Correlation between semen analysis by motile sperm organelle morphology examination and sperm DNA damage

Regression analysis of 538 semen samples demonstrated that percentages of normal nuclear sperm and all spermatozoa with abnormalities of nuclear form at high magnification had significant negative correlation with percentages of DNA fragmentation. On the other hand, there was a positive correlation between percentages of spermatozoa with nuclear vacuoles and those with DNA fragmentation. (Fertil Steril® 2010;■:■–■. ©2010 by American Society for Reproductive Medicine.)

As recent studies have demonstrated that intracytoplasmic morphologically selected sperm injection (IMSI), based on sperm normality as defined by the motile sperm organelle morphology examination (MSOME), improves ICSI outcome (1–11), attention has been given to the existence of a correlation between sperm morphologic abnormalities observed at high magnification (>×6,000), particularly as to the presence of nuclear vacuoles (12, 13), and DNA damage. Nevertheless, the significance of each particular nuclear sperm form observed at MSOME in relation to DNA alteration has yet to be determined. To better comprehend the diagnostic/prognostic value of morphologic analysis of semen by high magnification, the present study aimed to evaluate the correlation between the MSOME classification and sperm DNA damage.

Joao Batista Alcantara Oliveira, M.D., Ph.D.[a,b,c]
Fabiana Cagnoto Massaro, B.S.[a]
Ricardo Luis Razera Baruffi, M.D.[a,b,c]
Ana Lúcia Mauri, B.S.[a]
Claudia Guilhermino Petersen, Ph.D.[a,b]
Liliane F.I. Silva, B.S.[a,b,c]
Laura D. Vagnini, B.S.[c]
José Gonçalves Franco, Jr, M.D., Ph.D.[a,b,c]
[a] *Center for Human Reproduction Prof Franco Junior, Ribeirao Preto*
[b] *Department of Gynecology and Obstetrics, Botucatu Medical School São Paulo State University, Botucatu*
[c] *Paulista Center for Diagnosis, Research and Training, Ribeirao Preto, Brazil*

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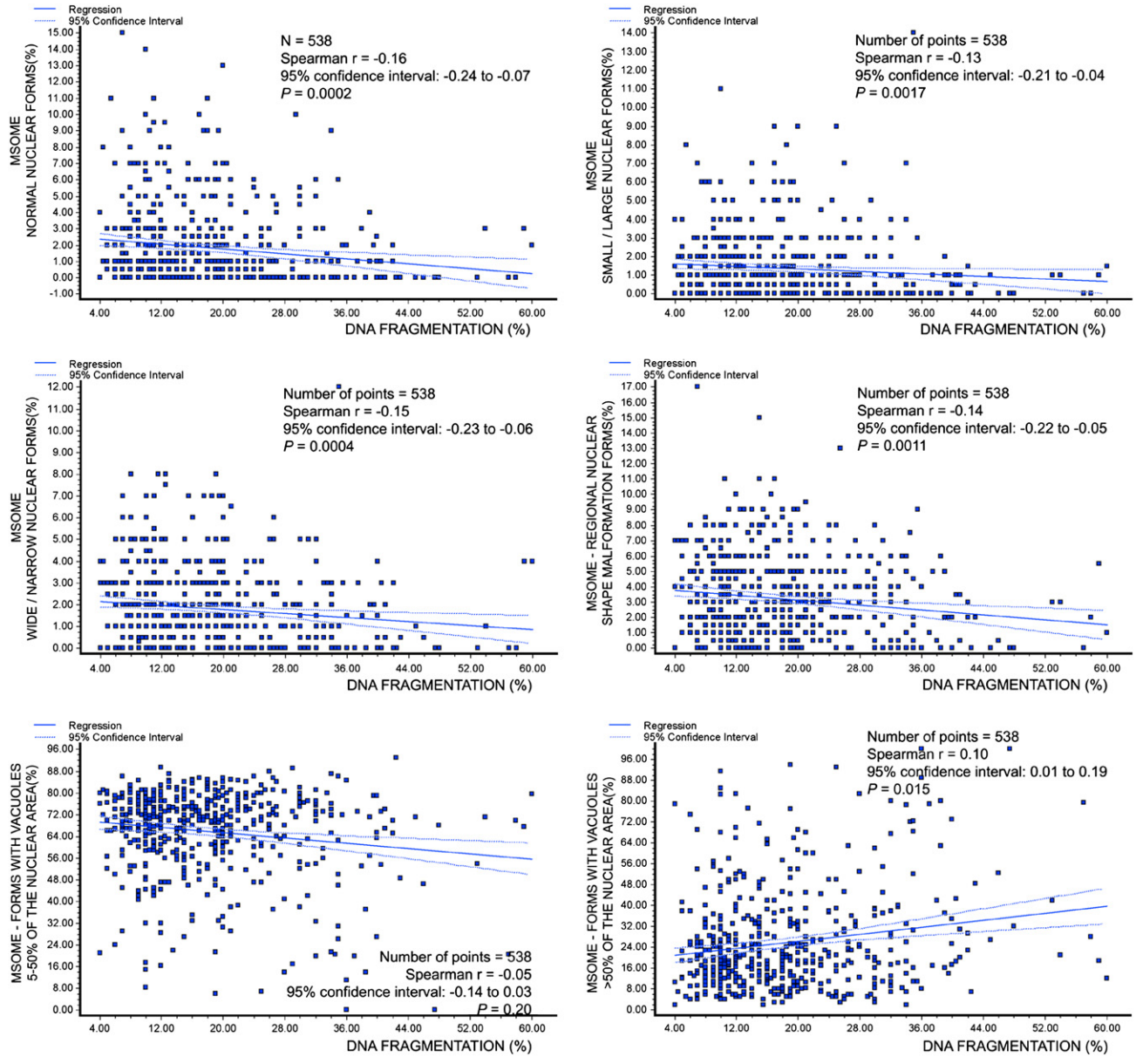
Reprint requests: Prof. J. G. Franco, Jr, Av. Prof. João Fiusa, 689–CEP 14025-310, Ribeirão Preto–SP–Brazil (FAX: 551639111100; E-mail: franco@crh.com.br).

Semen samples were obtained from 538 men from an unselected group of infertile couples. This study received internal Institutional Review Board approval. A portion of each semen sample was processed for MSOME and the remainder analyzed for DNA damage. Determination of morphology by MSOME was carried out as previous described (12, 14). At least 200 motile spermatozoa per patient were evaluated at ≥×8,400 magnification by inverted microscope equipped with Nomarski differential interference contrast optics, and the percentages of the following spermatozoa forms were determined: normal nuclear spermatozoa (smooth, symmetric, and oval nucleus measuring 3.28 ± 0.20 μm in width and 4.75 ± 0.20 μm in length, with absence of vacuoles occupying >4% of nuclear area) (7); abnormalities of nuclear form (spermatozoa with small or large oval nuclear forms [length ≤4.19 μm or ≥5.31 μm] (15), spermatozoa with wide or narrow nuclear forms [width >3.7 μm or <2.9 μm] (3), and spermatozoa with regional shape abnormality of nuclear form [extrusion or invagination of the nuclear chromatin] (3)); abnormalities of nuclear chromatin content (spermatozoa with vacuoles occupying 5%–50% of the nuclear area and spermatozoa with large nuclear vacuoles [vacuoles occupying >50% of the nuclear area]). Sperm cells with any severe abnormality (e.g., pin, amorphous, tapered, round, multinucleated head, double tail) easily identified at low magnification (×200–×400) were not assessed in this study. Spermatozoids that presented more than one alteration were classified as being the most severely altered (3, 4) (small/large < wide/narrow < regional shape abnormality < with vacuoles occupying >4% of the nuclear area). DNA damage was measured by DNA fragmentation analysis using the terminal deoxyribonucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay as previous described (12, 16). At least 200 spermatozoa in randomly selected areas on microscope slides were evaluated using a fluorescent microscope, and the percentage of spermatozoa with fragmented DNA (TUNEL-positive cells) was determined. Correlations were performed using the Spearman rank correlation test. The level of significance was set at $P<.05$.

The average age of the men was 37.4 ± 6.2 years, 34.6% had fathered at least one child (or a pregnancy that had ended in miscarriage), 14.1% had varicocele; 12.5% were smokers, 62.8% regularly used alcohol, and 13.6% regularly took vitamin

FIGURE 1

Relationship between percentages of DNA fragmentation and motile sperm organelle morphology examination (MSOME). Individual data points, regression line, and confidence interval are shown for each sperm form.



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supplements. According to MSOME, in samples examined, the mean incidence of morphologically normal nuclear spermatozoa was 1.8 ± 2.5% and the incidence of each abnormal form was: large/small spermatozoa 1.4 ± 1.7%; wide/narrow spermatozoa 1.8 ± 1.8%; spermatozoa with regional disorder 3.2 ± 2.7%; spermatozoa with vacuoles occupying 5%–50% of the nuclear area 65.7 ± 16.3%; and spermatozoa with vacuoles occupying >50% of the nuclear area 25.9 ± 19.2%. The mean DNA fragmentation was 18.4 ± 10%. Regression analysis demonstrated different results depending on the sperm form considered. First, there was a significant negative correlation between percentage of DNA fragmentation and percentage of normal nuclear sperm forms ($P<.05$; $r = -0.16$). In the same manner, percentages of sperm with abnormal nuclear form also presented significant negative correlations with percentages of DNA fragmentation, as follows: spermatozoa with small or large oval nuclear forms ($P<.05$, $r = -0.13$); spermatozoa with wide or narrow nuclear forms ($P<.05$, $r = -0.15$); and spermatozoa with regional shape abnormality of nuclear form ($P<.05$, $r = -0.14$). On the other hand, in relation to sperm with abnormalities of nuclear chromatin content, there was not a significant correlation between percentage of DNA fragmentation and percentage of spermatozoa with vacuoles

occupying 5%–50% of the nuclear area ($P>.05$; $r = -0.05$). However, there was a significant positive correlation between the percentage of DNA fragmentation and the percentage of spermatozoa with large nuclear vacuoles ($P<.05$, $r = 0.10$). Figure 1 summarizes these results.

Success in human reproduction depends on, among other factors, the integrity of sperm DNA. Clinical evidence now shows that sperm DNA damage is detrimental to reproductive outcomes and that the spermatozoa from infertile men possess substantially more DNA damage than do those of fertile men (17). The present study evidenced a significant negative correlation between normal nuclear sperm levels at MSOME evaluation and DNA fragmentation levels. This finding corroborates the result of a previous study by our group (12), in which a relatively low DNA fragmentation percentage (15.9%) was found in normal nuclear spermatozoa selected by high magnification. However, negative correlation with DNA fragmentation was not exclusive of normal nuclear sperm forms. The forms with alterations in nuclear dimensions (small/large or wide/narrow) presented a significant negative correlation with DNA fragmentation levels very close to those presented by normal nuclear forms. These findings indicate safety, from the perspective of DNA fragmentation, in using spermatozoa with these nuclear alterations. In addition, the sperm form with regional disorders (extrusion and/or invagination of chromatin), that theoretically would have a greater possibility of DNA damage, also presented negative correlation with DNA fragmentation level near that observed with normal nuclear form. In a study comparing normal nuclear sperm forms with spermatozoa exclusively with chromatin extrusion (18), similar levels were also observed between groups as to DNA fragmentation, although those with extrusion presented more DNA denaturation than normal ones. In this context, the spermatozoa with altered nuclear form appear to present a prognosis as favorable as that of normal nuclear sperm in relation to the possibility of DNA fragmentation.

On the other hand, the identification of abnormal chromatin content represented a change in the correlation with DNA fragmentation, in that this parameter was not significantly correlated with sperm showing vacuoles occupying 5%–50% of the nuclear area but presented a significant positive correlation with those presenting large nuclear vacuoles (>50% of the nuclear area). These results, besides indicating the detrimental effect of nuclear vacuole presence on sperm quality, demonstrate that the extent to which the nucleus is compromised (by vacuoles) reflects the extent of sperm DNA damage. These data corroborate the findings of other studies. Berkovitz et al. (3, 4), who graded the severity of nuclear morphologic alterations while highlighting the presence of large vacuoles, suggested that vacuolization of the sperm nucleus reflects some underlying chromosomal or DNA defects. Berkovitz et al. (5) and Bach et al. (19) reported that the presence of vacuoles provokes harm to embryo development, reduction in the pregnancy rate and increase in the miscarriage rate. Vanderzwalmen et al. (8) demonstrated that nuclear vacuoles negatively affect the percentage of embryos that reach the blastocyst stage in ICSI cycles. Franco et al. (12) showed an association between large nuclear vacuoles and both the presence of DNA fragmentation and denaturation in the spermatozoa. In addition, Garolla et al. (13) showed that the presence of nuclear vacuoles affects mitochondrial function, chromatin status, and aneuploidy rate.

The present data support recent studies that propose classifications for defining semen quality based on analysis at high magnification, with special emphasis on the number and extension of nuclear vacuoles (8, 20, 21). But despite the diagnostic and prognostic advantages of these classifications, concerns are raised from the clinical point of view regarding individuals that present nuclear vacuoles in 100% of their spermatozoids (in our sampling, ~10% of men). In this situation, obtaining success in the reproduction processes should depend on the possibility (but not certainty) of correction, by oocytes, of probable damage in sperm DNA (20, 22). Furthermore, incomplete or incorrect repair of sperm DNA damage also can lead to impairment in the reproductive process and, in turn, in the offspring. On the other hand, the existence of a correlation would imply that strategies to reduce sperm DNA damage would serve as alternatives for diminishing the percentage of vacuolated spermatozoa (23–26).

In conclusion, the results of the present study show that both normal and abnormal nuclear forms, under high-magnification analysis, appear to be equally favorable from a DNA fragmentation point of view. The only sperm type that correlates with a high rate of DNA fragmentation is the category of sperm with >50% vacuolated nucleus. Based on clinical/laboratory findings on the repercussions of possible DNA damage in offspring (27), and given that sperm nuclear vacuoles are evaluated more precisely at high magnification by MSOME (15), the present results support the routine use of MSOME for ICSI and as a criterion for semen analysis with potential clinical repercussions.

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