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Semen characteristics in consecutive ejaculates with short abstinence in subfertile males

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Dr Gulam Bahadur is a past member of the HFEA. His main involvement is in andrology with special emphasis on diagnostic analyses, male cancer patients, counselling, sperm donor recruitment and sperm freezing, and recovery rates following cryopreservation. Important ways to optimize pregnancy rates in simple first-line treatment intrauterine insemination is being researched and applied within a number of UK NHS Trust Hospitals. Dr Bahadur has made significant contributions to the understanding of semen qualities in subfertile males, cancer patients, adolescent cancer patients and produced one of the first reports on ovarian tissue freezing for cancer patients.

Abstract This study reports the favourable semen characteristics of 73 subfertile oligozoospermic men with short abstinence periods up to 40 min. Semen characteristics were compared between initial and consecutive ejaculate showing improved semen parameters: progressive grade A spermatozoa, morphology and sperm concentration. Median concentrations in initial and consecutive ejaculates were 10 million/ml and 17 million/ml, respectively. The second sample had a higher median normal morphology (7% versus 6%, $P < 0.001$). The median of non-progressive spermatozoa (Grade C) was significantly lower in the consecutive sample than the initial sample (0% versus 5%, $P < 0.01$). Medians for slow progression spermatozoa (B grade) and immotile spermatozoa (D grade) were lower in the consecutive samples (20% versus 13%, $P < 0.01$ and 60% versus 50%, $P < 0.001$, respectively). The median for rapid motility (Grade A) was significantly higher in the consecutive sample than the first (30% versus 5%, $P < 0.001$). Overall median progressive motility as benchmarked by the WHO 2010 criteria was significantly higher in the consecutive sample (43% versus 25%, $P < 0.001$). Semen analyses of consecutive semen samples collected 30 min (mean) apart in oligozoospermic men should be checked routinely for diagnostic purposes and for managing potential subfertility treatment. 

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Introduction

Abstinence between 2 and 7 days before diagnostic semen analyses is recommended in the World Health Organization (WHO, 2010) guidelines (WHO, 2010). The data derived from fertile males in a number of countries over three continents represents sound reference distributions of semen characteristics of fertile men, providing an appropriate tool to measure semen characteristics to evaluate a patient's prospects for fertility (Cooper et al., 2010; Esteves et al., 2012). At least two semen analyses should be performed at least 3 months apart and such timespans may not always be practical when treating patients. However, when it comes to subfertile men it is unclear how the recommendations for abstinence can be applied to gain an optimal sample to benefit the couples clinically. Clinics generally rely on the 3 days abstinence provided in the WHO 1999 guidance (WHO, 1999) or the 2–7 days from the WHO manual 2010, without questioning what this period could be for specific subfertile men.

Old reports suggest the commonly held belief that sperm count is inversely related to frequency of intercourse. In normozoospermic men, sperm counts were shown to decrease significantly with sequential ejaculation. A nearly 60% decrease in the total motile sperm count of the second ejaculate compared with the first has been reported (Olderid et al., 1984). Similarly, a correlation between sperm count and frequency of ejaculation confirmed that sperm concentration, volume and total sperm count decreased with frequent ejaculation, but concluded that to increase the chance of fertilization it might be more efficient to have intercourse every other day rather than daily (Levin et al., 1986).

The semen characteristics can be affected considerably by abstinence times and the semen characteristics will define first-line treatment options. A cost-effective analysis shows that if only cost per live birth is considered for each treatment, above a pre-wash total motile sperm count (TMSC) of three million intrauterine insemination (IUI) is more cost-effective than IVF. Below a pre-wash TMSC of three million, intracytoplasmic sperm injection (ICSI) is more cost-effective than IUI (Moolenaar et al., 2015). Therefore, it is important to secure sufficient total progressive sperm count (TPMS) numbers if first-line IUI treatment is to be efficiently managed (Bahadur et al., 2015).

Clues to sperm quality and abstinence times for subfertile men can be found embedded in studies where the objective was to gain a sample with optimal motile sperm parameters for treatment purpose. The 3–4 day abstinence period before IUI was originally based on maximizing TMSC in an ejaculate (Freund, 1963). Semen parameters vary depending on the abstinence period, with some groups suggesting better sperm characteristics can be obtained for the purposes of fertility treatment with much shorter abstinence periods of less than 1 day (Levitas et al., 2005). One study has used a 1 h abstinence period, whereby a consecutive ejaculate, within 1 h of the first in cases of unacceptable ejaculate quality (compared with previous occasions) or very poor semen characteristics, has shown the second sample to be superior in quality to the first and was therefore used for the fertilization process (Bar-Hava et al., 2000). Studies appearing in non-mainstream fertility literature, in an IVF setting support the benefits of short abstinence time (Sugiyam et al., 2008). Data also exist

on how semen quality is affected by environmental factors and the method of sample production (Elzanaty and Malm, 2008; Jørgensen et al., 2001) and therefore it is important to recognize and control for these factors.

There is no specific report on the diagnostic quality of semen from subfertile men with short abstinence, and we questioned whether the potential worth of a consecutive ejaculate can help men with oligozoospermia. The aim of this study was therefore to report on the diagnostic semen analyses and compare the semen characteristics between the initial ejaculate and a consecutive ejaculate obtained within 40 min in men with oligozoospermia being profiled for IUI treatment.

Materials and methods

In 73 couples undergoing IUI in our reproductive unit within a large NHS teaching hospital between June 2013 and April 2014, oligozoospermic men were profiled before treatment regarding their semen analyses with a view to checking whether five million motile and progressive (Grade A and B) spermatozoa were available for an IUI procedure. Where the initial sample was deemed unsuitable (<five million motile spermatozoa) the patient was asked to provide a second ejaculate within 40 min in order to assess whether the TPMS increased to > five million per ml. An abstinence time of 2–7 days for the initial ejaculate was compliant with the WHO 2010 standard (WHO, 2010). The scope of this report was restricted to reporting the semen characteristics between the initial and consecutive ejaculate only. The WHO 2010 semen analyses criteria were adhered to for the semen analyses profiling, but the WHO 1999 criteria were retained for the motility assessment, as this allowed for discrimination of the Grade A (rapid) and B (slow) spermatozoa, which was equally important to understand the outcomes within the IUI programme. Progression was classified as:

- (i) Grade A: rapid progressive movement. At least 25 $\mu\text{m/s}$ at 37°C (25 μm is approximately equal to five head lengths or half a tail length).
- (ii) Grade B: progressive motile with moderate to poor progression.
- (iii) Grade C: twitching spermatozoa with minimal forward progression. Non-progressive motility. <5 $\mu\text{m/s}$ at 37°C.
- (iv) Grade D: immotile.

Morphology assessment was performed on Diff Quick stained slides (Medion Diagnostics AG, Bonnestrasse 9, CH-3186 Duding, Switzerland). Viscosity was reported as follows: samples leaving the pipette in small discrete drops were graded as normal viscosity and samples forming threads longer than 2 cm were graded as abnormal or viscous samples.

All semen samples were produced by masturbation within the clinic environment, thereby minimizing factors that may contribute to semen variations caused by method of production or by environmental change. Men were counselled beforehand on the possible benefits of producing a consecutive ejaculate, produced in the same environment by masturbation. The short abstinence time was dictated by how long it took men to provide the consecutive ejaculate.

Semen samples were initially analysed using a Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel) immediately after liquefaction to assess whether a "consecutive ejaculate" was required and to facilitate the speed for

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