Perspectives on the assessment of human sperm chromatin integrity

Gianpiero D. Palermo, M.D., Ph.D., Queenie V. Neri, M.Sc., Tyler Cozzubbo, B.Sc., and Zev Rosenwaks, M.D.

The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, New York

Apoptosis plays a significant role in regulating germ cell development by removing damaged germ cells from seminiferous tubules, thereby safeguarding the genome of a given species. The unique chromatin-packing process of the spermatozoon has important implications for both the development of male infertility screening tests and understanding of sperm chromatin characteristics, which may affect assisted reproductive technology outcomes. Sperm deoxyribonucleic acid (DNA) integrity tests have been proposed as a means to assess male gamete competence. Although these assays are currently gaining popularity, and are more often used as a supplement to traditional semen analysis, the point at which DNA damage occurs during spermiogenesis, and to what degree, remains to be elucidated. Here, we examined current studies of DNA fragmentation, to understand its origin and import, as well as its impact on pre- and post-implantation development. As the DNA fragmentation index is strongly correlated with the motility characteristics of a semen specimen, controlling for this factor may be helpful. Utilization of more sensitive assays, possibly on the actual spermatozoa used for insemination, may generate healthier conceptuses. (Fertil Steril® 2014;102:1508–17. ©2014 by American Society for Reproductive Medicine.)

Key Words: ICSI, sperm DNA fragmentation, follow-up of children, TUNEL, SCSA, SCD, COMET

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Apoptosis is a major feature of male germ cell development that orchestrates the production and function of these cells from the early stages of gonadal differentiation to the moment of fertilization. Apoptosis in male germ cells occurs during fetal life in pro-spermatogonia/gonocytes when the testes are differentiating and adjustments need to be made to achieve the optimal ratio of germ cells to Sertoli cells. The regulatory role for apoptosis in the male germ line is very similar to that in the differentiating female gonad, where a wave of apoptosis eliminates germ cells that are missing the somatic cell counterpart needed to form primordial follicles (1).

Genetic perturbance of the germ line through overexpression of *SPATA17* (2); or deletion of key genes involved in the regulation of spermatogenesis, such as *GRTH/DDX25* (3); Jmjd1a demethylase (4); or several molecular chaperones (5); increases the level of apoptosis observed in the male germ line. Apoptosis seems to occur predominantly at the pachytene spermatocyte stage with the Fas/FasL system acting as a major mediator of this process (6, 7). Apoptosis plays a significant role in regulating germ cell development by removing damaged cells in the seminiferous tubules, thereby safeguarding the genome of a given species. Sperm cells with abnormal chromatin resemble apoptotic cells not only in terms of the loss of their reproductive potential but also their chromatin changes, which, as in apoptosis, are characterized by increased deoxyribonucleic acid (DNA) sensitivity to denaturation (8).

In somatic cells, the apoptotic pathway begins with reactive oxygen species (ROS) generation in the mitochondria and culminates in DNA fragmentation, with consequent cell death. On the other hand, because of its physical architecture and the condensed nature of its chromatin, ROS generation occurs in the mature spermatozoon's midpiece and results in a loss of fertilization competence owing to compromised motility (1). More extensive DNA fragmentation may occur subsequently as a result of endonuclease activation in the sperm's chromatin, which completes the destruction of the cell (9). Moreover, the presence of apoptotic markers such as phosphotidylserine on the surface of the flawed cell triggers incoming phagocytes to silently remove targeted cells (7, 10). Unresolved DNA "nicks" from chromatin remodeling that occurs during spermiogenesis are the only other mechanism for inducing DNA damage in human spermatozoa (11).

Received August 1, 2014; revised October 2, 2014; accepted October 6, 2014; published online October 25, 2014.
G.D.P. has nothing to disclose. Q.V.N. has nothing to disclose. T.C. has nothing to disclose. Z.R. has nothing to disclose.
Reprint requests: Gianpiero D. Palermo, M.D., Ph.D., The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, 1305 York Avenue, Suite 720, New York, New York 10021 (E-mail: gdpalerm@med.cornell.edu).

Fertility and Sterility® Vol. 102, No. 6, December 2014 0015-0282/$36.00
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http://dx.doi.org/10.1016/j.fertnstert.2014.10.008

Regardless of the origin of sperm DNA damage, most studies have been performed with the assumption that the critical value is the proportion of spermatozoa displaying DNA fragmentation in a patient's sample (which is revealed by assays such as the sperm chromatin structure assay [SCSA]), rather than the DNA damage per spermatozoon (which can be revealed in the alkaline Comet assay [12, 13]). DNA damage in mature spermatozoa seems to be strongly linked to subfertility. Further, for those damaged spermatozoa that do successfully fertilize an oocyte, the consequent effect of suboptimal paternal DNA integrity on the developing conceptus has become a subject of intense investigation (14, 15). Here, we share our perspective on the DNA status assays that are carried out on the male gamete, and the repercussions they may have for counseling and treating infertile men with this condition.

BUILDING AWARENESS

Because the integrity of the sperm genome is of paramount importance to embryo development, it has been proposed that DNA breakage may subtly contribute to infertility in a way that is not revealed by simple morphological evaluation of spermatozoa. Semen containing a high proportion of cells with fragmented DNA has been blamed for a lower rate of fertilization (13, 16), poorer embryo development (17, 18), and reduced implantation rates (17, 18). Independent investigators have concluded that semen exhibiting a high percentage of damaged spermatozoa with denatured DNA (>30%) has very low fertility potential (19).

These findings may have important clinical implications for assisted reproductive techniques (ART), particularly intracytoplasmic sperm injection (ICSI), in which the use of spermatozoa with damaged DNA may lead to paternal transmission of a defective genome (20). This possibility has been discussed in reports linking abnormal embryo development to poor semen quality, and it is now assumed that a significant proportion of infertile men have clinically important levels of DNA damage in their spermatozoa (19, 21), often accompanied by a higher frequency of chromosomal defects (22).

The sperm DNA fragmentation index (DFI), as assessed by the SCSA, has been reported to affect the likelihood of pregnancy, whether generated naturally or via ART (19). This finding was true even for men with apparently normal semen characteristics but with proven DNA fragmentation (21), showing that the standard assessment of only the male gamete is inadequate. These early reports on the validity of measures of human spermatozoa DNA fragmentation, although greeted with skepticism, have raised interest among reproductive specialists, particularly because of the recognized limitations of standard semen analysis and the need to address idiopathic forms of infertility. Although most reports have provided evidence for the inverse relationship in clinical outcomes of programmed intercourse and intrauterine insemination (IUI) treatments, the effect of sperm DFI on ART pregnancies, particularly in cases involving ICSI, remains elusive (19).

Interest in assessing the role and effect of DNA fragmentation on spermatozoa has induced the European Society of Human Reproduction and Embryology Special Interest Group in Andrology to take the lead in synthesizing the current status of the field and provide recommendations for future work (23). The group stated that although no chromatin assay is definitive, the majority of current tests assess the most-accessible sites identified as containing improperly stabilized protamines and histone-bound DNA. Moreover, the investigators recognized that although the DNA fragmentation in unprocessed semen correlates with IUI outcome (24), the prepared and selected sperm population that was actually used for insemination had a low-to-normal DFI (25). Thus, using a selected population of sperm cells with a normal DFI originating from raw ejaculate with a >30% DFI may still have a negative impact on treatment outcome, suggesting that other concurrent factors have an influence (23).

This observation was supported by another study that investigated whether the impact on fertilization could be the result of a simple concordance with a semen parameter such as motility. In 136 couples undergoing in vitro fertilization (IVF) who met the inclusion criteria, sperm motility was a predictor of poor fertilization with IVF, but DFI seemed to have a greater impact (16). Finally, the Special Interest Group in Andrology advised that the interpretation of any test that measures DFI may be confounded by the presence of dead cells, particularly when these tests are performed on unprocessed semen. As explained earlier, cells that have undergone apoptosis contain fragmented DNA and may bias the overall results (23).

In a systematic review and meta-analysis of the relationship between sperm DNA damage and pregnancy outcome, it was reported that higher DNA fragmentation was associated with lower pregnancy rates and an increase in pregnancy losses (17–19, 26, 27). Although this effect on outcome was relevant to natural conception and IUI treatments, the correlation had only a modest influence on IVF pregnancy outcomes. This finding is consistent with another meta-analysis (28) showing no correlation between DFI and inferior outcomes in ICSI insemination. Overall, these findings suggest that the results of DFI assays should be used by physicians to strongly recommend to couples in which a man has a high DFI that they use ICSI insemination, thereby mitigating the effect of the DFI on treatment outcomes (26).

Other studies have shown clearly that an increased sperm DFI is associated with a higher incidence of pregnancy loss with ART, whether IVF or ICSI (18, 26), owing to impaired embryo/blastocyst development (29, 30). This effect has been confirmed by independent investigators in a larger meta-analysis that included 16 studies, with both IVF and ICSI cycles, involving a range of 18 to 637 couples. The investigation demonstrated, in almost every study, a relatively high risk of miscarriage in pregnancies that resulted from sperm samples with a high DFI.

Finally, the authors found that the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was most strongly associated with accurately tracking this risk. In fact, a European multicenter study demonstrated a substantially higher miscarriage risk when the woman's age was >35 years, and the man's >40 years (31). This result

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