Tips and tricks for optimizing sperm preparation and Minimizing DNA fragmentation in the andrology process

The integrity of sperm DNA is essential for healthy embryo development as chromatin abnormalities or DNA damage may result in lower fertilization rates in IVF, impaired implantation rates and an increased incidence of abortion[1]. Sperm DNA fragmentation can be caused by intrinsic factors but also occurs as a result of extrinsic factors including storage temperatures, handling conditions, and time between ejaculation and preparation among others. Appropriate semen processing is critical for successful infertility treatment, therefore **ORIGIO has worked with leading experts to compile the following tips and tricks to optimize sperm preparation and minimize DNA fragmentation during sperm processing.**

Abstinence period:
It has been suggested that the man abstains from sexual activity for just one day before sample production for treatment as this has been shown to reduce DNA fragmentation [2, 3]. Patients might also be encouraged to ejaculate frequently prior to treatment so that spermatozoa spend the minimum period of time within the epididymis, generally accepted to be the site where DNA damage occurs *in vivo*.

Time between ejaculation and sample preparation:
As DNA fragmentation is known to increase, it is recommended that the time between sample production and the start of preparation is kept as short as possible and so should begin immediately after full liquefaction. If a sample must be produced at home, it should ideally be delivered to the laboratory within one hour and should be kept at 15-25°C; it should not be allowed to get too cold or too warm.

Viscous semen samples
To obtain satisfactory sperm yield from highly viscous semen samples it is recommended to dilute the viscous sperm sample 1:1 with a sperm washing medium (e.g. ORIGIO Sperm Preparation Medium or SAGE Quinn´s Sperm Washing Medium) followed by repeated pipetting[19].

Sperm DNA fragmentation assay:
Sperm DNA is not evaluated by a standard semen analysis but can provide essential information which can be used to guide couples to treatment that is appropriate for their particular needs.

Sperm preparation:
It is advised to use media containing anti-oxidants *(e.g. taurine, EDTA, citrate and HSA)* as oxidative stress is known to be the main mechanism causing DNA fragmentation[17].

Simple Sperm washing:
Simple washing should be avoided whenever possible since damage can be caused by reactive oxygen species (ROS) generated by non-viable spermatozoa and leukocytes. Additionally, the presence of large numbers of non-viable spermatozoa in the prepared sample can inhibit capacitation[4].

Gradient preparation and Swim-Up:
Rates of DNA damage are decreased by both swim-up and density gradient centrifugation, though it has been suggested that the gradient technique gives more stable sperm in terms of rate of DNA fragmentation[5]. Motile sperm obtained by density gradient centrifugation are shown to have a higher mitochondrial membrane potential and a lower rate of DNA fragmentation, to generate lower levels of ROS, and are more viable than those in whole semen[6].

Centrifugation time:
This should be kept as short as possible (15-20 min) in order to minimize the production of ROS by leukocytes and non-viable sperm cells. Longer centrifugation time also increases the temperature and affects the quality.[6]

Number of washes after density gradient:
It is important to wash samples twice following density gradient centrifugation; this removes any colloidal particles and has been shown to give a higher yield of rapidly progressive sperm[7].

Storage and temperature:
Sperm DNA damage is enhanced by prolonged incubation, especially at 37°C. The DNA fragmentation rate at room temperature is found to be significantly lower than that at 37°C after 24 h[8]. Handling and preparation should therefore always take place at room temperature, preferably in the dark, and for the shortest amount of time before insemination or use in treatment.

Sperm preparation for HIV positive men:
It is recommended to add an additional swim-up step of sperm from the pellet resulting from the density gradient. The swim-up step into clean media further separates the sperm from any leukocytes in the pellet, resulting in a cleaner sperm sample for insemination [20].

Zika Virus - precautions
Men with possible exposure to the Zika virus but without clinical illness consistent with Zika virus disease should wait at least 8 weeks after exposure to attempt conception. At present no data or recommendations are available for purifying sperm away from these viruses in semen; all counselling, precautions and procedures recommended for other viruses should be followed if there is any suspicion of infection[20].

Choose the most motile sperm for IVF and ICSI:
Choose the most motile fraction of a prepared semen sample for fertilization since an inverse correlation is found between sperm motility and the degree of sperm DNA fragmentation in patients with clinical varicocele and other conditions[13].

IVF vs ICSI in men with high sperm DNA damage:
Reports[16] show a decreased live birth rate after IVF for men with high sperm DNA fragmentation, whilst live birth rates are unaffected when ICSI is used. It may be preferable to perform ICSI for men with high sperm DNA fragmentation, though this needs more investigation[16].

Sperm Selection for ICSI:
It is known that apparently morphologically normal sperm from men with poor semen quality are at higher risk of being aneuploid[10] so the usual approach of choosing sperm for injection using motility and morphology is not completely effective. The additional use of hyaluronan binding is known to reduce the risk of selecting a sperm with fragmented DNA and improve embryo quality[11] and has been shown to reduce the risk of early pregnancy loss[12].

Cryopreservation:

Timing: Frozen-thawed sperm exhibit different dynamics of DNA fragmentation compared with fresh samples with a more rapid increase in the percentage of DNA damaged spermatozoa[14]. If cryopreservation is required, it is recommended that sperm be used in treatment as soon as possible after thawing.

Osmotic shock: The osmotic shock phenomenon is characterized by the increased coiling of the sperm tail and can results in loss of progressive motility. It is therefore important to allow for gradual osmotic adjustment by slowly mixing the Sperm Freezing medium with your sperm sample.

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