Role of Life Style Factors with Respect to Semen Quality and ART

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ABSTRACT
One of the most painful problems of marital life is infertility. There are various causes of infertility such as endometriosis, ovulatory disorder, chromosomal abnormalities, semen quality and idiopathic infertility. The current approaches in the treatment of infertility, the problem could be resolved to some extent by adopting modern treatment and procedures as well as healthy lifestyle. In last few years remarkable changes have been observed in our environment, diet and lifestyle. The effect of lifestyle factors on human reproductive potential might be varying because of circumstances or individual susceptibility. The incidence of male infertility is one of the rise probably due to environmental factors, psychosomatic disorders, stress as well as personal habits including medications. Special attention has been devoted in the scientific literature that is well established as health risks, viz. smoking, alcohol and obesity. Other factors that are being considered in the literature are abuse of drugs, genital health heat stress, physiological stress and effects of cellular phones. Assisted reproductive technology provides a means to bypass their woes. Several techniques exist depending on the extent of the infertility.

Keywords- ART, BMI, PAHs, IUI, IVF, ICSI, IMSI, MSOME.

Introduction
In recent years, infertility has become the subject of significant media attention and public discussions, particularly in light of new advances in the technology of assisted reproduction. It has been proposed that a man who fails at fertility is likely to evaluate himself as sexually inadequate and may experience temporary impotence and decreased sexual desire. In India, approximately 15 percent of couples are unable to initiate a pregnancy without some form of assistance or therapy. These patients are said to be “primary infertile”. In approximate 2 percent of these couples there are factors other than seminal abnormalities which prevent conception. It is imperative that these groups of patients are properly diagnosed and treated to enable them to achieve conception. Male infertility contributes to about 50 percent of total infertility problem according to large world health organization [WHO] study of more than 8,500 couples[66]. As is expected there is a wide variety of causes associated with it. It seems logical and cost effective to address male factors first, and managed together with the evaluation and management of the female partner.

Life Style Factors
Smoking
Cigarette smoking is known for formation of cancer [14]. There is also considerable evidence that smoking adversely affect male reproductive health.
Some studies have found the effect of same on sperm volume \[1\]. It is indicative that smoking has harmful effect on human male fertility \[2\]. Tobacco effects can be observed at both, microscopic and molecular levels. Microscopically there is an effect on sperm concentration, motility and morphology \[3-10\]. At the molecular level there is increased risk of sperm aneuploidy \[11,12\]. Furthermore, maternal smoking during pregnancy may have an adverse and irreversible effect on semen quality in male descendants \[13\], besides a high risk of birth defects and childhood cancers in the offspring \[14\].

**Alcohol**

Alcoholism has been long associated with reproductive health disturbances \[15\] sperm quality is seems to be deteriorate progressively with increasing level of alcohol intake \[16\]. chronic alcohol consumption has a detrimental effect on male reproductive hormones and one semen quality \[18\]. A case control study is conducted in Japan showed that alcohol intake was significantly more common in infertile men than in non drinkers \[19\]. An alcohol exposure in “in-vitro”induces reduction of sperm motility and morphology and the response is dose-related \[20\].

**Obesity**

A common observation in the western world is the increased average body mass index (BMI) in the general population that has resulted in an increased prevalence of obesity. Several studies have associated with lower semen parameters due to obesity \[21,22\]. In a follow up study in couples enrolled in the agricultural health study in USA \[23\] found a dose-response relationship between infertility and male BMI and that association was similar for older or younger men. Other authors have found that semen parameters are affected in men BMI above or below normal levels \[24-29\]. Maternal BMI may also have effect on the semen quality in sons of overweight mothers.

**Recreational Drug Use & Genital Heat Stress**

Cocaine use for five or more years was more common in men with sperm motility, low concentration or large proportion of abnormal forms \[30\]. Normal sperm production depends on an optical testicular temperature maintained below body temperature which is typically around 34-350 C \[31\]. Several experimental studies have shown that heat exposures may reduce semen quality \[32-34\]. In observational studies it has been found that individuals involved in activates that increase scrotal temperature have poor sperm morphology \[35\]. It has also been studied that the type of underwear used lead to increase in the scrotal temperature \[36\] and results indicated that scrotal temperature in volunteers wearing wool trouser and shirt fitting to body size were significantly higher for tight versus loose fitting.

**Psychological Stress**

The impact of psychological stress on semen quality is an area of great interest in which the further research is needed. Motility and morphologically normal spermatozoa decrease in healthy subjects undergoing examination stress \[37\]. In male involved in IVF procedures, the quality of the semen sample obtained on the same day, when eggs retrieval was performed was significantly worse than the first sample analyzed in the same patients collected before undergoing IVF procedures. The decline in the semen quality in the second sample was attributed to the psychological stress involved in that clinical process \[38,39\].

**Cellular telephone Use**

There has been increasing concern about the possibility that the use of cell phones could affect our health and male reproductive system. As cell phones produce radio frequency electromagnetic waves, and few observational studies have shown that the prolonged usage of cell phones have negative effects of sperm parameters like sperm count, motility, viability and normal morphology \[40-42\].

**Occupational and Environmental factors**

Pesticides \[43-44\], several heavy metals like lead or cadmium \[45-47\] and several air pollutants (PAHs, dioxins) \[48,49\] have been shown to compromise the reproductive male function. Several studies have explored and compared men’s semen parameters and occupational exposures in male partners of infertile couples attending infertility clinics \[46, 50,54-
Association has been found between welder and reduced sperm count and motility. In other case-controlled studies infertile men had been more frequently exposed to organic solvents, electromagnetic fields and heavy metals than fertile men. Several solvents affect human semen quality, proportionally to the amount and time of exposure. Semen quality in workers exposed occupationally to hydrocarbons like toluene, benzene and xylene lead to existence of anomalies in viscosity, liquefaction capacity, sperm count, sperm motility and proportion of sperm with normal morphology compared with unexposed males.

The below Table shows Studies investigated by WHO worldwide on influence of above life style factors on semen quality.

**Table 1. Influence of life style factors on semen quality**

| S. No | Life style factor            | Total Infertile Men Population |
|-------|------------------------------|-------------------------------|
| 1     | Alcohol                      | < 42%                         |
| 2     | Smoking                      | < 13%                         |
| 3     | obesity                      | < 2.7%                        |
| 4     | Recreational Drugs           | < 1%                          |
| 5     | Genital Heat Stress          | < 2%                          |
| 6     | Psychological Stress         |                               |
| 7     | Occupational and Environmental factors | < 0.3%                        |

**Assisted reproductive technology**

With the growing trend of male factor infertility and desire for infertile couples to reproduce, assisted reproductive technology provides a means to bypass their woes. Several techniques exist depending on the extent of the infertility.

**IUI (IntraUterine Insemination)**

Intrauterine insemination as an infertility therapy that has regained its use due to the improvement of the sperm preparation and washing procedures. This technique is used when there is semen with a low concentration of sperm, and involves artificial insemination after induced ovulation. IUI is cheaper, simpler, and less invasive than the more sophisticated assisted reproductive methods like IVF and ICSI. IUI is therefore, often offered to couples as a first line approach in cases of, antisperm, antibodies or idiopathic infertility.

**IVF (In Vitro Fertilization)**

The development of IVF procedures as a novel infertility therapy directly caused the establishment of a model where certain sperm variables such as morphology could be studied. The model for the sperm morphology mainly based on the appearance of a normal sperm cell. This technique is used when complete lack of sperm count in ejaculation. IVF attempts to imitate fertilization outside of the human body, by placing sperm in the vicinity of the oocyte and allowing fertilization to proceed with the help of various reagents. Logistic regression analysis was used to determine the threshold for fertilization using sperm morphology as contributing factor.

**ICSI (IntraCytoplasmic Sperm Injection)**

ICSI involves mechanical breeching of the oocyte and injection of single sperm in to the cytoplasm. When comparing ICSI with other ART procedures it has relatively lesser amount of limiting factors, because it requires the least amount of available sperm and can utilize immature and immotile sperm for the procedure. Recently a new method of Intracytoplasmic morphologically selected sperm injection has been proposed based on motile sperm organellar examination that results in a high pregnancy and decreased incidence of abortion.

**MSOME (Motile sperm organelle Morphology examination)**

Sperm functional morphology criteria based on real time observation of individual motile sperm cells under high magnification were developed. MSOME being able to detect subtle sperm morphological malformations, which might remain unnoticed during standard microinjection, and allow the identification of spermatozoa with the best morphology, was introduced to improve the ICSI success rates.

**IMSI (Intracytoplasmic Morphologically Selected Sperm Injection)**

MSOME evaluation coupled with conventional ICSI gave rise to new micromanipulation technique.
called intracytoplasmic morphologically selected sperm injection. Which is the currently one of the most debated issues in the ART field.

Materials and methods
The study was carried out among the 221 male (Age group of 20 - 45 years). Semen samples were obtained by masturbation within 3-6 days of sexual abstinence in a wide-mouth sterile container. Morphology of sperms were evaluated as per WHO criteria [64]. Semen sample were observed using 40x magnifications under the light microscope (OLYMPUS BX41). Sperm morphology was checked in semen samples using papanicolaou staining method [65]. A total of two hundred sperms were counted and categorized as below:

i. Morphologically normal sperm: Sperm with an oval head followed by distinct midpiece region, normal tail and clear well defined acrosomal area (~40-70% of the head area).

ii. Morphologically defective sperms: Head shape abnormality, midpiece defect, tail and multiple defects.

All the cells which did not present any overlap with debris or other cells were considered for analysis. Images were analyzed with ImageJ open source software [66] using custom macros to implement the procedure described below.

1. If the image is in RGB form, it is transformed to a gray scale image.
2. Then filtered using unsharp mask filter to increase the intensity of the sperm cells
3. Thresholding is done on the image such that shape of the all sperm cells will be appeared.
4. Image is calibrated to measure the various parameters on sperm head.

The above figures show analysis on sperm sample which is taken from system microscope first RGB image is converted to 8 bit gray scale. Then Thresholding is made on the image to differentiate sperm cells with respect to background. Next particle analysis is achieved to remove the noise and produce a mask on all sperm cells available in the image. Finally using ROI manager labelling and measurement is carried out on each sperm cell. Morphometric measurements carried out on human sperm

Results
The general characteristics of the study population such as area of residence, educational status and dietary habits have been shown in the following figure 4 to figure 7
Table 2: Comparison of semen parameters with respect to their lifestyle habits (Values are in Mean ± S.E)

| Parameter                  | Sperm Count (millions/ml) (n=196) | Normal morphology (%) (n=196) |
|----------------------------|-----------------------------------|-------------------------------|
| Smokers (n=54)             | 33.70 ± 1.29                      | 14.65 ± 0.32                  |
| Non-smokers (n=142)        | 49.96 ± 3.22                      | 16.23 ± 0.24                  |
| Chewers (n=168)            | 36.60 ± 1.88                      | 13.65 ± 0.51                  |
| Non-chewers (n=163)        | 39.84 ± 2.40                      | 15.89 ± 0.54                  |
| Alcoholic (n=54)           | 34.23 ± 1.61                      | 15.61 ± 0.40                  |
| Non-alcoholic (n=167)      | 40.46 ± 4.44                      | 16.47 ± 1.02                  |

Conclusions

The growing body of literature showing that wide variety of substances adversely affects the semen quality. In order to characterize risk assessment in better way it might be useful to revise the ways to report the damage to sperm quality and quantity the amount of toxic exposures on similar male endpoints. Progress in medicine can only be achieved if efforts are made for better understanding of the etiology of male infertility. Future development should proceed in the areas on blastocyst transfer, maximizing the implantation window, better sperm selection methods and the understanding the role of capacitation in ART successes. IMSI can be recognized as a promising technique that has fostered deeper understanding of the mechanisms interfering with male fertility potential in both natural and assisted reproduction.

The present data suggest that lifestyle factors such as tobacco smoking or chewing and/or alcohol consumption have some role in deteriorating male reproduction. Therefore, awareness programmes should be conducted towards specific group who has having such habits. In addition, the person who has habit of tobacco chewing or smoking and/or alcohol consumption undergoing for infertility treatment should be advised about the adverse effect of such habits on male reproductive function.

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