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Characterizing trends in semen indicators and potential risk factors for a male cohort seeking subfertility care in Dhaka, Bangladesh

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2015

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Abstract

Characterizing trends in semen indicators and potential risk factors for a male cohort seeking subfertility care in Dhaka, Bangladesh

By Eshita Sharmin

Alarming decreases in global trends of sperm quality indicators – sperm concentration, percentage of sperm motility, sperm density, and normal sperm morphology – have been observed over the last few decades. Because comprehensive research on male reproductive function in Bangladesh is lacking, the purpose of this study is to characterize infertility in Bangladeshi males through temporal trajectories of semen parameters and risk factor assessment. Retrospective, cross-sectional semen data was collected at the Centre for Assisted Reproduction (CARE) of BIRDEM General Hospital from 2000 to mid-2016 (n = 13811). Additionally, a mixed-methods approach informed risk factor influence on semen analysis reports from May-August 2016 (n = 72). Age distribution was significantly correlated with annual changes in median sperm count and motility over time ($p < .0001$). Adjusted median regression analyses for total motility and rapid linear (RL) motility indicate strong effects of confounding from age and duration of abstinence ($p < .0001$). When concentration diagnosis frequencies were adjusted by WHO 2010 parameters, normozoospermia frequency increased from 66.7% to 68.1%, while mild oligozoospermia decreased from 4.4% to 3.1%. Multiple regression analyses for the risk factors showed significant association of secondary subfertility with semen parameters: concentration

(48.9[15.1-82.7], $p < 0.006$), RL motility (14.4[4-24.8], $p < 0.01$), and total motility (20.3[8.9-31.7], $p < 0.001$). Hormonal imbalance impacted total motility with a regression coefficient of -28.8[-53.5- -4.1], $p < 0.026$. Participants aged 42-64 years had significantly lower concentration values than participants of all other ages (-64.8[-104.9,-24.7], $p < 0.003$). RL motility among participants aged 33-35 years was significantly different than those in other age groups ($p < 0.01$). Findings from this study indicate a relationship between increasing age and decreasing semen quality, as well as the existence of a temporal decline in semen parameters for Bangladeshi males seeking subfertility care. While this study sets the foundation for similar work in South Asia, future studies could be improved by quantifying risk factor measurements more effectively by conducting assays on biospecimens in addition to semen analysis. Moreover, expanding testing to male partners in couples facing normal fertility outcomes would provide substantial data to be used as a control.

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Definition of Key Terms and Abbreviations

Asthenozoospermia	-	an ejaculate with <40% of spermatozoa demonstrating progressive motility
Azoospermia	-	absence of spermatozoa in a semen sample
BIRDEM	-	Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine, and Metabolic Disorders General Hospital in Dhaka, Bangladesh
BMI	-	body mass index
BSMMU	-	Bangabandhu Sheikh Mujib Medical University
CARE	-	Centre for Assisted Reproduction at BIRDEM General Hospital
DBP	-	disinfection by-products
Endocrine disruptors	-	substances with the potential to adversely affect endocrine physiology and various body systems
Fecundity	-	the physiological capacity to conceive (often the term fertility is incorrectly used to refer to capacity)
Fertility	-	actual birth performance, can relate to total births including live births and "stillbirths," but usually refers to live births only
FSH	-	follicle-stimulating hormone
GDP	-	gross domestic product
Infecundity	-	also known as "sterility;" opposite of fecundity
LH	-	luteinizing hormone
*Normozoospermia**	-	an ejaculate with sperm concentration of >20 x 10^6 spermatozoa/mL, progressive sperm motility of >50%, or at least 25% of spermatozoa with linear progressive motility and ≥30% of morphologically normal spermatozoa
Oligozoospermia	-	an ejaculate with sperm concentration of <20 x 10^6 spermatozoa/mL

Primary Subfertility	-	experienced by couples who have not conceived after at least 1 year of unprotected coitus
RL Motility	-	rapid linear motility
ROS	-	reactive oxygen species
Secondary Subfertility	-	experienced when couples can no longer conceive after at least one child is conceived, regardless of the birth outcome
Teratozoospermia	-	an ejaculate with <30% of morphologically normal spermatozoa
WHO	-	World Health Organization

*The definitions for normozoospermia, oligozoospermia, asthenozoospermia, and teratozoospermia in this listing are based on WHO 2010 Criteria. These definitions, however, are not relevant to the entirety of the study population. Disparities between WHO 1999 and 2010 criteria will later be described in the text.

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Chapter I: Introduction

Background of the Problem

Decreases in semen parameters and the subsequent rapid increase of male infecundity observed worldwide is a public health crisis that has manifested over the last two to three decades (see Table 1.1 for complete source listing). Semen analysis data from Israel, Japan, Denmark, and Belgium, among emerging studies from additional regions, have indicated different parameters that are suffering from decline. For example, longitudinal and cross-sectional studies in Israel report decreased total sperm motility per ejaculate and percent motility [1,2]. Another retrospective analysis of healthy Belgian men also showed a significant decline in total sperm motility but also an increase in immotile sperm counts from 1977-1995 [3]. Apart from deducing overall trends of decline, semen studies have shed light on exposures that affect semen quality.

Research by the andrology community reports that that reduced semen parameters are associated with heredity, pre-existing health conditions (i.e. mumps, diabetes, thyroid disorders, testicular cancer), genital procedures (i.e. vasectomy, varicocele), drug use (i.e. tobacco, alcohol), and endocrine disruptors found in the environment and occupational settings [2,4]. However, exposure complexity of the stated risk factors fuels inconclusive debate regarding the severity of association on semen outcomes. In agreement with sperm density reviews from 1934-1996, a study highlighting Japan and Denmark as having the lowest semen indicators in the world concluded that regional disparities may arise in varying exposures, such as environmental factors or ethnicity-influenced genetic predisposition to testicular cancer incidence [4]. Findings from the Belgium study counter previous understandings of the effect of tobacco use and alcohol consumption on semen quality because decreasing trends of these habits were correlated with

increasing trends of defunct sperm. Such an observation may be due to confounding where the effect size of tobacco use and alcohol consumption did not strongly influence semen outcomes [3]. As more contradictory findings have called for focused sperm motility research, recent findings suggest that changing environmental patterns worldwide may be the underlying cause for negative health conditions that lead to the deterioration of sperm quality [3,5].

As there is a lack of adequate research on male infecundity in the context of Bangladesh, its burden remains a silent challenge for affected couples. A study conducted by Bangabandhu Sheikh Mujib Medical University (BSMMU) in 2007 found that about 62% of couples seeking infertility care faced primary infertility, or lack of conception since beginning of marriage; 38% experienced secondary infertility, meaning that they conceived at least once in the past, independent of birth outcome [6,7]. In 2010, an estimate of approximately three million Bangladeshi couples (5% of total married couples) classified as subfertile, and 60% of these cases were attributable to reproductive dysfunction in the male partner [8]. Moreover, semen analysis results from the BSMMU study indicated that oligozoospermia, or sperm concentration of $<20 \times 10^6$ spermatozoa/mL, caused couple infertility in 33.3% of cases [6,7]. A Bangladeshi study from the late 90's deduced that azoospermic male partners make up nearly a fifth of men seeking infertility care [9]. Beyond acting as a barrier to conception, reduced semen quality has had multiple effects on society in this context.

Holding women's health at the focal point of subfertility care and research has resulted in a lack of knowledge on andrology and ignorance of male reproductive dysfunction [10]. Male reproductive dysfunction is a physically, socially, and emotionally debilitating condition that has significantly impacted the quality of family life across Bangladesh. Prestige in the Bangladeshi culture is family-centric; individuals who bear offspring and sufficiently provide for their

families are held with high regard in society. Moreover, giving birth to male children is also a favorable asset in this context. Inability to conceive therefore concocts a stressful situation in which multiple family and community members become engrossed in the affected couple's marital issues and provide misplaced commentary on their sexual health. Bangladesh's patriarchal society naturally shames female partners when couple subfertility arises. Furthermore, society holds the widely-accepted misconception that infertility is only attributable to the female partner's inability to conceive. Implications of such misconceptions include social encouragement for men to remarry, family unrest, victimization of wives through unconsented polygamy and divorces by husbands, and in some cases, suicide or homicide [11].

Purpose and Aims of the Study

The purpose of this study is to characterize male reproductive function in Bangladesh by deriving the male population's semen quality and describing causes that may be associated with observed outcomes. The following aims were specified to fulfill the purpose of this study:

1. Establish temporal trajectories of sperm parameters in Bangladeshi males who are seeking infecundity care at an assisted reproduction facility in Dhaka, Bangladesh
2. Identify known risk factors from medical records of subset within the Aim 1 cohort to evaluate correlation with resulting sperm qualities

Significance Statement

Though there is existing literature that confirms temporally declining semen parameters in diverse nations around the world, little research has been done on the issue and interconnected health behaviors in the context of South Asia and Bangladesh. This study is integral to

strengthen evidence-based subfertility care for South Asian populations and developing nations similar to Bangladesh. Findings may be used to encourage preventative measures against health behaviors that lead to reduced fecundity in males. Moreover, knowledge of male reproductive dysfunction will increase awareness to reduce gender inequalities in which women are shamed for couple subfertility.

Chapter II: Review of Literature

Aging

Irrespective of region, literature regarding the relationship between aging and semen quality agrees unanimously that reproductive dysfunction declines throughout a male's lifespan. This inverse relationship accounts for decreased sperm motility, percentage of motile sperm, semen volume, sperm density, and normal morphology, even while controlling for confounding factors [5,12,13]. Stone et al. found that the typical age threshold before immediate decline in sperm concentration and motility is 34 years, while the threshold for sperm morphology decline is after 40. After age 45, ejaculate volume is also jeopardized; the overall ratio of male-bearing sperm decreases significantly after age 55 [14]. Thus, age range 34-55 years is when semen quality most suffers, and the margin of decline progressively worsens with each year [15]. Studies in Colombia, Korea, and India have comparable results, which indicate that the semen parameter changes attributable to increasing age are universal across all contexts [16-18].

A study in Bangladesh sampling 1,121 male partners indicated significant association of increasing male age on semen parameters ($p<.01$ for decreasing sperm motility and rapid linear (RL) motility; $p<.05$ for decreasing semen volume) [19]. Additional reports from India and Pakistan indicate similar findings for total motility and rapid linear motility as described in the Bangladesh study but with the exception of sperm concentration remaining stagnant and unaffected by increasing age [20].

Loss of reproductive function at a young age is preventable and continues to be a challenge for treatment when aged men seek care at acute stages. Increasing paternal age, or a male partner's age at first conception, has strong implications on sperm parameters and couple fertility status. Studies from infertility clinics comparable to BIRDEM indicate that the mean

age of male patients seeking care is roughly 35 years, and duration of couple subfertility averages 6-10 years of marriage [9,21]. Decreased testicular function due to age sets the stage for assisted reproduction outcomes, incidence of preterm births, and spontaneous abortion; additional research is required to understand the burden and biochemical mechanisms for such adverse birth outcomes [22-24]. Older men who are attempting to conceive are advised to take dietary antioxidant supplements and make lifestyle changes to retain reproductive function to some degree [25]. Abstinence duration also impacts seminal parameters because short time periods between ejaculation is associated with decreasing semen quality [26].

History of Illnesses and Procedures

While most literature from Bangladesh and similar contexts make conflicting statements regarding association of health conditions with male fecundity, multiple studies from other contexts have found correlation and/or causation of reduced semen parameters with health conditions. As semen quality is impacted by life-course exposures, male factor subfertility has observable comorbidity with chronic illnesses such as cardiovascular disease, diabetes, and metabolic syndromes such as overweight and obesity [27,28]. These conditions can reduce testosterone levels and increase estradiol, thus affecting spermatogenesis and subsequent reproductive function [21,29,30]. Hormonal imbalance history such as thyroid and other endocrine disorders are also important indicators for potential irregularities in spermatogenesis [31].

The presence of various malignancies (i.e. hernia, varicocele) and irritation in the testicular region heavily impact semen parameters [9,21]. As previously discussed, decreasing sperm density with increasing age is a trend that coincides with incidence of testicular cancer

[13,26]. Males diagnosed with testicular cancer do not have semen parameters that qualify for diagnosis of normozoospermia in comparison to males without testicular cancer. In addition, rapid linear motility especially suffers for individuals with testicular cancer in comparison to other malignancy and non-cancer groups [32]. Testicular tumors and other malignancies such as Hodgkin lymphoma have shown correlation with reduced semen concentration [33]. Undergoing treatment for malignancies increases abnormal semen quality due to effects from radiation and post-surgical conditions [34]. Orchiectomy, bariatric surgery, and other pelvic and genital surgeries typically have severe, lasting effects on reproductive dysfunction for the remainder of the patient's life course [35-37].

Systemic infections also play an important role in determining reproductive capability in males. Accumulation of pathogens that stimulate immunoglobulin G systemic responses is correlated with impaired semen parameters [38]. In the case of *Helicobacter pylori*, semen levels of inflammatory cytokines are increased, which leads to overall sperm damage [39]. Other conditions that lead to similar mechanisms affecting spermatozoa include HIV infection and chronic bacterial prostatitis [40,41]. Mumps is the only detected risk factor from childhood that is agreed to lead to complete sterility in adulthood due to altered protein activity in the Sertoli cells of testes [37,42]. The occurrence of all of these medical conditions and procedures from over the patient's life course should be well-documented in order to detect root causes and provide optimal treatment.

Drug Use: Tobacco and Alcohol

Like aging, there is consensus across literature that smoking negatively impacts semen quality, particularly by decreasing motility and lowering concentration [26,29,43-45]. Semen

quality is significantly reduced by progressive frequently of cigarette smoking and overall duration of smoking in a male's life course [45-47]. Moreover, effects of smoking impact testosterone, prolactin, and follicle-stimulating hormone (FSH) regulation in the body, which can be detected in semen sampling [48,49]. FSH and luteinizing hormone (LH) levels are higher in smokers than non-smokers, while testosterone levels have an inverse relationship with frequency of smoking [50,51]. As a result, smokers are more likely to be diagnosed with oligozoospermia, asthenozoospermia, and teratozoospermia in comparison to non-smokers [48,49].

A study from Iran stated that with each increase of one cigarette per day, sperm motility decreases by 1%. Within a year of smoking, 800,000 sperm are lost [21]. A cross-sectional study from a Bangladeshi infertility clinic found that 50% of men were smokers, and 20.7% of the men smoked 10 or more cigarettes a day. Both non-smokers and smokers suffered from secondary infertility. However, findings were not deemed reliable due to study limitations and lack of similar data from this region [52]. Because cultural smoking habits are similar in Bangladesh and the low-resource contexts described, data from these contexts can be used to inform smoking-associated male subfertility in Bangladesh.

Unlike smoking status, alcohol consumption may or may not have implications on semen quality. Studies in the United States and Europe found that moderate alcohol intake is associated with higher levels of testosterone than normal and does not adversely affect semen quality [53]. However, there appears to be a strong association between total alcohol consumption and total testosterone levels, especially in young males who consume greater than 20 units of alcohol weekly. Similar to smoking, progressive alcohol consumption to levels of alcoholism and binge drinking heavily impact testosterone levels and spermatogenesis; a study using ethanol levels observed in sera after heavy drinking (300 mg/dL) found irreversible damage to spermatozoa

[54,55]. A meta-analysis of studies spanning Western countries agrees that there are detrimental effects of heavy alcohol intake on semen quality but minimal to no effects on semen for males with moderate drinking behaviors [56].

Reducing smoking and alcohol consumption may improve semen parameters. Alcohol withdrawal leads to rapid improvement of semen parameters because maturation arrest of germinal cells in the testicles is reversed. In a longitudinal study reported by Sermondade et al., normozoospermia was observed for a patient followed over six years after just three months of alcohol withdrawal; the patient was previously diagnosed with severe teratozoospermia, oligoasthenozoospermia, cryptozoospermia, and azoospermia [57]. Hosseini et al. found that an Iranian cohort experienced 14-26% increased sperm concentration, 8-27% improved sperm motility, and 5-20% regained sperm morphology from completely ceasing smoking activities. Moreover, a third of couple subfertility was reversed in this cohort without any additional fertility treatment [58]. Currently no literature characterizes the impact of secondhand smoke exposure on semen quality, but its burden may be harmful to males, as will be explained in the section on endocrine disruptors.

Endocrine Disruptors from Environmental and Occupational Exposures

Meta-analyses consisting of male infecundity studies indicate an overall decline in human sperm quality since the 1950s, especially from high environmental exposures to endocrine disruptors in industrialized nations [59]. Endocrine disruptors are classified as substances with the potential to adversely affect endocrine physiology and various body systems. They mimic other hormones in the body and bind to receptors in the testes, which results in less testosterone binding, thus altering reproductive hormone secretion and hormonal control during

spermatogenesis [60-62]. Frequent exposure to highly toxic substances specifically containing BPA, DDT, DES, dioxin, PBC, phthalates, and phytoestrogens leads to male factor infertility [63]. Such substances can be produced from everyday products like plastic, metal cans, detergents, pesticides, cosmetics, etc. [60]. Environmental pollution is a major source of reactive oxygen species (ROS), which behave as endocrine disruptors that are most damaging of substances to semen quality [26]. In addition, by-products formed from chlorination of drinking water have a similar effect in the body. Formation of disinfection by-products (DBP) in chlorinated water and the body's absorption rather than excretion of DBPs leads to high levels of spermatotoxicity [64,65].

Similarly, occupational exposures to toxins from organic solvents, lead, and radiation act as endocrine disruptors to alter reproductive hormone secretion and hormonal control during spermatogenesis [61,62]. The correlation between lead and semen quality was analyzed through blood and semen samples from a cohort of Mexican males who had chronic occupational lead exposure. Results showed that the exposed group had higher lead concentrations and lower sperm quality (decreased sperm concentrations, lower motility, lower viability, and abnormal morphology) than the non-exposed group. Thus, chronic exposures, including in-utero or early exposures, to endocrine disruptors result in the pathogenesis of subsequent sperm abnormality [26,66]. Regional differences in semen quality can be observed in varying environmental exposures, as seen deduced by multiple European studies [67,68]. However, the associations between region, endocrine disruptors, and sperm count are inconclusive because the quality of data and nature of confounding when trying to quantify the exposure is too difficult [29]. Thus, no causative agent has been deduced.

Bangladeshi males are especially vulnerable to spermatotoxicity due to poor environmental qualities and increasing demand for factory jobs that have little consideration for employee health outcomes. Bangladesh's main GDP contributors are the industry and service sectors, 30.4% and 53.6% respectively. Out of the 81.5 million Bangladeshi nationals in the total labor force, a majority are involved in these sectors [69]. Employees have high levels of exposure to endocrine disruptors because factories are inconsistently? regulated and house pollutants from fossil fuel consumption, electricity, and fabricated metal production [70]. Furthermore, manpower outsourcing by the Bangladeshi government has sent over 5.4 million men to India, Saudi Arabia, Kuwait, and other countries to perform cheap labor in high-risk industries; workers frequently travel between Bangladesh and their workplace, leaving wives and families behind [71,72]. Domestic concerns are substantial because the most populous city, Dhaka, has been classified by the World Health Organization (WHO) as the third most polluted megacity in the world and thus remains a major source of reactive oxidative species that behave as endocrine disruptors [73]. Based on the WHO's ranking and current knowledge of the effect environmental factors have on semen quality, exploring semen quality trends in Bangladesh is integral to understanding how this exposure may provide an added challenge of preventing male infertility in this context.

Chapter III: Methods

Study Participants

BIRDEM CARE serves individuals and couples who are either curious of their fertility statuses or have been unsuccessful in conception for an extended period. Most CARE patients reside in Dhaka, but services are extended to patients who visit from different regions of Bangladesh, as well as from other countries. When couples come to CARE for their initial visit, male partners undergo semen analysis in the CARE Andrology Lab as a process of elimination before female partners are tested via laparoscopy. In addition, one-on-one intake assessments are conducted with male partners in private to inform the background of each case. Questions listed on the intake sheet incited responses regarding various life course exposures that have previously been stated to affect semen quality. Participation in the cross-sectional study is voluntary. Patients are required to provide informed consent signatures for their test results to be added to the semen study database, and they are given a hard copy of results after testing. Furthermore, patients are offered specialized counseling services on a case-by-case basis if hospital staff deems that non-clinical factors are critically impacting the overall well-being of both or one partner.

Semen Analysis Procedures

Semen collection and analysis methods are based on the WHO's Laboratory Manual for the Examination and Processing of Human Semen (4th and 5th ed.) [74]. Study participants are prompted to provide a semen sample via masturbation or intercourse after at least 3-5 days of abstinence in order to maximize sperm concentration [26,29]. The amount of semen the

participant provides is one of the indicators for normative semen quality, and based on global trends, normal values have been declining (Table 3.1) [75].

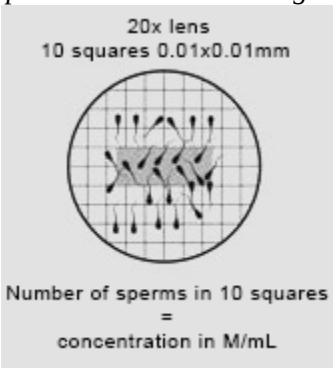
Table 3.1. *WHO reference values for normal semen characteristics, 1999-2010 [75]*

Semen Parameters	**WHO 1999**	**WHO 2010**
Volume (mL)	≥ 2	1.5
Concentration ($x10^6$/mL)	≥ 20	15
Total sperm count ($x10^6$)	≥ 40	39
% Total motility	≥ 50	40
RL motility*	≥ 25% (Grade A)	≥ 25% (A + B) or 32% (A)
% Normal morphology	14	4

*Grade A = rapid linear motility (> 25 μm/s), Grade B = slow linear motility (5-25 μm/s)

Samples are tested after a 30-minute to 1-hour resting period to allow for liquefaction. Testing should not be conducted after one hour because semen quality may be compromised from dehydration or temperature change effects. After liquefaction and gentle swirling, 2-3 drops of the semen sample are placed onto a Makler counting chamber for observation via a phase-contrast microscope (10X magnification). The grid on the base of the Makler chamber (0.01 mm x 0.01 mm) is used to quantify sperm qualities.

Figure 3.1. *Counting sperm concentration using a Makler chamber [76]*



Concentration of sperm is calculated by estimating the total number of spermatozoa in a strip of 10 squares either horizontally or vertically; the resulting count represents the number of sperm in millions per milliliter. Counts <15 x $10^6/mL$ must be recalculated as the sum of spermatozoa in the entire grid divided by 10 for the final count. Among these sperm, the fastest moving ones are classified as Grade A and can be distinguished by swift tail movements, resulting in easy movement through semen. Grade B sperm move slowly, while Grade C sperm do not show any movement. Rapid linear motility is calculated by summing the percentages of Grade A and Grade B sperm. Morphology is typically analyzed by viewing the sample under moderate to high magnification. The number of sperm which differ from the normal tadpole shape or swim abnormally are considered abnormal. The percentage is calculated using a similar process as motility. Based on the magnification, an estimate count from the visible grid is set in a proportion equivalent to the 10 x 10 grid.

Study Design

Two studies were conducted to address the aims outlined in this thesis. Aim 1 is fulfilled through the evaluation of cross-sectional data collected from participant semen samples in the Centre for Assisted Reproduction (CARE) at the Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine, and Metabolic Disorders (BIRDEM) General Hospital from January 2000 to August 2016 (n = 13811). Semen parameters of interest include concentration (x $10^6/mL$), % total motility, % RL motility, and % normal morphology. Because qualitative surveys were conducted in conjunction with semen analyses but not databased, survey responses were recorded for patients visiting CARE from May-August 2016 (n = 72). Aim 2 is

informed via a mixed-methods approach because the availability of both semen analysis and exposure data allowed for regression to express effects in all interviewees.

The total 17-year study cohort originally consisted of 13,954 participants. 143 participants were excluded from the Aim 1 analysis due to incomplete semen parameter data. Missing values for concentration existed in 6187 datasets, so concentration diagnosis (azoospermia, normozoospermia, and oligozoospermia) was used in lieu of quantitative concentration for analysis. These datasets were not excluded. Of the interviews in the database from May-August 2016, 72 had corresponding semen analysis data recorded. The remainder were excluded for the Aim 2 analysis. Out of the 72 datasets analyzed, some had missing values for duration of marriage (n=3), duration of couple infertility (n=6), and/or type of infertility (primary or secondary) (n=3). These datasets were not excluded from analysis.

Statistical Methods

All analyses were conducted using SAS® statistical software, version 9.4. Normal and non-normal variables were reported as mean ± standard deviation (SD) and median (interquartile range (IQR)), respectively, for baseline analyses. For data addressing Aim 1, baseline characteristics were represented by year of testing. Significance between annual means and medians were determined by parametric one-way ANOVA tests (significance = $p < .05$). Concentration diagnoses were adjusted based on WHO 2010 criteria in order to account for missing data in the concentration variable. Azoospermia, motility, and RL motility were regressed by age of patient at the time of testing and duration of abstinence prior to testing to control for confounding. Odds ratios (OR) and 95% confidence intervals (CI) were reported for azoospermia adjusting for age and abstinence. Aim 2 baseline characteristics were stratified by

concentration diagnosis. Quartiles were represented for age, smoking status, and semen parameters. Parameters were then correlated by the risk factors in step-wise multiple regression analyses.

Chapter IV: Results

SECTION A: Manuscript - "Decline in semen parameters of Bangladeshi males attending in tertiary care hospital from 2000-2016"

Author Contributions

Dr. Nusrat Mahmud initially proposed the study objectives and design, secured ethical clearance for the study, drafted the discussion portion, and approved the final version for publication. Eshita Sharmin is the primary contributor to writing, editing, and preparing the manuscript for submission to the *Indian Journal of Urology*. In addition, Eshita Sharmin led data interpretation and analysis. Md. Arif Mamun was the primary data collector, which included conducting semen analyses and maintaining the study database. Zayan Shamayeen conducted literature review, assisted with data analysis/interpretation and cleaning, prepared references, and actively participated in writing and editing the final manuscript. Natalie Rivadeneira was responsible for regression modelling in the data analysis. Dr. Roger Rochat actively edited the manuscript and provided insight into the study design and flow of analysis. Dr. Akanksha Mehta provided critical insight of urology epidemiology for the analysis (variable measures, stratified analysis) and discussion. All authors reviewed and consented to the final draft proposed for publication.

Abstract

Introduction: The objective of this study was to analyze longitudinal changes in sperm parameters of Bangladeshi men. We hypothesized declined semen parameters for this population.

Methods: We analyzed retrospective, cross-sectional semen data from males aged 15-64 years at an infertility clinic in Dhaka, Bangladesh from January 2000 to June 2016 (n = 13954). Exclusion criteria included samples missing one or more semen parameters (n = 143). WHO normal criteria and semen analysis procedures were used to evaluate parameters of the remaining 13,811 specimens. Datasets with missing concentration values (n = 6187) were excluded from raw concentration analyses. Age and duration of abstinence at testing were recorded and analyzed as confounders. Data were imported into SAS® 9.4 statistical software. Temporal significance was investigated using one-way ANOVA for motility parameters and Chi-square test for raw concentration. Logistic regression analyzed the effects of confounders on azoospermia and raw concentration, while median regression modelling adjusted confounders for concentration, total motility, and RL motility.

Results: Age distribution was significantly correlated with annual parameter changes (concentration, total motility, and RL motility, ($p < .0001$)). Adjusted total motility and RL motility declined by 20% from their maximum values to end of the study period ($p < .0001$). Raw concentration lacked clear trends and was unaffected by adjustment. Azoospermia increased by 18% between the 2000-2010 and 2011-2016 participants (OR = 0.16[0.14-0.16]).

Conclusion: In agreeance with the hypothesis, Bangladeshi males attending this clinic have experienced declines in semen parameters (total motility and RL motility) and increased frequency of azoospermia.

Introduction

Decreases in national and regional trends of sperm quality indicators - sperm count, percentage of sperm motility, sperm density, and normal sperm morphology - have been

explored globally over the last two to three decades.[1] Longitudinal and cross-sectional studies in Israel showed that the average sperm parameters in the nation have dropped over the last twenty-five years, as seen in significant decreases of total motile sperm counts per ejaculate and percent motility.[2,3] A retrospective analysis of semen in healthy Belgian men showed a significant decrease in motile sperm and increase in immotile sperm from 1977-1995.[4] Another study highlighted Japan and Denmark as having the lowest semen indicators in the world. This study, as well as a review on all sperm density studies done from 1934-1996, concluded that while geographical location of nations may result in regional disparities for semen quality, parameters have declined for overall and in both regions.[5,6]

While male semen data is available for most of the global community, South Asian countries lack research studies. A 2007 study conducted by the infertility unit at the Bangabandhu Sheikh Mujib Medical University (BSMMU) found that about 62% of couples attending the infertility unit faced primary infertility, while 38% experienced secondary infertility. Semen analysis results from this study indicated that among the male partner, oligozoospermia, or sperm concentration of $<20 \times 10^6$ spermatozoa/mL, caused couple infertility in 33.3% of cases.[7,8] In 2010, an estimated 3 million Bangladeshi couples were subfertile and for 60% of those couples, the male partner was responsible.[9]

The objective of this study was to analyze changes in semen quality of a subset of the Bangladeshi male population attending an infertility clinic between 2000 and 2016. Through this study, we hoped to establish whether there is an observable decline of semen parameters in Bangladeshi males, as determined by trends recorded for motility, morphology, and concentration. Based on trends observed in the global community, we hypothesized that there is a temporal decline in semen parameters for the study population.

Materials and Methods

Ethical Approval

The Ethical Review Committee (ERC) of the Diabetic Association of Bangladesh (BADAS) approved the protocol of this study (memo no. BADAS-ERC/EC/16/0091). Participants were required to provide signed consent for their analysis results to be included in the study database and received signed analysis reports for their personal records.

Study Population and Participants

Data collection for this study was conducted in the Centre for Assisted Reproduction (CARE) at Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine & Metabolic Disorders (BIRDEM) from January 2000 to June 2016. CARE is one of the largest infertility clinics in Bangladesh and a major clinic for infertility referrals. A majority of patients at CARE reside in Dhaka, Bangladesh, but services are also provided to patients from other regions in the country and those visiting from overseas. Upon intake, male partners are tested via semen analysis to determine whether their sperm has normal function or abnormal qualities (see procedure in "Semen Analysis Procedures").

The overall study population consisted of n = 13,954 participants. 143 participants were excluded from analysis due to having data sets with one or more semen parameters missing. 6,187 data sets were missing in the quantitative concentration dataset, but raw concentration was still analyzed with missing values excluded. There is also not any data available for 2006, so the study population includes male participants who visited BIRDEM CARE from 2000 to 2005, then 2007 to June 2016.

Semen Analysis Procedures & Calculations

All semen analyses were conducted by a single lab technician who used the same type of lab materials for the entire duration of the study period. The methods used for semen analysis are outlined in WHO's Laboratory Manual for Examination and Processing of Human Semen (4^{th} and 5^{th} ed.).[11] Participants provided semen samples through masturbation or intercourse at the on-site masterbatorium. 3-5 days of abstinence prior to sampling was advised, and duration was recorded. Samples were liquefied for 30 minutes then gently swirled before 2-3 drops were extracted. Drops were loaded onto a Makler counting chamber (0.01 mm x 0.01 mm grid) and observed under a phase-contrast microscope at 10X magnification. Concentration was found by estimating the total number of spermatozoa (in millions per milliliter) in 10 consecutive grid squares then multiplying by 10. If the count is less than 15 M 10^6/mL, the sum of spermatozoa in the whole grid can be divided by 10 to give the concentration reading. The sperm with rapid, streamline motion in the semen were grouped as Grade A. Grade B sperm moved slowly, and Grade C sperm lacked movement. Total motility was calculated as Grade A sperm + Grade B sperm. Rapid linear (RL) motility only accounted for the % of Grade A sperm.

The lab technician determined morphology by adjusting the microscopic view to a higher magnification so that physical characteristics of the spermatozoa were visible. The count of sperm that were not in the normal tadpole shape or swim abnormally were considered to have abnormal morphology. The percentage of sperm with abnormal morphology were estimated based on the magnification of the grid, proportional to the overall 10x10 Makler chamber grid.

Statistical Analysis

The dataset (n = 13,811) was imported from an electronic database into SAS® 9.4 statistical software (Cary, North Carolina) for analysis. Because normality tests presented semen parameters to be severely skewed, median (interquartile range (IQR)) were reported for parameter baseline. Age, duration of abstinence, and liquefaction were reported as mean ± standard deviation (SD). Significance of difference between annual means and medians was found via parametric one-way ANOVA tests, while raw concentration significance was determined via Chi-square distribution analysis. $p < .05$ deemed statistical significance. Raw concentration data that presented as missing in SAS due to missing and/or incomplete records (n = 6,187) were excluded in the logistic regression analyses of this variable. To control for confounding, concentration, motility, and RL motility were adjusted by median age of patient at the time of testing and median duration of abstinence prior to testing via median regression modelling.

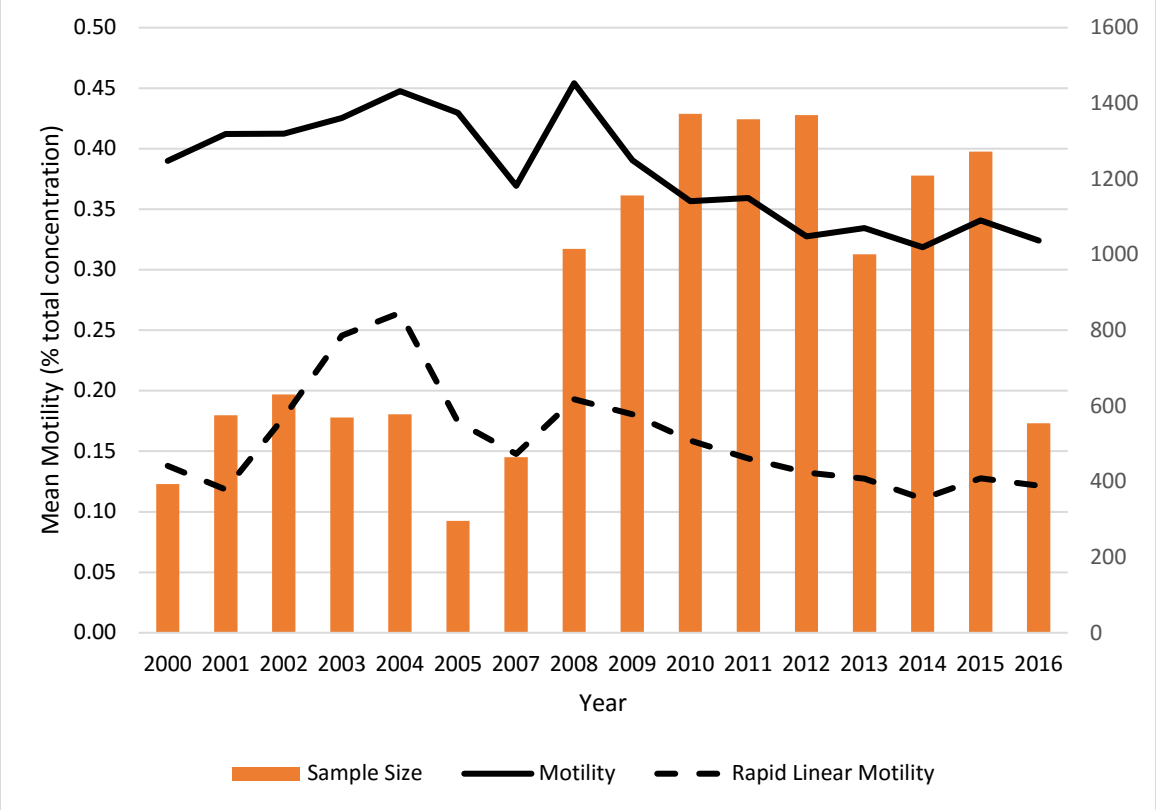
Due to the incompleteness of the raw concentration dataset, qualitative azoospermia concentration diagnosis was evaluated in logistic regression analysis because it is more definitive. Other diagnoses such as normozoospermia and oligozoospermia were not used in analysis because WHO criteria changes between 1999 and 2010 affected diagnosis frequencies.[10] To account for the criteria change, concentration diagnosis frequencies were adjusted separately based on WHO 2010 criteria. Morphology reporting in the dataset was inconsistent with WHO grading criteria and therefore omitted from analysis beyond baseline.

Results

Baseline semen characteristics, age, and duration of abstinence of the study population (n = 13811) are recorded in Table 4.1.1. The average age of participants was 35.4 ± 6.6 years, and

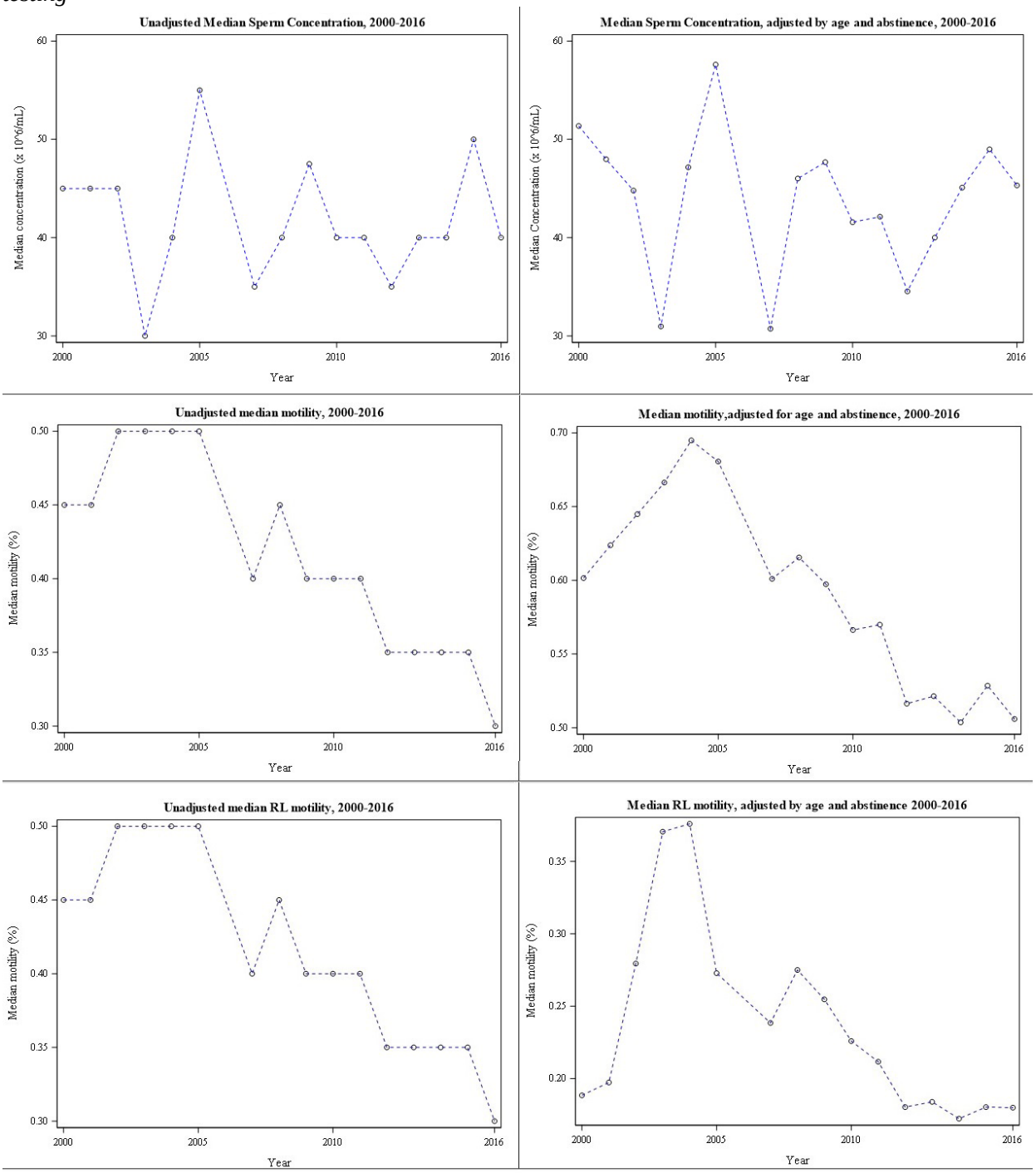
age distribution was significantly correlated with annual parameter changes ($p < .0001$). Duration of abstinence ($p = .05$) and liquefaction ($p = .07$) remained unchanged in annual comparisons, both averaging around 5.2 ± 3.8 days and 1.0 ± 0.08, respectively. All semen parameters (concentration, total motility, RL motility, and normal morphology) appeared to vary drastically ($p < .0001$) over time. Total motility is severely reduced from the beginning of the study period to the end, where the peak median of 50% decreases to consistent 30% median motility in the study population (Figure 4.1.1). RL motility is similar, where the median becomes a consistently low value of 10% from 2011-2016. There is an association between declining motility parameters and increasing annual sample size.

Figure 4.1.1. *Annual mean motility and rapid linear motility presented as % of total concentration and compared to annual sample sizes*



In order to control for potential confounding from age and duration of abstinence, adjusted medians for total motility and RL motility were estimated using median regression models. Unadjusted versus adjusted plots for raw concentration and both motility variables can be seen in Figure 4.1.2. The unadjusted median graphs simply indicate annual medians, while adjusted median graphs control for age and abstinence. Crude total motility medians decrease temporally, as previously described, but upon adjustment, median estimates per year from 2004 experience a steady decline. This observation is different from the trends stagnancy from 2009-2011 and 2012-2015. RL motility follows a similar suit where the parameter begins to decline from 2004 then is reduced drastically from 2008-2016. Raw concentration medians lacked clear trends and remain unchanged upon adjustment.

Figure 4.1.2. *Median regression models of unadjusted and adjusted raw concentration, total motility, and RL motility parameters confounded for age at the time of testing and duration of abstinence prior to testing*



Concentration diagnosis frequencies for normozoospermia, azoospermia, and oligozoospermia (severe, moderate, mild) are impacted by changing WHO parameters (Table 4.1.2). WHO 1999 parameters apply to datasets from 2000-2010, while 2010 parameters were standardized at BIRDEM CARE for the duration of 2011-2016. With this change, normozoospermia reduced from $\geq 20 \times 10^6$/mL to $>15 \times 10^6$/mL. Oligozoospermia parameters decreased to values outlined in Table 4.1.2.

Table 4.1.2. *Concentration diagnoses (normozoospermia, azoospermia, oligozoospermia) adjusted by WHO 2010 parameters*

Diagnosis	**WHO 1999***	**WHO 2010†**	**Overall (Crude)**	**Overall (Adjusted)**
Normozoospermia	4888 (71.2)	4330 (64)	9218 (66.7)	9402 (68.1)
Azoospermia	857 (10.9)	1008 (14.9)	1865 (13.5)	1865 (13.5)
Oligozoospermia‡ Severe Moderate Mild	 678 (7.8) 311 (4.8) 314 (5.4)	 740 (10.9) 379 (5.6) 306 (4.5)	 1418 (10.3) 700 (5.1) 610 (4.4)	 1418 (10.3) 700 (5.1) 426 (3.1)
Total, n (%)	n = 7048 (51)	n = 6763 (49)	n = 13811 (100)	n = 13811 (100)

*Participants from 2000-2010; normozoospermia parameter is $\geq 20 \times 10^6$/mL; mild oligozoospermia is 10-20 x 10^6/mL
†Participants from 2011-2016; normozoospermia and mild oligozoospermia parameters reduced to $>15 \times 10^6$/mL and 10-15 x 10^6/mL, respectively
‡Severe ($<5 \times 10^6$/mL) and moderate (5-10 x 10^6/mL) oligozoospermia parameters are consistent for both WHO 1999 and 2010 groups

Because frequencies were adjusted to WHO 2010 parameters, normozoospermia frequency increased from 66.7% to 68.1% of the study population, while mild oligozoospermia diagnosis decreased from 4.4% to 3.1%. Azoospermia and moderate and severe oligozoospermia remained constant between crude and adjusted calculations for median years of age (35 years) and median duration of abstinence (5 days). Odds ratio (OR) of azoospermia adjusting for age and duration of abstinence is 0.16[0.14-0.16] (Table 4.1.3).

Table 4.1.3. *Results of logistic regression of azoospermia among study participants*

Parameter	Estimate	Standard Error	Pr > ChiSq	OR	95% CI
Intercept	-1.8632	0.0251	<.0001	0.16	(0.14, 0.16)
Median age	-0.0197	0.00452	<.0001	0.98	(0.97, 0.99)
Median number of days of abstinence	0.0293	0.00673	<.0001	1.03	(1.02, 1.04)

OR for males at median age to present normozoospermia is 1.03 times higher ($p < .0001$) than men of other age groups (Table 4.1.4). Holding age constant, OR for males who have abstained from sex for 5 days and present normal sperm concentration in semen analysis is 1.02 times higher ($p = .008$) than men who have abstained less than five days. At median age and duration of abstinence, the likelihood of males expressing normozoospermia is 2.2 times higher than for all other males.

Table 4.1.4. *Results of logistic regression of raw sperm concentration among study participants*

Parameter	Estimate	OR	Standard Error	Wald Chi-Square	*p*-value*
Intercept	0.7916	2.21	0.0281	795.462	<.0001
Median age	0.0318	1.03	0.00485	43.086	<.0001
Median number of days of abstinence	0.0218	1.02	0.00825	7.0216	.0081

*From Chi-square distribution

Discussion

In response to the research question, declining semen quality is apparent for Bangladeshi males in our study population from 2000-2016. Our semen analysis data supports the decline of semen parameters by indicating that annual medians of total motility and RL motility decrease in both unadjusted and adjusted analyses. Trends and magnitude of decline in these parameters are

clearer upon confounding for age and duration of abstinence. Increase in azoospermia also is contingent upon temporal factors when regressed by age and duration of abstinence.

A unique observation to note is that the overall frequency of normozoospermia increased upon adjusting for WHO 2010 concentration diagnosis, though this finding does not indicate that the actual frequency of fertile males increased. The criteria change is solely responsible for the shift in normozoospermia. Given the trends observed in parameters, it would naturally be predicted that frequency of normal men would decrease. Decreasing the parameter from ≥20 x 10^6/mL to >15 x 10^6/mL resulted in more participants qualifying for normozoospermic classification. Because the benchmark for azoospermia remained constant between WHO 1999 and WHO 2010 parameters, frequencies can be more reliably compared. The increase in frequency of azoospermic participants from 10.9% of the 2000-2010 subset to 14.9% of the 2011-2016 indicates a significant decrease in concentration across the population. This finding is consistent with past meta-analyses from international literature that describe semen concentration trends over the last few decades.[1] More recent studies on concentration trends also have exposed decreased parameters in countries not previously studied, such as Israel, India, and New Zealand.[2,12,13] However, adjusted and unadjusted raw concentration analyses do not indicate a clear trend of decline for our study population.

When observing the trends of decline as we have in our findings, it is imperative to inquire about potential causes for the burden of male infertility to increase so rampantly. Because our data does not include measures for risk factors, we can simply extrapolate root causes based on available literature. The potential risk of endocrine disruptors on semen quality has actively been debated amongst the global community as the most influential cause of male infertility.[1] Industrialization is peaking this concern because more individuals, especially in

developing nations, are becoming exposed to harmful toxicities, thus resulting harmful effects such as male infertility. Regions of widespread industrialization generally experience higher rates of oligozoospermia than other areas.[14] Occupational exposure to toxicants such as organic solvents and pesticides also stem from industrialization and may have degenerative effects directly on reproductive organs or on hormonal balance that is crucial for growth, sexual development, and physiological functions.[15,16] Environmental factors can also affect male reproductive tract development and other physiological functions when exposure to endocrine disruptors is normalized in unsanitary, polluted environments.[17] Conclusions from our study are applicable to other developing nations where endocrine disruptors present through industrialization and environmental factors are prevalent.

The longevity of data collection and impact of CARE as a major infertility clinic in Bangladesh provide strength to this study and show that studies of this nature are feasible despite Bangladesh being a low-resource setting. Moreover, semen analysis readings and methodology should be consistent because the analyses were conducted by a single observer who used the same type of lab materials for the entire duration of the study period.

Conversely, several improvements were needed in the study design and data set. Although it was previously described that normozoospermic and azoospermic participant frequency increased with time, variation in sample size is evident in the fluctuation of patients attending CARE during the second half of the study period. Therefore, the increase in frequency of azoospermic individuals may have offset the expected decrease in normozoospermic individuals. Moreover, there appeared to be a positive trend in motility parameters between 2000 and 2004, which may have been affected by substantially-reduced sample size compared to

post-2008 data. The parameters would not have been affected by WHO criteria changes since the new normal cut-offs were posted in 2010.

Among the correlated dataset, outliers who do not reside in Dhaka or have drastically different life course exposures are not eliminated. Therefore, the effect of confounding due to influential risk factors (i.e. exposure to toxins, pre-existing health conditions, environmental factors, drug use) is not clear. All study participants were from a single clinic, thus limiting generalizability of our results. Moreover, we were unable to follow single participants over time and determine whether multiple data sets represented a single participant due to a lack of patient identifiers. Significant measurement bias exists where WHO 2010 semen parameters have been deemed as unreliable from emerging studies because they are determined by the world population at large, thus potentially not providing a true measure for the burden of infertility as differed regionally.[10] An absence of raw concentration counts for 6,187 participants makes it difficult to assess whether the lab technician's classification of oligozoospermia versus normozoospermia is consistent over time. Though consistently reported by a single technician, measurement bias also exists where human error affects accuracy of semen parameter readings.

This study provides a rationale for conducting prospective studies on male infertility in the context of Bangladesh and neighboring South Asian countries. As we established the trend of decline in sperm parameters in a clinic population, the next step might be to determine whether this is also true for the overall population and, if so, then to evaluate reasons for observing such a decline. Controlled studies tracking life course exposures of males in Bangladesh that are supplemented with extensive patient history, semen data, lifestyle factors, and effects of xenobiotics on reproductive hormones would help describe how the burden of male infertility may be reduced and prevented. There is a need for global action to solidify an

understanding of declining semen holistically in order to combat specific causes for the prosperity of future generations. Moreover, improvement of WHO parameters to provide a clearer definition for male infecundity as varied by context would improve treatment regimens of male partners significantly.

Manuscript References

1. Carlsen E, Giwermman A, Keiding N, Skakkeback NE. Evidence for decreasing quality of semen during past 50 years. *BMJ* 1992; 305:609-613.
2. Almagor M, Ivnitzki I, Yaffe H, Baras M. Changes in semen quality in Jerusalem between 1990 and 2000: a cross-sectional and longitudinal study. *Archives of Andrology* 2003; 49(2):139-144.
3. Haimov-Kochman R, Har-Nir R, Ein-Mor E et al. Is the quality of donated semen deteriorating? Findings from a 15 year longitudinal analysis of weekly sperm samples *Israel Medical Association Journal* 2012; 14(6): 372-377.
4. VanWaeleghem K, De Clercq N, Vermeulen L, Schoonjans F, Comhaire, F. Deterioration of sperm quality in young healthy Belgian men *Human Reproduction* 1996; 11(2): 325-329.
5. Iwamoto T, Nozawa S, Yoshiike M et al. Semen quality of 324 fertile Japanese men *Human Reproduction* 2005; 21(3): 760-765.
6. Swan SH, Elkin EP, Fenster L. The question of declining sperm density revisited: an analysis of 101 studies published 1934-1996 *Environmental Health Perspectives* 2000; 108(10): 961-966.

7. Akhter S, Alam H, Khanam NN, Zabin,F. Characteristics of infertile couples. *Mymensingh Med. J.* 2011; 20:121-127.
8. Čipak A, Stanić P, Đurić K, Serdar T, Suchanek E. Sperm morphology assessment according to WHO and strict criteria: method comparison and intra-laboratory variability *Biochemia Medica* 2009; 19(1): 87-94. http://dx.doi.org/10.11613/BM.2009.009
9. Bashed MA, Alam GM, Kabir MA, Al-Amin AQ. Male Infertility in Bangladesh: What Serve Better-Pharmacological Help or Awareness Programme?. *International Journal of Pharmacology* 2012; 8:687-694. DOI: 10.3923/ijp.2012.687.694
10. Esteeves SC et al. Critical appraisal of World Health Organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men *Urology* 2012; 79(1): 17.
11. World Health Organization. WHO laboratory manual for the examination and processing of human semen 2010.
12. Adiga SK, Jayaraman V, KGUDaKP. Declining semen quality among south Indian infertile men: A retrospective study. *J.Hum Reprod Sci* 2008; 1:15-18.
13. Shine R, Peek J, Bridsall M. Decline sperm quality in Newzealand over 20 years *NZ Med J* 2008; 1287:50-56.
14. Romero-Otero J, Medina- Polo J, Gracia-Gomez B, Lora –Pablos D et al. *Urology* 2015; 85(6):1333-1338.
15. Kumar S. Occupational exposure associated with reproductive dysfunction *Journal of Occupational Health* 2004; 46: 1-19.
16. Vigeh M, Smith DR, Hsu P-C (2011). How does lead induce male infertility? *Iranian Journal of Reproductive Medicine* 2011; 9(1): 1–8.

17. Jorgensen N, Andersen A, Eustache et al. Regional differences in semen quality in Europe *Human Reproduction* 2001; 16(5): 1012-1019.

SECTION B: Risk Factor Analysis

Data for the Aim 2 cohort was organized by risk factor and stratified by concentration diagnosis (Table 4.2.1). Duration of marriage, duration of infertility, district of residence, alcohol consumption status, smoking status, sexual dysfunction, cardiovascular disease, diabetes, hormonal imbalance, malignancy, mumps, urinary tract infection, systemic infections, and surgeries were found to be insignificant predictors of the semen parameters. The four semen parameters – volume, concentration, total motility, and RL motility – were then stratified by concentration and represented via quartiles (Table 4.2.2). There was no evidence of significant parameters pertaining to volume; therefore, volume was omitted in further analyses.

Table 4.2.2. *Distribution of semen parameters for interviewed cohort from BIRDEM CARE (May 2016 to August 2016) by concentration diagnosis*

				Distribution by Quartiles			
Semen Parameters	**Concentration**	**Mean ± SD**	**Median (IQR)**	**10th**	**25th**	**75th**	**90th**
Volume (mL)	Overall	2.1 ± 0.6	2	2	2	2	3
	Normozoospermia	2.2 ± 0.5	2	1.5	2	2	4
	Oligozoospermia	2.1 ± 0.5	2	1	2	2	3
	Azoospermia	2.1 ± 0.6	2	1	2	2	3
	Other	2.1 ± 0.8	2	0	2	2	4
Concentration (x 10^6/mL)	Overall	69.1 ± 67.0	50 (112)	0	8	120	160
	Normozoospermia	116.6 ± 51.1	120 (81)	30	69	150	230
	Oligozoospermia	8.2 ± 8.6	7 (4)	0.2	5	9	35
	Azoospermia	0.03 ± 0.08	0	0	0	0	0.2
	Other	78.1 ± 66.4	50 (55)	15	35	90	270

Total motility (%)	Overall	31.3 ± 23.1	30 (45)	0	10	55	60
	Normozoospermia	55.7 ± 12.5	60 (5)	8	55	60	65
	Oligozoospermia	19.3 ± 8.7	20 (10)	4	15	25	35
	Azoospermia	0	0	0	0	0	0
	Other	22.7 ± 13.1	25 (25)	0	10	35	40
RL motility (%)	Overall	17.2 ± 21.4	10 (25)	0	0	25	60
	Normozoospermia	39.8 ± 19.8	37.5 (40)	10	20	60	65
	Oligozoospermia	4.6 ± 6.9	2.5 (5)	0	0	5	25
	Azoospermia	0	0	0	0	0	0
	Other	6.0 ± 5.6	5 (10)	0	0	10	20

Step-wise multiple regression analyses were conducted to find adjusted regression coefficients and 95% CI for the significant predictors of the remaining three semen parameters (Table 4.2.3). Secondary subfertility was associated with these significant parameters: sperm concentration (48.9[15.1-82.7], $p < 0.006$), RL motility (14.4[4-24.8], $p < 0.01$), and total motility (20.3[8.9-31.7], $p < 0.001$). Hormonal imbalance impacted total motility with a regression coefficient of -28.8[-53.5- -4.1], $p < 0.026$ (Table 4.2.3). Participants in the age range 42-64 years had significantly lower concentration values than participants of all other ages (-64.8[-104.9,-24.7], $p < 0.003$). RL motility among participants in the age range of 33-35 years was significantly different than those in other age groups ($p < 0.01$).

Table 4.2.3. *Mean parameter estimates of significant risk factors for BIRDEM CARE participants (May 2016 to August 2016), as reported by adjusted regression coefficients via multiple regression analysis*

Risk Factor	**Estimate**	**Standard Error**	***p*-value***	**95% CI**
Concentration (x 10^6/mL)				
Secondary Infertility	48.9	17.3	0.006	(15.1, 82.7)
Age (42-64)	-64.8	20.5	0.003	(-104.9, -24.7)
% Total Motility				
Secondary Infertility	20.3	5.8	0.001	(8.9, 31.7)
Hormonal Imbalance	-28.8	12.6	0.026	(-53.5, -4.1)

% RL Motility				
Secondary Infertility	14.4	5.3	0.01	(4.0, 24.8)
Age (33-35)	15.9	6.3	0.01	(3.6, 28.2)

*probability value determined using F-score

Chapter V: Culminating Discussion

Discussion

The two analyses provide insight into the growing concern of declining semen quality in the Bangladeshi male population. In the Aim 1 analysis, the study population expressed decreasing semen parameters that were especially contingent upon temporality. These trends were significantly marked in concentration, total motility, and RL motility ($p < .0001$). The increase in frequency of azoospermic participants from 10.9% to 14.9% after adjustment is a trend that has especially been noted in other contexts [59]. Based on this clinic study, Bangladesh may be no exception to the temporal reduction of sperm parameters experienced worldwide and follows similar trends to those observed in low-resource contexts [1,77,78].

Importantly, the change of WHO criteria from 1990 to 2010 impacts the understanding of semen parameters relative to "normal" at a given time point. The parameter for normozoospermia reduced from $\geq 20 \times 10^6$/mL to $>15 \times 10^6$/mL, while oligozoospermia parameters also decreased to the values listed in Table 4.1.2 [75]. The decrease of normal values determined by WHO indicates that overall semen decline became normalized, so clinical practice contingent on semen analysis required adjustment. In our dataset, frequencies of concentration diagnoses for normozoospermia and all levels of oligozoospermia were impacted by adjustment to WHO 2010 criteria, such as the increase in frequency of normozoospermia (Table 4.1.2). As azoospermia is a more definitive concentration measure which was not impacted by shifting normal values, it was utilized in median regression analysis.

While confounding for age and duration of abstinence before testing for the azoospermic subset during median regression analysis, significant relationships between these risk factors appeared to influence the tested parameters (OR = 0.16[0.14-0.16]). These findings are in

agreement with the literature explored, which indicates that there is an inverse relationship between aging and semen quality despite the presence of confounders [5,12,13,19]. The risk factor analysis also indicated that the highest age range (42-64 years) resulted in lowest concentration values (-64.8[-104.9,-24.7], $p < 0.003$). Our findings also indicated the ages of 33-35 as the point of initial descent in parameters. Stone et al. also reported that decline begins roughly at age 34, while steeper declines can be observed beginning from age 40 then reaches severity at around age 55 [14,15]. Short duration of abstinence was previously found to lower semen quality during shorter intervals between each ejaculation, so the significant association observed in our study is compliant [26].

Secondary subfertility appeared to be most consistently associated with all the measured semen parameter outcomes (sperm concentration = $p < 0.006$, RL motility = $p < 0.01$), total motility = $p < 0.001$), with the exception of volume. As previously described, increasing paternal age, especially when associated with first conception, has serious implications for couple fertility. Secondary subfertility typically is associated with higher paternal age in the Bangladeshi context and thus implies testicular dysfunction, which causes the fetus to be more susceptible to negative birth outcomes [22].

Limitations & Biases

Several limitations exist in the nature of the data set provided that affected the validity of the study. Most importantly, the data collected reflects a participant pool consisting of only male partners from couples facing subfertility, so there is internal selection bias present in the datasets. While correlation of participants attending the single infertility clinic creates a data pool with homogenous inclusion criteria, generalizability of our results is hindered. The lack of a control

group is a limitation that frequently exists in comparable semen analysis studies that rely on infertility clinic cohorts [29]. In order to provide a true representation of the burden of male reproductive dysfunction in Bangladesh and better estimates for the measure of effect as differed in the subfertile population, it would be beneficial to sample male partners in couples with a normal fertility status. Without an adequate control group, calculating relative risk from the identified exposures cannot be reported between the infecund cohort of males and normal males.

While a single technician conducted all the semen analyses evaluated in the Aim 1 analysis, it is unclear whether the technician's data was holistically representative of WHO measurements. This discrepancy initially arose when evaluating the morphology data, which did not reflect WHO-compliant grading [74]. Moreover, missing concentration values were compromised by using qualitative concentration diagnoses in Aim 1, and the qualitative groupings were used for stratification in Aim 2. Because numerical values for concentration were not available to confirm whether correct diagnoses were reported in compliance with WHO criteria, potential measurement error inherently remains in the dataset. Studies evaluating the WHO 2010 semen parameters have determined them to be unreliable, which presents measurement error in the semen parameters of both the Aim 1 and Aim 2 analyses. Because the WHO data is determined by the world population at large, it is not possible to provide a standard measure for the various semen parameters without accounting for regional differences [75].

Although normozoospermia and azoospermia frequencies increased over time in the Aim 1 analysis, these fluctuations could also be attributable to variation in sample size. There was a fluctuation of patients attending CARE in 2008 that remained consistent for the remainder of the study. We were also unable to follow the participants over time and determine whether multiple data sets represented a single participant due to a lack of patient identifiers, thus the data is

skewed due to correlation. Therefore, the increase in frequency of azoospermic individuals may have offset the expected decrease in normozoospermic individuals depending on reasons for seeking care. Population size also severely hindered the observability of effects in the Aim 2 analysis because one of the three months of data collection was Ramadan. During Ramadan, males are reluctant to provide semen samples because masturbation and sexual intercourse disqualify a day's fast. Moreover, a large proportion of the sample size (>40 surveys) was discarded due to incomplete semen parameter data on the intake sheet. Similar outcomes were seen in a comparable study done by Naher et al. in the late 1990s. While attempting to characterize risk factors, they were unsuccessful due to severe gaps in their data and population size. Like in our study, they were only able to deduce that a majority of male partners seeking infertility care were between the ages of 30 and 35, and the average duration of infertility after marriage was 6-10 years [9].

Reporting bias exists in the Aim 2 analysis where the participants were fearful that the information provided during assessment would be repeated in the presence of their spouse or linked to their record. For example, it is a taboo to admit to alcoholism in Bangladeshi society because it is a predominantly Muslim country. Similarly, it is likely that some male partners were reluctant to share tobacco usage and previous health conditions with honesty. Recall bias is also strong in this population because BIRDEM CARE has a large proportion of low-literacy, low socioeconomic status patients. They responded to questions about medications, previous illnesses, and surgeries based on memory and may have misreported or misinterpreted the questions asked. Residence data was recorded to inform environmental factors, which did not provide a strong indication of life-course exposures or regional variance, especially with the presence of both urban and rural centers within the districts of Bangladesh. Given the political

and social climate in Dhaka during the past summer, participants were reluctant to respond extensively on their history of residence. Moreover, participants provided their occupation, but exposures to toxicity could vary in the same profession depending on quality of workplace and location. Occupation data was omitted from analysis for this reason. Measurement for endocrine disruptors was not possible because the BIRDEM Andrology Lab was not equipped to conduct these assays or collect additional biospecimens. Thus, the effect of confounding due to influential risk factors could not be observed in the study cohort.

Implications & Recommendations

This study sets a foundation for male infecundity studies in Bangladesh and related contexts. Longevity of data collection that is consistent and closely compliant to WHO procedures indicates the feasibility of such a study in Bangladesh and provides unique insight that has not previously been explored. As the Aim 1 analysis has established a trend of decline in semen parameters with minimal concern, the next step for future studies would be to evaluate risk factors in more depth. Conducting a prospective cohort study to track life-course exposures supplemented with extensive patient history, semen analysis data, lifestyle factors, and effects of xenobiotics on reproductive hormones would be beneficial to holistically understand how exposures impact male infecundity and should be adjusted accordingly [29].

The limitations presented in our Aim 2 analysis introduce the necessity for biospecimen testing that goes beyond semen analysis. Given the cost and time-effective methods of semen analysis and immunoassays, such testing would be feasible in a low-resource context like Bangladesh [78]. Developing baseline data that indicates causation of endocrine disruptors on semen outcomes could inform future programs that are directed toward regulating workplace

toxicity and encouraging preventive behaviors. Additionally, establishing interventions to prevent and detect male infecundity is highly favorable to the social context of Bangladesh because infertility is stigmatized to be a result of a woman's reproductive dysfunction. Openly acknowledging male factor infertility would improve outcomes for female partners and problem-solving approaches when faced when couple subfertility [11].

Conclusion

Findings from this study provide support for the relationship of increasing age and decreasing semen quality, as well as the existence of a temporal decline in semen parameters for Bangladeshi males seeking subfertility care. Future studies could be improved by quantifying risk factor measurements more effectively, such as conducting assays on biospecimens in addition to semen analysis. Moreover, expanding testing to male partners in couples facing normal fertility outcomes would provide substantial data to be used as a control.

References

1. Almagor, M., Ivnitzki, I., Yaffe, H., & Baras, M. (2003). "Changes in semen quality in Jerusalem between 1990 and 2000: a cross-sectional and longitudinal study," *Archives of Andrology*, Vol 49, No. 2, pp. 139-144.
2. Haimov-Kochman, R., Har-Nir, R., Ein-Mor, E. et al. (2012). "Is the quality of donated semen deteriorating? Findings from a 15 year longitudinal analysis of weekly sperm samples," *Israel Medical Association Journal,* vol. 14, no. 6, pp. 372-377.
3. VanWaeleghem, K., De Clercq, N., Vermeulen, L., Schoonjans, F., and Comhaire, F. (1996). "Deterioration of sperm quality in young healthy Belgian men," Human Reproduction, vol. 11, no. 2, pp. 325-329.
4. Iwamoto, T., Nozawa, S., Yoshiike, M. et al. (2005). "Semen quality of 324 fertile Japanese men," Human Reproduction, vol 21, no. 3, pp. 760-765.
5. Aleisa, N.A.S. (2013). "Semen characteristics of fertile and subfertile men in a fertility clinic and correlation with age," Journal of King Saud University - Science, vol. 25, 63-71.
6. Akhter, S., Alam, H., Khanam, N.N., & Zabin, F. (2011). Characteristics of infertile couples. Mymensingh Med. J., 20: 121-127.
7. Čipak, A., Stanić, P., Đurić, K., Serdar, T., & Suchanek, E. (2009). Sperm morphology assessment according to WHO and strict criteria: method comparison and intra-laboratory variability. *Biochemia Medica*; 19(1): 87-94. http://dx.doi.org/10.11613/BM.2009.009
8. Bashed, M.A., Alam, G.M., Kabir, M.A., & Al-Amin, A.Q. (2012). Male Infertility in Bangladesh: What Serve Better-Pharmacological Help or Awareness Programme?. International Journal of Pharmacology, 8: 687-694. DOI: 10.3923/ijp.2012.687.694

9. Naher, A., Begum, H., Mannan, M., Azad, A., "Role of male factors in infertility and analysis of semen", Journal of preventive and socail medicine, vol 18, no.1, pp. 16-21, 1999
10. Rokeya Anwar, B., Fatima, P., Afza, N., Tarannum, T., Begum, N., Umme Kulsum, S., Parveen, S., "Etiologic Factors of Infertility in a Referral Hospital (BSMMU, Bangladesh)", Journal of Medicine, vol 14, no. 2, pp. 110-113, 2014
11. Papreen, N., Sharma, A., Sabin, K., Begum, L., Ahsan, S.K., & Baqui, A.H. (2000). Living with infertility: Experiences among urban slum populations in Bangladesh. Reprod Health Matters, 8:33-44
12. Johnson, S. L., Dunleavy, J., Gemmell, N. J., & Nakagawa, S. (2015). Consistent age-dependent declines in human semen quality: A systematic review and meta-analysis. Ageing Research Reviews, 19, 22-33. doi:10.1016/j.arr.2014.10.007
13. Swan, S.H., Elkin, E.P., and Fenster, L., "The question of declining sperm density revisited: an analysis of 101 studies published 1934-1996," Environmental Health Perspectives, vol. 108, no. 10, pp. 961-966, 2000.
14. Stone, B. A., Alex, A., Werlin, L. B., & Marrs, R. P. (2013). Age thresholds for changes in semen parameters in men. Fertility and Sterility, 100(4), 952-958. doi:10.1016/j.fertnstert.2013.05.046
15. Matsuda, Y., Shimokawa, K., Katayama, M., Shimizu, H., & Chiba, R. (2004). Action Of Physiologically Active Materials In Human Semen During Aging. Archives of Andrology, 50(3), 131-137. doi:10.1080/01485010490425278

16. Maya, W. C., Berdugo, J., & Jaramillo, Á C. (2009). The effects of male age on semen parameters: Analysis of 1364 men attending an andrology center. The Aging Male, 12(4), 100-103. doi:10.3109/13685530903322841

17. Mukhopadhyay, D. et al. (2009). Semen quality and age-specific changes: a study between two decades on 3,729 male partners of couples with normal sperm count and attending an andrology laboratory for infertility-related problems in an Indian city. Fertility and Sterility, 93(7):2247-2254.
DOI: http://dx.doi.org/10.1016/j.fertnstert.2009.01.135

18. Kim, S. K., Kim, Y. S., Cho, I., & Min, S. K. (2015). Semen parameters from 2002 to 2013 in Korea young population: A preliminary report. Korean Journal of Urology Korean J Urol, 56(12), 831-837. doi:10.4111/kju.2015.56.12.831

19. Hossain, M.M., Fatima, P., Rahman, D., & Hossain, H.B. (2012). Semen parameters at different age groups of male partners of infertile couples. Mymensingh Med J, 21(2):306-15.

20. Alam, W., Mohammad, Khan, M. S., Akhtar, J., Bibi, R., Shabbir, A., Naeem, M., Qazi, B. M. (2011). Trend of Fertility Potentials With Increasing Age in Pakistani Males. Journal of Postgraduate Medical Institute, 25(1), 9-13.

21. Mahboubi, M., Foroughi, F., Ghahramani, F., Shahandeh, H., Moradi, S., Shirzadian, T., "A case-control study of the factors affecting male infertility", Turkish Journal of Medical Sciences, Vol 44, no. 5, pp. 862-865, 2014

22. Sharma, R., Agarwal, A., Rohra, V. K., Assidi, M., Abu-Elmagd, M., & Turki, R. F. (2015). Effects of increased paternal age on sperm quality, reproductive outcome and

associated epigenetic risks to offspring. Reprod Biol Endocrinol Reproductive Biology and Endocrinology, 13(1). doi:10.1186/s12958-015-0028-x

23. Suer, E., Gulpinar, O., & Yaman, O. (2012). The effects of aging on fertility in men. Türk Üroloji Dergisi/Turkish Journal of Urology Turkish Journal of Urology, 38(1), 40-43. doi:10.5152/tud.2012.009
24. Silva, L. F., Oliveira, J. B., Petersen, C. G., Mauri, A. L., Massaro, F. C., Cavagna, M., . . . Franco, J. G. (2012). The effects of male age on sperm analysis by motile sperm organelle morphology examination (MSOME). Reprod Biol Endocrinol Reproductive Biology and Endocrinology, 10(1), 19. doi:10.1186/1477-7827-10-19
25. Belloc, S., Hazout, A., Zini, A., Merviel, P., Cabry, R., Chahine, H., . . . Benkhalifa, M. (2014). How to overcome male infertility after 40: Influence of paternal age on fertility. Maturitas, 78(1), 22-29. doi:10.1016/j.maturitas.2014.02.011
26. Cocuzza, M., and Esteves, C. S., "Shedding Light on the Controversy Surrounding the Temporal Decline in Human Sperm Counts: A Systematic Review," The Scientific World Journal, vol 2014, pp. 1-10, DOI: 365691
27. Motamedzadeh, L., Khalili, M. A., Razaghian, M., Halvaei, I., & Ghasemzadeh, J. (2014). Effect of systolic and diastolic blood pressure on sperm parameters in men attending to infertility clinic. Iranian Journal Of Reproductive Medicine, 12121.
28. La Vignera, S., Condorelli, R. A., Di Mauro, M., Lo Presti, D., Mongioì, L. M., Russo, G., & Calogero, A. E. (2015). Reproductive function in male patients with type 1 diabetes mellitus. Andrology, 3(6), 1082-1087. doi:10.1111/andr.12097

29. Fisch, H., Declining Worldwide Sperm Counts: Disproving a Myth, Urologic Clinics of North America, Volume 35, Issue 2, May 2008, Pages 137-146, ISSN 0094-0143, http://dx.doi.org/10.1016/j.ucl.2008.01.001.

30. Bhattacharya, S. M., Ghosh, M., & Nandi, N. (2014). Diabetes mellitus and abnormalities in semen analysis. Journal Of Obstetrics & Gynaecology Research, 40(1), 167-171. doi:10.1111/jog.12149

31. Mclachlan, R. I., Wreford, N. G., O'donnell, L., Kretser, D. M., & Robertson, D. M. (1996). The endocrine regulation of spermatogenesis: independent roles for testosterone and FSH. Journal of Endocrinology, 148(1), 1-9. doi:10.1677/joe.0.1480001

32. Bussen, S., Sütterlin, M., Steck, T., & Dietl, J. (2004). Semen parameters in patients with unilateral testicular cancer compared to patients with other malignancies. Archives Of Gynecology & Obstetrics, 269(3), 196-198. doi:10.1007/s00404-003-0493-x

33. Caponecchia, L., Cimino, G., Sacchetto, R., Fiori, C., Sebastianelli, A., Salacone, P., & ... Rago, R. (2016). Do malignant diseases affect semen quality? Sperm parameters of men with cancers. Andrologia, 48(3), 333-340. doi:10.1111/and.12451

34. Djaladat, H., Burner, E., Parikh, P. M., Hays, K., & Beroukhim Kay, D. (2014). The Association Between Testis Cancer and Semen Abnormalities Before Orchiectomy: A Systematic Review. Journal Of Adolescent & Young Adult Oncology, 3(4), 153-159. doi:10.1089/jayao.2014.0012

35. Lazaros, L., Hatzi, E., Markoula, S., Takenaka, A., Sofikitis, N., Zikopoulos, K., & Georgiou, I. (2012). Dramatic reduction in sperm parameters following bariatric surgery: report of two cases. Andrologia, 44(6), 428-432. doi:10.1111/j.1439-0272.2012.01300.x

36. Sermondade, N., Massin, N., Boitrelle, F., Pfeffer, J., Eustache, F., Sifer, C., & ... Levy, R. (2012). Sperm parameters and male fertility after bariatric surgery: three case series. Reproductive Biomedicine Online (Elsevier Science), 24(2), 206-210. doi:10.1016/j.rbmo.2011.10.014

37. Aduloju, O. P., & Adegun, P. T. (2016). Factors predictive of abnormal semen parameters in male partners of couples attending the infertility clinic of a tertiary hospital in south-western Nigeria. South African Journal Of Obstetrics & Gynaecology, 22(2), 57-61. doi:10.7196/SAJOG.2016.v22i2.1082

38. Moretti, E., Figura, N., Campagna, M. S., Iacoponi, F., Gonnelli, S., & Collodel, G. (2017). Infectious Burden and Semen Parameters. Urology, 10090-96. doi:10.1016/j.urology.2016.10.032

39. Moretti, E., Figura, N., Campagna, M. S., Gonnelli, S., Iacoponi, F., & Collodel, G. (2015). Sperm Parameters and Semen Levels of Inflammatory Cytokines in Helicobacter pylori–infected Men. Urology, 86(1), 41-47. doi:10.1016/j.urology.2015.02.068

40. Yonggang, S., Chengcheng, L., Dong, C., Guangwei, H., & Shanhong, Y. (2014). The Effect of Chronic Bacterial Prostatitis on Semen Quality in Adult Men: A Meta-Analysis of Case-control Studies. Scientific Reports, 1-6. doi:10.1038/srep07233

41. Certain Semen Parameters Decrease after HIV-1 Infection. (2005). Fertility Weekly, 7-8.

42. Wang, X., Ying, P., Diao, F., Wang, Q., Ye, D., Jiang, C., . . . Li, C. (2013). Altered protein prenylation in Sertoli cells is associated with adult infertility resulting from childhood mumps infection. The Journal of Experimental Medicine, 210(8), 1559-1574. doi:10.1084/jem.20121806

43. Nadeem, F., Fahim, A., & Bugti, S. (2012). Effects of cigarette smoking on male fertility. Turkish Journal Of Medical Sciences, 42(S2), 1400-1405. doi:10.3906/sag-1107-25

44. Pant, P. R. (2013). Abnormal Semen Parameters among Men in Infertile Couples. Nepal Journal Of Obstetrics & Gynaecology, 8(1), 53-55.

45. Ashtary-Larky, D., Ghaffari, M. A., Noorbehbehani, M., & Alipour, M. (2016). Association of Smoking With Semen Quality andμ-Calpain Level in Normospermia: A Case-Control Study. Journal Of Family & Reproductive Health, 10(1), 15-20.

46. Sharma, R., Harlev, A., Agarwal, A., & Esteves, S. C. (2016). Cigarette Smoking and Semen Quality: A New Meta-analysis Examining the Effect of the 2010 World Health Organization Laboratory Methods for the Examination of Human Semen. European Urology, 70(4), 635-645. doi:10.1016/j.eururo.2016.04.010

47. Zhi Hong, Z., Hai Bo, Z., Lei Lei, L., Yang, Y., Hong Guo, Z., & Rui Zhi, L. (2013). Decline of semen quality and increase of leukocytes with cigarette smoking in infertile men. Iranian Journal Of Reproductive Medicine, 11(7), 589-596.

48. Asare-Anane, H., Bannison, S. B., Ofori, E. K., Ateko, R. O., Bawah, A. T., Amanquah, S. D., & ... Ziem, J. B. (2016). Tobacco smoking is associated with decreased semen quality. Reproductive Health, 131-6. doi:10.1186/s12978-016-0207-z

49. Al-Turki, H. A. (2015). Effect of smoking on reproductive hormones and semen parameters of infertile Saudi Arabians. Urology Annals, 7(1), 63-66. doi:10.4103/0974-7796.148621

50. Mitra, A., Chakraborty, B., Mukhopadhay, D., Pal, M., Mukherjee, S., Banerjee, S., & Chaudhuri, K. (2012). Effect of smoking on semen quality, FSH, testosterone level, and

CAG repeat length in androgen receptor gene of infertile men in an Indian city. Systems Biology In Reproductive Medicine, 58(5), 255-262. doi:10.3109/19396368.2012.684195

51. Al-Matubsi, H. Y., Kanaan, R. A., Hamdan, F., Salim, M., Oriquat, G. A., & Al Hanbali, O. A. (2011). Smoking ractices in Jordanian people and their impact on semen quality and hormonal levels among adult men. Central European Journal Of Public Health, 19(1), 54-59.
52. Fatima, P., Debnath, B. C., Hossain, Md. M., Rahman, D., Banu, J., "Effect of smoking on blood and semen lead level in male partners of infertile couple", Journal of Chittagong Medial College Teacher's Association, vol 19, no. 2, pp. 3-6, 2008
53. Alcohol and Male Reproductive Health. (2014). Fertility Weekly, 5-6.
54. Jensen, T. K., Swan, S., Jørgensen, N., Toppari, J., Redmon, B., Punab, M., & ... Andersson, A. (2014). Alcohol and male reproductive health: a cross-sectional study of 8344 healthy men from Europe and the USA. Human Reproduction, 29(8), 1801-1809.
55. Donnelly, McClure, Kennedy, Lewis, & Lewis, S. M. (1999). Direct effect of alcohol on the motility and morphology of human spermatozoa. Andrologia, 31(1), 43-47. doi:10.1046/j.1439-0272.1999.00242.x
56. Ricci, E., Al Beitawi, S., Cipriani, S., Candiani, M., Chiaffarino, F., Viganò, P., & ... Parazzini, F. (2017). Semen quality and alcohol intake: a systematic review and meta-analysis. Reproductive Biomedicine Online (Elsevier Science), 34(1), 38-47. doi:10.1016/j.rbmo.2016.09.012
57. Sermondade, N., Elloumi, H., Erthaut, I., Mathieu, E., Delarouzière, V., Ravel, C., & Mandelbaum, J. (2010). Progressive alcohol-induced sperm alterations leading to spermatogenic arrest, which was reversed after alcohol withdrawal. Reproductive

Biomedicine Online (Reproductive Healthcare Limited), 20(3), 324-327. doi:10.1016/j.rbmo.2009.12.003

58. Hosseini, M., Jahan-abadi, Z., Yonsefi, A., & Alborzi, M. (2014). Stopping smoking improves semen parameters in smoker infertile men. Iranian Journal Of Reproductive Medicine, 12118.
59. Carlsen, E., Giwercman, A., Keiding, N., & Skakkebaek, N.E. (1992). "Evidence for decreasing quality of semen during past 50 years," British Medical Journal, Vol. 305, No. 6854, pp. 609-613.
60. Endocrine Disruptors. (n.d.). *National Institute of Environmental Health Sciences*. Retrieved June 02, 2016, from http://www.niehs.nih.gov/health/topics/agents/endocrine/
61. Kumar, S. (2004). "Occupational exposure associated with reproductive dysfunction," Journal of Occupational Health; 46: 1-19.
62. Vigeh, M., Smith, D. R., & Hsu, P.-C. (2011). How does lead induce male infertility? Iranian Journal of Reproductive Medicine, 9(1), 1–8.
63. Wright, K. (2017). "Could endocrine disruptors like BPA be disrupting your fertility?" IntegraMed America, Inc. Retrieved from http://attainfertility.com/article/bpa-toxin.
64. Hauser, R., Chen, Z., Pothier, L., Ryan, L., & Altshul, L. (2003). The relationship between human semen parameters and environmental exposure to polychlorinated biphenyls and p,p'-DDE. Environmental Health Perspectives,111(12), 1505–1511.
65. Zeng, Q., Wang, Y., Xie, S., et al., "Drinking-Water Disinfection By-products and Semen Quality: A Cross-Sectional Study in China," Environmental Health Perspectives, vol 122, no. 7, pp. 741-746, 2014.

66. Morán-Martínez, J., Carranza-Rosales, P., Morales-Vallarta, M., A. Heredia-Rojas, J., Bassol-Mayagoitia, S., Denys Betancourt-Martínez, N., & M. Cerda-Flores, R. (2013). Chronic environmental exposure to lead affects semen quality in a Mexican men population. Iranian Journal of Reproductive Medicine,11(4), 267–274.

67. Jorgensen, N., Andersen, A, Eustache, et al., "Regional differences in semen quality in Europe," Human Reproduction, vol 16, no. 5, pp. 1012-1019, 2001

68. Skakkebaek, N.E., Andersson, A.-M., Juul, A. et al. (2011). “Sperm counts, data responsibility, and good scientific practice,” *Epidemiology*, Vol. 22, No. 5, pp. 620-621.

69. The World Factbook: Bangladesh. (2016). Retrieved June 03, 2016, from https://www.cia.gov/library/publications/the-world-factbook/geos/bg.html

70. Figure TS.2 - AR4 WGIII Technical Summary. (n.d.). Retrieved June 03, 2016, from http://www.ipcc.ch/publications_and_data/ar4/wg3/en/figure-ts-2.html

71. Bangladesh Economy Profile. (2014). Retrieved June 03, 2016, from http://www.indexmundi.com/bangladesh/economy_profile.html

72. Smith, J. (2016). *Imperialism in the Twenty-First Century: Globalization, Super-Exploitation, and Capitalism’s Final Crisis*. NYU Press.

73. WHO’s Urban Ambient Air Pollution database - Update 2016. (2016). Retrieved June 05, 2016, from http://www.who.int/phe/health_topics/outdoorair/databases/AAP_database_summary_results_2016_v02.pdf?ua=1

74. *WHO laboratory manual for the examination and processing of human semen*. (2010). Geneva: World Health Organization.

75. Esteves, S.C. et al. (2012). “Critical appraisal of World Health Organization’s new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men,” *Urology,* Vol. 79, Issue 1, pp 17.

76. Adiga SK, Jayaraman V, KGUDaKP. Declining semen quality among south Indian infertile men: A retrospective study. *J.Hum Reprod Sci* 2008; 1:15-18.

77. Sperm Counting Chambers. (2009). MMC Soft. Retrieved from http://www.mmcatalog.com/en/sperm/SpermCountingChamber.html

78. Shine R, Peek J, Bridsall M. Decline sperm quality in New Zealand over 20 years *NZ Med J* 2008; 1287:50-56.

79. Sharma, S., Zapatero-Rodríguez, J., Estrela, P., & O’Kennedy, R. (2015). Point-of-Care Diagnostics in Low Resource Settings: Present Status and Future Role of Microfluidics. *Biosensors*, *5*(3), 577–601. http://doi.org/10.3390/bios5030577

Appendix – *Additional Figures & Tables*

Table 1.1. *Literature pertaining to temporal changes in semen parameters and/or the burden of male infertility in Bangladesh*

Reference	Trial Period	Population	Type of Data Collection	Study Design	Results	Limitations
Adiga et al. (2008)	1993-2005	Indian males who visited the infertility clinic for semen analysis (n = 7770)	Clinic-based	Retrospective study	- Average sperm density significantly lower in 2004-2005 than 1993-1994 (26.61 ± 0.71 10^6/mL vs. 38.18 ± 1.46 10^6/mL) - Motility & normal morphology also significantly lower in 2004-2005 than 1993-1994 (47.14% vs. 61.16% & 19.75% vs. 40.51%, respectively) - Incidence of severe oligozoospermia from 2002-2005 & 1993-1997 had a significant inverse relationship ($p < .001$)	- Selection bias – participants were infertile and do not represent the general population - Data was not available on risk factors, only semen analysis results
Akhter et al. (2011)	January to December, 2007	Infertility clinic patients at BSMMU (n = 3184) in Dhaka, Bangladesh	Clinic-based	Observational study	- Male factor fertility in 13% of couples tested - Oligozoospermia was the most common cause (33.3%) of subfertility in males	- Selection bias – limited to patients attending for care rather than random sampling - No comparison to a control group - Statistical analysis limitations; primarily descriptive reporting
Almagor et al. (2003)	1990-1999	Israeli male partners who underwent IUI (n = 2638); (n = 417) in longitudinal study	Clinic-based	Cross-sectional & longitudinal study	Significant declines in concentration and motility: - Concentration = -5.2 x 10^6 ± 0.9 x 10^6/mL each year ($p < .0001$) - Motility = -0.5 ± -0.14% ($p = .0003$)	- No control group of healthy males - Only accounts for IUI male partners - Retention for longitudinal study (16%)
Anwar et al. (2013)	December, 2004 to March, 2005	Couples (n = 100) at infertility unit of BSMMU in Dhaka, Bangladesh	Clinic-based	Cross-sectional study	- Primary (56%) & secondary (44%) infertility cases - 25% cases due to both male & female factors, 3% male factor only, 15% unknown - 82% normozoospermia; 28% oligozoospermia, asthenozoospermia,	- Selection bias – only from one clinic & small sample size = weak effect size and generalizability - Semen analysis reporting not compliant with WHO guidelines

					oligoasthenozoospermia, & teratozoospermia	
Bashed et al. (2012)	2004-2011	ITRC data in Bangladesh (n = 9000 couples)	Clinic-based	Cross-sectional & longitudinal study	- 60% of infertility cases due to male factor - 40% azoospermia, 34% oligozoospermia, 5% asthenozoospermia, 1% teratozoospermia	- Selection bias – no control group or data collection from community - % of cases analyzed not generalizable as prevalence of burden
Carlsen et al. (1992)	1938-1991	61 papers (n = 14,947 men) in MEDLINE and Cumulated Index Medicus and Current List	--	Systematic review	- Linear regression showed significant decrease in mean sperm count = 113 x 10^6/mL (1940) to 66 x 10^6/mL (1990) ($p < .0001$) & seminal volume = 3.40 mL to 2.75 mL ($p = .027$) - Suggests an overall decline in semen quality for over 50 years that could be associated with testicular cancer, cryptorchidism, & hypospadias	- Methodological bias – inconsistent data collection and semen analysis methods among the various articles; varying duration of abstinence - Selection bias – manually omitted articles based on various criteria - Geographical & racial differences that skew results were all analyzed together = heterogeneity in articles
Haimov-Kochman et al. (2012)	1995-2009	Hired, healthy Israeli semen donors (n = 58) provided weekly semen samples (n = 2182)	Clinic-based	Retrospective longitudinal cohort study	Because of donor criteria changes, semen rejection increased due to decreased average semen parameters from start to end of the study period: - Concentration = 106 ± 25 x 10^6/mL to 68 ± 14 x 10^6/mL ($p < .0001$) - Motility = 79 ± 4.3% to 66 ± 4.5% ($p < .0001$) - Total motile sperm count per ejaculate = 66.4 ± 18.2 x 10^6/mL to 48.7 ± 12 x 10^6/mL ($p < .005$)	- Selection bias - recruitment criteria predetermined for educated, healthy men - Criteria changes for semen collection resulted in inconsistent parameters - Inconsistency in collection and retention (differing # of samples for each participant, only from individuals residing in Jerusalem during testing = varied exposure)
Naher et al. (1999)	1999	Male partners attending care at facility in Dhaka, Bangladesh (n = 260)	Clinic-based	Cross-sectional study	- 6-10 years of infertility following marriage for 63.1% of subjects - 2-3 mL volume of samples in 81.2% - 63.9% normozoospermic, 20.8% azoospermic, & oligozoospermic	- Selection bias – only comparing individuals in clinic = difficult to generalize - Motility and RL motility not differentiated or quantitatively provided

					(7.6% severe, 5% moderate, 2.5% mild), 3.9% necrozoospermia - 13% had relevant illnesses in the past - Increasing # of pus cells & abnormal sperm motility strongly correlated	- Pus cell finding not reliable due to lack of detail on UTI relationship to semen quality
Romero-Otero et al. (2015)	1985-2009	Fertile men in Spain who already have 2 children & visited a clinic for semen analysis before vasectomy (n = 992)	Clinic-based	Retrospective study	- All semen parameters changed significantly - Mean (SD) of concentration from 1985-1990, 1990-2000, & 2000-2009 = 27.7 (22.97), 20.73 (14.79), & 20.18 (20.79) x 10^6/mL ($p < .0001$) - RL motility for the same periods = 53.19 (20.35), 47.22 (15.84), & 40.57 (15.15), $p < .0001$ - Normal morphology for the same periods = 67.69 (10.24), 58.87 (14.67), & 51.02 (15.76), $p < .0001$ - Logistic regression indicated that age had no significant effect in the variation of semen parameters at the cut-points analyzed, except for normal forms at percentile 25 ($p = .001$) - Multivariate analysis indicated trends for decline in sperm concentration, RL and slow linear motility, & % normal morphology ($p = .001$ - $.002$)	- Selection bias – results not representative of the general population because participants sought care; disregards the increasing burden of infertility, which would significantly impact mean (SD) results - Data for additional indicators that may confound results was not reported
Shine et al. (2008)	1987-2007	Initial semen analyses from males presenting as sperm donors in New Zealand (n = 975)	Clinic-based	Retrospective study	- Linear regression indicated that mean concentration decreased from 1987 to 2007 by 2.5% yearly (110 x 10^6/mL vs. 50 x 10^6/mL, $p < .001$ - Volume reduced significantly from 3.7 mL to 3.3 mL ($p < .001$)	- Selection bias – only males interested in being donors rather than from the general population - No confounding factors reported or controlled for

					- No significance for duration of abstinence	
Swan et al. (2000)	1934-1996	Studies from Carlsen et al. plus a few more (n = 101)	--	Systematic review	- Significant declines of sperm density in the US Europe/Australia while controlling for abstinence, time, age, % men with proven fertility, specimen collection method - Non-Western countries did not show trends of sperm density effects - Results between Carlsen et al.'s study and the extended study of additional sources shows consistent results; thus, reported trends are not based solely on article choice, so power of articles & study is strong	- Very limited data from non-Western countries that was difficult to draw conclusions from
Van Waeleghem et al. (1996)	1977-1996	Healthy, young Belgian males willing to donate sperm (n = 416)	Clinic-based	Retrospective cohort study	- Concentration decreased by -12.4 x 10^6/mL (p = .035) - Strong temporal decrease for total motility (r = -0.33, p < .0001) & rapid linear motility (52.7 to 31.7%, r = -0.42, p < .0001)	- Selection bias – targeting only young males from one university in Belgium - Quadratic modeling did not report significant sperm quality decline after 1990

Table 2.1. *Literature describing risk factors that impact semen quality*

Reference	Trial Period	Population	Type of Data Collection	Study Design	Results	Limitations
AGING						
Alam et al. (2011)	2006-2009	Males at an NIH lab in Pakistan (n = 250), aged 21-50	Clinic-based	Cross-sectional study	- Does not conclude an age threshold - Concentration declined with increasing age = .047 x 10^6/mL each year ($p > .05$) - Motility declined with increasing age ($p > .05$)	- Selection bias – non-probability sampling - Small sample size
Aleisa (2013)	2013	Fertile (n = 49), subfertile (n = 160), and unknown status (n = 76) males from a fertility clinic in Saudi Arabia	Clinic-based	Cross-sectional study	- Inverse correlation between age, sperm motility, and semen volume consistent among all groups	- Selection bias – not randomized; only representative of one clinic in one city
Cardona et al. (2009)	2008	Males attending andrology center in Colombia	Clinic-based	Retrospective study	- Association of increasing age with reduced semen parameters ($p < .05$)	- Selection bias – only males at facility, no control group - No confounding for other risk factors
Hossain et al. (2012)	2010	Males seeking care in Bangladesh (n = 1121), aged 25-55	Clinic-based	Cross-sectional study	- Significantly decreased with increasing age: semen volume (r = -.070, $p < .05$), motility (r = -.115, $p < .01$), RL motility (r = -.107, $p < .01$) - No significant difference between smokers and non-smokers	- Selection bias – only males who attended for care; no comparison with healthy aged men - No confounding for regional disparities
Johnson et al. (2015)	--	90 studies (n = 93839 participants)	--	Systematic review and meta-analysis	- Statistically-significant declines in semen volume, % motility, progressive motility, & normal morphology while controlled for confounding	- Selection bias – heterogeneity of sources, methodologies, etc.
Kim et al. (2015)	2002-2013	Young Korean males attending care for infertility, varicoceles, or	Clinic-based	Cross-sectional study	- Significant change in overall parameters from 2002-2003 to 2012-2013 groups, without regards to 2007-2008 group;	- Selection bias – not homogeneous; came to clinic for different causes; no normal group to compare to; severe cases

		other related problems (2002-2003, n = 160; 2007-2008, n = 162; 2012-2013, n = 194)			overall lack of change when all groups factored in - pH unaffected - Negative correlations of parameters (volume, motility, morphology) with increasing age	manually omitted to prevent skewing of data - Mean age in each group was different - Regional variation for participants was not considered
Mukhopadhyay et al. (2010)	1981-1985 2000-2006	Indian males attending clinic in Calcutta, India (n = 3729)	Clinic-based	Prospective study	- Significant decline in sperm motility & semen volume of study period - No overall change in concentration noted - Age-related change in parameters confirmed	- Selection bias – only clinic patients who had <20 x 10^6/mL concentration & lacked pathological disorders - No confounding for other risk factors
Silva et al. (2012)	--	One semen sample from each male in (n = 975) couples seeking care in Brazil	Clinic-based	Cross-sectional study	- Among 3 age groupings (< 35, 36-40, > 41), no difference in normal sperm concentration for the younger 2 groups - % of abnormal sperm significantly higher in eldest age group due to large nuclear vacuoles ($p < .05$) - Regression analysis results of significantly decreasing normal sperm with increasing age (r = -.10, $p < .05$) & significantly positive correlation of % spermatozoa with large nuclear vacuoles & increasing age (r = .10, $p < .05$)	- Selection bias – samples only selected among patients seeking care - Does not mention effects of confounding factors
Stone et al. (2013)	January, 2007 to December, 2012	American males from southern California (n = 4822 samples)	Clinic-based	Retrospective study	- Immediately after age 34, total motility and total concentration declined - Normal morphology declined after 40 years of age - Ratio of male-bearing sperm declined only after age 55	- Selection bias – males only attending clinic - No confounding for other risk factors; all males were from a similar region in California, but environmental impact was not defined
HISTORY OF ILLNESSES AND PROCEDURES						
Aduloju et al. (2016)	January, 2012 to	Nigerian males attending tertiary care (n = 443)	Clinic-based	Retrospective study	- Risk factors significantly impacted abnormal semen outcomes: smoking ($p = .025$),	- Selection bias – no normal comparison group; participant

	December, 2015				mumps (p = .04), groin surgery (p = .017) - 38.2% had abnormal semen = 34.8% oligozoospermia, 26.9% asthenozoospermia, 3.4% azoospermia	pool only selected from those seeking care
Bhattacharya et al. (2014)	January, 2010 to May, 2012	Males seeking care at infertility clinic in India (n = 118); non-diabetic couples (n = 66) vs. couples with diabetic males (n = 52)	Clinic-based	Prospective study	- Significant differences found between semen parameters from the non-diabetic and male diabetic groupings: volume of ejaculate (p = .004), total cells per ejaculate (p = .01), % total motility (p < .0001), % RL motility (p < .0001), % normal morphology (p = .02) - Concludes diabetes mellitus affects spermatogenesis and can influence male infertility	- Selection bias – only selected males seeking care - While non-diabetic couples formed the "control" group, they were still seeking care for infertility status, and confounding was not mentioned in the analysis
Bussen et al. (2003)	--	Males suffering from unilateral testicular cancer (n = 16) vs. diagnosed with other malignant tumors (n = 21) in Germany prior to treatment	Clinic-based	Cross-sectional study	- Normozoospermia found in five of the malignant tumor patients (p = .047) - Concentration significantly decreased for cancer patients = 18.7 ± 22.3 x 10^6/mL vs. 35.6 ± 31.3 x 10^6/mL (p = .03) - % RL motility significantly less in cancer patients = 1.1 ± 2.0% vs. 4.7 ± 5.6% (p = .02) - % normal morphology significantly lower in cancer group = 16.2 ± 6.0% vs. 26.1 ± 18.0% (p = .03)	- Selection bias – only males seeking care and diagnosed were tested - No control group from this context - Small sample size
Caponecchia et al. (2016)	2015	Cancer-positive patients (n = 236) and healthy, fertile patients (n = 102)	Clinic-based	Cross-sectional study	- Type of cancer impacts semen parameters (i.e. for testicular tumors & Hodgkin lymphoma, sperm concentration significantly lower); other cancers show no impact on semen parameters	- Selection bias – participants only selected from those who attend clinic for infertility care

Djaladat et al. (2014)	1983-2010	Peer-review articles reporting semen parameters before orchiectomy in adults with testicular germ cell tumors (n = 6 papers)	--	Systematic review	- After quality of paper and risk of bias were assessed, papers indicated semen abnormalities (count, motility, morphology) in men with the testicular tumors prior to orchiectomy	- Selection bias – limited to papers available in English & peer-reviewed only; small sample size - Publication bias – papers may have been excluded because they did not indicate an association or were not accepted for publication
La Vignera et al. (2015)	--	Patients with diabetes mellitus I (n = 32) and healthy males (n = 20)	Population-based	Retrospective study	- Diabetes positive patients had lower RL motility than negative patients = 10.0 (7.0-12.75) vs. 45.0 (42.0-47.75), $p < .01$ & higher % of sperm with abnormal mitochondrial function = 47.0 (43.0-55.0) vs. 2.0 (1.0-5.0), $p < .01$ - Correlation analysis indicated RL motility association with fasting glucose (r = -0.68, $p <$ .01)	- Selection bias – only patients from a single clinic - Small sample size - No confounding mentioned for other risk factors
Motamedzadeh et al. (2014)	--	Infertile men aged 27-45 years (n = 40) who attended clinic in Iran; excluded patients with varicocele, advanced age, and smoking status	Clinic-based	Retrospective study	- Significant positive correlation between systolic blood pressure and sperm concentration (Pearson Correlation: 0.3, p = .049) & RL motility (Pearson Correlation: 0.3, p = .02) - Insignificant correlation between blood pressure, total motility, & normal morphology	- Selection bias – cases only from single clinic; exclusion criteria does not cover the vast array of conditions that may affect semen parameters, and no further measure for confounding is analyzed - Small sample size
Sermondade et al. (2012)	--	Case studies (n = 3) of three patients who underwent bariatric surgery and severe weight loss	Clinic-based	Prospective case studies	- Semen parameters severely worsened immediately after surgery but no azoospermia (extreme oligoasthenoteratozoospermia) - Could be explained by deleterious effects of obesity, toxicity in the body, and/or nutritional deficiencies	- Selection bias – cases specifically selected based on patient histories at clinic - Small sample size

Shang et al. (2014)	1998-2013	Relevant studies describing the effect of chronic bacterial prostatitis on semen (n = 7)	--	Meta-analysis	- Sperm vitality, total motility, & % RL motility of patients with chronic bacterial prostatitis were significantly lower than controls ($p < .05$) - No effect on semen volume, concentration, or liquefaction	- Selection bias – small sample size generated after determined exclusion & inclusion criteria (i.e. lack of control data) & small sample sizes within the studies - Recall bias because included studies were case-control - Limited statistical power of the study because only 2 studies analyzed reported association - Confounding not measured / inadequately measured
DRUG USE: TOBACCO AND ALCOHOL						
Al-Matubsi et al. (2011)	October, 2008 to October, 2009	Both male & female Jordanian smokers (n = 804, n = 530 male, n = 274 female); of those, n = 111 semen analyses & n = 93 matched controls	Survey-based	Cross-sectional study	- Smokers had significantly lower sperm concentration and motility ($p < .001$) and higher testosterone & LH than non-smokers	- Small sample size for semen analysis - No confounding for other risk factors and health conditions
Al-Turki (2015)	January, 2010 to December, 2012	Saudi Arabian males who sought care at an infertility clinic (n = 279)	Clinic-based	Retrospective study	- Primary infertility more common in smokers than non-smokers ($p < .0001$) - Parameters in smokers vs. non-smokers = volume (2.8 ± 1.35 mL vs. 3.08 ± 0.76 mL, $p < .008$, CI < -0.123), RL motility (31.5 ± 23.1% vs. 40.05 ± 25.43%, $p = .002$, CI < -3.2962), concentration (119.52 ± 114.12 x 10^6/mL vs. 139.71 ± 104.82 x 10^6/mL, $p = .07$, CI < 1.4657)	- Selection bias – only males who sought care; no normal patients identified as control - No indicator for duration and frequency of smoking in the study population
Asare-Anane et al. (2016)	January, 2010 to April, 2011	Ghanaian males of males seeking care at a hospital (n = 140; n = 95 smokers & n = 45 non-smokers);	Clinic-based	Cross-sectional study	- Smokers had significantly lower semen volume, concentration, motility, normal morphology, testosterone, & FSH ($p < .05$) - Smokers had a higher risk of developing oligozoospermia,	- Process for selecting matched controls is unclear

		infertility care seekers excluded & matched controls designated			asthenozoospermia, & teratozoospermia (OR = 3.1, 4.2, & 4.7; $p < .05$)	
Ashtary-Larky et al. (2016)	June, 2013 to March, 2014	Iranian males who classified as normozoospermic and were seeking care (n = 117); divided into smokers (n = 50) and non-smokers (n = 67)	Clinic-based	Case-control study	- Volume, concentration, motility, & morphology significantly lower among smokers than non-smokers - Increasing frequency and longer duration of smoking also led to significantly lower parameters	- Selection bias – only picked from pool of males seeking care - Information bias – self classification into smokers and non-smokers
Jensen et al. (2014)	1996-2007	Coordinated study (n = 8344) of American & European males from four states and four cities, respectively (n = 1872) and young men with unknown fertility status from the general population in six European countries (n = 6472)	Clinic-based	Cross-sectional study	- No consistent association between semen parameters and alcohol consumption - Linear association between total alcohol consumption and testosterone levels - Alcohol intake not significantly associated with FSH or LH	- Low participation rate - Information bias & measurement error / misclassification– males had to recall their alcohol intake in the previous week, and that served as a marker for intake up to 3 months before - No control for confounding - Recruitment methods were slightly different based on location of study population (US vs. Europe, the two population subsets)
Hosseini et al. (2014)	September, 2009 to September, 2013	Male partners from Iranian couples seeking care (n = 235); includes smokers (n = 123), where (n = 78) gave up smoking	Clinic-based	Cross-sectional study	- Discontinuing smoking resulted in a 14-26% (19% median) increase in sperm concentration, 8-27% (17% median) improvement in motility, & 5-20% (14%) improvement in morphology - 28.2% conception success rate and 20.5% resulted in births	- Selection bias – study cohort not selected from the general population; no control group - Information bias – second testing relied on patient self-report of whether they discontinued smoking - No control for confounding, thus findings are not generalizable
Mahboubi et al. (2014)	--	Iranian males seeking care (n =	Clinic-based	Case-control study	- As 1 cigarette increases per day, 1% decrease in sperm motility;	- Selection bias – males only attending clinic

		269), separated into fertile (n = 161) and infertile (108) groupings			with each 1 year increase of cigarette smoking, 800,000 sperm are lost - Statistically-significant relationship between male infertility and hernia, varicocele, occupation, & BMI	- Reported statistics for smoking not generalizable due to sample size
Mitra et al. (2012)	--	Indian males who classify as smokers (n = 178) vs. non-smokers (n = 126)	Survey-based	Cross-sectional study	- Smoking habits associated with lower sperm mortality ($p < .001$) & increased morphological defects ($p < .0001$) - Binary logistic regression analysis indicated significant positive correlation ($p = .006$) - FSH & LH levels higher in smokers; testosterone significantly decreased with increasing smoking habit	- Selection bias – inconsistent recruitment methods - No control for confounding factors
Nadeem et al. (2012)	January, 2010 to May, 2010	Males seeking care in Hyderabad, India (n = 130) and married >1 year	Clinic-based	Observational study	- 33.3% of nonsmokers had < 5% sperm motility & 25.8% had < 3% normal morphology - Motility < 5% in smoking groups = 18.8% in light smokers, 31.2% in moderate smokers, and 50% in heavy smokers - Morphology < 3% in smoking groups = 25% in light smokers, 35% in moderate smokers, and 40% in heavy smokers	- Selection bias – participants only from clinic despite being selected from the clinic at random - No association statistics indicated; just descriptive statistics for correlation
Pant (2013)	March, 2007 to July, 2011	Nepali males seeking care at infertility clinic after 3 days of abstinence (n = 630)	Clinic-based	Prospective study	- 20% had a semen abnormality = 39% azoospermia, 47% oligozoospermia, 14% asthenozoospermia - Majority of males with semen abnormality were Brahmin - Smoking, alcohol, & varicocele possible contributing factors	- Selection bias – participants only from clinic - Small sample size reported for risk factor analysis data
Ricci et al. (2016)	1995-2015	15 cross-sectional articles (n =	--	Systematic review	- Alcohol intake has detrimental effects on semen volume (0.25	- Heterogeneity of results, even in similar populations

		16,395 men enrolled)			mL, [0.07-0.42]) & normal morphology (1.87%, [0.86-2.88%]) - Marked differences with increasing intake frequency - Moderate consumption did NOT adversely affect semen parameters	- Classification of alcohol use & measures of intake not consistent across studies - Not all variables were normally distributed, so they had to be converted to medians & IQR for the meta-analysis
Sharma et al. (2016)	January, 2010 to August, 2015	20 studies (n = 5865 participants)	--	Systematic review and meta-analysis	- Cigarette smoking is associated with reduced concentration (mean difference (MD) = -9.72 x 10^6/mL; 95% CI: [-13.32,-6.12]), motility (MD = −3.48%; 95% CI: [−5.53, −1.44]), & morphology (MD = −1.37%; 95% CI: [−2.63, −0.11]) - Effect size higher in infertile men than the general population & in moderate/heavy smokers than mild	- High heterogeneity in studies analyzed - Small number of participants in each study & varied confounders stated - Methodological rigor lacking in low-resource contexts and smaller studies as opposed to large studies - Publication bias – studies that did not qualify for publication - Manual exclusion of studies that did not have adequate data for mean & SD calculation - Selection bias – for selection of controls in each study
Zhang et al. (2013)	2007-2010	Chinese men who visited hospitals in network with Jilin University for care (n = 1512)	Clinic-based	Retrospective study	- Smokers faced a significant decrease in semen volume (p = .006), rapid progressive motility (p = .002) and sperm viability (p = .019) - pH (p = .789) and sperm concentration (p = .297) were not statistically significant - % normal morphology decreased significantly in smokers (p = .003) & worsened with increasing frequency of smoking	- Selection bias – participants by referral rather than randomly sampled from population
ENDOCRINE DISRUPTORS FROM ENVIRONMENTAL AND OCCUPATIONAL EXPOSURES						
Cocuzza & Esteves (2014)	1985-2013	Two studies: 24 articles (n = 107,701) vs. 20	--	Systematic review	- No evidence for semen concentration decline in analysis with all 24 articles, but the 20	- Difficult to control confounders in the highly variable nature of semen, selection criteria, &

		articles (n = 79,884)			articles report an unambiguous decline in concentration - Studies reporting no decline or an increase in sperm concentration comprised about 30% more participants than studies that reported a decline	comparability of populations from different time periods, quality of lab methods for semen analysis, & regional variability
Hauser et al. (2003)	January, 2000 to October, 2001	Male partners in subfertile couples seeking care in Massachusetts (n = 212)	Clinic-based	Cross-sectional study	- Dose-response relationships seen among PCB-138 & motility (OR per tertile, adjusted for age, abstinence, and smoking, and *p*-value for trend were, respectively, 1.00, 1.68, 2.35, and $p = 0.03$) & morphology (1.00, 1.36, 2.53, $p = 0.04$)	- Selection bias – only men from the clinic rather than general population - Within groups, congeners were summed using concentration, but there was not a measurement for differential activities - Misclassification bias – groupings were based on PCB activity in animals and not humans & on general biological activity rather than specifically testicular toxicity
Jorgensen et al. (2001)	October, 1996 to June, 1998	Fertile men from four European cities (n = 1082)	Clinic-based	Cross-sectional study	- Lowest sperm concentration found in Danish men, followed by French and Scottish men; Finnish men had the highest concentrations - Scottish men had the highest proportion of motility, followed by Finnish, Danish, & French; significant differences between French/Scottish ($p = .003$) & French/Finnish ($p = .002$) - No significant differences in morphology - Seasonal variation in concentration (summer 70% of winter) & total sperm count (summer 72% of winter) - Winter/summer sperm concentrations for each population: Finnish (132/93),	- Selection bias – recruitment methods slightly differed in each city - Complexity of exposures and lack of control for confounding made environmental implications inconclusive - Participation rates differed by city

					Scottish (119/84), French (103/73), & Danish (98/69)	
Moran-Martinez et al. (2013)		Mexican males (n = 47); exposed group resided within 500 m radius of metallurgic zone (n = 20) vs. non-exposed group residing 15-18 km from the zone (n = 27)	Population-based	Cross-sectional study	- Lead concentration significantly greater in the exposed group = PbB (10.10 ± 0.97 µgdL-1 vs. 6.42 ± 0.38 µgdL-1, $p < .01$) & PbS (3.28 ± 0.35 vs. 1.76 ± 0.14 µgdL-1, $p = .043$) - Significant correlation between PbS & PbB concentration in the exposed group (r = 0.573, $p = .038$) - Overall semen quality was lower in the exposed than unexposed group - Specific significant differences in exposed vs. unexposed = concentration (43.98 ± 6.26 vs. 68.78 ± 8.51 x 10^6 cell/mL, $p < .01$), motility (49 ± 7 vs. 67 ± 4%, $p = .029$), viability (36.32 ± 3.59 vs. 72.12 ± 1.91%, $p < .01$), & abnormal morphology (67 ± 18 vs. 32 ± 12%, $p < .01$)	- Selection bias – recruitment methods unclear as to whether males seeking care were also included or if enrollment was primarily by survey / at random in the community - Information bias – exclusion is based on self-reports of health behaviors by those surveyed
Zeng et al. (2014)	April, 2011 to May, 2012	Men seeking semen analysis at infertility clinic in China (n = 2009)	Clinic-based	Cross-sectional study	- Exposure to drinking water DBPs contributes to decreased semen quality, as seen in trichloroacetic acid measurements in urine samples	- Selection bias – only from clinic attendees and not random males from the general population - Limited sample size made the effect of exposure ambiguous - Complexity of the exposure from confounding by the presence of other chemicals makes exposure assessment especially challenging for analyzing drinking water DBP & reproductive health

Table 4.1.1. *Baseline semen analysis results of male patients attending BIRDEM CARE (n=13811) in Dhaka, Bangladesh, annually from 2000-2016**

Year	Age in years, mean ± SD	Duration of Abstinence in days, mean ± SD	Liquefaction, mean ± SD	Concentration† (x 10^6/mL), median (IQR)	% Total Motility, median (IQR)	% RL Motility, median (IQR)	% Normal morphology, median (IQR)	Total, n (%)
2000	34.7 ± 5.4	4.8 ± 2.6	1.0 ± 0.1	45 (90)	45 (30)	10 (20)	60 (30)	393 (2.9)
2001	34.8 ± 5.3	4.9 ± 2.0	1.0 ± 0.04	45 (58)	45 (25)	10 (15)	80 (15)	575 (4.2)
2002	34.9 ± 5.2	5.2 ± 2.2	1.0 ± 0.06	45 (90)	50 (30)	20 (25)	80 (15)	630 (4.6)
2003	35.6 ± 5.7	5.3 ± 2.3	1.0	30 (85)	50 (30)	30 (35)	80 (15)	569 (4.1)
2004	35.1 ± 5.4	5.1 ± 2.3	1.0 ± 0.1	40 (90)	50 (25)	30 (30)	75 (20)	578 (4.2)
2005	35.2 ± 5.2	4.9 ± 2.0	1.0 ± 0.06	55 (110)	50 (30)	20 (25)	60 (20)	296 (2.1)
2007	35.2 ± 5.3	5.0 ± 2.4	1.0 ± 0.2	35 (90)	40 (35)	15 (20)	50 (10)	464 (3.4)
2008	35.2 ± 5.5	5.1 ± 7.8	1.0 ± 0.09	40 (110)	45 (35)	20 (25)	50 (10)	1015 (7.4)
2009	35.4 ± 5.5	5.2 ± 3.4	1.0 ± 0.06	48 (114)	40 (35)	15 (23)	50 (20)	1156 (8.4)
2010	35.5 ± 5.8	5.2 ± 3.1	1.0 ± 0.1	40 (90)	40 (40)	15 (25)	50 (20)	1372 (9.9)
2011	35.5 ± 5.7	5.3 ± 3.7	1.0 ± 0.1	40 (90)	40 (35)	10 (20)	45 (20)	1358 (9.8)
2012	35.8 ± 5.5	5.2 ± 3.4	1.0 ± 0.07	35 (70)	35 (45)	10 (20)	45 (20)	1369 (9.9)
2013	35.8 ± 5.8	5.5 ± 4.0	1.0 ± 0.04	40 (80)	35 (40)	10 (20)	55 (15)	1001 (7.3)
2014	35.2 ± 5.8	5.1 ± 3.2	1.0 ± 0.05	40 (90)	35 (45)	10 (20)	50 (15)	1209 (8.8)
2015	35.2 ± 12.6	5.3 ± 3.7	1.0 ± 0.04	50 (75)	35 (40)	10 (20)	50 (15)	1272 (9.2)
2016	35.2 ± 5.9	5.5 ± 3.9	1.0 ± 0.07	40 (90)	30 (45)	10 (20)	50 (15)	554 (4.0)
Overall	35.4 ± 6.6	5.2 ± 3.8	1.0 ± 0.08	40 (90)	40 (40)	10 (25)	50 (20)	13811 (100)

*Missing data for 2006; *p*-values indicating significance between annual values were $p < .0001$ for age, concentration, total motility, rapid linear motility, and normal morphology; $p = .05$ for duration of abstinence and .07 for liquefaction

†Missing values for numerical concentration (n = 6187) in dataset; concentration represented for all datasets (n = 13811) by recorded diagnosis frequencies in Table 2

Table 4.2.1. *Characteristics of interviewed male participants (n=72) at BIRDEM CARE, May-August 2016*

	Total (n = 72)		**Normozoospermia (n = 27)**		**Oligozoospermia (n = 14)**		**Azoospermia (n = 10)**		**Other[c] (n = 21)**	
Risk Factor	**n (%)**	**Mean ± SD**	**n (%)**	**Mean ± SD**	**n (%)**	**Mean ± SD**	**n (%)**	**Mean ± SD**	**n (%)**	**Mean ± SD**
Age (yrs.)										
Overall	72 (100)	37.1 ± 7.2	27 (37.5)	35.0 ± 4.7	14 (19.4)	41.9 ± 6.1	10 (13.9)	34.4 ± 9.1	21 (29.2)	37.8 ± 8.4
27-32	24 (33.3)	30.5 ± 1.6	10 (13.9)	30.8 ± 1.8	1 (1.4)	28.0	7 (9.7)	30.3 ± 1.8	6 (8.3)	30.7 ± 1.2
33-35	15 (20.8)	34.5 ± 0.7	9 (12.5)	34.3 ± 0.9	3 (4.2)	35.0	0	0	3 (4.2)	34.7 ± 0.6
36-41	15 (20.8)	37.7 ± 1.6	5 (6.9)	38.8 ± 1.8	0	0	2 (2.8)	36.5 ± 0.7	8 (11.1)	37.4 ± 1.3
42-64	18 (25)	47.3 ± 5.9	3 (4.2)	44.3 ± 1.2	10 (13.9)	45.3 ± 2.0	1 (1.4)	59.0	4 (5.6)	51.8 ± 9.3
Duration of marriage (yrs.)[a]	69 (95.8)	8.2 ± 6.0	26 (36.1)	7.3 ± 4.3	14 (19.4)	10.3 ± 7.2	10 (14.5)	9.6 ± 9.7	19 (27.5)	7.1 ± 4.2
Duration of infertility (yrs.)[a]	66 (91.7)	5.2 ± 5.2	25 (34.7)	4.3 ± 3.3	13 (18.1)	6.9 ± 5.6	10 (15.2)	7.1 ± 9.2	18 (27.3)	4.3 ± 4.2
Type of infertility[a]										
Primary subfertility	45 (65.2)	--	13 (18.8)	--	10 (14.5)	--	9 (13)	--	13 (18.8)	--
Secondary subfertility	24 (34.8)	--	13 (18.8)	--	4 (5.8)	--	1 (1.5)	--	6 (8.7)	--
District of residence*										
Dhaka City	41 (56.9)	--	14 (19.4)	--	10 (13.9)	--	6 (8.3)	--	11 (15.3)	--
Non-Dhaka	31 (43.1)	--	13 (18.1)	--	4 (5.6)	--	4 (5.6)	--	10 (13.9)	--
Alcohol status										
1 = YES	2 (2.8)	--	1 (1.4)	--	0	--	0	--	1 (1.4)	--
2 = NO	70 (97.2)	--	26 (36.1)	--	14 (19.4)	--	10 (13.9)	--	20 (27.8)	--
Smoking status										
1 = YES	39 (54.2)	3.5 ± 5.4	14 (19.4)	3.2 ± 5.0	8 (11.1)	5.3 ± 6.4	5 (6.9)	1.3 ± 2.0	12 (16.7)	3.7 ± 6.2
Rare (0.2-2)	12 (16.7)	0.7 ± 0.6	5 (6.9)	1.1 ± 0.6	1(1.4)	0.2	2 (2.8)	0.2	4 (5.6)	0.4 ± 0.4
Occasional (2-4.8)	10 (13.9)	3.7 ± 0.8	3 (4.2)	3.7 ± 0.8	1 (1.4)	2.5	3 (4.2)	4.2 ± 0.6	3 (4.2)	3.7 ± 0.8
Frequent (4.8-10)	11 (15.3)	8.1 ± 1.8	5 (6.9)	7.1 ± 1.4	4 (5.6)	9.8 ± 0.5	0	0	2 (2.8)	7.3 ± 2.5
Nonstop (10-22.5)	7 (9.7)	16.9 ± 3.1	2 (2.8)	17.5 ± 0.7	2 (2.8)	16.5 ± 2.1	0	0	3 (4.2)	16.8 ± 5.0
2 = NO	32 (44.4)	0	12 (16.7)	0	6 (8.3)	0	5 (6.9)	0	9 (12.5)	0
Sexual dysfunction										
Erectile dysfunction	4 (5.6)	--	0	--	2 (2.8)	--	0	--	2 (2.8)	--
Premature ejaculation	4 (5.6)	--	1 (1.4)	--	1 (1.4)	--	0	--	2 (2.8)	--
Medical history										
Cardiovascular disease	3 (4.2)	--	1 (1.4)	--	2 (2.8)	--	0	--	0	--

Diabetes	13 (18.1)	--	7 (9.7)	--	2 (2.8)	--	0	--	4 (5.6)	--
Hormonal imbalance	3 (4.2)	--	0	--	0	--	2 (2.8)	--	1 (1.4)	--
Malignancy[d]	6 (8.5)	--	3 (4.2)	--	1 (1.4)	--	1 (1.4)	--	1 (1.4)	--
Mumps	11 (15.3)	--	4 (5.6)	--	1 (1.4)	--	2 (2.8)	--	4 (5.6)	--
Urinary tract infection	2 (2.8)	--	0	--	0	--	0	--	2 (2.8)	--
Systemic Infections[e]	6 (8.5)	--	4 (5.6)	--	1 (1.4)	--	1 (1.4)	--	0	--
Surgeries / procedures										
1=YES	25 (34.7)	--	8 (11.1)	--	9 (12.5)	--	2 (2.8)	--	6 (8.3)	--
Pelvic	8 (11.1)	--	2 (2.8)	--	4 (5.6)	--	0	--	2 (2.8)	--
Other	17 (23.6)	--	6 (8.3)	--	5 (6.9)	--	2 (2.8)	--	4 (5.6)	--
2=NO	47 (65.3)	--	19 (26.4)	--	5 (6.9)	--	8 (11.1)	--	15 (20.8)	--

[a] Missing values for duration of marriage (n=3), duration of infertility (n=6), and type of infertility (n=3)

*Dhaka residence includes participants from the main Dhaka city area and Old ("Puran") Dhaka; non-Dhaka residence includes participants from Barisal, Bogra, Comilla, Gopalganj, Kishoreganj, Konabari, Kushtia, Madaripur, Mymensingh, Narsingdi, Narayanganj, Noakhali, Pabna, Rajbari, Rajshahi, Rangpur, and Tangail districts plus one participant residing in Italy

[c] "Other" diagnosis includes patients classified as gross/severe/general asthenozoospermia and necrozoospermia

[d] Includes hernias and tumors

[e] Includes chicken pox, typhoid, jaundice, ands rabies