Seminal reactive oxygen species, a novel biochemical assay for testing male fertility?

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Infertility is defined as a failure to achieve a positive pregnancy test over 12 months of regular unprotected sex. It is a devastating condition affecting 15% of couples and is socially marginalizing. Despite significant focus on the female, nearly half of cases are in fact due to poor sperm function in the male partner. It is therefore surprising that despite the existence of numerous diagnostic tools and management options currently available for female infertility, very few exist for their male counterparts. So, what can we do to diagnose men with impaired fertility promptly and direct couples to effective management strategies?

Male infertility

Couples who are unable to conceive naturally are routinely referred by their GP for hospital investigations to determine whether either partner has impaired fertility. Approximately half of assisted reproduction treatments (ART) are related to male infertility and the Human Fertilisation and Embryology Authority reports that this number more than doubled between 2009 and 2013. Factors which can impair male fertility are numerous, ranging from infection, testicular pathologies and genetic disorders to endocrine or other systemic diseases. Lifestyle features such as cigarette smoking, illicit drug use, alcohol consumption, a high fat diet or increased scrotal temperature play an additional negative role on male reproduction. However, despite improvements in diagnostic examination, the cause remains unknown in 40% of cases.

Diagnosis and current limitations

Currently, the only NHS diagnostic tool for male infertility is semen analysis. Semen analysis involves the assessment of spermatozoal concentration, motility and morphology under specific World Health Organization criteria (Table 1). WHO criteria for the evaluation of human semen provide reference values for seminal characteristics and are associated with a couple's likelihood to achieve pregnancy within 12 months. Results above or below the reference values are essentially a standardized guide regarding a man's fertility status. However, changes in male fertility are not detected with standard semen examination in 15% of couples. In these cases, couples find the lack of diagnosis distressing as no explanation can be given for their presentation.

Table 1. Lower reference limit and their 95% CI for semen parameters from fertile men whose partners had a time-to-pregnancy of 12 months or less [WHO Criteria, Cooper et al. (2010)].

| Parameter                       | Lower reference limit (95% CI) |
|---------------------------------|--------------------------------|
| Semen volume (ml)               | 1.5 (1.4–1.7)                 |
| Sperm concentration (106 per ml or M/ml) | 15 (12–16)                  |
| Total motility (PR* + NP**, %)  | 40 (38–42)                    |
| Progressive motility (PR, %)    | 32 (31–34)                    |
| Sperm morphology (normal forms %) | 4 (3.0–4.0) %               |

PR* progressively motile, NP** non-progressively motile

Conventional semen analysis can only evaluate male reproductive capacity to a certain extent, therefore it is clinically important to develop novel methods for the assessment of seminal quality. Over the last few years the role of seminal reactive oxygen species (ROS) have gained considerable interest because early diagnosis of high oxidative stress could potentially guide couples to effective therapeutic approaches. ROS detection could be a key factor when advising couples about optimal sperm quality before natural or assisted conception.
ROS are generated in seminal plasma from endogenous sources such as leukocytes or immature spermatozoa, and are physiologically required for sperm movement and fertilization of the oocyte. However, in men with testicular pathology such as varicoceles (enlargement of blood vessels in the scrotum), sexually transmitted infections (STIs) or metabolic imbalance due to high energy diets or toxic environmental factors, ROS production is disproportionate (Figure 1).

Once excessive ROS production surpasses the antioxidant capacity of free radical scavengers within the semen oxidative stress is generated, sperm plasma membrane fatty acids undergo lipid peroxidation by ROS and multiple DNA defects can occur. DNA is damaged via fragmentation both in the sperm nucleus as well as the mitochondria and, as a result, sperm become dysfunctional and can impede fertilization of the oocyte, affect embryo development or lead to pregnancy loss and miscarriage.

**How can we practically measure seminal ROS?**

Indirect assays to measure ROS include analysis of sperm chromatin and evaluation of DNA damage via fragmentation. The Department of Andrology at Hammersmith Hospital recently developed a direct assay based on chemiluminescence. Light is emitted when luminol (5-amino-2,3-dihydro-1,4-phtalazinedione) is oxidized. Mean chemiluminescence is measured over a 10-minute period and compared to a negative control to eliminate background variation that may confound readings. Results are reported as relative light units per second (RLU/sec). A raised ROS is defined as >3.8 RLU/sec/106 sperm.

**Clinical implications and future avenues**

Testing for ROS can be particularly relevant in men with genitourinary infections, varicocele or exposure to harmful lifestyle factors. For example, increased seminal oxidation due to STIs can be managed with antibiotics and metabolic causes such as high fat diet or smoking can be eliminated with lifestyle changes. In addition, men with varicocele exhibiting high ROS through testing could be at substantial risk for DNA damage and may benefit from surgical varicocele repair. Finally, ROS levels may be higher in couples with recurrent miscarriage, therefore seminal ROS measurement may have diagnostic and therapeutic potential for couples with unexplained recurrent pregnancy.

Male infertility is increasingly viewed as a marker of a man’s general health. Biochemical assays such as seminal ROS may open new avenues for evaluating and treating couples with infertility. It is critical to build links with specialist clinicians to develop new services, and for further laboratory research to improve the diagnostic tools available to help a couple start a family.

**Further reading**

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- Jayasena C (2017) Setting up a reference lab: What you need to know. Endocrinologist www.endocrinology.org/endocrinologist/124-summer17/next-generation/setting-up-reference-lab/

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