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Human semen can be air-dried prior to testing for sperm DNA fragmentation with the Halosperm® G2 kit

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ABSTRACT

Objective: To explore a method of semen storage prior to assessment of sperm DNA fragmentation. **Methods:** This study examined a simplified alternative of air-drying semen on a microscope slide and reconstituting in seminal plasma prior to assessment of sperm DNA fragmentation using the halosperm® G2 kit. **Results:** It showed that semen could be air-dried and stored overnight at room temperature with no detrimental effect on DNA quality. A significant correlation between results existed for 20 semen samples both air-dried and snap-frozen in liquid nitrogen ($r=0.982$, $P=0.000$). A mean difference between the results of only -1.98% confirmed the effectiveness of air-drying compared to snap-freezing. **Conclusions:** Future studies to refine this technique are required on the effect of extrinsic factors such as the choice of reconstituting medium, and stability over an extended time-frame at different temperatures.

1. Introduction

There is a wide range of tests available to assess sperm DNA fragmentation^[1,2], but it is increasingly apparent that much work is yet to be done on the standardisation of tests and their clinical interpretation^[2-4], together with the development of external quality assurance schemes^[4]. At a practical level, the tests to detect sperm DNA integrity often require specialised equipment, though one test that requires only standard laboratory technology is the Halosperm® test^[5], a commercially-available kit based on the sperm chromatin dispersion test^[6].

In an effort to make the processing of samples more efficient, semen samples are often frozen in liquid nitrogen or on dry ice for dispatching to a central specialist laboratory or stockpiling for batch analysis^[7-10]. However, the use of

liquid nitrogen or dry ice is not always convenient or can be costly to transport around. A simplified method of sperm storage and/or transportation would therefore be an advantage. DNA has been successfully extracted in a forensic setting from saliva that has been air-dried^[11], and fluorescence in situ hybridisation can be done on semen air-dried on microscope slides. The present study has therefore investigated the use of air-drying as a possible means of storing human semen prior to testing for sperm DNA fragmentation.

2. Materials and methods

Twenty men attending Fertility North for fertility investigations each produced a semen sample by masturbation into wide-mouthed 60 mL universal containers. Semen samples were held in an incubator (Memmert GmbH, Germany) at 37 °C for a maximum of 1 h from production until analysis. For each semen sample at the time of analysis, a 0.5 mL aliquot was placed in a 1.8 mL Cryotube™ vial (Nunc, Denmark), plunged in to

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liquid nitrogen and held prior to testing for sperm DNA fragmentation the following day. At the same time, a 40 μL aliquot of the sample was dispensed on a microscope slide and placed on a 37 °C warming stage (Minitüb GmbH, Germany) for a maximum of 15 min until dry, and the slide was kept overnight at room temperature prior to testing. Sperm DNA fragmentation was assessed using the Halosperm® G2 in vitro diagnostic test kit (Halotech DNA SL, Spain). In essence, samples were mixed with an agarose cell support before being applied to a proprietary coated slide. Following exposure to a denaturing agent and then a lysis solution, the sperm were stained and assessed. A total of 300 sperm were counted per sample, and the proportion judged to have fragmented DNA was expressed as a percentage. Both frozen and air-dried sperm were analysed for sperm fragmentation at the same time the following day. Frozen samples were removed from the liquid nitrogen and thawed at room temperature ready for analysis. Air-dried samples were reconstituted by adding 40 μL of homologous seminal plasma that had been centrifuged to remove cellular material and then stored at 4 °C. The dried spot was gently mixed with the seminal plasma until the attached sperm were resuspended, and the reconstituted semen then placed in a 0.5 mL conical tube (Eppendorf, Germany).

The association between the results for both preparations of sperm for each sample was determined firstly by the Pearson correlation coefficient (r) calculated using the Excel add-in StatistiXL (Nedlands, Western Australia), and secondly with a difference plot^[12].

3. Results

The relationship between the Halosperm® results with frozen and air-dried semen is shown in Figure 1. A scatterplot (Figure 1a) showed there was a significant correlation ($r=0.982$, $P=0.000$) whilst a Bland-Altman difference plot (Figure 1b) confirmed close agreement with an average difference of only -1.98% and a coefficient of repeatability of 8.26% .

4. Discussion

The financial and practical benefits of storing and transporting air-dried semen without the need for cryogenics are considerable. Air-drying has been found suitable for preserving cellular DNA from saliva for forensic purposes^[11], and more relevantly fluorescence *in situ* hybridisation has been done on semen air-dried on microscope slides. Having shown air-drying to be a viable option for the short-term storage of semen prior to sperm DNA fragmentation testing, a full validation of the air-drying technique would therefore now seem warranted to investigate formally the effects of extrinsic factors such as the nature of the reconstituting

fluid, and stability over time at different temperatures. The development of suitable strategies for samples to be analysed with different tests (eg. sperm chromatin structure assay^[13]), as well as the feasibility of distributing dried semen to participants in an external quality assurance programme^[4], is also of importance. In summary, the use of air-dried semen to store and transport semen prior to testing for sperm DNA fragmentation is feasible. Further studies are now required to refine this technique, and to develop reliable air-drying methods suitable for other sperm DNA diagnostic tests.

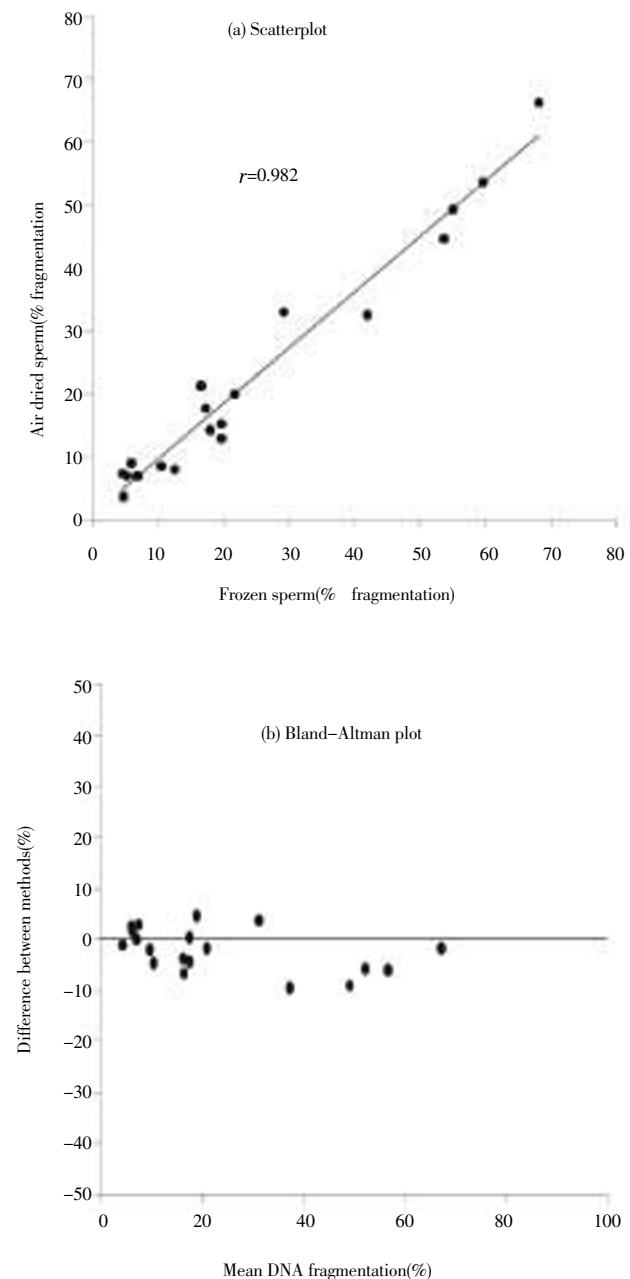


Figure 1. A comparison between Halosperm® G2 results for 20 semen samples stored both by snap freezing in liquid nitrogen and air-drying on a microscope slide. Air-dried samples were reconstituted with an equal volume of homologous seminal plasma. The comparison was made by (a) a scatterplot, and (b) a Bland-Altman plot.

Conflict of interest statement

We declare that we have no conflict of interest.

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