the mature spermatozoa is highly compromised due to the Base Excision Repair Route (BER) being truncated. In the mature spermatozoa only the first enzyme of the route (OGG1) is present. Consequently, reduced activity of the enzymes of the BER route both during spermatogenesis and in the mature spermatozoa may be detrimental for fertility. The objective of our study was to investigate the correlation between two representative SNPs of those enzymes, SNPs *OGG1* Ser326Cys (rs1052133) and *XRCC1* Arg399Gln (rs25487) and male infertility. 313 seminal samples from infertile patients and 80 from donors with proven fertility were included in the study. All samples were subjected to a regular sperm analysis and genotyped using the PCR-RFLP system. We found significant differences in the genotype frequencies between patients and donors for the *XRCC1* Arg399Gln polymorphism ($\chi2(2)=8.7$, $P=0.013$), with the Gln allele showing a protective role and for the *OGG1* Ser326Cys polymorphism between normozoospermic and non-normozoospermic patients ($\chi2(2)=12.67$, $P=0.002$) with the Cys allele showing a detrimental effect over concentration. In conclusion, our study shows that polymorphisms in the genes coding for the DNA damage repair enzymes may be associated with poor sperm parameters and male infertility.

Introduction

Reactive oxygen species (ROS) are free radicals that have at least one unpaired electron (Rahal, Kumar et al. 2014). ROS originate as by-products of the metabolism of oxygen and are normally neutralized by the antioxidant systems. However, occasionally an elevated production of ROS or a failure in the antioxidant systems leads to an increase in ROS concentration. Under this situation elevated levels of ROS can cause both DNA damage and lipid peroxidation (Agarwal, Virk et al. 2014). One of the main effects of oxidative stress affecting sperm DNA quality is the oxidation of guanine bases producing 8-hydroxy-2´-deoxyguanosine (8-OHdG) which is a highly

mutagenic element leading to a G:C → T:A transversion after replication (Wood, Wood et al. 1992).

The Base Excision Repair System (BER) is the DNA repair pathway that detects and repairs the presence of 8OHdG residues in the DNA (Olsen, Lindeman et al. 2005). Two principal enzymes in this pathway are the 8-oxoguanine glycosylase 1 (OGG1) and the X-ray repair cross complementing group 1 (XRCC1). The OGG1 is the first enzyme in the route and the one that detects the presence of the 8OHdG residues and eliminates them, producing an abasic site while the XRCC1 is a scaffold enzyme that attracts the rest of the implicated enzymes to the newly abasic site and coordinates them, enhancing the effectiveness of the pathway.

In contrast to somatic cells, in the mature spermatozoa the BER route is truncated, being only the OGG1 enzyme active (Smith, Dun et al. 2013). Due to this situation, a proper functioning of the BER pathway during spermatogenesis is essential in order to obtain mature spermatozoa with the lowest levels of 8-OHdG residues possible, as the remaining 8-OHdG residues cannot be completely repaired in the mature spermatozoa. Moreover, as the OGG1 enzyme is active in the mature spermatozoa and the rest of the pathway is truncated, its activity on the remaining 8-OHdG residues leads to the presence of abasic sites in the DNA of the fertilizing spermatozoa which may be detrimental to the fertilization process (González-Marín, Gosálvez et al. 2012).

However, little information is available regarding the relationship between polymorphisms in those critical enzymes and the consequences for male infertility. The objective of our study was to investigate the correlation between two representative SNPs located in two key enzymes of the BER pathway, SNPs *OGG1* Ser326Cys (rs1052133) and *XRCC1* Arg399Gln (rs25487) and male infertility.

Materials and methods

2.1.) Subjects and sample collection

The study was approved by the Bioethics Committee of the University Autonoma of Madrid (CI 60-1058). A total of 393 subjects were included in the study. The patient cohort consisted of 313 individuals undergoing an assisted reproduction treatment that showed no evidence of any other fertility related diseases, such as prostate cancer, cryptorchidism, varicocele, diabetes, seminal infections or karyotype abnormalities. The control cohort included 80 individuals with proven fertility who had already fathered at least one child. Each subject donated 1mL of semen obtained by masturbation after at least 4 days of abstinence (the regular abstinence period applied in the collaborating clinic).

2.1) Seminal analysis

To get a general overview of the seminal quality in our population a regular sperm analysis was performed for each sample according to World Health Organization (WHO) guidelines (World Health Organization 2010). The included parameters were volume, concentration, motility, morphology and vitality. The individuals included in the patient cohort were classified into normozoospermic and non-normozoospermic according to cut-off reference values for seminal characteristics as published in the WHO 2010 laboratory manual for the examination and processing of human semen (for non-normozoospermic volume ≤ 1.5mL, concentration ≤ 15 mill mL-1, motility ≤ 40%, morphology ≤ 4% and vitality ≤ 58%).

2.2) Sperm DNA fragmentation

Sperm DNA fragmentation was assessed for all samples in the patient cohort using the Sperm Chromatin Dispersion Assay (SCD) in the form of the Halosperm Kit (Halosperm Kit, Halotech DNA, Madrid, Spain) and in accordance with the manufacturer's guidelines. Briefly, seminal samples were included in an agarose microgel on a microscope slide and they were next subjected to a lysis solution and a deproteinization solution. After being washed and dehydrated with alcohols all samples were stained with SYBR-Green (SYBR-Green 10000x, Cat No S7563, Thermo Fisher Scientific, Braunschweig, Germany), mounted with Vectashield (Vectashield

Mounting Medium, Cat No H-1000, Vector Laboratories, Burlingame, USA) and observed with fluorescence microscopy (Leica DMRB, Leica-Mycrosystems, Wetzlar, Germany).

2.3) DNA extraction

DNA extraction was performed using the phenol-chloroform method with proteinase-K treatment as described elsewhere with minor modifications (Yuan, Kuete et al. 2015). Briefly, 500 µL of liquefied semen was centrifuged at 1800 rpm and the pellet re-suspended in 100 µL of seminal plasma. This sample was then incubated overnight in a solution consisting of 8 µL 10 mg mL-1 proteinase K (Proteinase K Recombinant PCR Grade, Cat No 03 115 887 001, Roche, Mannheim, Germany), 8 µL of 1M DTT and 100 µL extraction buffer (20mM Tris-Cl, 20mM EDTA, 200mM NaCl, 4% SDS). Thereafter, 216 µL of phenol (Phenol/Chloroform/Isoamyl alcohol 25:24:1, Cat No 327115000, Acros Organics, New Jersey, USA) was added to the semen sample and the mixture agitated by a mechanical shaker for 2 min after which it was centrifuged at 8000 rpm for 10 min. The upper aqueous phase was then recovered and the same operation performed with 200 µL of chloroform:isoamil 24:1 (Chloroform reagent grade - Cat No CL02031000, Scharlab, Spain; Isoamylalcohol >98% - Cat No 818969, Merck, Munich, Germany). The upper aqueous phase was then recovered once more to which 250 µL of cold (-20 ºC) 100% ethanol was also added. This solution was shaken gently until the DNA precipitated and the sample was left to stand overnight at -20 ºC. The DNA was then washed with cold (-20 ºC) 70% ethanol and air-dried. To eliminate contaminating RNA, the pellet was re-suspended in 50 µL of TRIS-EDTA buffer with 0.5 µL of 1 mg mL-1 RNAse (RNase DNase free, Cat No 11 119 915 001, Roche, Mannheim, Germany) and incubated for 2 h at 37 ºC. Finally, the concentration of the extracted DNA was determined using a NanoDrop ND-1000 (Thermo Scientific, Braunschweig, Germany) before diluting to a concentration of 50 ng µL-1 and stored at 4 ºC.

2.4) Genotype analysis

The *OGG1* Ser326Cys and *XRCC1* Arg399Gln genotypes were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method according to the protocols by Sobczuk et al (Sobczuk, Poplawski et al. 2012) and Lunn et al (Lunn, Langlois et al. 1999) respectively. The PCR protocol was performed as follows: initial denaturation (94 °C, 10 min) followed by 35 cycles of denaturation (94 °C, 30 s), annealing (melting temperature, 30 s) and elongation (72 °C, 30 s). The PCR products were then digested with the restriction enzymes SatI and MspI respectively (both FastDigest Restriction Enzymes, Cat Nos SatI-FD1644 and MspI-FD0544, Thermo Scientific, Braunschweig, Germany) by incubation at 37 ºC for 45 min. The primer sequences and the restriction enzymes are shown in table 1.

2.5) Statistical analysis

Statistical analysis was performed using the SPSS 22 software for Windows (SPSS Inc., Chicago. USA). Adjustment of continuous variables to normal distribution was checked using the Kolmogorov-Smirnov test. A goodness-of-fit Chi square test was used to determine the Hardy-Weinberg equilibrium of the observed genotype frequencies. Associations between categorical variables (genotype frequencies and odds ratios) were assessed using the Chi-square test. Continuous variables (seminal parameters) were compared between genotypic groups using the non-parametric test Kruskal-Wallis. A P value <0.05 was considered to be statistically significant.

Results

We obtained data from 393 subjects: 313 infertile patients and 80 controls with proven fertility. Table 2 describes the demographic and clinical profile of the participants. The genotype frequencies of the studied polymorphisms are shown in table 3. Both cohorts were in Hardy-Weinberg equilibrium for both polymorphisms. We only found significant differences between patients and donors for the *XRCC1* Arg399Gln polymorphism ($\chi2(2)=8.7$, $P=0.013$). Similarly, as showed in figure 1, we only got statistically significant odds ratios of infertility for the SNP *XRCC1*

Arg399Gln and the genotypes Arg/Arg (OR=2.223, 95% CI=1.296-3.813, $\chi 2(1)=8.67$, P=0.003) and Arg/Gln (OR=0.555, 95%CI=0.337-0.912, $\chi 2(1)=5.47$, P=0.019).

When we compared the values of the traditional sperm analysis between genotypes we obtained significant differences for the parameter concentration and the SNP *XRCC1* Arg399Gln (H=8.43, P=0.015) (see table 4). Pairwise comparisons revealed significant differences between genotypes Arg/Arg and Gln/Gln (H=-43.23, P=0.047, r=-0.131) and differences approaching significance between genotypes Arg/Arg and Arg/Gln (H=-28.98, P=0.056, r=-0.159). We also obtained differences approaching significance for the SNP *OGG1* Ser3226Cys and the parameter morphology. Moreover, for the SNP *OGG1* Ser3226Cys we obtained the highest values in all parameters for the genotype Ser/Ser while for the SNP *XRCC1* Arg399Gln we obtained the highest values in all parameters for the genotype Gln/Gln. In concordance, we obtained the lowest levels of sperm DNA fragmentation for genotypes Ser/Ser for the SNP *OGG1* Ser3226Cys and Gln/Gln for the SNP *XRCC1* Arg399Gln.

When we classified the patients cohort according to its diagnosis (normozoospermic and non-normozoospermic) we obtained statistically significant differences in the genotype frequencies between those two subgroups for SNP *OGG1* Ser326Cys ($\chi 2(2)=12.67$, P=0.002) (see table 5).

Nine possible combinations were derived from the observed genotypes, of which two of them (Ser/Ser-Arg/Arg and Ser/Ser-Arg/Gln) represented more than 50% of the occurrences both in controls and in cases (see table 6). The general distribution was not significantly different between infertile patients and fertile controls ($\chi 2(8)=11.61$, P=0.169). Only two combinations showed a statistically significant association with male fertility status: Ser/Ser-Arg/Arg (OR=1.915, 95% CI=1.005-3.648, $\chi 2(1)=4.003$, P=0.045) and Ser/Ser-Arg/Gln (OR=0.531, 95% CI=0.315-0.894, $\chi 2(1)=5.765$, P=0.016).

Discussion

As mentioned earlier, BER is a DNA repair pathway that detects the presence of 8-OHdG modified nucleotides in the DNA and repairs them. Two principal enzymes in this pathway are OGG1 and XRCC1. The aim of this study was to find a relationship between two representative SNPs of those enzymes, SNPs *OGG1* Ser326Cys and *XRRC1* Arg399Gln, and male infertility.

OGG1, coded in chromosome 3p26, is the first enzyme which works in the BER DNA repair system. It recognizes oxo-G:C base pairs and catalyses the expulsion of the oxoG leaving an abasic site in the DNA (Norman, Verdine et al. 2000). The OGG1 is the only enzyme of the BER route present in the mature spermatozoa and its activity is essential for the repair of the oxo-G:C base pairs. The oocyte, although being able to repair part of the DNA damage from the spermatozoa post fecundation, has very low concentration of this particular enzyme. It has been shown that semen of infertile men has an increased level of oxo-G:C base pairs (Dantzer, Bjørås et al. 2003). The Ser326Cys polymorphism is a C to G transversion in exon 7 in an α-helix domain (Kohno, Shinmura et al. 1998). Several studies have associated the Cys allele with a reduced repair capacity (Hill, Evans 2006), (Smart, Chipman et al. 2006). Specifically, the group of Kohno et al found a strong association with the Ser allele having 7 times higher enzyme activity levels compared to the enzyme activity produced by the Cys allele (Kohno, Shinmura et al. 1998).
To our knowledge there is only other published study for this polymorphism related to infertility; they also observed a higher frequency of genotypes Ser/Cys and Cys/Cys in patients than in donors although their frequencies are not similar to ours (Ji, Yan et al. 2013). These differences can be a consequence of the differences in the number of subjects studied (they had 620 patients and 480 donors) or of the different ethnicity of the subjects (Asiatic versus Caucasian). In our study, we could not find statistically significant differences in the genotype frequencies between patients and donors; in contrast, we have observed statistically significant differences in the genotype frequencies between normozoospermic and non-normozoospermic patients, with the latter showing a reduction in the frequency of the Ser/Ser genotype. In addition, although not being statistically significant, the only genotype showing an odds ratio of infertility

under 1 (protective role) was the genotype Ser/Ser. Moreover, we obtained the highest sperm parameters and the lowest sperm DNA fragmentation levels for the Ser/Ser genotype with differences approaching a significant value for parameter morphology. Consequently, our observations are concordant with the hypothesis that the Cys allele correlates with a reduced repair capacity and point to the idea that it has a major impact on sperm morphology. A reduced activity of the enzyme OGG1 may correlate with a higher presence of oxo-G:C base pairs and thus with a defective spermiogenic procedure generating morphologically abnormal spermatozoa.

XRCC1, coded in chromosome 19q13.2, is a key enzyme in the BER DNA repair system whose function is to attract other enzymes to the sites where the DNA is damaged (Thompson, West 2000), (Radicella, Boiteux et al. 2001). It has been shown that its expression in the testes is higher than in other tissues suggesting that it may play an important role during spermiogenesis to repair damage from the meiosis in the DNA (Ahmed, de Boer et al. 2010), (Walter, Lu et al. 1994). In contrast, the XRCC1 enzyme is not present in the mature spermatozoa. Consequently, XRCC1 enzyme deficiency during spermiogenesis would lead to the presence of damage in the DNA of the mature spermatozoa and this damage will pass to the embryo, as the mature spermatozoa has a truncated BER route and thus cannot repair it. In the Arg399Gln polymorphism there is an Arg to Gln substitution in codon 399 which is located in the poly ADP ribose polymerase (PARP) binding domain (Saadat, Ansari-Lari 2009).

In our study we observed significant differences in the genotype frequencies between patients and donors with an increased presence of the heterozygous Arg/Gln genotype in fertile donors. Our results are consistent with those of Gu et al (Gu, Liang et al. 2007), (Gu, Ji et al. 2007) but there are other studies which offer an opposite conclusion (Zheng, Wang et al. 2012) or even no correlation between this polymorphism and idiopathic male infertility (Gashemi, Khodadadi et al. 2017). These differences may be as a consequence of the ethnic background of the

population. Both the Gu et al and Zheng et al studies were conducted with Asiatic populations (China) while ours was conducted with a Caucasian population (Spain). Moreover, our genotype frequencies are consistent with those observed by Silva et al in Caucasian women (Portugal) (Silva, Moita et al. 2007). However, when we compared the sperm parameters between genotypes we obtained the highest values in all parameters for genotype Gln/Gln with significant differences in the case of the parameter concentration. This may indicate that the Arg allele might have an impact on fertility with the Arg/Arg genotype correlating with an important reduction in sperm concentration. A possible explanation for this observation could be that this variant of the enzyme would be less active, leaving more abasic sites in the sperm DNA and thus increasing the activation of apoptotic processes which lead to a reduction in sperm concentration. In concordance with this idea, Manente et al have recently correlated the presence of elevated levels of sperm DNA fragmentation with an increase in apoptosis and with the diagnosis of oligo and necrozoospermia (Manente, Pecoraro et al. 2015).

When combining the studied polymorphism we obtained nine possible options but only two of them showed a significant association with the male fertility status (Ser/Ser-Arg/Arg and Ser/Ser-Arg/Gln). In both cases the observed odds ratios were similar to those observed for the *XRCC1* Arg399Gln polymorphism alone. Thus we hypothesise that the effect of the presence of a polymorphism is more important for the XRCC1 enzyme than for the OGG1 one. That observation may be explained due to the lack of activity of the XRCC1 enzyme in the mature spermatozoa which makes the XRCC1´s activity levels during spermatogenesis critical.

We are aware that our study has both strengths and weaknesses. The main strengths are the relatively high number of individuals included in the study and that all subjects included in the donors’ cohort had proven fertility as they had already fathered at least one child. With respect to the study’s weaknesses, due to sample availability the number of individuals included in each cohort was not balanced, with a total of 313 patients but only 80 donors. In addition, although

sperm parameters are influenced by patient exposure factors such as BMI, smoking and diet, these aspects are difficult or virtually impossible to fully define in the individual patients and thus we have not considered them in our study.

In conclusion, our study shows that polymorphisms in the genes coding for the DNA damage repair enzymes are associated with lower enzyme activity, poor sperm parameters and male infertility. More research should be done including a broader spectrum of polymorphisms located in genes coding for the enzymes of the BER route in order to better understand their relation to male infertility.

AGARWAL, A., VIRK, G., ONG, C. and DU PLESSIS, S.S., 2014. Effect of Oxidative Stress on Male Reproduction. *The World Journal of Men's Health,* **32**(1), pp. 1-17.

AHMED, E.A., DE BOER, P., PHILIPPENS, M.E.P., KAL, H.B. and DE ROOIJ, D.G., 2010. Parp1-XRCC1 and the repair of DNA double strand breaks in mouse round spermatids. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis,* **683**(1-2), pp. 84-90.

DANTZER, F., BJØRÅS, M., LUNA, L., KLUNGLAND, A. and SEEBERG, E., 2003. Comparative analysis of 8-oxoG:C, 8-oxoG:A, A:C and C:C DNA repair in extracts from wild type or 8-oxoG DNA glycosylase deficient mammalian and bacterial cells. *DNA Repair,* **2**(6), pp. 707-718.

GASHEMI, H., KHODADADI, I., FATTAHI, A., MOGHINBEIGI, A. and TAVILANI, H., 2017. Polymorphisms of DNA repair genes *XRCC1* and *LIG4* and idiopathic male infertility. *Systems Biology in Reproductive Medicine,* **63**(6), pp. 382-390.

GONZÁLEZ-MARÍN, C., GOSÁLVEZ, J. and ROY, R., 2012. Types, causes, detection and repair of DNA fragmentation in animal and human sperm cells. *International Journal of Molecular Sciences,* **13**(11), pp. 14026-14052.

GU, A., JI, G., LIANG, J., XIA, Y., LU, N., WU, B., WANG, W., SONG, L., WANG, S. and WANG, X., 2007. DNA repair gene XRCC1 and XPD polymorphisms and the risk of idiopathic azoospermia in a Chinese population. *International journal of molecular medicine,* **20**(5), pp. 743-747.

GU, A., LIANG, J., LU, N., WU, B., XIA, Y., LU, C., SONG, L., WANG, S. and WANG, X., 2007. Association of XRCC1 gene polymorphisms with idiopathic azoospermia in a Chinese population. *Asian Journal of Andrology,* **9**(6), pp. 781-786.

HILL, J.W. and EVANS, M.K., 2006. Dimerization and opposite base-dependent catalytic impairment of polymorphic S326C OGG1 glycosylase. *Nucleic acids research,* **34**(5), pp. 1620-1632.

JI, G., YAN, L., LIU, W. and QU, J., 2013. OGG1 Ser326Cys polymorphism interacts with cigarette smoking to increase oxidative DNA damage in human sperm and the risk of male infertility. *Toxicology letters,* **218**(2), pp. 144-149.

KOHNO, T., SHINMURA, K., TOSAKA, M., TANI, M., KIM, S., SUGIMURA, H., NOHMI, T., KASAI, H. and YOKOTA, J., 1998. Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. *Oncogene,* **16**(25), pp. 3219-3225.

LUNN, R.M., LANGLOIS, R.G., HSIEH, L.L., THOMPSON, C.L. and BELL, D.A., 1999. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. *Cancer research,* **59**(11), pp. 2557.

MANENTE, L., PECORARO, S., PICILLO, E., GARGIULO, U., GARGIULO, P., DE LUCA, A. and POLITANO, L., 2015. Molecular Evidence of Apoptotic Pathway Activation in Semen Samples with High DNA Fragmentation. *IN VIVO,* **29**(2), pp. 289-294.

NORMAN, D.P.G., VERDINE, G.L. and BRUNER, S.D., 2000. Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature,* **403**(6772), pp. 859-866.

OLSEN, A., LINDEMAN, B., WIGER, R., DUALE, N. and BRUNBORG, G., 2005. How do male germ cells handle DNA damage? *Toxicology and applied pharmacology,* **207**(2), pp. 521-531.

RADICELLA, J.P., BOITEUX, S., HICKSON, I.D. and VIDAL, A.E., 2001. XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. *The EMBO journal,* **20**(22), pp. 6530-6539.

RAHAL, A., KUMAR, A., SINGH, V., YADAV, B., TIWARI, R., CHAKRABORTY, S. and DHAMA, K., 2014. Oxidative Stress, Prooxidants, and Antioxidants: The Interplay. *BioMed Research International,* **2014**, pp. 1-19.

SAADAT, M. and ANSARI-LARI, M., 2009. Polymorphism of XRCC1 (at codon 399) and susceptibility to breast cancer, a meta-analysis of the literatures. *Breast cancer research and treatment,* **115**(1), pp. 137-144.

SILVA, S.N., MOITA, R., AZEVEDO, A.P., GOUVEIA, R., MANITA, I., PINA, J.E., RUEFF, J. and GASPAR, J., 2007. Menopausal age and XRCC1 gene polymorphisms: Role in breast cancer risk. *Cancer detection and prevention,* **31**(4), pp. 303-309.

SMART, D.J., CHIPMAN, J.K. and HODGES, N.J., 2006. Activity of OGG1 variants in the repair of pro-oxidant-induced 8-oxo-2′-deoxyguanosine. *DNA Repair,* **5**(11), pp. 1337-1345.

SMITH, T.B., DUN, M.D., SMITH, N.D., CURRY, B.J., CONNAUGHTON, H.S. and AITKEN, R.J., 2013. The presence of a truncated base excision repair pathway in human spermatozoa that is mediated by OGG1. *Journal of cell science,* **126**(6), pp. 1488-1497.

SOBCZUK, A., POPLAWSKI, T. and BLASIAK, J., 2012. Polymorphisms of DNA Repair Genes in Endometrial Cancer. *Pathology & Oncology Research,* **18**(4), pp. 1015-1020.

THOMPSON, L.H. and WEST, M.G., 2000. XRCC1 keeps DNA from getting stranded. *Mutation Research - DNA Repair,* **459**(1), pp. 1-18.

62 WALTER, C.A., LU, J., BHAKTA, M., ZHOU, Z., THOMPSON, L.H. and MCCARREY, J.R., 1994. Testis
63 and somatic Xrcc-1 DNA repair gene expression. *Somatic cell and molecular genetics,* **20**(6), pp.
64 451-461.

65 WOOD, M.L., WOOD, M.L., ESTEVE, A., ESTEVE, A., MORNINGSTAR, M.L., MORNINGSTAR, M.L.,
66 KUZIEMKO, G.M., KUZIEMKO, G.M., ESSIGMANN, J.M. and ESSIGMANN, J.M., 1992. Genetic
67 effects of oxidative DNA damage: Comparative mutagenesis of 7,8-dihydro-8-oxoguanine and
68 7,8-dihydro-8-oxoadenine in Escherichia coli. *Nucleic acids research,* **20**(22), pp. 6023-6032.

69 WORLD HEALTH ORGANIZATION, 2010.
70 *WHO laboratory manual for the examination and processing of human semen.* 5º edn.

71 YUAN, H., KUETE, M., SU, L., YANG, F., HU, Z., TIAN, B., ZHANG, H. and ZHAO, K., 2015.
72 Comparison of three different techniques of human sperm DNA isolation for methylation
73 assay. Journal of Huazhong University of Science and Technology. Medical Sciences, **35**(6), pp.
74 938-942.

75 ZHENG, L., WANG, X., ZHOU, D., ZHANG, J., HUO, Y. and TIAN, H., 2012. Association between
76 XRCC1 single-nucleotide polymorphisms and infertility with idiopathic azoospermia in northern
77 Chinese Han males. *Reproductive BioMedicine Online,* **25**(4), pp. 402-407.

78

Figure legends

Figure 1: Forest plot of infertility risk for each genotype. Horizontal lines represent 95% CI. Each square represents the OR point. The vertical line is at the null value (OR=1). Statistical analysis: chi-square test.

Tables

Table 1: RFLP settings.

Polymorphism	**OOG1 Ser326Cys**	**XRCC1 Arg399Gln**
PCR primers	F 5`-GGAAGGTGCTTGGGGAAT-3`	F 5`-TTGTGCTTTCTCTGTGTCCA-3`
	R 5`-ACTGTCACTAGTCTCACCAG-3`	R 5`-TCCTCCAGCCTTTTCTGATA-3`
Annealing temperature	56 ºC	56 ºC
PCR product	200 bp	615 bp
Restriction enzyme	SatI	MspI
Restriction fragments	Ser/Ser 200	Arg/Arg 374,221

Ser/Cys 200,100	Arg/Gln 615,374,221
Cys/Cys 100	Gln/Gln 615

Table 2: Demographic and clinical profiles of the cohorts included in the study (Mean ± Standard deviation). Statistical analysis: Mann-Whitney U-test.

	Patients	**Donors**	***P*-value**
Volume (mL)	3.33 ± 1.36	3.19 ± 1.19	0.431
Sperm Concentration (X 10^6 mL^{-1})	26.93 ± 18.41	53.82 ± 18.92	<0.001
Motility (%)	38.93 ± 12.22	54.55 ± 6.36	<0.001
Morphology (%)	3.41 ± 1.87	6.41 ± 1.71	<0.001
DNA fragmentation (%)	19.69 ± 13.15	No data	No data

Table 3: Genotype frequencies of the *OGG1* Ser326Cys and *XRCC1* Arg399Gln polymorphisms among the cases and controls. Statistical analysis: Chi-square test.

	Patients		**Donors**		
Genotype	**n (obs)**	**Genotype frequency**	**n (obs)**	**Genotype frequency**	**P-value**
***OGG1* Ser326Cys**					
Ser/Ser	182	0.59	53	0.66	0.455
Ser/Cys	118	0.37	25	0.32	
Cys/Cys	13	0.04	2	0.02	
***XRCC1* Arg399Gln**					
Arg/Arg	144	0.46	22	0.28	0.013
Arg/Gln	129	0.41	45	0.56	
Gln/Gln	40	0.13	13	0.16	

Table 4: Extended analysis of traditional seminal parameters for each genotype. The data shown are the mean for volume (mL), concentration (mill mL-1), motility (%), morphology (%), vitality (%) and DNA fragmentation (%). Statistical analysis: Kruskal-Wallis test.

		Genotypes			p-value
OGG1 **Ser326Cys**		**Ser/Ser**	**Ser/Cys**	**Cys/Cys**	
	Volume (mL)	3.38	3.21	2.90	0.266
	Concentration (mill mL-1)	33.09	31.29	30.33	0.538
	Motility (%)	42.88	41.15	38.66	0.269
	Morphology (%)	4.20	3.73	3.66	0.056
	Vitality (%)	75.55	72.29	71.60	0.280
	Fragmentation (%)	19.19	20.44	19.87	0.688
XRCC1 **Arg399Gln**		**Arg/Arg**	**Arg/Gln**	**Gln/Gln**	
	Volume (mL)	3.29	3.29	3.34	0.957
	Concentration (mill mL-1)	28.81	33.99	38.09	0.015
	Motility (%)	40.92	42.20	45.28	0.159
	Morphology (%)	3.89	4.04	4.30	0.408
	Vitality (%)	73.22	74.33	75.76	0.666
	Fragmentation (%)	19.46	20.50	17.86	0.641

Table 5: Extended comparison of genotype frequencies of the *OGG1* Ser326Cys and *XRCC1* Arg399Gln polymorphisms between controls, normozosspermic patients and non-normozoospermic patients. Statistical analysis: Chi Square test.

			Genotypes			*p*-value
***OGG1* Ser326Cys**			**Ser/Ser**	**Ser/Cys**	**Cys/Cys**	
	Donors		0.66	0.32	0.02	0.002 (Donors vs Normo)
	Patients	**Normo**	0.74	0.24	0.02	
		Non-normo	0.52	0.43	0.05	
***XRCC1* Arg399Gln**			**Arg/Arg**	**Arg/Gln**	**Gln/Gln**	
	Donors		0.28	0.56	0.16	0.006 (Donors vs Normo)
	Patients	**Normo**	0.50	0.33	0.17	
		Non-normo	0.44	0.45	0.11	

Table 6: Distribution of the genotype frequencies for the 9 possible combinations of the *OGG1* Ser326Cys and *XRCC1* Arg399Gln polymorphisms. OR= Odds ratio.

Genotypes		Patients		Donors			
***OGG1* Ser326Cys**	***XRCC1* Arg399Gln**	**n (obs)**	**%**	**n (obs)**	**%**	**P-value**	**OR (95% CI)**
Ser/Ser	**Arg/Arg**	85	27.4	13	16.5	0.045	1.915 (1.005-3.648)
Ser/Ser	**Arg/Gln**	77	24.7	31	38	0.016	0.531 (0.315-0.894)
Ser/Ser	**Gln/Gln**	20	6.4	9	11.4	0.127	0.529 (0.231-1.212)
Ser/Cys	**Arg/Arg**	55	17.5	10	12.7	0.299	1.465 (0.710-3.023)
Ser/Cys	**Arg/Gln**	44	14	11	13.9	0.984	1.007 (0.494-2.054)
Ser/Cys	**Gln/Gln**	19	6.1	4	5.1	0.738	1.208 (0.399-3.656)
Cys/Cys	**Arg/Arg**	4	1.3	0	0	-	-
Cys/Cys	**Arg/Gln**	8	2.5	2	2.5	0.994	1.007 (0.210-4.836)
Cys/Cys	**Gln/Gln**	1	0.3	0	0	-	-