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Editorial

New Markers for the Assessment of Sperm Quality

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Fertilization is a step by step process that virtually starts at the gametogenesis and culminate with the first division of the zygote. A successful fertilization depends on gamete potential and quality. The last edition of World Health Organization manual [1] provides guidelines for a series of tests and the conditions that must be satisfied to optimize the fertilization competence of spermatozoa. Whether sperm fertilizing capability may be predicted from the evaluation of semen parameters as concentration, motility and morphology is at present matter of debate. Numerous studies aimed to correlate semen characteristics and fertilization potential concluded that basic spermiogram cannot provide complete diagnostic information because it does not assess sperm function [2,3].

In the last decades many tests have been developed in order to implement semen evaluation and better predict the sperm fertilizing ability. Among them zona free hamster egg penetration test, acrosome reaction assay, zona binding assay and hypoosmotic swelling test were applied. However it was soon clear that these could not provide a clear picture of the sperm fertilizing ability, highlighting the need to identify new markers of sperm function mainly related to DNA and membrane integrity and the metabolic activity [4].

Although new specific tests of sperm functional potential still lack standardized protocols and cutoffs, they have been introduced and widely applied in many of the IVF/andrology laboratories all over the world. Main tests related to different sperm function are at present: CASA software (computer assisted sperm analysis), chromatin condensation, DNA fragmentation, mitochondrial potential, lipid peroxidation. CASA, introduced in the clinical centers in the past decades, offers objective measurements of either sperm concentration and kinematic (Figure 1). Nonetheless the cost of the CASA machine, this technology is at present widespread applied since allows to achieve reproducible and comparable results thus avoiding the variability due to the operator evaluation.

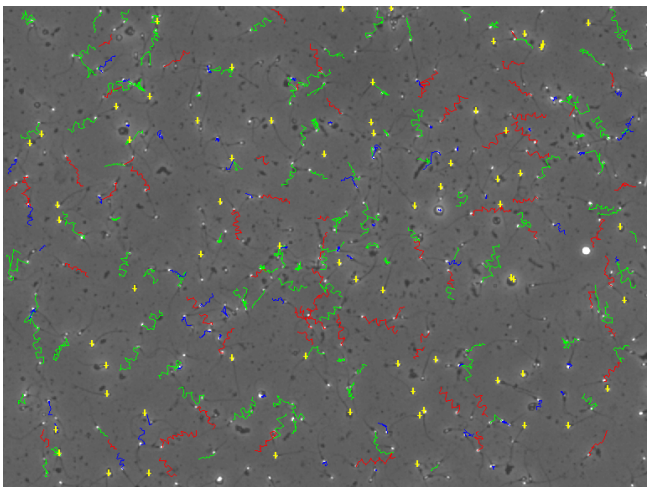

Figure 1: Representative image of a CASA software based analysis of sperm concentration and motility.
Different colors respectively indicate the following sperm movements: red rapid progressive, green slow progressive, blue *in situ* and yellow immotile

Sperm DNA damage is a factor which affects the functional potential of the spermatozoa and in long term embryo development and offspring production [5]. The loss of DNA integrity may have different etiologies from deficient packaging of chromatin to oxidative stress and apoptotic processes induced by adverse environmental conditions. The tests evaluating nuclear chromatin decondensation (aniline blue and chromomycin α) and DNA fragmentation (Tunel, SCD/Halo, Comet) have been shown to be good predictors of fertilization rate and possibly of the reproductive outcome although some differences in the sensitivity of different tests have been reported [6].

Sperm membrane integrity is also essential to be assessed. Plasma membrane represents the borderline between cell and the external environment and for gametes is the site where are allocated receptors and ligands responsible for sperm-oocyte interaction. Furthermore, membrane integrity is needed for sperm survival in the female genital tract, fluidity for sperm motion and the gamete membranes fusion.

Changes in the membrane structure may be associated with disarrangement of lipids and in particular to lipid peroxidation induced by oxidative stress. Test based on a oxidation sensitive fluorescent fatty acid analog (C11-Bodipy) is at present a reliable method to determine the damage of sperm plasma membrane generated by different environmental insults in animals [7,8].

Mitochondrial functionality and associated physiological events as intracellular calcium, have been related with sperm function [9,10]. ATP-related energy metabolism is at the base of a series of sperm activation processes, in particular inner mitochondrial membrane potential (IMM) is associated with the tail motility pattern and fertilizing ability. Specific MitoTracker dyes may identify the mitochondrial functioning, in particular JC1 is new generation fluorescent probe which differentiates the state of polarization of IMM.

Finally, since sperm contains specific and unique RNAs that are delivered in the oocyte at the time of fertilization, studies on sperm genomic expression pattern in sub-fertile men provided new hints on a possible use of RNAs profiles as clinical markers of sperm fertility competence [11].

Conclusion

A traditional spermiogram may report normal values but cannot evaluate cryptic sperm defects that, in turn, may affect either fertilization and embryo development. The fertility potential of a seminal sample results from a strict synergy of sperm functional

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competences. Therefore, to identify new markers of sperm quality may be essential to either add new tests to the conventional analyses and, consequently, to formulate new therapies aimed to alleviate male sub-fertility and support the management of an infertile couple.

Here we have described some of the recent biomarkers that appear to be promising tools to identify the fertilization competence of a sperm population.

Hopefully, in a close future these new markers will be associated with routine semen analysis, after careful quality control measurements and the establishment of evidence-based standard values [12].

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