

DNA fragmentation and the ultimate success of a pregnancy

Giuseppe Benagiano¹, Donatella Paoli², Francesco Lombardo², Jan J. Brosens³, Ivo A. Brosens⁴

¹Department of Gynecology, Obstetrics and Urology; ²Department of Experimental Medicine, Laboratory of Seminology-Sperm Bank, Sapienza University of Rome, Rome, Italy; ³Leuven Institute for Fertility and Embryology, Leuven, Belgium; ⁴Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, UK

Correspondence to: Giuseppe Benagiano. Department of Gynecology, Obstetrics and Urology, Sapienza University of Rome, Rome, Italy.

Email: giuseppe.benagiano@uniroma1.it.

Comment on: Agarwal A, Majzoub A, Esteves SC, et al. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. *Transl Androl Urol* 2016;5:935-50.

Submitted Dec 28, 2016. Accepted for publication Feb 06, 2017.

doi: 10.21037/tau.2017.03.46

View this article at: <http://dx.doi.org/10.21037/tau.2017.03.46>

Two very recent comprehensive reviews on sperm DNA fragmentation tests (1,2) have reopened the debate over their usefulness in improving pregnancy outcome.

In this regards, two considerations should be disentangled. First, spermatozoa are not simply carriers of paternal chromosomes, but play a role beyond fertilization. For instance, the spermatozoon transcribes genes critical for early embryonic development, inferring that integrity of sperm genome is essential for a successful gestation. Second, if sperm factors play a role in early embryonic development, are sperm DNA integrity tests useful as diagnostic and prognostic markers, especially in the context of recurrent pregnancy loss (RPL) (3) ?

The concept that sperm quality influences the success of a pregnancy is far from new but still subject of ongoing controversy: In the 1950s, Joel (4) linked RPL in five women to oligozoospermia in the partner, whereas Macleod and Gold (5) observed that semen characteristics were poorer in the partners of subjects with repeated abortions than in those of fertile couples. Then, in the 1960s, Furuholm *et al.* claimed that fathers of children who died in the perinatal period had “*a decreased concentration of spermatozoa and an increased percentage of morphologically abnormal cells*” in their semen (6). In a controlled investigation, they also showed “*a statistically highly significant increase in the percentage of abnormal spermatozoa*” in the male partners of women whose pregnancies had ended in a spontaneous miscarriage (7). Contradicting these findings, some twenty years later Homonnai *et al.* (8) concluded “*sperm concentration was significantly higher in the repeated and*

habitual abortion groups with a tendency to polyzoospermia”.

Explaining, at least in part, these contradictory early results is the notion that RPL is a multifactorial disorder caused by a multitude of factors, including uterine anomalies, hormonal imbalance, autoimmune diseases, thrombophilia, and free radical imbalance (9,10). Consequently, it has become commonplace to classify RPL as either “unexplained” or “idiopathic” miscarriage and, presumably, “explained” recurrent miscarriage. Yet, every diagnostic test currently in clinical practice lacks specificity, meaning that many women with normal pregnancies also test positive. Nevertheless, it remains standard practice to label a “positive” test as “causal”, ignoring the lack of clinical evidence, biological plausibility, or the absence of interventions that are even remotely effective. In addition, most women suffering either repeated implantation failure or multiple miscarriages ‘explained’ or not, will eventually achieve a successful pregnancy irrespective of treatment (11-13). For example, several randomized-controlled trials on RPL, defined here as three consecutive pregnancy losses, reported life-births rate of 65% or more in the placebo group (14,15). The situation is further complicated by the now confirmed observation that preclinical pregnancy loss may exceed 50% (16-18). Indeed, human fecundity rarely exceeds 35% and may be decreasing due to deteriorating semen quality (19).

It is for this reason that male factors may well be important in causing embryonic loss, but, mixed with all other variables, their effect may be masked. Factors that may influence the number, motility and morphological

features of spermatozoa include occupation, exposure to environmental toxins and smoking habits (20-22).

In the minds of many practitioners, intracytoplasmic sperm injection (ICSI) (23) renders detailed sperm analysis unnecessary. Yet, already in the nineties it was documented that embryo viability may be compromised when fertilization is achieved using an abnormal spermatozoon (24). Indeed, in 1997, Hamamah *et al.* (25) demonstrated a relationship between poor semen quality and poor embryonic development; and reported an increased incidence of abnormal sperm morphology in couples suffering from RPL. It has been shown that successful fertilization in humans requires centrosome restoration and microtubule-mediated motility and that the sperm introduces the centrosome (24-26).

Thus, while ICSI has improved dramatically the management of male factor subfertility, careful sperm analysis remains potentially a valid test when investigating persistent reproductive failure. The situation, however, is complex. In 2000, Wennerholm *et al.* (27) evaluated the outcome of 1,293 clinical pregnancies according to sperm quality. Their results indicate that sperm origin or quality is not associated with preterm birth, although there appears to be a correlation with multiple births. Also, perinatal mortality rates did not differ according to sperm quality. This led to the conclusion that obstetric outcome following ICSI is similar to that of conventional IVF and not influenced by sperm origin or quality. Carrell *et al.* (28) reported significantly higher aneuploidy rates in the sperm of partners of women with RPL when compared to general or fertile populations ($P < 0.005$). They concluded that, at least in some cases of RPL, partners have a significant increase of abnormal sperm morphology, chromosome aneuploidy or apoptosis. This finding appeared to be supported by a study using *in situ* hybridization (29).

More recently, an important study by Lin *et al.* (30) attempted to correlate sperm chromatin structure assay (SCSA) parameters, DNA fragmentation index (DFI) and high DNA stainability (HDS) with the outcomes following IVF and ICSI. No significant differences were found in fertilization rates, the number of good quality embryos and the likelihood of pregnancy between high, moderate, and low DFI or HDS groups. At the same time, men with HDS $>15\%$ had significantly increased miscarriage rates following IVF. A similar but non-significant trend was observed in the high DFI group.

Given the conflicting findings, Gil-Villa *et al.* (31) further investigated sperm characteristics in order to determine the

relationship with RPL using standard sperm parameters, lipid peroxidation of sperm plasma membranes, antioxidant capacity of seminal plasma, sperm chromatin integrity and DNA fragmentation tests. Following a full comparison, the investigators reported that RPL is associated with a higher incidence of teratozoospermia. In yet another study, sperm DNA fragmentation in seminal ejaculates in men whose partners had a history of RPL was compared to that of men with proven fertility (32). A significant difference was observed in sperm motility, but not in other parameters. However, the number of sperm with fragmented DNA was significantly increased in the group of men whose partners had RPL. The authors concluded that a higher incidence of DNA damage and poor motility can explain, at least in part, pregnancy loss in their partners.

Like sunshine after rain, these results were contradicted by a prospective study where the rate of DNA damage was measured in fresh and processed ejaculated sperm. Starting from the observation that rates of aneuploidy and the index of DNA fragmentation are higher in poor-quality sperm samples, Bronet *et al.* (33) assessed the relationship between sperm DNA fragmentation and aneuploidy rates in spermatozoa and embryos in couples suffering from RPL. They found no correlation between the extent of DNA fragmentation and the rate of aneuploidy in embryos or sperm, implying that sperm DNA fragmentation does not correlate with embryonic aneuploidies. Whether or not different methods to assess DNA fragmentation would have produced different results remains an open question.

We have tried to summarize this conflicting evidence in *Table 1*.

In the absence of unequivocal evidence, the question arises whether these tests are clinically useful in RPL, whether “explained” or not. Kumar *et al.* (34) applied receiver operating curve (ROC) analysis to semen samples from 45 patients whose spouses had “idiopathic” RPL and 20 normally fertile controls. As DNA damage was higher in RPL couples, the authors concluded that sperm DFI is useful in the management of affected couples. This conclusion appears to be supported by the findings of Carlini *et al.* (35), who also reported a correlation between increased sperm DNA fragmentation in men from couples reporting two or more spontaneous abortions and impaired reproductive capacity in terms of both rates of fertilization and of pregnancies with viable offspring. Clearly, the information gleaned from small studies needs to be interpreted with care, and large, well-designed prospective studies are urgently needed.

Table 1 Semen abnormalities and recurrent pregnancy loss

Author	Year	Number of cases	Outcome	Correlation
Joel (4)	1955	5	Repeated abortion	Oligozoospermia in the partner
Macleod & Gold (5)	1957	Not reported	Repeated abortion	Poor semen quality
Furuhjelm <i>et al.</i> (7)	1962	201	Miscarriage	Defective semen may be an etiological factor
Homonnai <i>et al.</i> (8)	1980	534	Repeated abortion	No evidence of poor semen quality
Hamamah <i>et al.</i> (25)	1997	Not reported	RPL	Suggested various factors
Carrell <i>et al.</i> (28)	2003	24	RPL	Some cases show chromosome aneuploidy and apoptosis
Bernardini <i>et al.</i> (29)	2004	20	RPL	Aneuploidy present in 10% makes interpretation difficult
Lin <i>et al.</i> (30)	2008	223	Not reported	Neither DFI nor HDS can provide independent information about embryo quality
Gil-Villa <i>et al.</i> (31)	2010	23	RPL	Pregnancy losses are probably not due to alterations in the sperm DNA package
Brahem <i>et al.</i> (32)	2011	31	RPL	Higher incidence of DNA damage and poor motility in partners
Bronet <i>et al.</i> (33)	2012	38	RPL	SDF is not related to chromosomal abnormalities in 154 embryos
Kumar <i>et al.</i> (34)	2012	45	RPL	SDF was found in approximately 26% of male partners
Carlini <i>et al.</i> (35)	2016	112	RPL	High SDF cannot yet be considered a predictive factor for the risk of RPL

RPL, repeated pregnancy loss; DFI, DNA fragmentation index; HDS, high DNA stainability; SDF, sperm DNA fragmentation.

Based on current evidence, the American Society for Reproductive Medicine (ASRM) Selective Practice Guidelines (36) emphasize that the relationship between sperm DFI and miscarriage following IVF or ICSI remains unproven. Therefore, there is no sound clinical basis as yet to recommend inclusion of sperm DNA integrity among routinely mandated tests.

One final technical comment relates to the fact that methods to evaluate chromatin integrity can only measure the percentage of cells with fragmented DNA and are based on the idea that the greater the fragmentation rate, the greater the chance that the sperm population is pathological (37). The problem is that no definite threshold of DNA damage beyond which a seminal sample can be considered pathological has been agreed and different studies provided different information, since stratifying results on the basis of the methodology applied gives different results (38). The majority of investigations reporting a significant impact following either IVF or ICSI, of the level of sperm DNA fragmentation on blastocyst and

embryo development and on miscarriage rate, utilized the TUNEL technique. On the contrary, using SCSA variable results have been obtained (39).

In conclusion, presently available sperm chromatin integrity tests represent a useful research tool allowing the study of chromatin structure, as well as of the origin and mechanisms of DNA damage. Implementation in clinical practice, however, is not yet supported because of a lack of robust evidence. Clearly, there is an urgent need for the standardization of the methods and for additional clinical studies on the impact of SDF on ART outcomes.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

- Agarwal A, Majzoub A, Esteves SC, et al. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. *Transl Androl Urol* 2016;5:935-50.
- Cissen M, Wely MV, Scholten I, et al. Measuring Sperm DNA Fragmentation and Clinical Outcomes of Medically Assisted Reproduction: A Systematic Review and Meta-Analysis. *PLoS One* 2016;11:e0165125.
- Kirkman-Brown JC, De Jonge C. Sperm DNA fragmentation in miscarriage - a promising diagnostic, or a test too far? *Reprod Biomed Online* 2017;34:3-4.
- Joel CA. The role of spermatozoa in habitual abortion. *Fertil Steril* 1955;6:459-64.
- Macleod J, Gold RZ. The male factor in fertility and infertility. IX. Semen quality in relation to accidents of pregnancy. *Fertil Steril* 1957;8:36-49.
- Furuhjelm M, Jonson B, Lagergren CG, et al. The quality of the human semen in relation to perinatal mortality. *Acta Obstet Gynecol Scand* 1960;39:499-505.
- Furuhjelm M, Jonson B, Lagergren CG. The quality of human semen in spontaneous abortion. *Int J Fertil* 1962;7:17-21.
- Homonnai ZT, Paz GF, Weiss JN, et al. Relation between semen quality and fate of pregnancy: retrospective study on 534 pregnancies. *Int J Androl* 1980;3:574-84.
- Larsen EC, Christiansen OB, Kolte AM, et al. New insights into mechanisms behind miscarriage. *BMC Med* 2013;11:154.
- Vaiman D. Genetic regulation of recurrent spontaneous abortion in humans. *Biomed J* 2015;38:11-24.
- Saravelos SH, Regan L. Unexplained recurrent pregnancy loss. *Obstet Gynecol Clin North Am* 2014;41:157-66.
- Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. *Hum Reprod* 1999;14:2868-71.
- Ogasawara M, Aoki K, Okada S, et al. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. *Fertil Steril* 2000;73:300-4.
- Coomarasamy A, Williams H, Truchanowicz E, et al. A Randomized Trial of Progesterone in Women with Recurrent Miscarriages. *N Engl J Med* 2015;373:2141-8.
- Pasquier E, de Saint Martin L, Bohec C, et al. Enoxaparin for prevention of unexplained recurrent miscarriage: a multicenter randomized double-blind placebo-controlled trial. *Blood* 2015;125:2200-5.
- Zinaman MJ, Clegg ED, Brown CC, et al. Estimates of human fertility and pregnancy loss. *Fertil Steril* 1996;65:503-9.
- Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *N Engl J Med* 1988;319:189-94.
- Wang X, Chen C, Wang L, et al. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. *Fertil Steril* 2003;79:577-84.
- Benagiano G, Farris M, Grudzinskas G. Fate of fertilized human oocytes. *Reprod Biomed Online* 2010;21:732-41.
- Edmonds DK, Lindsay KS, Miller JF, et al. Early embryonic mortality in women. *Fertil Steril* 1982;38:447-53.
- Parazzini F, Bocciolone L, Fedele L, et al. Risk factors for spontaneous abortion. *Int J Epidemiol* 1991;20:157-61.
- Bulletti C, Flamigni C, Giacomucci E. Reproductive failure due to spontaneous abortion and recurrent miscarriage. *Hum Reprod Update* 1996;2:118-36.
- Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992;340:17-8.
- Simerly C, Wu GJ, Zoran S, et al. The paternal inheritance of the centrosome, the cell's microtubule-organizing center, in humans, and the implications for infertility. *Nat Med* 1995;1:47-52.
- Hamamah S, Fignon A, Lansac J. The effect of male factors in repeated spontaneous abortion: lesson from in-vitro fertilization and intracytoplasmic sperm injection. *Hum Reprod Update* 1997;3:393-400.
- Schatten G. The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. *Dev Biol* 1994;165:299-335.
- Wennerholm UB, Bergh C, Hamberger L, et al. Obstetric outcome of pregnancies following ICSI, classified according to sperm origin and quality. *Hum Reprod* 2000;15:1189-94.
- Carrell DT, Wilcox AL, Lowy L, et al. Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. *Obstet Gynecol* 2003;101:1229-35.
- Bernardini LM, Costa M, Bottazzi C, et al. Sperm aneuploidy and recurrent pregnancy loss. *Reprod Biomed Online* 2004;9:312-20.
- Lin MH, Kuo-Kuang Lee R, et al. Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. *Fertil*

- Steril 2008;90:352-9.
31. Gil-Villa AM, Cardona-Maya W, Agarwal A, et al. Assessment of sperm factors possibly involved in early recurrent pregnancy loss. *Fertil Steril* 2010;94:1465-72.
 32. Brahem S, Mehdi M, Landolsi H, et al. Semen parameters and sperm DNA fragmentation as causes of recurrent pregnancy loss. *Urology* 2011;78:792-6.
 33. Bronet F, Martínez E, Gaytán M, et al. Sperm DNA fragmentation index does not correlate with the sperm or embryo aneuploidy rate in recurrent miscarriage or implantation failure patients. *Hum Reprod* 2012;27:1922-9.
 34. Kumar K, Deka D, Singh A, et al. Predictive value of DNA integrity analysis in idiopathic recurrent pregnancy loss following spontaneous conception. *J Assist Reprod Genet* 2012;29:861-7.
 35. Carlini T, Paoli D, Pelloni M, et al. Sperm DNA fragmentation in Italian couples with recurrent pregnancy loss. *Reprod Biomed Online* 2017;34:58-65.
 36. Practice Committee of the American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing: a guideline. *Fertil Steril* 2013;99:673-7.
 37. Zini A, Sigman M. Are tests of sperm DNA damage clinically useful? Pros and cons. *J Androl* 2009;30:219-29.
 38. Simon L, Brunborg G, Stevenson M, et al. Clinical significance of sperm DNA damage in assisted reproduction outcome. *Hum Reprod* 2010;25:1594-608.
 39. Tamburrino L, Marchiani S, Montoya M, et al. Mechanisms and clinical correlates of sperm DNA damage. *Asian J Androl* 2012;14:24-31.

Cite this article as: Benagiano G, Paoli D, Lombardo F, Brosens JJ, Brosens IA. DNA fragmentation and the ultimate success of a pregnancy. *Transl Androl Urol* 2017;6(Suppl 4):S539-S543. doi: 10.21037/tau.2017.03.46