

Assessment of male infertility causes in Mozambique: A Case study of working class patients by IVF at *Medicos Associados Clinica Cruz Azul* Laboratory Maputo

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Abstract

A total of 105 semen specimens from infertile patients from different economic backgrounds collected throughout the country, were analyzed at IVF laboratory *Medicos Associados Clinica Cruz Azul*, Maputo between 2008 and 2010. The semen from patients were collected at the laboratory and quality assessment was carried out through microscopic observation. The objective of this study was to: (a) evaluate semen specimen's prior IFV; (b) provide appropriate prognosis; and (c) find out alternative reproductive techniques to apply. Results revealed 17.14% with normo spermia; 36.2% with moderate oligospermia; 24.8% with severe oligospermia; 9.5% with presence of crystals consisting epithelial and germ cells within the seminal plasma specimens and 21.9% with high semen specimen viscosity (HSSV). These results indicate a significant level of infertility caused by both moderate and severe oligospermia, while the rest of the observed infertility is attributed to HSSV, followed by possible coital disorders which

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affected 17.14% of patients with standard semen parameters. Introduction of Intra-Cytoplasmic Sperm Injection techniques and counseling measures in the IVF laboratory are advised given their contribution in achieving both fertilization and acceptable conception rates, concurrently with observation of health care, towards keeping active and functional reproductive organs and standard semen quality..

Keywords: high semen specimen viscosity; infertility; oligospermia, liquefaction

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1 Introduction

Human infertility as cross-cutting factor affecting both women and men constitutes reason for separation of couples, in case effective treatment is not timely conducted. In the past decades couples affected by different categories of infertility had no alternative but separation, a fact which could be avoided through simple detailed semen or ova assessment aimed at disclosing and treating the cause of infertility. Nowadays, variable techniques targeting both men and women causes of infertility like microscopic semen specimens assessment prior artificial insemination; *in vitro* fertilization and/or intra-cytoplasmic sperm injection; embryo culture and respective transfer to recipient are available and broadly applied with success.

In vitro fertilization procedures are used as alternative measures towards management of diverse causes of infertility in already frustrated couples battling to have babies (Frimel et al., 2014). Assessment of infertility causes in both male and females is a necessary measure prior to *in vitro* fertilization procedures are attempted to allow determination of appropriate guidance on semen processing and correct prognosis.

Poor semen assessment prior insemination can reduce chances of fertilization and prolong infertility and occasionally lead to divorce (Bankani et al., 2012). Semen is normally ejaculated in liquid form and gets liquefied after 20-30 minutes (Gonzales et al., 1994). In case, a significant number of semen specimens assessed does not liquefy after the expected time due to semen quality variability resulting from the way/manner and timing of semen collection, Zhand et al. (2013) and Wang et al. (2014), advised that a semen test should be repeated at least twice or three times.

Male accessory glands secrete fluids containing proteins, which exert significant influence in the coagulation and liquefaction of semen. Hypo-function of these accessory reproductive organs like the prostate and seminal vesicles can result in seminal fluid abnormal viscosity (Honea et al., 1990; Du Plessis et al., 2013). Occasionally, semen keeps its viscosity for 30-45 min or even more (Menckveld et al., 1990), a fact considered abnormal. In this case,

possibly the semen producing mechanisms, more specifically the enzymatic process catalyzed by a proteolytic enzymes present in the prostatic secretions might be deficient or absent, which may cause semen liquefaction to fail (Honea et al., 1990; Du Plessis et al., 2013). Additionally, semen with high viscosity can result in prolonged infertility irrespective of high sperm density, a fact which can be attributed to the deficient movement of spermatozoa within the female reproductive tract towards the ovum further reducing the chance of fertilization. Semen with high viscosity makes it difficult for use in vitro manipulation with regards to separation of semen plasma and/or isolation of spermatozoa for further quality assessment and insemination or application in other assisted reproductive techniques.

In couples with normal semen and ova quality, infertility might be due to infrequent intercourse or wrongly timed intercourse, especially when one or both partners in a couple are addicted to alcohol, all of which are collectively referred to as coital disorders. Coital disorders may also include failure to ejaculate or retrograde ejaculation, impotence, failure to adjust to appropriate time for intercourse which can all lead or cause male infertility (Hamid et al., 2006). Hopefully, counseling, psychotherapy or administration of vasodilators, can be measures which may have significant effect on the treatment of this category of infertility if considered (Hamid et al., 2006)

Frequently, oligospermia which is regarded as low sperm count is a leading cause of infertility or subfertility among men (Frimel et al., 2014). While it only requires one sperm to fertilize the ovum, the odds of conception are such that it takes millions of sperms per milliliter of semen to actually achieve the goal of fertilization. A *normal* sperm count is above 20 million of sperm per milliliter of semen. Abnormal sperm morphology greater than 95% or specific sperm defects have been identified as cause of infertility due to failure in sperm-oocyte interaction (Rabbani et al., 2009), where this type of infertility is better treated through intra-cytoplasmic sperm injection (Gambera et al., 2010).

In assisted reproduction procedures, other causes of infertility like alcohol abuse; chronic cigarette smokers; oxidative stress have been identified as factors with detrimental effects on outcomes of natural fertilization and might be reasons which contribute to divorce (Bakani et al., 2012). Therefore, all indicated factors were not subject for these studies.

In this study, semen assessments were carried out at *Medicos Associados Clinica Cruz Azul*, a pioneer Clinic on Human Assisted Reproduction in Maputo, Mozambique, as a measure for further treatment of infertility. Given the high frequency of human infertility observed among the registered patients, the objective of this study was to assess semen specimens prior in vitro fertilization procedures. The study was also aimed at identifying alternative reproductive techniques to apply and come out with appropriate prognosis, while ensuring successful fertilization and conception rates.

2 Materials and Methods

Patients from different economic background countrywide were registered at *Clinica Cruz Azul* with variable causes of male infertility. A total of 105 semen specimens were provided by these patients for reproductive quality assessment. Procedures recommended by the World Health Organization (WHO) - manual for semen analysis, immuno-bead and sperm-cervical mucus penetration, were applied (WHO, 2010). Semen samples for assessment were collected twice or three times from a total of 105 patients and, depending on the severity of results of semen analyses, patients were occasionally advised to abstain from sexual activities for one or two weeks prior to collection of their semen. Semen collections were carried out in special sterile disposable plastic jars through masturbation in a special comfortable toilet containing hand washing facilities and bed (for occasional assistance when needed), near the IVF laboratory, WHO, (2010). Other semen samples were collected outside the clinic (at home), which was delivered to IVF laboratory within at least one (1) hour after collection and kept at constant temperature, not above 37°C. High viscosity (hyper viscosity) semen specimens (HVSS) were left 30-35 min for liquefaction.

Cases of patients with HIV; hepatitis B and C were reported prior IVF procedures and were excluded from this endeavor, while other cases of sexually transmissible diseases diagnosed earlier were treated separately prior semen collection for IVF. Semen samples were subject to quality analysis though microscopic (stereomicroscopy) observation (WHO, 2010) and thick samples were subject to dilution (v/v) with embryo culture medium (BlastAssist System - MediCult) 1:1, 1:2 or 1:3 ml depending on the level of semenviscosity, followed by centrifugation at 17,000 rpm to remove semen debris in order to facilitate manipulation and sperm count.

3 Results and Discussion

The results obtained in this study are summarized in the Table 1.

The presence of epithelial cells within the semen specimens might either reflect an infection of bulbourethral glands, seminal vesicles, swollen or infected prostatic glands (Hamid et al., 2006).

The presence of germ cells is an indication of an incomplete spermatogenesis cycle, without necessarily reflecting clinical disorders. It is obvious that irrespective of the category, oligospermia is not advisable in circumstances where couples attempt to achieve successful fertilization. Data from semen specimen assessments indicate both moderate and severe

Table 1: Semen characteristics in the Maputo sample of working class patients

Period	No. patients	Normo-spermia, 17.14% *	Mod. oligosp., 36.2% **	Sev. oligosp, 24.8% ***	Crystals; epithelial and germ cells, 9.5%	HSSV > 1cm 21.9%****
2008	36	9	9	8	1	10
2009	38	6	11	7	6	14
2010	31	3	8	11	3	9
Total	105	18	38	26	10	23

*Normospermia - refers to semen with technical parameters recommended by the WHO;

**Mod. olig. (moderate oligospermia) - refers to semen with number of spermatozoa not far below 20.000 million (WHO);

***Sev. olig. (severe oligospermia) - refers to semen with a number of spermatozoa far below the recommended by WHO;

**** HSSV refers to high semen specimen viscosity which was higher than 1cm in these specific cases (normally viscosity should not be high that 30mm)

oligospermia which may also reflect the effect of age regarded as responsible for reducing fertilization rates (Zhang et al., 2014), despite the need for a single spermatozoon for a successful fertilization to occur. Infertility caused by both moderate and severe oligospermia can be prolonged in case appropriate technical measures are not taken and can also result on divorces (Bakani et al., 2012).

High semen specimen viscosity is claimed to cause sperm tangling in the fibrous or mucoid mass in the semen, thus preventing sperm from migrating from the seminal plasma through cervical tract towards the fertilization site (Zavos, 1986; Duplessis et al., 2013). Studies by Paul et al. (1996) advocate the use of pentoxifyline to stimulate sperm motility, a phenomenon which is presumably resulting from triggering activation of proteolytic enzymatic activities. Studies on the dilution of seminal plasma towards isolation and identification of motile sperm have been carried out in order to improve semen liquefaction and reduce difficulties of manipulating high viscosity semen specimens.

Studies on the effect of diluting different samples of high viscosity semen specimens through addition of Blast Assisted System culture medium (v/v) 1:1; 1:2 and 1:3 ml to form a uniform suspension towards isolation of motile sperm from seminal plasma through disruption of the mucous material were carried out and results will be displayed in the next publication. Furthermore, outcomes on conception rates resulting from the use of isolated motile sperm from HSSV above 1cm for artificial insemination were also carried out in our laboratory and results will also be tabled out in the up-coming publication.

Interestingly, the manipulated semen was collected from patients from different economic background like mining, heavy industry, agriculture and other fields of significant importance for the country development, just to make few references. It is known that the field of agriculture occasionally applies pesticides that might be a contributing factor in reducing semen quality previously stated by Mehrpour et al. (2014). It is also evident that heavy smoking constitute a detrimental effect in patients leading to poor semen quality (Zhang et al., 2013). Furthermore, psychological stress can not be ignored as it may play a negative role on the reproductive performance (Hjollund et al., 2014) The inclusion and focus on all of the above indicated factors or the effect of economic activities to which patients were involved in this study, are facts which would have significantly contributed to better conclusions and provide cross-cutting recommendations to both patients and different employers.

4 Conclusion

1. Oligospermia constitutes a significant factor causing human infertility regardless of its category and an introduction of intra-cytoplasmic sperm injection techniques in the IVF Laboratory by the *Medicos Associados Clinica Cruz Azul* is a recommendable measure to positively alter the rates and trend of fertilization;
2. HSSV exert negative influence over human fertility and thus, care to health should be observed in order to keep active and functional reproductive organs to maintain standard semen quality;
3. A proper timing of intercourses is crucial in order to achieve fertilization by couples with normal semen quality and is herein recommended and alcohol abuse should concurrently be avoided;
4. Delay on semen liquefaction above 30-35 minutes should be considered as an abnormal phenomenon and due measures towards releasing active spermatozoa from the tick ejaculate should be taken.

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