

# Semen Parameters of Fertile Men Attending Tribhuvan University Teaching Hospital

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**Aims:** This study was done to analyze the semen parameters of fertile Nepalese men with a known time to pregnancy of less than or equal to 12 months and to compare the values obtained with the newer reference limit of WHO, 2010.

**Methods:** A hospital based prospective cross-sectional study was conducted at Tribhuvan University Teaching Hospital, Institute of Medicine, from 15th April 2012 – 15th April 2013. Sixty fertile men with a known time to pregnancy (TTP)  $\leq$  12 months were included in the study and semen collection and analysis done at the laboratory of Department of Pathology.

**Results:** The following one-sided reference limits, the fifth centile (with 95th percent confidence interval), were generated: semen volume, 1.0 ml (0.8-1.2); total sperm number, 25 million per ejaculate (11-39); sperm concentration, 10 million per ml (5-15); progressive motility, 15% (10-20); total (progressive plus non-progressive motility) motility, 30% (25 -35); vitality 50% (46-54); morphologically normal form, 99% (99.3- 99.4).

**Conclusions:** The study concluded that only a single semen parameter cannot be conclusive of fertility, therefore combination of all semen parameters is more predictive. In addition the study also concluded that the semen parameters of Nepalese fertile men were found lower as compared to reference limits of WHO.

**Keywords:** fertile men; semen parameters; time to pregnancy (TTP).

## INTRODUCTION

One in six couples seeks medical help because of infertility.<sup>1</sup> About in 20-30 % male factors remain to be the underlying cause of infertility.<sup>2</sup> With such current scenario of infertility worldwide, and semen quality being taken as a surrogate measure of male fecundity as well as fertility,<sup>3</sup> a set of standard semen parameters in a particular region or a country is of necessity. As per various studies the semen parameters obtained worldwide have been variable as per geographical and demographic variation.<sup>4</sup> Another important concern is the difficulty in interpretation of semen parameters of various labs, which are subjected to intra or inter lab variability.<sup>5,6</sup> To overcome such limitations, a study about evaluation of semen parameters in fertile Nepalese men has been realized and therefore

been conducted. Apart from determining the semen variables of Nepalese fertile men, the results obtained can also be compared with that of reference limits set by World Health Organization.<sup>3</sup>

For results to be acceptable as standard values, the study has greatly followed the principle set by WHO in collecting, processing and analyzing the samples. Beside this as addressed by WHO (2010), the concept of recent fathers with known time to pregnancy,<sup>3</sup> the study has also considered TTP of less than 12 months as an important inclusion criteria of the individuals. TTP- a well-known and standardized epidemiological index<sup>7</sup> is defined as numbers of months (or cycles) from stopping the contraception to achieving the pregnancy or simply as duration taken to become pregnant.

The study has followed conventional method for determining sperm concentration and motility. It has taken in consideration the abstinence period of >48 hours and <7 days as one of the standard of inclusion

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criteria.<sup>8</sup> When the duration of abstinence is more than 7 days, sperm motility, i.e. the proportion of spermatozoa with rapid progressive motility, may decline.<sup>9</sup> If the duration of abstinence is less than 48 hours, sperm concentration may be reduced, but motility will probably not be affected.<sup>9</sup> The debate of possible decline in semen quality<sup>4,10</sup> has been a known fact. As per WHO, reference values of human semen characteristics,<sup>3</sup> newer values have even lowered as compared to before. Our aim was to determine the various semen variables of fertile Nepalese men.

## METHODS

This study was conducted at Tribhuvan University Teaching Hospital, Institute of Medicine, from 15th April 2012 – 15th April 2013. Sixty fertile men with a known time to pregnancy (TTP)  $\leq$  12 months were included in the study. During antenatal visits at outpatient department (OPD) females were inquired about duration of marriage and the time taken to become pregnant. With this basic information gathered if TTP was less than 12 months, couple was informed about the study and their willingness to volunteer for the study was decided and written consent in Nepali language was taken. Special instructions regarding cost, technique of collection and duration of abstinence were explained and inquired respectively. The lab reports available were collected from Department of Pathology.

Semen was collected in a sterile plastic container and analysis was done within 60 minutes of collection.

For microscopic examination, collected semen was diluted with following diluting fluid (Sodium bicarbonate 5 g, Formalin 1 ml, Distilled water 100 ml) and microscopic examination was done in following sequential order.

*Sperm Motility:* It was determined by finding the percentage of motile sperm cells against dead cells. According to the quality of sperm motility it was graded as; non motile (grade 0), Twitching movement (grade I), Non progressive motility (grade II), Slow linear movement (grade III), Rapid linear movement (grade IV). As WHO lab manual of semen analysis-2010,<sup>8</sup> this study has also categorized motility under two headings as total motility which is summation of twitching motility, non- progressive

motility, slow linear movement and rapid linear movement. The second is progressive motility, which is a summation of slow linear movement and rapid linear movement.

*Sperm Count:* With the help of pipette, 0.02 ml of well mixed seminal fluid mixed with 0.38 ml of diluting fluid in a test tube and charge into a Neubauer haemocytometer chamber which was allowed to settle for two minute and counted in the 4 corner squares (1 sq. mm).

*Morphology:* One separate slide was used for routine morphology evaluation. Papinocolau staining method for morphology evaluation was used. Following fixation of smear in propanol, staining in following sequence - Haematoxylin staining for ten minutes, H<sub>2</sub>O - 6-8 dips, 1%acid alcohol- 1 dip, 70% alcohol- 6-8 dips, 1% lithium carbonate for 1 minute, 70% alcohol- 6-8 dips, 80 % ethyl alcohol- 6-8 dips, OrangeG- 6 dips for 5 minutes then 90% alcohol- 6-8 dips, EA 50 for 5 minutes after that 70 % alcohol- 6-8 dips, absolute alcohol- 6-8 dips, xylene + alcohol- 6-8 dips, xylene I -5 minutes, xylene - II -5 minutes, DPX mounting was done.

Microscopic examination of the prepared slide for morphology was done. Abnormal spermatozoa were seen for four abnormalities viz. head, neck/ midpiece or tail abnormalities. The study has followed liberal approach of morphology estimation. As stated by MacLeod et al<sup>11</sup> in liberal approach the most distorted forms are considered as truly abnormal. The 5th edition of the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of human semen has followed Strict (Tygerberg) Criteria in estimation of normal morphology (%). In contrast to the liberal approach; in strict criteria- the definition for a morphologically normal spermatozoon was based on biological evidence obtained from spermatozoa found at the level of the internal cervical os.<sup>12</sup> One of the most important aspects of the strict evaluation criteria is that the range allowed for these minute normal biological variations has to be kept as small as possible.<sup>12</sup> The total number of abnormalities recorded were added together and divided by the total number of normal spermatozoa and percentage of abnormal morphology semen was detected. In addition, the number of germ cells, pus cells and RBC were noted. Semen samples were discarded after completion of

semen analysis.

A structural questionnaire was used on all participants, which were fulfilled up by the researcher. After the primary data collection, daily entry of the data was done into the master chart. Data analysis was done by computer program “statistical package for social science” (SPSS) version 19. The 95% confidence interval (CI) were calculated by calculating 5th centile  $\pm$  (1.96  $\times$  standard error). As the numbers of examined ejaculates were limited i.e. 60 in number, descriptive analysis was done.

Written informed consent was taken. The study was approved by ethical board of Institute of Medicine, Tribhuvan University Teaching Hospital.

## RESULTS

The one sided reference limits, the fifth centile (with 95 percent confidence intervals) of the data calculated were: semen volume, 1.0 ml (0.8-1.2); total sperm number, 25 million per ejaculate (11-39); sperm concentration, 10 million per ml (5-15); progressive motility 15% (10-20); total (progressive plus non progressive motility) motility, 30% (25-35); vitality, 50% (46-54); morphologically normal form, 99% (99.3-99.4). Semen parameters obtained from the study were compared with the latest reference limits published by WHO, which is tabulated as below.

<b>Table 1. Semen parameters of the study and WHO, 2010.</b>		
<b>Semen parameters</b>	<b>Study parameters</b>	<b>WHO, 2010 parameters</b>
	<b>5 centile (95%CI)</b>	<b>5 centile (95%CI)</b>
Semen volume (ml)	1.0 (0.8 - 1.2)	1.5 (1.4 - 1.7)
Total sperm number (million/ejaculate)	25(11 - 39)	39 (33 - 46)
Sperm concentration (million/ml)	10 (5 - 15)	15 (12 - 16)
Progressive motility (%)	15 (10 - 20)	32 (31 - 34)
Total motility (%)	30 (25 - 35)	40 (38 - 42)
Vitality (%)	50 (46 - 54)	58 (55 - 63)

The interesting aspect of the study results were its close resemblance with the study by Haugen et al.<sup>13</sup> The 5<sup>th</sup> centile value of both studies has been tabulated in Table 2.

<b>Table 2. Semen parameters of the study and the Norwegian fertile men.</b>		
<b>Semen parameters</b>	<b>Study parameters (n=60)</b>	<b>Norwegian fertile men<sup>13</sup> (n=82)</b>
Semen volume(ml)	1.0	1.7
Sperm concentration (million/ml)	10.0	10.6
Total sperm number (million/ejaculate)	25.0	22.3
Rapid progressive motility (%)	15.0	15.2
Total motility (%)	30	33.2

## DISCUSSION

In a developing country like Nepal where infertility itself is a social stigma leading to devastating psychosexual consequences, its proper diagnosis is of utmost importance. For a more confirmatory diagnosis, the laboratory reports available should also be interpreted more carefully and accurately. As semen analysis remains to be the surrogate marker of male fertility, its correct interpretation is very important. Though the reference limits of various semen parameters published by WHO has universally been accepted as reference values, but the current study has detected exceptions to it. For example, five individuals (8%) of total had semen concentration <15 million/ml which as per reference value of WHO are subjected to as below range i.e. sub-fertile. On the other hand the study has detected that even these individuals were fertile. Such difficulty in regards to interpreting results obtained is common. Such situations judge many number of individual

as sub-fertile. As semen parameters are subjected to geographical variation,<sup>4</sup> a standard set of values of a particular country or region to great extent can guide in appropriate management of infertile couple. As per the results of current study, the 5<sup>th</sup> centile (95% CI) of all the semen parameters (except morphology) were found to be lower as compared to reference limit of WHO.

In the Table 3 the study result were compared with various studies in fertile men worldwide.

The current study value was found closer to Swiss<sup>14</sup> and Indian<sup>17</sup> population. Though there was disparity in mean value but the 5<sup>th</sup> centile value of current study was similar as Haugen et al.<sup>13</sup> The values of Australian men<sup>15</sup> were found to be much higher than current study.

Sperm motility - is the percentage of progressively motile sperm in the ejaculate. The normal lower limit is 32% as per WHO, 2010. As per this current study the progressive motility was found to be 15%; which is even lower than the newer limit set by WHO,

**Table 3. Study results from fertile men from various part of world.**

<b>Semen parameters (mean value)</b>	<b>Present study (n=60)</b>	<b>Crazzolora et al<sup>14</sup> (n=34)</b>	<b>Haugen et al<sup>13</sup> (n=82)</b>	<b>Ombelet et al<sup>16</sup> (n=144)</b>	<b>Pal et al<sup>17</sup> (n=324)</b>	<b>Stewart et al<sup>15</sup> (n=225)</b>
Volume (ml)	2.24	2.6	3.9	3.1	3.2	3.8
Sperm concentration (million/ml)	40	60	94	53.1	68.2	108
Total sperm count (million/ ejaculate)	90	160	355	149		368
Progressive motility (%)	44	36	34	16	40.95	
Total motility (%)	59	42	53	53		53(10)

Semen volume and pH – As per WHO, 2010 the lower limit of normal semen volume is 1.5 ml and pH should be more than 7.2.<sup>8</sup> In the current study semen pH in all ejaculate was alkaline. Around 12% had semen volume less than 1.5 ml. While comparing the various studies; the mean semen volume value of the study made a close resemblance with that of Crazzolora et al.<sup>14</sup> Unlike other studies (Haugen et al,<sup>13</sup> Stewart et al,<sup>14</sup> Ombelet et al<sup>16</sup>) the mean semen volume was found lower in this study.

Sperm concentration – is defined as number of sperms per ml in the total ejaculate. The normal lower limit is 15 million/ml as per WHO, 2010.<sup>8</sup> In this study the value was found to be 10 million/ml which is lower than that of WHO. In the current study, 5 ejaculate (12%) had sperm concentration less than 15 million/ml. Comparing the mean sperm concentration with the other studies, the value of current study was found to be the lowest of all above the study listed above.

2010.<sup>8</sup> The study results obtained also showed that 12 numbers (20%) of participants had value lower than 32%. In perspective to various fertile populations worldwide the mean progressive motility in Nepalese was found to be slightly higher than Swiss<sup>14</sup> as well as Norwegian population<sup>13</sup> and it made much closer resemblance with that of Indian population.<sup>17</sup> The mean progressive sperm motility of the current study is higher than that of Ombelet et al.<sup>16</sup>

Sperm Morphology– The lower reference limit for normal morphology is 4% or more as per WHO, 2010 using strict criteria. The current study result obtained in regards to morphology were almost was 99% normal as per the liberal criteria. As the current study has followed liberal approach in normal morphology estimation, this could not be compared with that WHO 2010, which has followed strict criteria.

Vitality: As per WHO, 2010<sup>8</sup> the lower limit is 58% and the study results obtained was 50 %.

## CONCLUSIONS

The study concluded that only a single semen parameter cannot be conclusive of fertility. The combination of all semen parameters was more predictive than single parameter. It also concluded semen parameters of Nepalese men were lower as compare to reference limits by WHO, 2010. As study is of small sample size, to validate the result obtained

from the study, we recommend a larger study of similar kind in the other part of the country.

## DISCLOSURE

The authors report no conflicts of interest in this work.

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