

What is known already: Sperm with fragmented DNA may be morphologically normal and therefore still be selected for fertilization following standard sperm analysis. Assessment of spermatozoa under high magnification provides details about the presence and size of the human sperm vacuoles. Their presence has been already shown to correlate with abnormal chromatin condensation. Studies evaluating the association of sperm morphology and DNA fragmentation have found contradictory results. Currently there is no non-invasive test allowing for determination of spermatozoa DNA damage.

Study design, size, duration: Semen samples were collected from ten partners (mean age 33.6 ± 4.20) of women undergoing IVF treatment at INVICTA Fertility Clinic in August 2015. Standard semen parameters were obtained according to the guidelines of the WHO 2010. The semen samples were prepared using the swim-up method. Men had a normal karyotype, no AZF microdeletions, no CFTR gene mutations. Exclusion criteria were history of varicocele, cryptorchidism, presence of sperm infections, anti-sperm antibodies, smoking and obesity.

Participants/materials, setting, methods: MSOME analysis was performed and spermatozoa were graded into four groups (I-IV) according to Vanderzwalmen's criteria, assigning the best quality spermatozoa, without vacuoles to Grade I. A total of 1869 motile spermatozoa were analyzed by the Neutral Comet assay. Comets were categorized into five classes (0–4) assigning the spermatozoa without DNA fragmentation to Class 0.

Main results and the role of chance: Spermatozoa from MSOME Grade I presented the highest number of spermatozoa without nuclear DNA damage, than Grade II, III, IV groups ($p < 0.05$) as determined with the neutral Comet assay (dsDNA damage).

Grade I spermatozoa presented significantly lower DNA decondensation than Grade II, III, IV ($p < 0.05$).

Our results suggest that even MSOME Grade I spermatozoa shows some DNA fragmentation. In that group about 10% of the spermatozoa presented DNA decondensation, but hardly any (<0.2%) had high degree of DNA fragmentation. Additionally, our results suggest that even MSOME Grade IV spermatozoa normozoospermic patients consist of approximately 32% spermatozoa that have non fragmented nuclear DNA.

Limitations, reasons for caution: The study was limited by relatively small number of normozoospermic patients.

Wider implications of the findings: Our study confirmed the association between nuclear vacuoles and chromatin damage. However, the observation of nuclear vacuoles in the sperm without precise DNA fragmentation investigation is not sufficient for optimal sperm selection for ICSI. New sperm selection methods allowing for low/noninvasive determination of DNA fragmentation are still needed.

Trial registration number: Not applicable.

P-088 Conventional sperm analysis: is it telling us the whole story?

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Study question: Can a comprehensive assessment of sperm quality including gene expression evaluation of protamines reveal male factor functional defects affecting embryo development and ART outcomes?

Summary answer: Altered gene expression ratio of protamine 1 and 2 in human sperm samples is associated to defective embryo development and reduced blastocyst rate.

What is known already: Male infertility impacts 50% of infertile couples. Conventional semen analyses, while helpful, may fail to identify subtle sperm defects, which could influence ART outcomes. A considerable number of studies have reported the utility of evaluating additional sperm parameters to identify these subtle functionality deficiencies. Recent studies have associated aberrant sperm gene expression protamine ratios (mRNA-P1/P2 ratio) and male infertility. Protamines are sperm-specific nuclear proteins essential for packaging the paternal genome in the sperm nucleus. The normal mRNA P1/P2 ratio has been described as 1:1. Variations in this proportion may have implications in sperm function, fertilization and embryo development.

Study design, size, duration: A total of 30 human sperm samples from couples with suspected male factor and at least one previous failed IVF-cycle seeking ART at our center between December 2014 and December 2015 were included

in this retrospective study. Comprehensive semen quality analyses including concentration, motility, morphology, apoptosis, sperm DNA fragmentation (SDF) and mRNA-P1/P2 ratio were performed in all samples after 2 days of abstinence. The impact of semen quality parameters on embryo development was explored.

Participants/materials, setting, methods: Conventional semen parameters (concentration, motility and morphology) were analyzed based on WHO criteria (2010). Apoptotic cell levels were evaluated by flow cytometry using annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) assay. SDF was assessed using SCSA methodology and mRNA P1/P2 ratio was evaluated by qRT-PCR (Ferticert® test). The impact of semen parameters on embryo development (fertilization rate, blastocyst rate and good morphological quality blastocyst rate) was explored.

Main results and the role of chance: A significant correlation was found between mRNA-P1/P2 ratio and blastocyst rate (Rho Spearman test, $R = 0.680$, $p < 0.05$). Low mRNA-P1/P2 ratio was associated with reduced blastocyst rate and high mRNA-P1/P2 levels were linked to high blastocyst yield. No other sperm parameter was found to significantly correlate with either fertilization or embryo development rates. Normal P1/P2 ratio is established between 0.92–1.08 values; levels below and above this range are considered to be altered.

Samples with low mRNA-P1/P2 ratio showed a blastocyst rate significantly lower when compared to normal mRNA-P1/P2 ratio samples (46.89 ± 8.15 vs 61.69 ± 2.28 , $p = 0.034$). High mRNA-P1/P2 samples showed a blastocyst rate significantly higher than controls (93.94 ± 6.06 versus 61.69 ± 2.28 $p = 0.034$).

mRNA-P1/P2 ratio correlated with the percentage of early apoptotic cells (Rho Spearman test, $R = 0.576$, $p < 0.05$). Samples with low mRNA-P1/P2 levels showed lower apoptotic live sperm levels compared to samples with normal mRNA-P1/P2 ratios (15.35 ± 1.11 vs 28.88 ± 11.69 , $p = 0.001$). No differences in any of the other semen quality parameters studied were found between the groups.

These results are highlighting the importance of evaluating mRNA-P1/P2 ratio in male factor suspected cases in order to identify functional deficiencies undetected in a conventional spermogram.

Limitations, reasons for caution: A higher number of samples would be necessary in order to improve the statistical power. Further data regarding clinical outcomes must be included to confirm the predictive value of the analyzed parameters. Female factor controlled cases (ovodonation cycles) would help confirm the conclusions drawn in the present study.

Wider implications of the findings: Gene expression protamine ratio assessment is a reliable and valuable biomarker that offers extra information about sperm functionality. The implementation of this technique as part of the routine spermogram performed in IVF-centers could be very useful to identify male factor infertility cases and improve diagnosis and ART-treatment of infertile couples.

Trial registration number: None.

P-089 Men born small for gestational age may be at risk of developing male-factor subfertility

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Study question: In males accepted for infertility intervention, are non-optimal birth characteristics associated with a higher risk of male-factor infertility in adulthood?

Summary answer: Men needing infertility treatment are often born with non-optimal birth characteristics suggesting that being born SGA may be a risk factor for infertility in males.

What is known already: Being born with low birth weight, prematurely or SGA increases the risk of developing diseases in adulthood, such as those associated with metabolic syndrome. An association between non-optimal birth characteristics and the reproductive ability in adulthood has been demonstrated in some previous studies, whereas other studies have not been able to find an increased risk of infertility. Men conceiving by ICSI are more often born SGA than men becoming fathers by conventional IVF, suggesting that intrauterine growth restriction may be a risk factor of male factor infertility in adulthood.

Study design, size, duration: Retrospective cohort study of a clinical sample of 1,152 men born in Sweden between 1973 and 1986, partners in couples who following clinical evaluation and diagnosis were accepted for infertility