# Protamine 1 to protamine 2 ratio correlates with dynamic aspects of DNA fragmentation in human sperm

*Agustín García-Peiró, B.S.,*[a,b] *Juan Martínez-Heredia, Ph.D.,*[a] *María Oliver-Bonet, Ph.D.,*[a] *Carlos Abad, M.D.,*[c] *María José Amengual, Ph.D.,*[d] *Joaquima Navarro, Ph.D.,*[a] *Celine Jones, B.S.,*[e] *Kevin Coward, Ph.D.,*[e] *Jaime Gosálvez, Ph.D.,*[f] *and Jordi Benet, Ph.D.*[a]

[a] Departament de Biologia Cel·lular, Fisiologia i Immunologia, Bellaterra, Spain; [b] Càtedra de Recerca Eugin-UAB, Universitat Autònoma de Barcelona, Bellaterra, Spain; [c] Servei d'Urologia, Sabadell, Spain; [d] UDIAT, Consorci Hospitalari Parc Taulí, Sabadell, Spain; [e] Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom; and [f] Departamento de Biología, Unidad de Genética, Universidad Autónoma de Madrid, Madrid, Spain

**Objective:** To investigate the relationship between the protamine 1 to protamine 2 (P1/P2) ratio and the rate of sperm DNA fragmentation in sperm samples from human males with proven fertility and three different cohorts of male patients.
**Design:** P1/P2 ratio was analyzed using acid-urea polyacrylamide acid-urea gels electrophoresis (PAGE). Sperm DNA fragmentation using sperm chromatin dispersion methodology was analyzed after 0, 4, 8, and 24 hours of incubation at 37°C.
**Setting:** University medical school and hospital.
**Patient(s):** A total of 32 human males: six with proven fertility, seven carriers of chromosome reorganizations, nine clinical varicocele patients, and ten subclinical varicocele patients.
**Intervention(s):** None.
**Main Outcome Measure(s):** P1/P2 ratio, sperm DNA fragmentation (SDF) and the rate of sperm DNA fragmentation (rSDF).
**Result(s):** P1/P2 ratio correlated with SDF and rSDF. Statistical differences were detected between fertile controls and patients for the three pathologies studied. rSDF yielded information that differed from baseline SDF. No differences were detected for P1/P2 ratio among patient groups, in reference to the three pathologies studied.
**Conclusion(s):** SDF and rSDF correlates with P1/P2 ratio in human sperm, and statistical differences were detected when fertile controls were compared with three different cohorts of patients. (Fertil Steril® 2011;95:105–9. ©2011 by American Society for Reproductive Medicine.)

**Key Words:** Spermatozoa, protamine, P1/P2 ratio, DNA integrity, infertility, DNA damage

The protamines are the most abundant nuclear proteins present in mammalian sperm. Human sperm express two types of these key nucleoproteins: protamine 1 (P1) and the protamine 2 (P2) family of proteins (P2, P3, and P4). Under normal conditions, fertile and healthy human sperm express P1 and P2 in almost identical amounts (1). However, studies of infertile human males have shown that the P1/P2 ratio deviates from that seen in normal fertile controls (2). One of the predominant functions proposed for these important sperm proteins is preserving DNA integrity in the sperm head by preventing attack from exogenous or endogenous agents (3, 4). Of particular note is that a correlation exists between P1/P2 ratio and the incidence of sperm DNA fragmentation (SDF), suggesting that this observation may be of use for clinical diagnosis in infertility clinics (5, 6).

Previous studies have established that the integrity of sperm DNA is a highly limiting factor for the correct transmission of paternal genetic information to the developing embryo (7). Because it has been further demonstrated that sperm samples from different human males can exhibit differing dynamic properties of SDF, evaluation of the rate of SDF (rSDF; SDF measured between two consecutive time points) could provide accurate information pertaining to nuclear sperm vulnerability and differential susceptibility to DNA

Received April 22, 2010; revised June 8, 2010; accepted June 16, 2010; published online July 29, 2010.
A.G-P. has nothing to disclose. J.M-H. has nothing to disclose. M.O-B. has nothing to disclose. C.A. has nothing to disclose. M.J.A. has nothing to disclose. J.N. has nothing to disclose. C.J. has nothing to disclose. K.C. has nothing to disclose. J.G. has nothing to disclose. J.B. has nothing to disclose.
A.G-P. and J.M-H. contributed equally to this work.
Supported by the Fondo de Investigación Sanitaria (FIS) (PI051834, PI080623), Generalitat de Catalunya (2009 SGR 1107), Ministerio de Ciencia y Tecnología (MCYT) (BFU 2007-66340/BFI), and Càtedra de Recerca Eugin-UAB.
Reprint requests: Jordi Benet, Ph.D., and Agustín García-Peiró, B.S., Departament de Biologia Cel·lular, Fisiologia i Immunologia, Unitat de Biologia Cel·lular i Genètica Mèdica, Facultat de Medicina, Universitat Autònoma de Barcelona, Bellaterra, 08193, Spain (TEL: 34-93-581-1773; FAX: 34-93-581-1025; E-mail: agusti.garcia@uab.es, jordi.benet@uab.es).

0015-0282/$36.00
doi:10.1016/j.fertnstert.2010.06.053

Copyright ©2011 American Society for Reproductive Medicine, Published by Elsevier Inc.

damage. This observation has already been demonstrated in a variety of mammalian species (8–10).

The present study aimed to determine the dynamics underlying sperm DNA fragmentation and, in particular, its potential relationship with protamine content. To achieve these objectives, P1/P2 ratio was compared with baseline SDF and rSDF in sperm from healthy fertile human males and in groups of males diagnosed with three differing pathologies of the reproductive system. In addition, we assessed the possibility that further understanding of the dynamic aspects of sperm DNA damage could provide key additional information about sperm health and subsequent potential embryo quality that is not apparent from analyses of baseline SDF alone, and such knowledge could be of value to clinical diagnosis.

## MATERIALS AND METHODS

### Sample Selection

Semen samples from 32 individuals (six control donors of proven fertility, seven patients who were carriers of structural chromosome reorganization, nine patients with clinical varicocele, and 10 patients with subclinical varicocele) were obtained by masturbation after 3 days of sexual abstinence. Fresh ejaculate was allowed to liquefy and was then cryopreserved. Written informed consent was given by all patients, and the study was approved by the institutional ethics committee.

### Extraction of Sperm Proteins

An aliquot of semen sample containing $14 \times 10^6$ spermatozoa was washed three times with Ham's F10 1x (GibCo, Grand Island, NY). Sperm cells were then resuspended in 200 $\mu$L of 1 mM phenylmethylsulphonyl fluoride (Sigma-Aldrich, St. Louis, MO). Sediment was then resuspended in 200 $\mu$L of 20 mM EDTA, 1 mM phenylmethylsulphonyl fluoride, and 100 mM Tris–HCl (pH 8) and processed as described previously (6). Finally, each sample was resuspended in 20-$\mu$L buffer of 5.5 M urea, 20% $\beta$-mercaptoethanol, and 5% acetic acid.

### Separation and Analysis of Sperm Proteins

Acid-urea polyacrylamide gels electrophoresis was performed on a Mini-protean System (Bio-Rad, Hercules, CA) using gels containing (final concentrations) 0.9 M acetic acid, 2.5 M urea, 15% acrylamide, 0.09% bis-acrylamide, 0.53% ammonium persulfate, and 0.53% TEMED (N,N,N,N-tetramethyl-ethylenediamine; Amersham Biosciences, Uppsala, Sweden). Gels were prerun for 1 hour at 150 V before loading. Samples were then loaded onto the gel (2.5 $\mu$L per sample) and separated by electrophoresis for one additional hour at 150 V in 0.9 M acetic acid buffer. Gels were stained with a solution of BioSafe Coomassie Blue G-250 (Bio-Rad) following the manufacturer's instructions. Finally, gels were scanned using a GS-800 scanner (Bio-Rad), and band intensity quantified with Quantity One software (Bio-Rad).

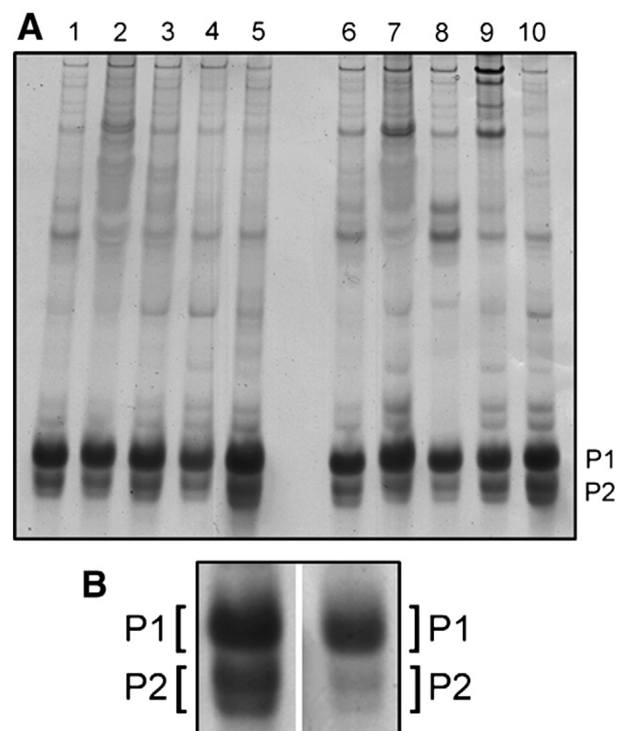
### Sperm DNA Fragmentation and the Dynamics Underlying Fragmentation

An aliquot of each sperm sample was incubated at a physiologic temperature of 37°C in phosphate-buffered saline buffer. SDF, defined as the proportion (%) of fragmented sperm present among the total number of spermatozoa in the analyzed sample, was assessed after 0, 4, 8, and 24 hours of incubation (t0, t4, t8, and t24). Samples were assessed for SDF immediately after thawing (t0), and the corresponding value was considered as the baseline for each sample—SDF (t0). Values for rSDF were determined according to the following equation: $rSDF = (SDF_{t8} - SDF_{t0}) \div 8$ hours.

The Halosperm Kit (Halotech DNA SL, Madrid, Spain), which is based on the sperm chromatin dispersion test (SCDt), was used to determine sperm DNA fragmentation following commercial instructions (11). Slides were stained for fluorescence microscopy using propidium iodide (2.5 $\mu$g/mL in Vectashield; Vector Laboratories, Burlingame, CA). For this study, 200 spermatozoa were scored per experimental point.

**FIGURE 1**

Analysis of protamine 1 (P1) and protamine 2 (P2) ratio. (**A**) Proteins extracted from spermatozoa, separated on a polyacrylamide-acetic-urea gel and stained with Coomassie blue. Lanes 1–5 and 6–10 correspond to different sperm samples from clinical and subclinical varicocele patients, respectively. (**B**) Example from a fertile donor sample with normal P1/P2 ratio (*left*) and a patient sample with a highly altered P1/P2 ratio (*right*).




*García-Peiró. P1/P2 ratio and sperm DNA fragmentation. Fertil Steril 2011.*

### Statistical Analysis

Data analyses were performed using the Statistics Package for the Social Sciences software, version 15 (SPSS, Inc., Chicago, IL). Values were compared using the Kruskal-Wallis test. Correlations were studied using the Pearson test. The level of significance was established at 95% of the confidence interval to be considered as statistically significant.

## RESULTS

### Determination of P1/P2 Ratio

Figure 1 shows analysis of P1 and P2 in human sperm samples. P1/P2 ratio was 1.2 $\pm$ 0.48 (average $\pm$ standard deviation) and ranged from 0.5 to 1.9 in fertile control sperm (Table 1), compared with 1.85 $\pm$ 0.68 (range, 1–4.68) in sperm obtained from a group of patients suffering pathologies of the reproductive system (Fig. 2A). P1/P2 ratio was significantly higher in the group of patients than in the controls ($P$=0.023). There were no significant differences in sperm P1/P2 ratio when compared between the three pathologic conditions. P1/P2 ratio was 1.7 $\pm$ 0.46 (range, 1–2.41), 2.12 $\pm$ 1.19 (range, 1.41–4.68), and 1.72 $\pm$ 0.34 (range, 1.25–2.34) in patient groups with rearranged genome, clinical varicocele, and subclinical varicocele, respectively (Fig. 2B).

Download English Version:

https://daneshyari.com/en/article/3932613

Download Persian Version:

https://daneshyari.com/article/3932613

Daneshyari.com