New 2010 WHO Standards (5th Edition) for the Evaluation of Human Semen

(2010 WHO Guidelines for Semen Analysis)

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During the course of our lives, a variety of factors affect our physical and mental health. Some of these factors impact our reproductive system leading to subfertility or infertility. Nearly 15% of couples will experience difficulty conceiving during their reproductive lives. Both men and women are more or less equally affected. Therefore, it can be stated that men are contributory to the failure to conceive in 50% of the times (in 7-8% of couples) either alone or along with their female partners.[1]

1. Anderson JE, Farr SL, Jamieson DJ, Warner L & Macaluso M. (2009). Infertility Services Reported by Men in the United States: National Survey Data. Fertil Steril, 91, 2466–2470.

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With the 7-8% rate, it can be stated that the prevalence of male subfertility/infertility is similar to that of Type 1 and 2 diabetes combined[2]

Semen analysis is the cornerstone for evaluating men for subfertility or infertility. However, the test has been shown to be ineffective in accurately predicting the fertility status of men. One of the main reasons for this lack of predictability is the fact that we often utilize the test inappropriately and often for the wrong reason. Semen analysis should not be used for differentiating fertility from infertility. It , however, can be used to assess the status of the reproductive system relating it to the degree of difficulty one may have fathering a child. An methodologically performed semen analysis can provide very important information about the reproductive system of the man from hormonal status to the patency of the reproductive tracts.

2. http://www.diabetes.org/diabetes-basics/statistics/

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There are many reasons for this low predictive power of semen parameters. Three important ones are: 1) Lack of technology to precisely determine various semen parameters and associate the numbers with the sperm function. 2) Inherent variability in semen parameters among men. 3) Absence of valid reference (normal) values specific to the fertile population distinct from those who have lower potential for fertility. The methodologies used to establish the reference thresholds (“normal values”) have also contributed to the unreliability of the reference thresholds. For example, we have accepted semen samples for testing to establish reference thresholds with sexual abstinences between 2 to 5 or 7 days. This loose criterion contributes significantly to the variation in sperm concentration and motility. A better approach is to adhere to a

strict abstinence period (i.e., 24 or 48 hours for all participants) and determine the testicular output (i.e., the output of sperm or the number of motile sperm or the number of motile sperm with good morphology and perhaps with intact DNA per hour or per 24 hour).

The technology and techniques to associate the readings with the function (i.e., morphology and pregnancy outcome or morphology and status of DNA) have improved but need to be improved further. Although at this time the idea may be implausible, but our knowledge of male infertility would improve tremendously if we are able to devise a method to separate a group of sperm similar in characteristics and functions and assess their effectiveness using various assisted conception methods.

The variability in semen quality is inherent in men and little we can do to rectify the situation. An exception would be, as noted above, if we shift our focus from looking at the numbers (basic semen parameters) to assessing the attributes of a particular population of sperm within a semen sample produced within a specific timeframe.

No note for slide 5

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Since 1950, several major studies have been carried out in an attempt to establish reference ranges (thresholds) for major semen parameters in fertile and subfertile men. Equally many studies have also been conducted to assess the validity of these thresholds. The studies carried out by John MacLeod in 1949-1950, presented in 1950 and published in 1951 as well as follow up studies carried out later by MacLeod and colleagues became the basis for establishing the threshold values for major semen parameters in the 1st edition (1980) of the World Health Organization (WHO) manual and subsequently in the 2nd, 3rd and 4th editions.

In 2001, Guzick et al published their findings on the validity of WHO thresholds for sperm concentration, motility and morphology in distinguishing fertile from subfertile men. Similar to many other published investigations, Guzick's study showed that the established threshold values for semen parameters lack predictive power and the values obtained for fertile and subfertile populations overlap significantly. In an attempt to refine and redefine the values, to make them more distinct in the 2 populations and make them more predictive, WHO created a task force to undertake this difficult task. The task took a good part of the 10 years since the latest edition of the WHO manual (4th edition, 1999) to establish a new set of guidelines which was published in 2010 (WHO 5th edition).

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John Macleod Study[3]:

In 1949-50, MacLeod undertook a major study in order to assess the difference between fertile and subfertile men in regard to their semen characteristics. MacLeod evaluated 1000 men whose wives were pregnant at the time of semen analysis as well as 800 men who were seeking treatments for subfertility. All motility and morphology evaluations were performed by a single person in a blind fashion.

3. John McLeod, Fertil Steril (1951), Semen quality in one thousand men of known fertility and in eight hundred cases of infertile marriage,2(2):115-39.

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Overall, he found that the data from the two groups overlapped significantly making it difficult to distinguish them from each other. However, upon further scrutiny, he was able to find some differences which was found to be significant using simplistic and inadequate statistical evaluations. It was noted that only 5% of fertile men had sperm concentrations below 20 million per mL while 17% of the infertile (subfertile) men had less than 20 million sperm per mL semen. This simplified characterization and comparison of the two groups became the basis for establishing the reference threshold for sperm concentration.

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Related to sperm motility, as you see in next slide, only 18% of fertile men had less than 50% whereas this number for the subfertile men was 31%. Semen volumes and percent normal forms (routine morphology) did not appear to be significantly different between the 2 groups although some differences were recognized.

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The study carried out by MacLeod had some major shortcomings which may have contributed to the establishment of values unable to distinguish fertile from subfertile men. While obtaining semen samples from men whose wives were pregnant and the blind nature of the study are highly advantageous, the study was riddled with design flaws and incorrect assumptions. Some of the problems with the study were:

1. Semen were collected by masturbation or coitus interruptus
2. Only one semen sample was obtained from each man participating in the study
3. In the fertile group, if semen quality was poor, a second sample was collected and examined. This was not done for the infertile (subfertile) group

4. Sperm motility was assessed at 5 hours or less post collection. It is believed that the production of motility inhibitors (i.e., byproducts of polyamines; amines conversion/oxidation to aldehydes toxic to sperm) can impact sperm motility starting after one hour post collection.

5. Fertility was not clearly defined. In other words, although the fertile group were men who had pregnant wives, it did not take into consideration the length of time it took for the couples to achieve pregnancies. The subfertility was not clearly defined either. The subfertile group consisted of men with short, medium and long duration of infertility. The status of their wives were not known.

6. Valid statistical methods in order to assess the influence of a particular semen parameter on the pregnancy outcome were not carried out. Only percentages of various categories (i.e., the percentage of men with sperm concentration below 20 million per mL or the percent motile between 10-30% between the 2 groups) were compared and found to be statistically different.

7. Morphological evaluations were performed loosely

8. Follow up studies on the “infertile” group showed that some were successful in fathering children

About 300 men in the subfertile group were found to be azoospermic. They were not included in this group for statistical analyses. The author did not elaborate if these men were absolutely azoospermic (following centrifugation of semen samples and observation of pellets). With this number, azoospermia among the subfertile men evaluated was found to be very high.

No notes for slide 11 and 12

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With the exception of sperm morphology, the reference values for major semen parameters remained the same for many years following the publication of the 1st edition of WHO manual for the evaluation of human semen. Sperm morphology got special attention throughout the years and its reference threshold decreased significantly with each new edition of the WHO manual.

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Clinical reference limits or ranges (from general population or from fertile men) are needed in order to be able to compare the results obtained from subfertile or infertile men with the ranges obtained from the clinical reference ranges. For many years

following establishment of reference ranges for semen parameters, studies had been published disputing the diagnostic value of the established ranges. In 1991, Guzick et al published the results of a controlled study of 696 fertile men and 765 men who were attending fertility clinics at 9 sites across the US in an attempt to assess the validity of the established WHO criteria in distinguishing the fertile and infertile/subfertile population and to determine if new thresholds can be established differentiating the two populations. The infertile couples were the participants in intrauterine inseminations combined with ovulation induction and all female partners had no female factor infertility. The duration of infertility for the couples was 12 months minimum with a mean duration of 43 months. The fertile population consisted of men whose wives were pregnant at the time of semen collection or had fathered children during the previous 2 years. Fertile men with an established cause of recent infertility (i.e., unable to father a child during the previous 12 months or having varicocelectomy or vasovasostomy) were excluded. The ages for the men were between 20 to 55 and for the women between 20 and 40 years. The fertile and the infertile populations were age-matched as closely as possible. Two specimens from each of the fertile participants approximately 2-3 weeks apart were obtained for evaluation. The infertile population provided at least 6 semen samples and the two semen samples collected closest to each other were used for the study. The investigators use highly involved and appropriate statistical methods to analyze their data. Their investigation showed that the WHO threshold were not able to distinguish fertile from infertile population. However, their studies yielded new ranges for sperm concentration and motility as well as for sperm normal morphology (Strict Criteria) that could differentiate the fertile from subfertile populations. There were also values for sperm morphology, concentration and motility that fell in between the fertile and subfertile groups. They classified these ranges as “indeterminate.” The authors cautioned that although there were more infertile men in the subfertile ranges, there were some fertile men who had semen parameters falling in this range. Therefore, the subfertile ranges need only to be used as a guide for evaluating men for infertility.

<u>Some</u> of the shortcomings of the Guzick’s study:

1. The fertile population was not as uniformly defined as it should have been (a mixed population of men recently fathering a child or during the previous 2 years)

2. The subfertile population was not made of truly subfertile men. Despite the claim that the female partners of the subfertile group had no female factor infertility, they may indeed have been contributing to the subfertility due to undetermined factors.

3. The time frame (abstinence) between the 2 semen samples collected by the fertile population varied significantly

4. The age of men varied significantly
5. Two semen samples out of 6 collected by the subfertile group were included
6. Assisted conception were used to assess the fertility status of the subfertile group whereas the fertile group made of men who were successful via natural conception

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Some positive aspects of the Guzick's study:

1. The fertile population was better defined
2. Two semen samples per individual were obtained for the study
3. The status of the female partners were more clearly defined
4. Valid statistical methods were utilized to evaluate the data

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Although the fertile population was defined, the population consisted of men who had fathered children during previous 24 months (1, through 24). The semen parameters may vary significantly among this group of men. The subfertile population consisted of men who were not truly infertile as some fathered children following IUIs. Although the authors sated that the woman partners had no female factors, these could be some unrecognized factors present in some of these partners. Similar to MacLeod's study, the abstinence with not strictly enforced and contributed to the variation observed among men from both populations. This variation may have been resulted in different presentations in the two populations. The investigators used only 2 samples from the 6 collected by the subfertile group. The choice of the 2 samples varied and depended on how close the 2 samples collected from each other. The authors included the 2 samples collected closest to each other in regard to the number of days of abstinence. It is recognized that as men age their hormonal levels (i.e., testosterone and ration of testosterone to estardiol) change. With this change, comes the consequences such as lower sperm production/concentration. With abstinence varying significantly, testicular output (i.e., number of sperm output per 12, 24 or 48 hour) should have been determined. Although the authors attempted to match the men in the 2 groups based on age, the variation of the age in the 2 groups was too broad.

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The study carried out by Guzick et al, revealed some expected and some interesting findings. It was found that, overall, there was an overlap of data for major semen

parameters obtained from fertile and subfertile populations. It was concluded that the 1999 WHO guidelines (thresholds) were not able to distinguish between the two populations. However, their statistical evaluations revealed a new set of thresholds which could clearly delineate the fertile from subfertile groups. Men with sperm concentrations of <13.5 million/mL semen, sperm motilities of <32% and normal morphologies of <9% (Strict Criteria) were clearly in the subfertile category distinct from men with sperm concentrations above 48 million per mL semen, sperm motilities above 63% and with normal sperm morphologies of above 12%. Calculation of odds and likelihood ratios are very useful tool for evaluating the value of a particular laboratory test (i.e., semen analysis) for its intended use. They use the sensitivity and specificity to assess if the results of a test such as semen analysis can reveal if a condition (i.e. infertility), exists. Of course, infertility is a multifactorial condition. Nevertheless, the ratios can provide additional information about the utility of the analysis. Finding a parameter with the odds ratios of 5 or above is significant. The study found good odds ratios for sperm concentration of below 13.5 million/mL semen and motilities below 32%. On the other hand, the ratio for normal morphology of <9% was not as strong. This perhaps reveals that the 9% threshold is too high for distinguishing the subfertile population. The 2010 WHO guidelines has established a threshold of 4% which will be discussed later. Looking at the odds ratios for fertility, we can clearly see that having a high sperm concentration, motility and morphology are not as revealing (for fertility) as having low numbers (for subfertility).

They also concluded that there were a group of men with semen characteristics between the two fertile and subfertile ranges. Semen parameters from these men could not noticeably reveal the fertility status of these men.

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This slide also shows some interesting findings from the Guzick's study. As the number of abnormal semen parameters increases, the odds ratio for infertility increases too. With one abnormality, the odds ration was between 2.2-2.9, for two abnormalities the ratio was between 5.5-7.2 and for three abnormalities it increased to 15.8. For a single abnormality, sperm morphology had a higher odds ratio for infertility than did sperm concentration or motility. I wonder if the morphology evaluators were more strict, it could have even higher odds ratio.

No notes for slide 19

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The TTP $\leq$ 12 months group consisted of men who participated in prospective and retrospective studies. Data from a total of 1953 semen samples from 5 studies in 8 counties on 3 continents were combined and analyzed.

The unscreened group (men of unknown fertility) were men from general population who donated semen samples for hormonal contraceptive research studies. A total of 965 semen samples from 7 studies in 5 countries on 3 continents were combined and evaluated.

The group considered fertile of unknown TTP (NOTTP) were men who fathered children in the past but TTP was not known for these men. These men had female partners with high, normal, moderately or severely impaired fecundity. A total of 817 data points from two studies in

two countries on two continents and from two multinational WHO studies (1990, 1996) were combined and analyzed.

The screened men were those whose semen samples met the criteria of being normal based on WHO 1999 criteria. These men were either participants in screening phase of contraceptive research studies or those attending infertility clinic. A total of 934 data points from 4 studies in 4 counties on 3 continents and 2 multinational WHO studies in 1990 and 1996 were combined and studied.

In order to be able to combine the data from different laboratories, we must ascertain that all methodologies and guidelines to analyze semen sample for basic semen analysis were the same. The authors claim that all participating laboratories were adhering to the WHO 1987, 1992 and 1999 standards for the analyses and all required guidelines were followed and data were generated under similar conditions. It must be noted that determinations of semen volume, sperm concentration and motility has remained more or less the same throughout the years. However as noted, the sperm morphology assessment is the only criterion which has been revised several times. As a result, morphology readings were only obtained from very few laboratories which have been experts in assessing the morphology based on Strict Criteria and, in some cases, the slides generated by these laboratories were read at a specific expert laboratory. In addition, some morphology readings were reviewed by the Tygerberg group who developed the Strict Criteria to make certain that the readings were within the specified guideline. The data that were utilized to calculate various statistical parameters for the 2010 guidelines were from laboratories that have been participating in various external and internal quality control measures to comply with the minimum requirements.

“Data on semen volume, sperm concentration, total sperm number per ejaculate, motility, vitality and normal morphology were included only if

they were generated from complete semen samples, obtained following 2–7 days of sexual abstinence.” Cooper et al 2009. Only one semen sample from each individual was included in data analyses. If more than one semen sample were provided, the data

from the first collection were used. Neubauer, or alike chambers and dilution methods were used for sperm counting.

The age of the men providing the samples ranged from 17 to 67 years with only a handful of participating men being over the age of 45.

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With these results available, the next step was to establish thresholds or reference intervals associated with the population of men with TTP < 12 months which could indentify the fertile men. Traditional calculation of mean and $\pm$ 2standard deviations applicable to clinical laboratory tests are not suitable for semen parameters simply because many of semen parameters are not normally distributed and the reading s are in the form of percentages. The log transformation of the data obtained is not an easy task either. Employing nonparametric methods and use of calculations such as median and interquartile ranges have been suggested (MacLeod, 1950, 1951 and Pasqualotto, 2006). However, none of these can effectively serve as reference limits. The lower reference limit for major semen parameters from fertile men have also been suggested. However, there has not been agreement on which value (2.5^{th} , 5^{th} , 10^{th} , 15^{th} or 16^{th} centile) to use. Yet, other investigators such as Guzick have proposed use of classification and regression as well as receiver operating characteristics curves to establish reference limits to distinguish the fertile from infertile populations.

James Boyd of the University of Virginia, authored a very interesting paper related to the 2010 WHO guidelines for analysis of human semen. The paper was published in Asian Journal of Andrology (2012). I highly recommend that you read this paper which describes fundamental theories behind establishing threshold values (reference intervals) and alternatives to reference intervals. The next paragraph directly quotes or paraphrases the Boyd comments in the article noted. “Generally, reference intervals (or normal ranges) for majority of lab tests is defined as the threshold values between which 95% healthy individuals would fall. In case of semen analysis, men with proven fertility would be used as the population to be used. This is particularly true and appropriate because Cooper 2009 showed that several semen parameters from fertile men (TTP<=12 months) were superior to those of unscreened men in the general population. Thus, the idea of using this group makes sense.” However, we must make sure that the population chosen truly represent the population that is needed to be used as the reference population. In case of fertile men, we must make sure that they are not consist of men who have fathered children naturally within a year and those who have father within 2 years plus those who fathered following some medical interventions. “The acceptance of 95% of the results would result in exclusion of 2.5% of individuals with the highest results and 2.5% of individuals with the lowest results. In cases where the upper or lower results may not be clinically significant, one may be able to accept

one-sided reference intervals which exclude 5% of the results from the opposite end. Since logically higher number of sperm or percent motility may not be pathologic, one sided reference intervals with 5th centile as the lower threshold limit makes sense."

"Recently, for some tests the definition of reference intervals has changed from a pure statistical representation (95% of the data) to the clinical outcome This perhaps makes more sense for semen parameters too. Another method of defining reference intervals could be based on the genetic susceptibility or genetic marker which may be found in some individuals with male factor infertility. Similarly, we may define different reference intervals for men of older age, those with certain hormonal imbalance and those with higher days of abstinence."

The best, though impractical, would have been to collect appropriate documentation of fertility and time to pregnancy for each reference interval.

The likelihood of regional differences in semen analysis results that have an underlying biological basis cannot be ignored.

Pre-analytical variable can influence the outcome of semen analysis. Examples are age, diet, social habits, medications taken and health condition all can influence the readings of their semen.

Analytical phase such as the methods used to test semen can also influence the results. We all know that reading of semen parameters vary greatly from lab to lab.

Reference Intervals and test interpretation:

As a specific example, because reference intervals are statistically derived with respect to only the healthy population, they cannot be used to rule in or rule out specific conditions such as male infertility.

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If the data are normally distributed, generally the mean and $\pm$ 2SD of a series of data is calculated and is used to establish reference range. If the data are not normally distributed, the central 95% boundaries are determined by removing the lowest and the highest 2.5% of the observations/readings (in this case for the 4 populations). The data spread in both sides of the mean encompassing 95% of the data (47.5% above the mean and 47.5% below the mean) are considered as the range of values encompassing 95% of the data distributed on both sides of the mean (95% confidence intervals, 95% CI, two sided). The committee in charge of establishing the reference values for major semen parameters, argued that there is no scientific evidence that the values in the higher end of the spectrum (above +47.5%, the remaining +2.5%) are pathological and should be omitted from consideration. In other words, these is no scientific evidence

that men with the highest number of sperm or the best morphologies have less fertility potential or should be considered as outliers. The committee decided to include this +2.5% end of the data and rather remove the lowest 5% of the data from consideration for establishing the "normal" thresholds for semen parameters in the fertile group (5^{th} centile, one sided). This threshold, the lowest 5^{th} centile of the data for each of various sperm parameters, was considered as the lowest threshold considered acceptable to be considered "fertile." Therefore, the reference values for "normal" semen parameters in the newest edition of the WHO manual (WHO 5^{th} edition, 2010) for the examination of human semen do not have ranges (i.e., sperm concentration between 20 to 200 million/mL semen). Rather, the limit is set at the lower 5% of data from the fertile group (men who fathered children during the previous 12 months) and is labeled lower threshold (or reference) limit for each parameter. The 95% confidence interval (95% CI) then is calculated using the lowest 5% of the data obtained for each parameter. For example, sperm morphology of 4% is considered as the lowest acceptable threshold for being considered in the "normal" category. The 95% CI of the lowest 5% of the data below 4% which has been calculated to be 3-4% is still considered acceptable to be considered as a part of the fertile group. In other words, a semen sample with a morphology of 3% is considered to fall in the "normal" category although at the lowest acceptable level. As noted, the 2010 edition has no upper limit for any of the semen parameters.

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The TTP $\leq$ 12 months group consisted of 1953 semen samples from 5 studies in 8 counties in 3 continents.

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"Figure 1 Box and whisker plots of semen analysis data. Semen volume, sperm concentration, total sperm numbers per ejaculate, total percentage motility, percentage progressive motility and percentage normal morphology from fathers with time-to-pregnancy 12 months (TTP , 12, black), unscreened men from the general population (UNSCR, red), fathers with no known time-to-pregnancy (No TTP, green) and screened men selected for normozoospermia (SCR, yellow). The boxes represent the quartiles and the lines within them are the medians; the whiskers extend from the 10^{th} to the 90th centiles and the dots represent the 5th and 95th centiles. *significantly different from fathers with TTP of $\leq$ 12 months." Cooper et al, 2009

This slide represents the bulk of findings from the study of 4500 men and particularly from 1953 semen samples from men with TTP $\leq$ 12 months (fertile population with known TTP). Men with TTP $\leq$ 12 months had semen parameters significantly different from men from general population (red), fertile men of unknown TTP (green) and men

considered normozoospermic based on WHO 1999 guidelines. Men with TTP $\leq$ 12 months had higher semen volumes and sperm concentrations leading to higher total number of sperm per ejaculate. Compared to other groups, these men also had a higher total number of motile sperm and morphologically normal sperm per ejaculate.

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This slide represents the values obtained for reference population or values proposed to be used as reference thresholds from various groups. The values proposed by the 5th (2010) edition of WHO are in some respect similar to what Guzick et al found back in 1991 and different from the 1999 WHO standards. In it interesting to note that Guruswamy et al also presented similar findings from his small study back in 2005. They obtained 62 semen samples from 47 healthy donors with proven natural fertility and single ejaculates from 406 patients to assess where the semen parameters fall based on 1999 WHO criteria. The investigators calculated the 3rd centile for sperm concentration, motility and morphology for the fertile donors. Based on 1999 WHO standards, 30 (48%) fertile donors and 331 (81.5%) men assessed for infertility had one or more abnormal sperm parameters. Then the authors reclassified the normality of the readings from the semen samples based on the 3rd centile. The 3rd centile values for sperm concentration, motility and normal morphology in fertile semen samples were $11x10^6$/mL, 27%, and 12%, respectively. “The application of these values resulted in a significant reduction in the number of samples diagnosed with semen parameter(s) below reference values among fertile donors (19% vs. 48%, $P < 0.0001$) and men assessed for infertility (45% vs. 81.5%, $P < 0.0001$).Guruswamy et al (Abstract presented at the 2005 annual meeting of the American Society of Andrology).

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This is a table of centiles for men with TTP $\leq$ months. The 5th centile has been chosen as the lower threshold limit for various semen parameters. Parameters falling below the 95% CI from this 5th centile are considered low or abnormal and are flagged in semen analysis reports which have adapted WHO 1020 guidelines. It is a good idea to have this table handy as one may want to know where a particular patient may fall compared to men with TTP $\leq$ months. Remembering the thresholds for the subfertile group established by Guzick’s study and comparing to the centiles in this table, we note that the subfertile group had sperm concentrations in the 5th centile region, total sperm motilities in the 2.5 centile and sperm progressive motilities in the 5th centile. For normal morphology, the Guzick’s subfertile group (< 9% normal forms) fall below the 25 centile based on WHO 2010.

No notes for slides 27 and 28

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The laboratories practiced different methods of sperm incubation for liquefaction and for the assessment of sperm motility. Some incubated sperm at room temperature and understandably assessed the motility at room temperature. Yet, other laboratories employed 37 C conditions. Undoubtedly, determinations of sperm motility at 37 C can result in higher progressive motility closer to what the motility would be for a particular sample compared to motilities assessed at room temperature. Related to the use of counting chambers, it is known that sperm counting methods utilizing dilution of semen can provide different results compared to methods using undiluted semen. In addition, it also has been published that certain counting chambers such as Makler often overestimate sperm concentration reading compared to other chambers (Keel, 2009).

No notes for slides 30 through 35

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A good semen analysis reports must contain not only new 2010 WHO thresholds, but also other information making it easier for the healthcare provider to assess the status of the patient whose semen was evaluated. Notably, the report should contain:

Time collected and time analyzed which should be within 1 hour post collection in order to avoid the impact of sperm motility inhibitors (i.e., byproducts of polyamines oxidation).

The type of counting chamber (i.e., Makler) to provide the provider information about the potential for overestimation of the sperm concentration.

The temperature under which the sample was incubated for liquefaction and assessment

The days of sexual abstinence as semen samples collected from the same individual at different time with different abstinence periods may not be suitable for comparison.

Whether or not the collection was complete or a spillage occurred and, if an spillage occurred, which portion of the semen missed the container.

Complete information about the semen data, sperm data and morphology in separate section which make it easier for the provider to review. These include reporting total motility as well as progressive motility which should also include the percentages of sperm with rapid, medium and slow progression. The reference values referring to the lower 5th centile (lower threshold values) for major semen parameters should also be noted in the report. The values below the 95% CI should be flagged as low. For

readings with no established WHO standards, there would be a note in the reference section indicating that the threshold values have not been established.

No notes for slide 37

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Regardless of the methdology, analysis of semen should adhere to strict acceptable guidelines in order to provide the most reliable results. Not to discount the value of developing strict pre-analytical and post analytical guideline, the analytical phase of semen analysis include determination of semen volume and sperm concentration, motility and normal morphology as major parameters of semen critical for assessment of patients for their subfertility/infertility potential. Semen volume is assessed to the nearest 10^{th} fraction of a milliliter and it a good idea to use pipets to assess the volume. For sperm concentration, various methods of counting (dilution of semen or direct assessment without any dilution) as well as different chambers (Hemacytometer, Makler, MicroCell, Cell-VU, etc.) are used. Regardless of the methodology and the typo of chambers used, certain guidelines must be followed in order to provide the most precise and accurate results.

No notes for slides 39-41

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For example, for sperm concentration, you need to have duplicate readings and the variation or difference between the 2 readings must be within an acceptable limit. Semen is a suspension of cells and other particulate matters and a thorough mixing of the semen is necessary in order to obtain readings within acceptable difference from each other. Graphs or formula proposed in WHO manuals can be used for this purpose.

Example: You are evaluating duplicate readings of the same sample and the number of sperm counted to determine if your readings are acceptable. The first reading was 138 and the second reading was 112. Use the graph and answer whether or not your duplicate readings are acceptable

A. The readings are acceptable X

B. The readings are not acceptable and must be repeated

Alternatively, you can use the formula Y= 0.0551 X + 16.445 (X= two readings summed, Y+ the acceptable difference).

For example, if the sum of your 2 readings is 218 (you read 102 cells in one chamber and 116 in another from the same area of the chambers), plug in the 218 in place of X

and calculate the Y which will be 0.0551 x 218 + 16.455 or 12+16.455= 28. Your difference between the 2 reading should not be more than 28. The above example for the 2 readings show a difference of 16 which is acceptable. However, if one of your readings was 93 and the other 125, the sum will be 218 (the same), however, the difference is more than 28. In this case, you need to repeat the loading and reading of your sample.

Question: If your first reading is 165 and the second reading is 135. Using the equation Y= 0.0951 X + 16.455 what is the maximum acceptable difference between these duplicate readings?

A. The maximum acceptable reading is 45

B. The maximum acceptable reading is 33

No notes for slides 43 and 44

Slide 45

Similar to sperm concentration, you need to have sperm motility assessed in duplicates. You also can use the graphs from the WHO manual. An example is noted in this slide. To use the graph:

- Calculate % motility from your 1st reading. Note how many sperm were counted to assess the percent motility.
- Calculate % motility from your 2nd reading. Note how many sperm were counted to assess the percent motility.
- Average the two readings of motility.
- If the final averaged percent motility is more than 50%, subtract it from100% and plug in the result. If not, read directly from the curves.
- Depending on how many total sperm you counted to assess the motility, use the curves 100, 200 or 400. Plug in the total sperm (X axis). From the corresponding curve, calculate how much the difference should be (from the Y axis). If the total number of sperm counted is more than 200 but below 300, you can use the 200 curve.

An example:

- 1st reading: 125 sperm, motility= 65%

- 2nd reading: 146 sperm, motility= 52%
- Average the two percentages=58.5%
- This is more than 50%, then 100-58.5=41.5%
- Plug in 41.5 using the 200 curve
- The difference should be 9.5%
- The difference between 65% and 52% is 13% which is more than the acceptable difference of 9.5%. Readings are rejected. Repeat.

You are deciding if the duplicate readings for sperm motility are acceptable. Using the graph, decide if the readings of 115 sperm with a percent motility of 63% and 136 sperm with a percent motility of 52% are acceptable

A. The readings are acceptable

✓B. The readings are rejected and need to be repeated

No notes for slide 46

Slide 47

For sperm morphology, the current established method is the assessment based on the Strict Criteria. To highlight the importance of morphology, here I show you the morphology of sperm attached to surface of human egg (zona pellucida) preserved for the Hemizona Assay. The photo was taken from incubation of washed human semen with the hemizona. Looking at the photo although you may not see it with high resolution, you can appreciate the uniformity of the sperm morphology indicating that only sperm with this type of morphology binds to the surface of egg.

No notes for slide 48

Slide 49

A leukocyte, a spermatid and a sperm. Note the difference between the nuclei of a leukocyte (having bridges) and those of a spermatid (separated and mainly round).

Slide 50

Let’s take a look at a few semen analysis results to make the judgment about the nature of abnormality:

This slide shows the report for a semen sample with no sperm (azoospermia, if proven upon further analysis). There are a few other determined parameters that can help the provider narrow down the reason/cause of lack of sperm in the ejaculate.

- Semen volume is more or less normal
- Coagulation occurred and complete
- pH is basic (normal)
- No sperm in semen
- No sperm in the pellet
- No sperm in the re-centrifuged supernatant
- No sperm in the smear prepared from part of the pellet and the supernatant pellet

Most probable diagnosis is non-obstructive azoospermia (NOA). In obstructive azoospermia, seminal vesicles are either absent or blocked but in NOA they are present and contribute to the volume of semen. Therefore, volume of semen is near normal because seminal vesicles are contributing. Coagulation occurred because seminal vesicles secretions responsible for coagulation are present/contributing. pH also normal because basic secretions of the seminal vesicles are present/contributing. All required steps for evaluating the sperm for the presence of sperm have also been performed. Before making a judgment, a repeat analysis is performed. If similar results such as normal volume, pH and no sperm are noted, non-obstructive azoospermia (NOA) is the most likely diagnosis.

Slide 51

This slide represents a report for a man whose semen sample had no sperm. Similar to the previous slide, there are a few other determined parameters that can help the provider narrow down the reason/cause of lack of sperm in the ejaculate.

- Semen volume is very low (most of the times <1 mL)
- Coagulation does not occur. Semen looks watery upon ejaculation
- pH is acidic (abnormal)
- No sperm in semen
- No sperm in the pellet
- No sperm in the re-centrifuged supernatant

- No sperm in the smear prepared from part of the pellet and the supernatant pellet

The most probable cause in this case may be obstructive azoospermia although further investigation is needed.

You already know some of the causes of obstructive azoospermia: CF, Young's syndrome, DES exposure of mothers, obstruction of ejaculatory ducts due to inflammation/infection, etc.).

Slide 52

The latest WHO studies carried out to establish new thresholds for semen parameters have been fairly extensive. However, with our current scientific capability any attempt to make a clear cut line between fertility and infertility will be a failure. Fertility is a relative condition and our focus should shift to find methods to assess the degree of the problem combined with the fertility potential or chances of success. Along with the investigation of the man, we should not overlook the contribution of the woman partner to the difficulty and assess the status of both the male and the female jointly.

With all the comments made, there should remain no doubt that a correctly performed semen analysis can provide considerable information about the status and patency of the reproductive system.

No notes for the remaining slides.