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Pregnancy outcome following swim up preparation of both fresh and cryopreserved spermatozoa

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This study was designed to assess the impact of swim up preparation of both fresh and cryopreserved sperm on the pregnancy outcome in a private fertility centre in Lagos. A cross-sectional prospective analysis of 34 asthenozoospermic semen samples of men whose wives were undergoing assisted reproduction was studied. The basic semen parameters comprising of the volume, count, and motility of the sperm before and after swim up preparations with pregnancy outcome were measured. For fresh semen (n = 28, mean age = 37.0 ± 1.1 years, mean volume = 2.16 ± 0.1 ml), the sperm count decreased significantly (p<0.01) from the pre swim up value of 55.4 ± 3.10 to 44.6 ± 3.20 x 10⁶/ml post swim up. While, the motility increased significantly (p<0.01) from 39.6 ± 3.84 to 58.5 ± 4.29%. The percentage pregnancy outcome in the fresh semen was 66%. For the cryopreserved semen (n = 6, mean age = 41.0 ± 5.4 years, mean volume = 1.8 ± 0.1 ml), the sperm count decreased significantly (p<0.01) from pre swim up value of 35.6 ± 3.03 to 33.3 ± 4.33 x 10⁶/ml post swim up, while the motility increased significantly (p<0.01) from 25.1 ± 4.01 to 32.8 ± 6.18%. The pregnancy outcome of cryopreserved was 30%. The pregnancy outcome was higher with fresh than the cryopreserved semen. However, the motility was a significant indicator for the successful outcome. Swim up procedure improve the motility of both cryopreserved and fresh semen with a better pregnancy outcome in this study.

Key words: Cryopreserved sperm, fresh sperm, asthenozoospermia, swim up, sperm motility, pregnancy outcome, Lagos.

INTRODUCTION

According to the World Health Organization (WHO, 1991), infertility is a global problem affecting more than 80 million people worldwide. Most of those who suffer from infertility live in the developing countries where infertility services in general and Artificial Reproductive Technology (ART) in particular are not available (WHO, 2001). 40% of cases of infertility have been attributed to male factor (WHO, 1987) and the systemic treatments available for such couples with male infertility are limited.

However, assisted reproduction has shifted from the mere gynecological indications to anthropological indic-

ations during the past years. This has urged andrological scientists to understand the physiology of male germ cell better and to develop more sophisticated techniques to separate functional spermatozoa from those that are immotile, have poor morphology or are not capable to fertilize oocytes. Initially, starting from simple washing of spermatozoa, separation techniques based on different principles like migration, filtration or density gradient centrifugation have hence evolved (Henkel and Schill, 2003).

Many studies have been performed comparing the direct semen processing procedures (that is, simple wash, swim-up, etc.) with gradient separation techniques (Moohan and Lindsay, 1995). However, the demands on sperm separation techniques have increased with the expanding knowledge of sperm physiology and their

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genetic contribution to the embryo. Because of this, there has been rising concern over the safety of any sperm separation procedure with respect to the viability of the sperm and the long-term effects on any resulting pregnancy (Tucker and Jansen, 2002).

Therapeutic approach for managing male infertility include ART such as intrauterine insemination (IUI) either with fresh or cryopreserved sperm from donors, intra cytoplasmic sperm injection (ICSI) for couples with severe male factor infertility, *in vitro* fertilization-embryo transfer (IVF-ET), gamete intrafallopian transfer (GIFT) or zygote intra-fallopian transfer (ZIFT) with semen manipulation (Kazutomo et al., 1992).

Among the semen manipulation methods, swim-up preparation appears to be the most common and cheapest method of selecting viable sperm for most ART (Henkel and Schill, 2003). The swim up technique appeared to enhance the velocity and the number of morphologically normal sperm from normozoospermic samples (Ng et al., 1992).

Pregnancy outcome post swim up was 25% as compared to 21.4% in a combined swim up and test yolk buffer during IUI (Guido Ragni, 1998). Fertility is the primary goal in the field of reproductive medicine and assessment of semen quality which includes postwash total motile sperm count has been used as a potential screening at insemination to select patient for either IUI or IVF (Van Weert et al., 2000).

When semen quality is impaired, the incidence of fertilization is greatly reduced (Cohen et al., 1985; Tournaye, 1992). The number of motile spermatozoa obtained after selection techniques is the limiting factor for application of IVF versus (ICSI) procedure in cases of moderate to severe oligoasthenozoospermia (McDowell, 1986).

Spermatozoa viability is assessed in the laboratory by determining the percentage of progressive forward motile cells using a phase contrast light microscopy (McDowell, 1986; Kazutomo et al., 1992).

Sperm motility in any ejaculate is irreversibly but variably depressed by freezing and thawing. Hence, freezing caused a decrease in the percentage of progressively motile spermatozoa for all men. The extent of decrease varied widely among donors (Kolm et al., 1992; Tournaye, 2001).

However, the prospect of males that require the option of having their semen frozen could be affected in the future if they produce freezing sensitive spermatozoa (Stanley et al., 2001). Cryopreserved motile spermatozoa can be selected after thawing and with washing; few million of these active motile spermatozoa can be concentrated in a small insemination volume not exceeding 0.5 ml. Hence, 20 times more active spermatozoa may reach the uterine cavity from the use of any of the techniques (IUI, ZIFT, GIFT, etc) compared to the normal physiology of sperm migration (Kolm et al., 1992; Tournaye, 2001).

Washing of sperm before intrauterine instillation pre-

vent uterine contraction induced by presence of prostaglandin in the semen. But usually, there is production of reactive oxygen species in the semen sample, which may affect their sperm function (Aitken and Fisher, 1994). Studies showed that combining multiple semen characteristics in a linear score can be used as a predictor of male fertility potential (Bartovov et al., 2003; Shyam et al., 2004). Overall, literatures indicate that improving the number of motile spermatozoa at insemination improves fertility outcome, even in cases of borderline semen characteristics including those with sperm morphology below 5% (Ord et al., 1993; Luconi et al., 2004).

Donor sperm cryopreservation and insemination procedure is on the increase because of the popularity of assisted conception among the public. Here, we assessed the impact of swim up preparation of both fresh and cryopreserved asthenozoospermic patients' sperm on the pregnancy outcome in a private fertility centre in Lagos.

MATERIALS AND METHODS

Patients

Semen from 34 asthenozoospermic men attending our Fertility Center was studied. Six were frozen-thawed semen while 28 were treated as fresh before further processing was done. The ages of the patients ranged between 29 and 49 years. The semen analyses were carried by a single observer in the Andrology laboratory between April and September 2004.

Semen analysis

Semen samples were obtained by masturbation after 3 to 5 days of abstinence from sexual intercourse and prepared within one hour of ejaculation (according to WHO recommended procedure, 1999). However, samples with leukocytes and/or immature germ cell concentration were not included in the study.

After liquefaction at room temperature, analysis of semen samples before and after swim up preparation were done to determine the sperm count, percentage motility based on WHO laboratory manual guidelines (1999) using a Makler Counting Chamber [Sefi-Medual Institute, Haifai, Israel] (1985) and a phase contrast light microscopy at 37°C. At least, 200 spermatozoa in five different microscopic fields were evaluated from each sample.

Swim-up procedure

The liquefied semen sample was diluted with an equal volume of Ham's F-12 medium (GIBCO, Grand Island, New York, USA) at 37°C after the removal of 0.5 ml for initial analysis. The mixture was centrifuged for 10 min at 2800 x g and the supernatant was discarded carefully. Another 2.0 ml of F-12 medium was added to the test tube and mixed thoroughly. This was later centrifuged for 5 min at 2800 x g. Then the supernatant was discarded again and 1.0 ml of Ham's F-12 medium was placed over the sperm pellet in the centrifuge tube held at 5° angle for 30 min at 37°C. This was to allow the motile sperm to swim up into the medium. 1.0 ml of this mixed medium was then carefully aspirated without disturbing the

Table 1. Semen analysis of pre and post swim up preparation of the 34 male patients.

Parameter	Fresh sample (n=25)	Cryopreserved sample (n=6)
Age (years)	37.4 ± 1.08	41.0 ± 2.72
Volume (ml)	2.16 ± 0.14	1.8 ± 0.16
Initial (pre) sperm concentration (x10 ⁶ /ml)	55.4 ± 3.10	35.6 ± 3.03
Final (post) sperm concentration (x10 ⁶ /ml)	44.6 ± 3.20	33.3 ± 4.33
Initial (pre) motility (%)	39.6 ± 3.84	25.1 ± 4.01
Final (post) motility (%)	58.5 ± 4.29	32.8 ± 6.18

All values were expressed as mean ± SEM.

sperm pellet at the bottom of the test tube. 1000 ml of the upper medium phase were collected and checked for sperm count and motility.

Cryopreservation and thawing of sperm

Ejaculated spermatozoa were frozen in liquid nitrogen in a formal Lab Dewar 8124 tank by manual controlled freezing procedure (forma, Ohio, United States of America). Freezing of semen was performed by a drop-wise addition of a glycerol-based cryoprotectant medium sperm freezing medium: Medicult, Jyllingsø, Denmark) to the semen sample at 1:1 (v/v) with a continuous shaking in a cryotube. After a short equilibration of 10 min at 37°C on the laboratory bench, the cryotube was then manually frozen by 10 min exposure to liquid nitrogen vapour and then lowered into the liquid Nitrogen (-196°C) storage in the sperm bank (Gil-Salom et al., 1996). The cryotube was later thawed at room temperature in a water bath for 15 min. The cryoprotectant was removed by washing in 5 ml Ham's F-12 medium and the aliquots of the samples were then processed using swim up manipulation procedure as earlier described. Then the samples were finally analyzed for post-thaw seminal indices (parameters). Count and motility were assessed immediately.

Ethical statement

Consent of the patients was obtained with detailed information given before commencement of the study. Data protection and confidentiality were maintained all through the study.

Data analysis

Computerized analyses of the study data were expressed as the Mean ± S.E.M. Student's t-test analysis was used to compare pre-count with other semen parameters and correlation matrix were carried out using SPSS software package (SPSS, Inc., Chicago Illinois). P-values less than 0.01 were considered significant.

RESULTS AND DISCUSSION

For fresh semen (n = 28, mean age = 37.0 ± 1.1 years, mean volume = 2.16 ± 0.1 ml), the sperm count decreased significantly (p<0.01) from pre swim up value of 55.4 ± 3.10 to 44.6 ± 3.20 x 10⁶/ml post swim up, while the motility increased significantly (p<0.01) from 39.6 ± 3.84 to 58.5 ± 4.29% (Table 1). The percentage

pregnancy outcome in the fresh semen was 66%. For the cryopreserved semen (n = 6, mean age = 41.0 ± 5.4 years, mean volume = 1.8 ± 0.1 ml), the sperm count decreased significantly (p<0.01) from pre swim up value of 35.6 ± 3.03 to 33.3 ± 4.33 x 10⁶/ml post swim up, while the motility increased significantly (p<0.01) from 25.1 ± 4.01 to 32.8 ± 6.18%. The pregnancy outcome of cryopreserved was 30% (Table 1). The pregnancy outcome was higher with fresh semen.

However, the motility was a significant indicator for the successful outcome. The pre swim up preparation sperm concentration was closely and significantly related to the post swim up concentration (p<0.01) and to the percentage post motility (p<0.01). Hence, the pre-count and post motile sperm were found to be a better predictor of the quality and outcome of swim up preparation.

IUI is the most widely accepted assisted reproductive technique and successful for couples with oligospermia or severe male infertility because it is of reduced cost, depends on a few number of cycles to achieved pregnancy with a reduced possible risk to the conceptus and less invasive as compared to ICSI (which allows homologous conception for many couples with male infertility) (Luetjens et al., 1999; Tucker and Jansen, 2002). Therefore, the possibility of increasing sperm motility in cases of asthenozoospermia might increase the chances for IVF application (Hamberger et al. 1999).

Our results contribute to the growing evidence that the swim up technique improve the quality of post wash motile spermatozoa (Ng et al., 1992). In preparation for intrauterine insemination (IUI) or in vitro fertilization (IVF), the motile, and hopefully, the most fertilizable population of sperm must be separated from the surrounding milieu (Tucker and Jansen, 2002).

With the swim up technique, the result obtained in this current study for the fresh semen sample post motility and counts were similar to that of previous studies (Cohen et al., 1985; Ng et al., 1992; Smith et al., 1995).

However, it is of considerable importance to improve on the sperm quality by obtaining a high number of motile sperm by semen techniques (Ng et al., 1992; Okanlawon et al., 1997). This will also improve their fertilization and pregnancy rates post insemination (Guido Ragni, 1998; Gil-Salom et al., 1996) as obtained

in this present study of 66% for fresh and 30% for the cryopreserved semen pregnancy outcome.

The concentration of the overall sperm cells was significantly reduced due to the swim up effect (44.6 to 39.6 x 10⁶/ml). Since, sperm washing with swim up method removes all the debris, dead cells and seminal plasma containing prostaglandin that could have induced uterine contraction from the semen (Kazutomo et al., 1992). Interestingly, the percentage (%) motility of the spermatozoa in both samples were significantly increased ($p < 0.01$) because of the created room for more motile cells to swim up into the media (Cohen et al., 1985; Ng et al., 1992; Cormier and Bailey, 2003).

Semen Cryopreservation is an important tool in assisted reproductive technology. Study has shown that the fertility rate of the frozen thawed sperm may be reduced probably due to the triggering of the signal pathway that lead to capacitation by freezing and thawing of the spermatozoa (Cormier and Bailey, 2003). There has been evidence of analogous and significant male to male difference in sperm sensitivity to cryopreservation among human (Kliesch et al., 1996). Thus, the quality of the semen post thawed can vary despite the initial screening of donors for sperm quality (fresh semen) as reflected in our study. It is therefore not surprising that the concentration of the cryopreserved sample was reduced (Table 1).

There have been conflicting reports in the use of sperm motility characteristic as a predictor of fertility outcome of frozen-thawed semen (Marshbam et al., 1992; Sidhu et al., 1997). This study showed that the post thawed motility was enhanced by swim up method and sperm washing, which was found to be 32.8% compared to 25.1% for the initial (pre) freeze motility (Bartvov et al., 2003). This is contrary to the general believed that the freezing caused a decrease in the percentage of progressively motile spermatozoa from all men (Kolm et al., 1992; Kliesch et al., 1996; Tournaye, 2001).

However, it has been recently observed that specific characteristics such as velocity of progression and the actual pattern of sperm movement and motility index, rather than the gross percentage motility are important determination of fertilization (Okanlawon, 1997).

In addition, swim up procedure is important to improve the fertility potential of the frozen sperm now that cryopreservation of donor semen is made mandatory because of the era of HIV/AIDS pandemic (Shittu et al., 2005).

Conclusion

The swim up technique is useful for obtaining hyper-activated quality motile sperm because of its simplicity, rapid and cost effectiveness especially in our poor socio-economic environment where ART facilities are limited in the country.

Swim up procedure improves motility of both cryopreserved and fresh semen with a better pregnancy outcome in this study. Further study is in progress to compare the technique with other types of sperm separation techniques using cryopreserved sample especially and to appreciate ways of improving on the swim up technique to enhance the present the conception rate our centre.

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