SPERM SOURCE INFLUENCES THE EXTENT OF DNA FRAGMENTATION AND REPRODUCTIVE OUTCOME

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ABSTRACT

Objective: During the later stages of spermatogenesis, DNA breakage is physiologically required to allow tight chromatin compaction. While most spermatozoa undergo DNA repair, reactive oxygen species (ROS) are the main cause for DNA injury. We question whether sperm chromatin integrity differs among spermatozoa isolated from different sections of the male genital tract and how it may affect reproductive outcome.

Design: Over 44 months, men with high SCF in their ejaculates (n=79) underwent surgical sampling, often bilateral, from vas deferens, epididymis, and testis. SCF was assessed by TUNEL, and clinical outcome was recorded for each sperm source for men undergoing ICSI treatment.

Materials and Methods: Ejaculates processed in the standard fashion were assessed for SCF by TUNEL. Surgical samples were minced and prepared for SCF evaluation and were cryopreserved for later use with ICSI. DNA fragmentation was measured by TUNEL on specimens isolated from all sites. TUNEL was executed by utilizing a commercial kit (In Situ Cell Death Detection Kit, Roche). At least 500 spermatozoa were counted per site under fluorescent microscopy with an adopted threshold of 15%.

Results: Of the original 79 patients, 56 were treated by ART with an average SCF of 26.3± 11.8% (11.8-42.3) in their ejaculate. In 10 men aspiration of the vas deferens resulted in 19.9± 6.4% SCF (range 35-5.8) while in 42 men epididymal sampling yielded 15.8 ± 7.7% SCF (range 38.4-5.3) and in 79 the SCF on testicular spermatozoa was 11.5± 5.7% (range 31.2-1.5). The SCF progressively decreased as TUNEL was measured proximally from the ejaculate toward the vas deferens (*P*=0.02), the epididymis (*P*=0.005), and testis (*P*<0.001). A fertilization of 68.3% (298/436), 78.4% (105/134) and 59.0% (197/334) was achieved by ICSI using ejaculated, epididymal, and testicular spermatozoa, respectively. While the clinical pregnancy rate in ejaculated spermatozoa was only 16.7%, ART outcome with ICSI utilizing these surgical sources yielded a clinical pregnancy of 37.5%. Based on these preliminary findings a subgroup of patients (n=28), with SCF of 30.5 ± 17.4 bypassed the prerequisite cycle with ejaculated spermatozoa and opted to undergo TESE with ICSI. The clinical pregnancy rate achieved was 35.0% per cycle that translated to 50% per couple treated.

Conclusions: DNA integrity assessment on the spermatozoa isolated at different levels of the male genital tract evidenced that oxidative stressors progressively alter DNA integrity toward the ejaculate. Couples unable to achieve a pregnancy using ejaculated spermatozoa with compromised DNA may benefit from undergoing testicular retrieval for diagnostic and therapeutic purposes.

INTRODUCTION

During the later stages of spermatogenesis, DNA breakage is physiologically required to allow tight chromatin compaction. While most spermatozoa undergo DNA repair, reactive oxygen species (ROS) are the main cause for DNA injury. We question whether sperm chromatin integrity differs among spermatozoa isolated from different sections of the male genital tract and how it may affect reproductive outcome.

METHODS

Over 44 months, men with high SCF in their ejaculates (n=79) underwent surgical sampling, often bilateral, from vas deferens, epididymis, and testis. SCF was assessed by TUNEL, and clinical outcome was recorded for each sperm source for men undergoing ICSI treatment. Ejaculates processed in the standard fashion were assessed for SCF by TUNEL (Figure 1).

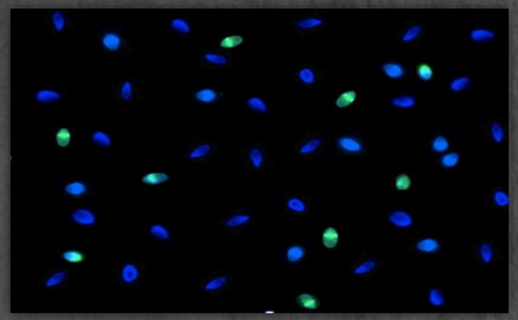

Figure 1
Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling (TUNEL) with DAPI counterstain (in blue). Green fluorescence indicate spermatozoa with SCF

RESULTS

Of the original 79 patients, 50 were treated by ART with an average SCF of 26.3± 11.8% (11.8-42.3) in their ejaculate (Table 1). In 10 men aspiration of the vas deferens resulted in 19.9± 6.4% SCF (range 35-5.8) while in 42 men epididymal sampling yielded 15.8 ± 7.7% SCF (range 38.4-5.3) and in 79 the SCF on testicular spermatozoa was 11.5± 5.7% (range 31.2-1.5). The SCF progressively decreased as TUNEL was measured proximally from the ejaculate toward the vas deferens (*P*=0.02), the epididymis (*P*=0.005), and testis (*P*<0.001). (Figure 2).

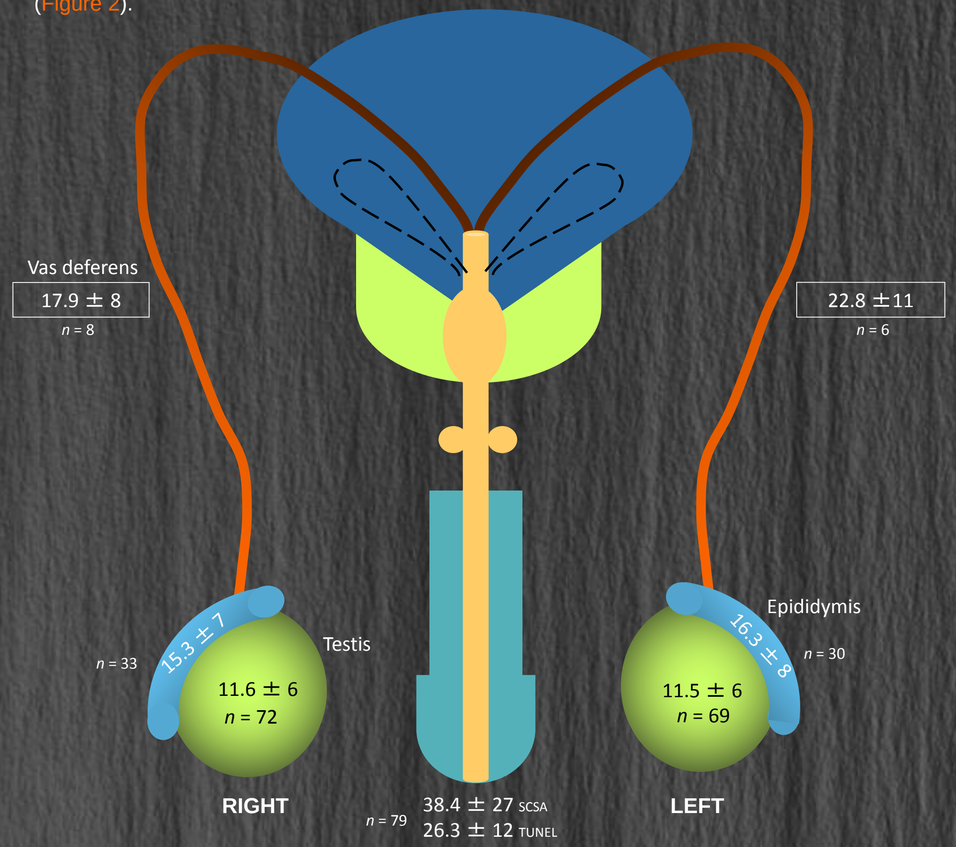

Figure 2 TUNEL result for each level of the male genital tract

Table.1 Parameters of semen analysis of men according to WHO 2010 criteria

No. of	Ejaculated Cycles	Surgical Cycles
Couples	21	29
Cycles	56	64
Female age (M yr ± SD)	37.9 ± 4	47.1 ± 12
Male age (M yr ± SD)	47.0 ± 11	53.4 ± 13

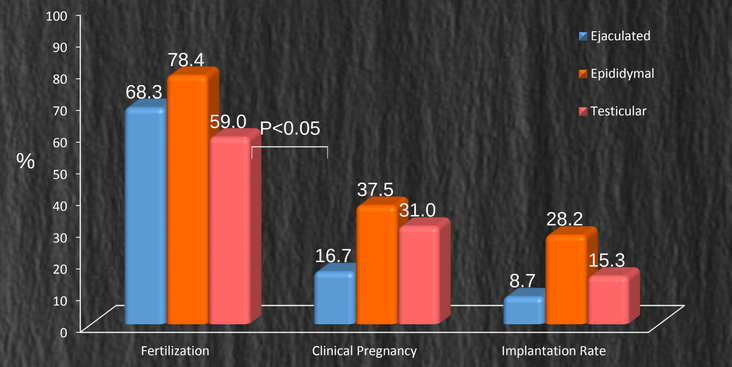

Figure 3 Fertilization, clinical pregnancy rate and implantation rates for ejaculates specimen when compared to surgically retrieved spermatozoa

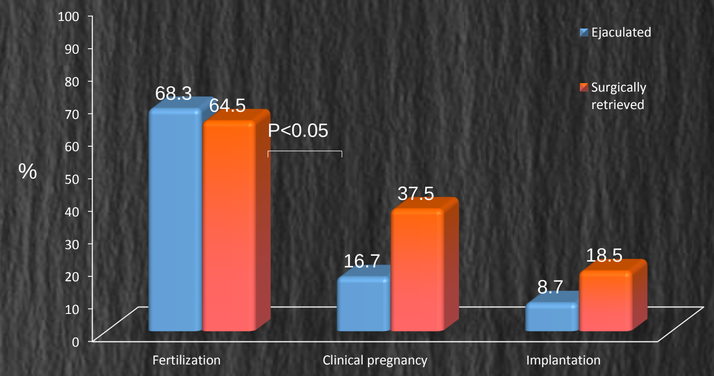

Figure 4 Fertilization, clinical pregnancy rate and implantation rates for ejaculates specimen when compared to surgically retrieved spermatozoa

CONCLUSIONS

DNA integrity assessment on the spermatozoa isolated at different levels of the male genital tract evidenced that oxidative stressors progressively alter DNA integrity toward the ejaculate. Couples unable to achieve a pregnancy using ejaculated spermatozoa with compromised DNA may benefit from undergoing testicular retrieval for diagnostic and therapeutic purposes.