From the Pages of History

History of Semen Analysis

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Chettinad Health City Medical Journal 2015; 4(1): 63 - 64

Semen analysis is an important tool in the diagnosis of male infertility. Through its cellular and chemical components, human semen can provide information on the functional properties of the organs producing this fluid i.e., the testis, epididymis, and accessory glands[1].

This important test has its origins traced back to 1677 when Anton Van Leeuwenhoek (Fig 1), a Dutch master craftsman, first observed human spermatozoa from an ejaculate, along with his student, Johan Ham. Using a simple microscope which he created, he observed millions of small motile 'animals', which he called animalcules (Fig 2 & 3). In his letter to the Royal Society of London, he illustrated the structure of spermatozoa quite accurately that in retrospect, his observations with the help of such a primitive microscope seem incredible[2]. It is interesting to know that this landmark letter to the Royal Society, which paved way for modern Andrology, was written and sent in the fear of being considered repugnant and even scandalous due to the nature of the sample. Leeuwenhoek was also the first to observe the serpentine motion of the animalcules and he also observed different shapes of spermatozoa across different species[3].

Fig 1: Anton van Leeuwenhoek (1632 - 1723))

Almost a hundred years later, it was Lazaro Spallanzani in 1771, after extensive work on artificial insemination, observed spermatic animalcules in various species, including humans and even documented the fertilizing capacity of the sperm[4].

While numerous spermatologists continued to work on spermatozoa characteristics during that period, it was Rudolf Wagner in 1837, who made substantial contribution by documenting his observations on spermatozoa of more than 400 species, including humans. Wagner, back in those days observed that, 'the motility of the sperm was greatest at the point of ejaculation and was less in sperm taken from vas deferens and even lesser or non-existent in sperm taken from testis[5]'.

Fig 2 - Simple microscope created by Leeuwenhoek

Further highlight was placed on spermatozoa through J.Marion Sim's work on post coital cervical mucus test. He made an important observation which is significant even today, that the presence of spermatozoa indicates that the male is not barren[6]. It was a landmark paper by Macomber and Sanders in 1929 that quantitatively assessed spermatozoa. From the study which involved 294 males, it was deduced that a 'normal' reference value of above 60 million/ml significantly increases the chances of pregnancy. They also tried to establish a method for counting spermatozoa with the help of a blood counting chamber[7].

The 'normal' values were lowered to 40 million/ml by Amelar and Williams[8] and subsequently brought down to 20 million/ml after the remarkable study done at that time by John MacLeod on 1800 men comparing sperm characteristics between fertile and infertile populations[9].

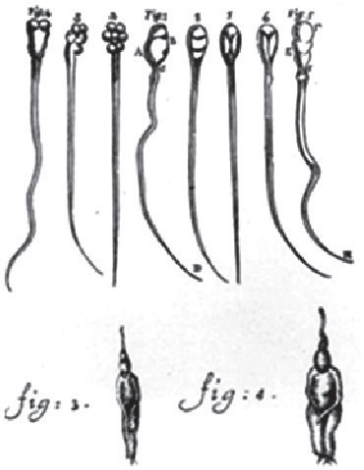

Fig 3 - Illustrations of spermatozoa by Leeuwenhoek

Further contribution to 'normal' values was done by Rune Eliasson who stated that it was not justified to discriminate a semen sample with 5million/ml sperm concentration as infertile[1].

Based on a number of studies done by the above mentioned pioneers of Andrology, the World Health Organisation(WHO) published its first manual on semen analysis in 1980[10], thereby helping establish uniformity in methods of evaluating spermatozoa worldwide. It has been updated periodically with the fifth edition currently being in use[11]. While the first few editions seem consensus- based, the fifth edition appears mostly evidence-based, despite the discrepancies and despite being limited to a select population. The introduction of CASA systems in 1990's paved way for extensive studies on sperm kinesiology and a relatively more subjective way to conduct semen analysis.

Spermatozoa Morphology: Morphology of spermatozoa requires a special mention as it has been the most debated issue in spermatology due to its heterogeneity and subjective nature of evaluation. Since 1900s studies have been conducted to equate normal morphology to sperm fertilization capacity.

Interestingly, until 1970's, it was only the head of the spermatozoa that was considered while assessing morphology. It was in 1971 that Rune Eliasson emphasized the importance of evaluation of the whole spermatozoon including the mid-piece and tail. Eliasson was one of the first to standardize this parameter through a classification system containing three groups – head, mid-piece and tail[12].

Evaluation of sperm morphology has seen two approaches – liberal and strict. The liberal approach as followed by MacLeod, considered all forms to be normal except those that were highly distorted, therefore no criteria was put forth for normal spermatozoa[13]. But in the strict criteria, as described by Menkveld and Kruger, even borderline forms are to be considered abnormal. A normal spermatozoa was defined on the basis of spermatozoa obtained from the internal cervical os (after coitus) and from those tightly bound to zona pellucida[14], thereby providing guidelines which were adopted by the WHO manual. The WHO manual in its first two editions followed the liberal approach after which it implemented the stricter criteria[12]; this would explain the dramatic reduction in the normal morphology reference value from 80%(1st edition)[10] to 4%(5th edition)[11].

In spite of the strict criteria description, the subjectivity of sperm morphology assessment makes it difficult to standardize this parameter, and bring uniformity across different labs; this results in a significant inter and intra observer variability. This is beautifully described by Eliasson using Edward Adelson's checker shadow example, as shown in fig 4[15,16].

Conclusion

While semen analysis is an important tool in diagnosing male infertility, it is to be remembered that reference ranges given by the WHO manual are not diagnostic cut-off values but only results obtained out of an observation of a fertile population.

Male fertility cannot be determined solely on the result of a semen analysis as there is no evidence stating the exact number and quality of sperm required for a man to be considered fertile. As Christopher De Jonge rightly said, semen analysis is still the subject of both commendation and condemnation[17].

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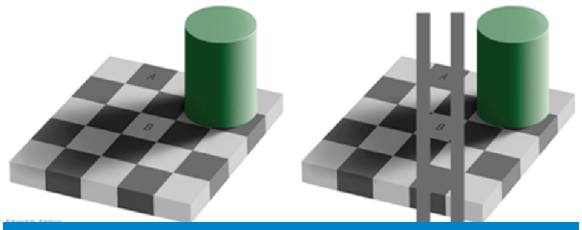

Fig 4 : Adelson's checker shadow optical illusion. In this checker - shadow image, the squares A and B have exactly the same shade of grey.