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A glimpse at male infertility based on semen analyses in Brunei Darussalam

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ABSTRACT

Introduction: Infertility is defined as the inability to conceive and the causes are equally divided between males and females. Considerable advances have been made in treatment of infertility amongst women, especially with the introduction of in-vitro fertilisation. However there is very little progress made in treating the male infertility. Male causes of infertility can be categorised into pre-testicular, testicular and post-testicular. This study looked at the causes of infertility amongst the males, based on the semen analysis in our local setting. **Materials and Methods:** From 2006-2008, 1,242 semen specimens were received for analysis. Subjects were instructed on proper semen collection using the World Health Organisation criteria, where the specimen is collected following three days of abstinence and sent to the laboratory within one hour of collection. Semen samples were examined microscopically for morphology, motility and the concentration. **Results:** Interestingly, 109 subjects (8.8%) had normal spermatozoa (normal analyses) and this included 38 patients (34.2%) whose initial analyses were abnormal. 57 (4.6%) subjects were azoospermic. 730 (58.8%) men had more than two abnormalities in spermatozoa and a further 217 (17.5%) men had abnormal morphology alone. Among patients with two or more abnormalities, a majority had three abnormalities and this was consistently seen in all age groups. There was a trend towards less severe abnormalities from 2006 to 2008. **Conclusions:** A majority had more than two abnormalities and abnormal morphology and they may not be able to father a child normally. However they may be able to have an offspring by assisted reproductive methods. Only a minority was azoospermic and they will not be able to father any children even with assisted reproductive techniques. Interestingly, 8.8% had normal analyses suggesting other causes of infertility such their female partners or improper techniques.

Keywords: Azoospermia, infertility, male infertility, sperm morphology, sperm motility

INTRODUCTION

The World Health Organisation's (WHO) definition of infertility is "when a couple has not been able to conceive, following regular sexual intercourse without using contraception over a period of one year".[1] The cause may either lie with the male or female partner. Hence it is necessary to regard the reproduc-

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whole and treat both partners as a single unit (infertile couple) rather than an infertile individual. The costs of investigation and management of the infertility are high and infertility have significant impacts affecting the family, society and nation as a whole.

In the past, infertility was blamed solely on the females. In the 1950's approximately 10% of the infertile couples was attributed to the males.[2] At present, this has risen to 40-50%.[3, 4] In the last few decades considerable advances have been made in treatment of infertility amongst women with the introduction of in-vitro fertilisation (IVF). However there is very little progress made in treating male infertility. Male infertility can be categorised into pre-testicular, testicular and post-testicular causes. This study retrospectively looked at the causes of infertility based on semen analysis in Brunei Darussalam over a three years period.

MATERIALS AND METHODS

During a three year period (2006 to 2008), the Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital Cytology Laboratory, received 1,276 semen samples for analysis. All cases were referred for analysis by the infertility clinic. 31 samples were excluded as these were specimens from patients who previously had vasectomy. Another three samples were unsuitable for analysis and were excluded leaving a total of 1,242 for the study.

Specimen collection: Patients were instructed on the proper method of semen collection using the WHO criteria by a member of the laboratory technical staff or a nurse and were requested to bring the sample to the laboratory within one hour of collection. They were also instructed on how to maintain the temperature of the collected specimen as close to the body temperature as possible and to avoid exposure to hot/cold temperature. The WHO criterion is that the semen samples should be collected by masturbation into a sterile container after sexual abstinence for three days. They were also advised not to use a condom for semen collection as condoms may contain spermicidal agents.

Semen analysis: Microscopic examination for sperm motility, sperm concentration and sperm morphology were evaluated according to the WHO guidelines for the examination of

Table 1: World Health Organisation reference values for normal semen parameters.

Parameters	**Normal reference value**
Volume	2.0 ml or more
pH	7.2 or more
Sperm concentration	20 x 10^6 spermatozoa/ml or more
Total sperm number	40 x 10^6 spermatozoa per ejaculate or more
Motility	50% or more motile (grades a+b) or 25% or more with progressive motility (grade a) within 60 minutes of ejaculation
Morphology	15% or more normal forms using the methods and definitions described in the WHO laboratory manual
Vitality	50% or more live spermatozoa
White cells	Fewer than 1 x 10^6 per ml

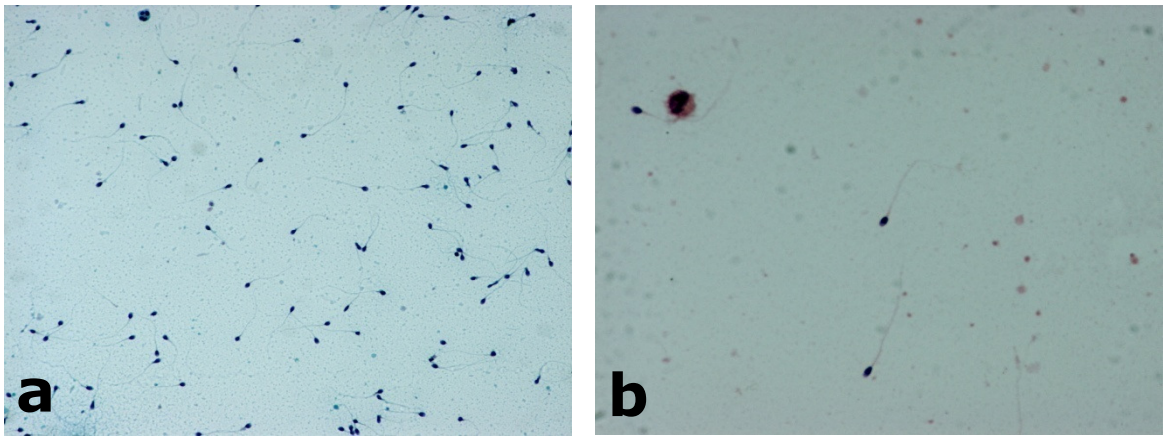

Fig. 1a) Normal semen sample showing normal spermatozoa and more than 20 spermatozoa per high power field (x40) and 1b) Oligozoospermia with few spermatozoa per high power field (x40).

human semen.[1] The reference values for the semen parameters are shown in table 1. Figures 1a and 1b show sperm concentration and sperm morphology. The nomenclature for some of the semen parameters are shown in table 2.

RESULTS

The ages of the patients ranged from 20-65 years. Almost all the patients are Malays except for twelve (n=12) Chinese, two (n=2) Ibans, one (n=1) Dusun, two (n=2) Indians and one (n=1) Filipino.

The breakdown of the semen analysis over the three year period is shown in table 3. Normal semen analyses accounted for 8.8% (n=109/1,242) ranging between 6.2 to 20.9% between the three years. This included 38 patients (34.2%) whose initial analyses were abnormal suggesting non-testicular cause of infertility. The most common abnormality was the morphology ranging from 7.5 to 37.4%. 58.8% (n=730) had more than two abnormalities. 4.6% were azoospermic with a range between three to five percent. Over the three years the proportions of samples with azoospermia and morphology abnormalities

Table 2: Nomenclature used in semen analysis.

Nomenclature	**Definitions**
Normozoospermia	Normal ejaculate as defined by the reference value
Oligozoospermia	Sperm concentration less than the reference values
Asthenozoospermia	Sperm motility less than the reference values
Teratozoospermia	Sperm morphology less than the reference values
Oligoasthenoteratozoospermia	All three parameters (concentration, motility and morphology) less than the reference values
Oligoasthenozoospermia	Sperm concentration and motility less than the reference values
Oligoteratozoospermia	Sperm concentration and morphology less than the reference values
Asthenoteratozoospermia	Sperm motility and morphology less than the reference values
Azoospermia	No spermatozoa in the ejaculate
Aspermia	No ejaculate

increased, while the proportions with concentration and motility problem have decreased. Overall the proportions with more than two abnormalities have remained consistent.

The results of the semen analysis based on age distribution are shown on table 4.The highest incidence of infertility was seen in those aged between 30-49 years.

Among patients with two or more abnormalities, a majority had three abnormalities (oligoasthenoteratozoospermia) and this was consistently seen in all age groups. Table 5 shows that analysis of cases with two or more abnormalities detected. Interestingly, there was shift towards less severe abnormalities from 2006 to 2008.

DISCUSSION

Interestingly, our study showed that 8.8% of the male patients evaluated for infertility actually had normal semen analyses. This suggests that this group of patients had other causes of infertility. These may include problems with their female partners and timing of coitus, resulting in failure of successful inseminations at the optimal time.

The presence of normal semen analyses is virtually diagnostic of normal semen assembly line; an intact hypothalamic-pituitary-testicular axis, testicular morphology and normal transport mechanisms. All these are equally important in determining male fertility. It is interesting to note that among our patients who had normal semen analyses, 38 actually had abnormal results in their initial analyses. The discrepancies may be due to several reasons and these include improper collection, and or delay in transport of the specimens to the laboratory. Sperm motility is affected by delay in analyses as well as the temperature of the external environment. Controversies remain whether one or two sample analyses are required before labeling someone as infertility. Some suggested that one properly collected sample is adequate, whilst others including the WHO guidelines stressed the importance of a second sample if

Table 3: Results of semen analyses over the three years (2006-2008).

Year		N	Normal	Azoospermia	Concentration	Motility	Morphology	>2 abnormalities
2006	Total	462	44 (9.5)	21 (4.5)	34 (7.4)	58 (12.6)	59 (12.8)	246 (53.2)
	1st time	162	31 (19.1)	12 (7.4)	7 (4.3)	49 (30.2)	33 (20.4)	30 (18.5)
	Repeat	300	13 (4.3)	9 (3.0)	27 (9.0)	9 (3.0)	26 (8.7)	216 (72.0)
2007	Total	386	48 (12.4)	18 (4.7)	8 (2.1)	16 (4.1)	70 (18.1)	226 (58.5)
	1st time	191	40 (20.9)	8 (4.2)	6 (3.1)	11 (5.8)	47 (24.6)	79 (41.4)
	Repeat	195	8 (4.1)	10 (5.1)	2 (1.0)	5 (2.6)	23 (11.8)	147 (75.4)
2008	Total	394	17 (4.3)	18 (4.6)	7 (1.8)	6 (1.5)	88 (22.3)	258 (65.5)
	1st time	195	12 (6.2)	9 (4.6)	5 (2.6)	5 (2.6)	73 (37.4)	91 (46.7)
	Repeat	199	5 (2.5)	9 (4.5)	2 (1.0)	1 (0.5)	15 (7.5)	167 (83.9)
Overall		1,242 (100)	109 (8.8)	57 (4.6)	49 (3.9)	80 (6.4)	217 (17.5)	730 (58.8)

Table 4: Results of semen analyses over the three years (2006-2008).

Age gp	Year	Normal	Azoospermia	Oligospermia	Asthenospermia	Teratospermia	>2 abnormalities
	2006						
20-29		10 (12.8)	14 (12.0)	19 (17.1)	23 (20.7)	28 (25.2)	17 (15.3)
30-39		27 (6.2)	5 (0.7)	170 (22.6)	193 (25.7)	22 (5.3)	158 (21.0)
40-49		7 (2.4)	0 (0.0)	67 (22.8)	78 (26.5)	76 (25.9)	66 (22.5)
50-59		0 (0.0)	0 (0.0)	15 (22.7)	20 (30.3)	16 (24.2)	15 (22.7)
60-69		0 (0.0)	0 (0.0)	4 (23.5)	5 (29.4)	4 (23.5)	4 (23.5)
	2007						
20-29		7 (4.9)	1 (0.7)	31 (21.7)	32 (22.4)	41 (28.7)	31 (21.9)
30-39		32 (4.6)	13 (1.9)	153 (21.8)	159 (22.6)	197 (28.0)	149 (21.2)
40-49		9 (5.5)	3 (1.8)	35 (21.2)	39 (23.6)	45 (27.3)	34 (20.6)
50-59		0 (0.0)	0 (0.0)	10 (25.0)	10 (25.0)	10 (25.0)	10 (25.0)
60-69		0 (0.0)	0 (0.0)	2 (25.0)	2 (25.0)	2 (25.0)	2 (25.0)
	2008						
20-29		3 (1.6)	1 (0.5)	40 (21.2)	31 (16.4)	68 (35.9)	46 (24.3)
30-39		9 (1.5)	16 (2.7)	121 (20.0)	106 (17.6)	205 (33.9)	147 (24.3)
40-49		6 (3.4)	0 (0.0)	37 (20.9)	35 (19.8)	56 (31.6)	43 (24.3)
50-59		1 (2.7)	0 (0.0)	8 (21.6)	7 (18.9)	11 (29.7)	10 (27.0)
60-69		0 (0.0)	0 (0.0)	2 (25.0)	2 (25.0)	2 (25.0)	2 (25.0)

Figures presented in absolute numbers and percentages in brackets

the results of the first sample is abnormal.[1, 5] Our result highlighted the importance of repeat analyses.

In our study, 4.6% of the samples had no spermatozoa at all (azoospermia). Azoospermia can be due to abnormalities in the hypothalamic-pituitary-testicular axis or ab-normalities in the testicular morphology and transport mechanisms. The causes of azoospermia can be divided to obstructive (post-
testicular) and non-obstructive (testicular).[2] Amongst the non-obstructive causes, 60% is due to hormonal imbalance (elevated testosterone and oestrogen levels).[6] Use of anabolic steroids, vascular trauma, vasculitis, smoking, excessive alcohol usage, usage of recreational drugs, radiation, retrograde ejaculation into the urinary bladder, pesticides in the environment and excess heat as seen in the uncent of the causes. All the others are categorised as idiopathic.

Obstructive (post-testicular) and destructive (testicular) lesions are important causes of male infertility. Infertility due to mumps orchitis is well known.[7, 8] Other causes such as trauma, infarction, bacterial infections such as brucellosis and parasitic infestations such as filariasis are extremely uncommon.[9, 10] In countries where tuberculosis remains endemic, it is important to consider this infection. All inflammatory lesions lead to scarring and obstruction of the tubules resulting in infertility.

The most common abnormality was the morphology with more than half having two or more abnormalities. Of concern is that a majority had three abnormalities (oligoastheno-teratozoospermia) and this was

seen in all age groups. Interestingly, there was a shift towards less severe abnormalities from 2006 to 2008. Further studies should reveal this shift is real and is so would suggest that the severities are becoming less.

In our study, 3.9% had sperm concentration that was below the reference value. This could be due to several reasons that include improper semen collection partial maturation arrest or partial obstruction. Repeat semen analyses may help to overcome the problem of improper semen collection. Testicular biopsies may help to detect the partial maturation arrest and partial obstruction. These men are not really infertile, although they may not be able to have natural fertilisation; they may be able to father children by assisted reproductive methods such as intrauterine insemination (IUI) or IVF.

In maturation arrest, the spermatozoa are either absent or scanty despite of normal anatomy. The cause of this condition is not known. Some consider elevated temperature as an important cause. Undescended testes, wearing tight pants or underpants and vigorous exercise such as cycling have been reported to cause infertility through raising the testicular temperature.[11, 12] Some of these subjects may
recover spontaneously. However, some may not recover even when the precipitating factors have been removed. Varicocele is one condition that has been associated with infertility through elevated of testicular temperature by engorged veins.[13] In our local setting, a recent study showed that varicocelectomies may improve male fertility. Among 39 patients who had varicocelectomies as part infertility management, three subsequently went on to father children.[14]

Table 5: Detail analysis of cases with two or more abnormalities (2006-2008).

Age group	Year	Oligo+ Asthenospermia	Oligo+ Teratospermia	Astheno+ Teratospermia	Oligoastheno+ Teratospermia	Total
	2006 (n=260)					
20-29		0 (0.0)	1 (5.9)	0 (0.0)	16 (94.1)	17 (6.5)
30-39		1 (0.6)	9 (5.7)	0 (0.0)	148 (93.7)	158 (60.8)
40-49		0 (0.0)	0 (0.0)	1 (1,5)	65 (98.5)	66 (25.4)
50-59		0 (0.0)	0 (0.0)	0 (0.0)	15 (100)	15 (5.8)
60-69		0 (0.0)	0 (0.0)	0 (0.0)	4 (100)	4 (1.5)
	2007 (n=226)					
20-29		0 (0.0)	0 (0.0)	0 (0.0)	31 (100)	31 (13.7)
30-39		1 (0.7)	1 (0.7)	3 (2.0)	144 (96.6)	149 (65.9)
40-49		0 (0.0)	0 (0.0)	2 (5.9)	32 (94.1)	34 (15.1)
50-59		0 (0.0)	0 (0.0)	0 (0.0)	10 (100)	10 (4.4)
60-69		0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	2 (0.9)
	2008 (n=248)					
20-29		1 (2.2)	17 (36.9)	7 (15.2)	21 (46.7)	46 (18.5)
30-39		2 (1.4)	20 (13.6)	10 (6.8)	115 (78.2)	147 (59.3)
40-49		2 (4.7)	8 (18.6)	6 (14.0)	27 (62.7)	43 (17.4)
50-59		0 (0.0)	0 (0.0)	0 (0.0)	10 (100)	10 (4.0)
60-69		0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	2 (0.8)

Figures presented in absolute numbers and percentages in brackets

Amongst the semen analysis parameters, sperm motility and morphology are the best criteria for demonstrating the fertilisation capacity of the male.[15] Sperm motility is important in the fertilisation process and provides a measure of the integrity of the sperm axoneme and tail structures as well as the metabolic machinery of the mitochondria. Sperm morphology is a measure of the integrity of the DNA packaging and quality of the spermatogenesis.[16] When detailed analysis of the sperm morphology alone is taken into account, sperm morphology is considered to be the best predictive factor in natural fertilisation, IUI and IVF.[17-19]

Normal sperm should have a smooth oval head, with well defined acrosome comprising comprising 40-70% of the sperm head. The length of the normal sperm head should be 5-6 μm and the diameter is about 2.5-3.5 μm. Furthermore, there should be no visible defect of the neck, mid piece or the tail. They should be less than a third of the head size or no cytoplasmic droplets.[19] In our study, 17.5% had spermatozoa with abnormal morphology. The abnormalities included double-headed, pin-headed, large-headed, and sperms with thin midpiece, with double tails and some with cytoplasmic droplets. The cause of teratospermia is not completely understood. However, some consider it is due to genetic defect. There are reports that showed detrimental effects of smoking on sperm morphology and minor effect on sperm concentration and motility.[20]

Smoking can cause low semen quality in many ways. Cigarette smoke itself contains high levels of superoxide anion, hydrogen peroxide and hydroxyl radicals which can rette smoke may induce inflammatory reaction in the male genital tract with release of chemical mediators which recruits and activate leucocytes.[22] These activated leucocytes can generate seminal oxidative stress in semen. Toxic metabolites in cigarette smoke may impair spermatogenesis resulting in abnormal spermatozoa.[23] According to the National nutritional status survey done in Brunei-Darussalam in 1996, 12.8% males were obese and 31.1% of males above 20 years age smoke and a further 13.5% had previously smoked[24] Both these factors have a negative effect on infertility. Obese people will have low sperm count by virtue of having high oestrogen levels in the fat depots. Furthermore, 21.7% of the obese and 8.7% of the overweight males had hypertension. Some of the antihypertensive drugs used can cause oligospermia.

There are several limitations with our study. First, we did not have details such as smoking habits, alcohol use, recreational drug use, drug use, history of orchitis, sexually transmitted disease, trauma to the testes, varicocele and previous surgery especially hernia operation. These data were not normally captured in our clinical worksheet. Second, it is also important to note that semen specimens in our study were not collected on-site. Therefore, this may lead to semen quality degradation leading to abnormal results.

In conclusion, a majority of our patients had more than two abnormalities and abnormal morphology. They may not be able to father a child normally. However they may be able to have offspring by assisted reproductive methods. Only a minority had azoospermia and they will not be able to father

techniques. A large proportion had more than abnormalities and a further 17.5% had morphological abnormality. This group will not be able to father children by natural means, but they may be able produce an offspring by selection of spermatozoa and by assisted reproductive treatment. Interestingly, 8.8% had normal analyses suggesting other causes of infertility such their female partners or improper techniques.

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