

# On the Origins of the Semen Analysis: A Close Relationship with the History of the Reproductive Medicine

Fernando Tadeu Andrade-Rocha

Lisa Andrology Lab  
Research Center, Santa Isabel  
Laboratory Medicine,  
Três Rios, Brazil

ABSTRACT

The spermatozoa were first seen in ejaculates in the 17<sup>th</sup> century. However, the basic mechanisms of human fertilization have been only fully understood after the discovery of ovum in 1827. As a result, the interest in developing technologies for semen analysis arose from the early 1900s. Indeed, standard methodologies for semen analysis were designed mostly along the first half of the 20<sup>th</sup> century. Before the 1930s, semen analysis was nearly unavailable clinically, since there were still no robust methodologies for assessing sperm characteristics, as well as to set up standard references that could be able to assess the reproductive capacity of men. However, joining some methodologies reported from 1910 up to 1930, standardization was attained and thereby semen analysis increasingly assumed its role in laboratory practice for investigating men in barren marriage. This article aims in reviewing historical backgrounds on the semen analysis, up to its insertion in laboratory practice. Emphasis is given to the major studies that contributed either directly or indirectly in developing the earliest routine for the semen analysis.

**KEYWORDS:** *History of medicine, human reproduction, infertility, semen, semen analysis, spermatozoa*

## INTRODUCTION

Semen analysis is mandatory in the diagnostic workup of infertility, since the early 1930s. Semen analysis provides valuable information for investigating disorders and pathologies affecting the male genital tract, such as varicocele, infections, and hormonal disorders, which often negatively impact male reproductive capacity. In this regard, earlier studies have extensively reviewed and discussed the key attributes and limitations of the semen analysis.<sup>[1-9]</sup>

Currently, semen analysis is based on the recommendations of the fifth edition of the World Health Organization (WHO) Manual for the Examination and Processing of Human Semen<sup>[10]</sup> that provides technologies and the reference values for evaluating semen parameters. They include standard procedures (macroscopic examination, initial microscopic examination, sperm count, motility, vitality, morphology, membrane integrity, assessment of leukocytes, immature germ cells, and testing for antibody coating of spermatozoa), optional tests (indices of multiple sperm

defects, pan leukocyte [CD45] immunocytochemical staining, interaction between spermatozoa and cervical mucus, computer-aided sperm analysis, and biochemical analysis), and research procedures (reactive oxygen species, human sperm-ooocyte interaction tests, human zona pellucida binding tests, assessment of the acrosome reaction, zona-free hamster oocyte penetration test, and the assessment of sperm chromatin). It became increasingly widespread worldwide since the publication of the first edition of the WHO manual in 1980.<sup>[11]</sup>

Upon looking, attentively at the laboratory practice, it can be assumed that semen analysis routine was born in the 20<sup>th</sup> century. Indeed, the first methodologies for semen analysis were reported as from the early past century. However, coming back to the past, it can be noticed that the history of semen analysis has many connections with old events that were landmarks in the history of the reproductive medicine, starting exactly

**Address for correspondence:** Dr. Fernando Tadeu Andrade-Rocha, Rua Dr. Oswaldo Cruz, 312 - Três Rios, RJ, 25.620-040, Brazil. E-mail: lisalabrescenter@gmail.com

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in the 17<sup>th</sup> century, when spermatozoa were first seen in ejaculates. Thenceforward, about 350 years passed involving laborious efforts of countless researchers for reporting techniques for semen analysis, as well as for investigating its clinical value in reproductive failures. As a result, many techniques have been readily inserted in laboratory practice. Sometimes, they had to be further optimized to improve its performance. Others have remained as optional tests, while some failed to reach its goals. Thus, either they have been seldom used or they have been forgotten. As a whole, they are part of the history of semen analysis.

This article aimed in reviewing historical backgrounds of the semen analysis, extending the reach from immemorial times up to the 1930s when the semen analysis became a part of laboratory practice for investigating male sterility. This study searched database MEDLINE (1966–2017) whenever necessary. Historical manuscripts published prior to 1966 were searched in the periodicals cited on the references and through cross-references. Aside from the historical approaches, the current study also reviews the applicability of the technologies used at the time.

## CONCEPTS ABOUT SEMEN IN ANCIENT TIMES

Concepts on the role of the semen in human reproduction date back into antiquity. Indeed, there is a range of information available about the semen reported by practitioners from ancient times. The Roman poet and philosopher Titus Lucretius Carus in his epic poem *De Rerum Natura* (On the Nature of Things) gave a lengthy explanation about the semen, mixing theories from different fields such as dreams, sex, sexual desire, heredity, and conception.<sup>[12]</sup> In book four, he briefly wrote about the production of semen and the ejaculation (1037–1048) and raised the assumption of a relationship between sexual desire and ejaculation (1049–1057). According to Lucretius, an external stimulus, presumably through the influx of simulacra into the eyes, is likely to produce the semen, with simultaneous stimulation throughout the body. Semen would be composed of particles coming from all over the body (*per membra atque artus decedit corpore toto*). Lucretius attributed to a system of vessels, especially in the spinal marrow, the transport of semen up to the genital organs, before the ejaculation. Likewise, he assumed that the physical changes of adolescence around the 14<sup>th</sup> years of life are responsible for initiating the production of semen.

Lucretius also wrote about the presence of seeds in both males and females for explaining the reproduction, as follows: both males and females produce fluids that are strong determinants for the procreation. They contain seeds that together can produce a child. Male and

female seeds have also generative power of the child characteristics. If the female's seed is more powerful, the child resembles mother's characteristics. On the other hand, if male's seed is dominant, the child resembles father's characteristics. When male and female seeds have no dominance, the child resembles both partners. This is surprising, since it provided genetic concepts of the reproduction, of which Lucretius had no knowledge. In his poem, Lucretius also comments about infertility: "Infertility is a fail of partners for matching your seeds." Unintended, he assumed the existence of both male and female gametes, in the same way that infertility can be accused by both genders.

These assumptions about conception, semen, seeds, and infertility are part of many ancient texts, mainly from practitioners from Greece and Rome, such as Democritus, Alcmaeon, Hippon, Empedocles, Hippocrates, Aristotle, Soranus, and Galen,<sup>[13-17]</sup> as well as Avicenna, a Persian physician.<sup>[18]</sup> In addition, there are many evidences coming from most ancient peoples such as Sumerians, Akkadians, and Egyptians, showing theoretical models about conception based on the contact of male and female seeds, including the contribution through the semen.<sup>[19,20]</sup> Although hypothetical and unproven, these ancient concepts remained influential for long, going throughout centuries up to the Middle Ages, because both sperm and egg have only been discovered in 17<sup>th</sup> and 19<sup>th</sup> centuries, respectively. Irrespectively the time they lived, they thought in the same way of the seed's theory for explaining the conception, although they had no knowledge about embryology and genetic and microscopic structures.

## THE DISCOVERIES OF THE SPERM, EGG, AND OF THE MECHANISMS OF FECUNDATION

Long afterward, painstaking researches in optics took in extending viewing of images beyond the reach of sight, mainly at the end of the 16<sup>th</sup> century. The availability of lenses with more powerful optical properties opened up the way for Hans and Zacharias Jansen build what is regarded as the earliest prototype of a compound microscope, most likely in 1591.<sup>[21]</sup> Thereafter, one can amplify biological images, although limited to no more than 10 folds to the original size.

In the mid-17<sup>th</sup> century, Antony van Leeuwenhoek improved the rudimentary microscopes hitherto existing and built a "potent" microscope, able to magnify up to 300 times. Readily, he was able to assess a wide range of biological structures, discovering a new microscopic world, previously unapproachable to the human eye. In spite of having incessantly studied both living and dead matter, Leeuwenhoek credited to Johan Ham, a student

from the Medical School of Leyden, the discovery of the spermatozoa. Ham noted male gamete in the semen of a man who was suffering from gonorrhoea. He called them animalcules spermatiques. In a letter addressed to the Royal Society of London in November 1677 (*de Natis è semine genitali Animalculis*), Leeuwenhoek reported the microscopic findings of Ham, which was published in the *Philosophical Transactions*, the journal of the society.<sup>[22]</sup> He depicted the seminal liquefaction, the prevalence of live animalcules that not remained alive after 24 h, motility patterns, and both head and tail characteristics. Despite being restricted and with no clinical purpose, this was surely the first attempt for semen analysis. Afterward, Leeuwenhoek devoted exhaustive efforts to investigate biological and morphological properties of spermatozoa in dogs, swines, rabbits, fishes, mollusks, amphibian, and birds, which have also been depicted in letters sent to the Royal Society of London.<sup>[23]</sup>

The discovery of spermatozoa raised an issue: what would be its biological function? Promptly, one thought that the spermatozoa would be seminal parasites. However, since the ancient times, it was well known that the man plays a pivotal role in reproduction and that a pregnancy takes place by coupling between man and woman. It was thought that the formation of the embryo relied on the combination of menstrual blood and semen, according to the Aristotelian theory, or through the combination of both female and male semen, according to the Galenic theory. However, shortly before the discovery of the spermatozoa, William Harvey has published in 1651 the book titled, *Exercitationes de Generatione Animalium*. In his book, translated from Latin into English by Willis,<sup>[24]</sup> he reported theories about spontaneous generation, embryogenesis, and conception. On one of his doctrines, he asserted: *Ex Ovo Omnia*, namely, all life came from an egg, opposing the theory of spontaneous generation, which has depicted that the living beings emerged from nonliving matter. In his book, Harvey provides consistent information about conception and embryo development, which opposed against the Aristotle's and Galen's theories. One should praise the theories reported by Harvey because he did not know the sperm and the egg, which were discovered later. In fact, Harvey provided a strong basis for the development of the modern embryology, although his theories were only acknowledged to long after.

After the discovery of the sperm, it was believed that the sperm head could keep a miniature of a preformed embryo inside (*homunculus*) that would grow after starting pregnancy (*Preformation Theory*). Supporters of the *homunculus* theory, such as van Leeuwenhoek,

Nicolas Hartsoeker, and Wilhelm Gottfried von Leibniz, formed what became known as *spermists*. On the other hand, disagreeing to the *spermism*, Jan Swammerdam, Lazzaro Spallanzani, Albrecht von Haller, Marcello Malpighi, and others claimed the *preformed embryo* inside the ovum (*ovists*). *Preformation theory* was advocated by *spermists* versus *ovists* from the late 17<sup>th</sup> century until the late 18<sup>th</sup> century, as a sole means for explaining the fecundation. There were still those who believed that the spermatozoa would be merely seminal parasites.

A study published by Caspar Friedrich Wolff in 1759<sup>[25]</sup> was the first step to overthrow the *Preformation theory*. He stated that at the beginning of development, the germ is nothing else than an unorganized material formed from the sexual organ of each parent, which gradually becomes organized following fertilization. This statement became known as *Epigenesis Theory*. First, this theory was hotly contested, but it consolidated itself over time, chiefly after the discovery of the ovum by Karl Ernst von Baer<sup>[26]</sup> It was also a remarkable discovery of Matthias Jacob Schleiden<sup>[27]</sup> and Theodor Schwann<sup>[28]</sup> that both vegetal and animal tissues are composed of cells and they are the morphological and functional units of all living beings (*cell theory*). Afterward, Remak, Virchow, and Kolliker<sup>[29]</sup> ascertained that all cells come from preexisting cells by cell division. Collectively, these findings provided a new way for understanding about embryonic development. Hence, *Preformation theory* was dropped entirely. Notwithstanding, it is also part of the history of the reproductive medicine.

It is worth to emphasize that the quarrel between *ovists* and *spermists* plus the assumption that spermatozoa could be seminal parasites averted the focus of researches on the sperm function in semen and its role in forming the embryo over decades, after the discovery of the male gamete. Likewise, the discovery of the ovum about 150 years later also hampered the sequence of the researches. Therefore, knowledge about reproductive biology has remained broadly unchanged throughout the 1700s. Accordingly, no one became interested in developing technologies for semen analysis, even for a simple sperm count.

Some issues remain unanswered about the discovery of the spermatozoa, which is historically credited to van Leeuwenhoek. First, there is a letter addressed by Christiaan Huygens to the *Journal des Sçavans* (Paris) and published in 1678<sup>[30]</sup> that reports the discovery of small animals in semen, but with no mention who made this discovery. Second, 15 days after the publication of the letter of Christiaan Huygens, Nicholas Hartsoeker



also reported in the same journal that he had observed the presence of tadpole-like animals in rooster semen.<sup>[31]</sup> Coincidentally, both were published in the same time of the letter of Leeuwenhoek. It is most likely that Huygens, as well as Hartsoeker, had only reported in these letters, the previous observations of Hamm and Leeuwenhoek, since they did not provide more information about it, unlike the detailed data reported by Leeuwenhoek, in his letter published in the *Philosophical Transactions*. Hence, no one knows sure who saw the sperm in the semen for the first time. Nevertheless, historical facts suggest that Leeuwenhoek has been indeed the first to report the discovery, despite having strongly credited to Ham, according to the one published in his letter addressed to the Royal Society of London. In addition, there is also a concrete information that Ham communicated his observations, especially to Fridericus Schrader at the Leyden University, on the presence of spermatozoa in rooster semen, in healthy men and in men who suffered from “virulent” gonorrhoea.<sup>[32,33]</sup> Seemingly, these observations were made at the time of the first contact between Ham and Leeuwenhoek.<sup>[33]</sup> Unfortunately, it seems that Ham has never published his discovery, although it was remarkable.

After the discovery of the spermatozoa, Leeuwenhoek and others believed that the sperm contained a preformed embryo (homunculus). They advocated that the homunculus initiates embryonic development after intercourse, although they did not know how this occurred. In the late 1700s, Lazzaro Spallanzani provided the first concrete evidence for a role of the sperm in the fecundation. Spallanzani covered the sex organ of male frogs with a strip of taffeta fabric for filtering the semen and recovered two fractions; the first, a fraction containing spermatozoa, which was retained by the filter; and the second, a filtered fraction devoid of spermatozoa.<sup>[34]</sup> He noticed that unfiltered residue if promptly added to water containing female frog eggs develops a new animal. He concluded that the contact between semen and the egg had a pivotal role in the reproductive process. In spite of the results of this classic experiment, Spallanzani was a strong supporter of the view that spermatozoa were seminal parasites, which he called *vermicelli spermatici* (spermatic worms). He believed that a “vapor” of the seminal fluid, and not the sperm, would trigger the preformed embryo growth into the egg (Spallanzani was an ovist). It is likely that Spallanzani has also carried out the first artificial insemination in dogs, using the nonfiltered semen fraction as mentioned above.<sup>[35]</sup> Although he incisively refused any role for sperm in fecundation, unknowingly, he established a definite role of the semen and of the

spermatozoa in the reproductive process, which is acknowledged as a historical landmark.

In 1824, Jean-Louis Prévost and Jean-Baptiste-André Dumas provided the most factual proof, of which spermatozoa were necessary for the fertilization in sexual reproduction.<sup>[36]</sup> In a wide series of investigations examining the semen of different kinds of animals, they found that: (1) Besides the semen, motile sperms are also found in testicular tissue fluids of sexually mature males in vertebrates and invertebrates. Thus, they provide a definite proof that spermatozoa were not seminal parasites; (2) Spermatozoa are not found in very young male that has not reached sexual maturity; (3) Placing a batch of frog eggs into distilled water, they observed that the eggs grew up, but they decomposed later on. On the other hand, on placing frog eggs in distilled water plus testicular fluid extracts, the eggs underwent sequential changes (early stages of development, including initial egg cleavage). This was the first report on early embryonic development; (4) Prevost and Dumas also investigated whether “vapor” from warmed semen in contact with gelatinous eggs might actually be responsible for fecundation, as proposed by Spallanzani. They observed that the fecundation did not occur. Nevertheless, when they placed the nonwarmed semen in contact with eggs, embryonic development occurred normally; (5) Finally, Prevost and Dumas repeated Spallanzani’s experiments: they filtered frog semen for removing spermatozoa. The filtrate became sterile. On diluting the sperm fraction that retained in the filter with distilled water, fertilizing capacity was recovered; (6) In addition, Prevost and Dumas also observed that eggs of mammals were probably fertilized in the oviduct.

With these experiments, Prevost and Dumas finally proved that spermatozoa were essential for the fecundation. They overthrew the Preformation theory and ruled out any likelihood of the sperm to be seminal parasites. Nevertheless, it is noteworthy that Prevost and Dumas solely have theorized about the penetration of sperm into the egg. The definite proof of this event was only reported in a letter sent by Martin Barry to the Royal Society of London.<sup>[37]</sup> Just before, Karl Ernst von Baer published “*De Ovi Mammalian et Homini Genesi*” identifying mammalian eggs, including human.<sup>[26]</sup> Soon after, the basic mechanisms of gametogenesis and of the fertilization process have been fully understood in many studies reported prior to 1900.<sup>[38-46]</sup> It is worth emphasizing that these factual proofs have only been achieved from 150 to 200 years after the discovery of spermatozoa by Ham and Leeuwenhoek.

## THE FIRST ATTEMPTS FOR SEMEN ANALYSIS BEFORE THE 20<sup>TH</sup> CENTURY

Once the basic mechanisms about fecundation were increasingly well-known from the second half of the 19<sup>th</sup> century, the male share in a childless marriage became unequivocal. Accordingly, the investigation of male partner became pivotal and the semen analysis was necessary. However, during the second half of the 19<sup>th</sup> century, there was no lab test for evaluating semen parameters, even for a simple sperm count. While some fertility disorders in women were well-known, the reproductive inability of men was just entailed in the investigation of their performance at the coitus, namely, focusing in investigating anatomical defects that could lead to abnormal deposition of the semen in the vagina.

Mantegazza was the first one to correlate semen characteristics with male fertility.<sup>[47]</sup> However, he solely gave emphasis to the analysis of semen volume as an indicator of fertility status of men. He found values ranging from 0.85 to 6.0 mL. In addition, Mantegazza also investigated the effect of temperature on the sperm motility and noted that exposure of the semen at temperatures ranging from 37°C to 47°C caused a progressive negative impact on the sperm motility. This was the first investigation reported on the effect of temperature on the sperm motility.

James Marion Sims gave a major contribution in this sense.<sup>[48]</sup> Sims introduced the analysis of the progressively motile sperm in the cervical mucus after the coitus, a lab test termed postcoital test. The examination was scheduled at the ovulation because mucus was plentiful facilitating the sperm penetration and its evaluation. He observed that if motile sperms were detected in cervical mucus, the man was not barren, as well as the cervix was not a cause of female sterility. According to Sims, the postcoital test had these purposes: (1) it must be sure that we have semen with spermatozoa; (2) it must ascertain if spermatozoa enter the uterocervical canal; (3) it must determine whether the secretions of this canal are favorable or not to the vitality of the spermatozoa. Postcoital test has been used for investigating barren couples for more than 100 years. Although too many contested today, it is still used in some instances.

Later, Alois Lode made the first attempt for counting spermatozoa.<sup>[49]</sup> He diluted the semen in a solution 2 per thousand of potassium hydroxide and the spermatozoa were counted in a Thoma-Zeiss chamber. He surveyed semen specimens of a dog for 1 month and found sperm concentrations ranging from 0 to  $176 \times 10^6/\text{mL}$  and 0–101  $\times 10^6/\text{ejaculate}$ . Lode also assessed semen volume, which ranged from 0.25 to

6.0 mL. It is noteworthy that he expressed both semen volume and sperm count in  $\text{mm}^3$ . In addition, Lode has also assessed semen specimens from three men and found sperm concentrations varying from 0 to  $135 \times 10^6/\text{mL}$  (results were also reported in  $\text{mm}^3$ ) and from 0 to  $551 \times 10^6/\text{ejaculate}$ . Based on these findings, Lode stated that dog semen had an average concentration of 55,778,000/ejaculate, whilst the human semen had an average concentration of 226,257,000 sperm/ejaculate. The latter is rather compliant with results usually found in routine semen analysis nowadays. It is important to stress that Lode most likely developed the first technology for sperm count. However, it was never used later.

## DEVELOPMENT OF TECHNOLOGIES FOR SEMEN ANALYSIS IN THE EARLY 20<sup>TH</sup> CENTURY

Benedict also carried out the sperm count in semen sample using a blood cell count chamber.<sup>[50]</sup> However, he was unable to achieve the expected outcomes. Benedict reported sperm count of three specimens varying from  $28.6 \times 10^6/\text{mL}$  up to  $593.8 \times 10^6/\text{mL}$  (the results were expressed in cubic millimeter) and from 286.0 million up to 2.672 million/ejaculate. Benedict concluded that the enumeration of spermatozoa has seldom been practiced. How useful either as an index of sexual or general health it is is not yet known. Nevertheless, he thought that his methodology could be useful in laboratory practice.

Gustaf Retzius has also provided significant remarks about the sperm analysis. He studied a large diversity of sperm from more than 400 species, including humans, using specimens from the epididymis, seminiferous tubules, and semen, which were examined after fixation with osmium tetroxide and Zenker's fixation.<sup>[51]</sup> Illustrations of Retzius were richly depicted in drawings published in the journal *Biologische Untersuchungen* created by him for publishing his works. He started the publication in the 11<sup>th</sup> volume in 1904, extending up to the 19<sup>th</sup> volume. Although he had added too little for developing the semen analysis, the notes and illustrations of Retzius gave a huge contribution about morphological characteristics of the sperm of many species.

Once it consolidated the point of view that men as well have trouble getting pregnant their partner, unhesitatingly, physicians and investigators agreed that the investigation of the male partner was needed in the diagnostic workup of infertility. However, men were only investigated if their partner did not present sterility. Likewise, the assumption of male sterility in a barren marriage arose a new issue: What could be done to evaluate men's fertility potential? Although available, the physical examination, marital history, performance of men in the coitus, and qualitative analysis of the

sperm concentration and motility in postcoital cervical mucus were disappointing. Thus, one would expect that only through a more accurate semen analysis, it would be possible to assess the male factor in sterile couples. However, first, it was necessary to develop a technique for assessing the potency of spermatozoa and second, to establish benchmarks based on the normal variations of fertile males. Nevertheless, technological development of the semen analysis was disappointing in the first decade of the 20<sup>th</sup> century and this laboratory test was almost disregarded.

In 1902, Martin *et al.* studied azoospermic men who were suffering from obliterating epididymitis, and for the first time, he gave emphasis on the importance in investigating male sterility through the semen analysis.<sup>[52]</sup> He said: hence, should an unfruitful marriage take place, the semen should be examined before submitting the woman to treatment at the hand of gynecologist; most important of all that treatment should be continued until microscopic examination shows that spermatozoa are again present in semen. In his study, they evaluated the sperm motility after surgery and performed a comprehensive analysis of the sperm morphology using semen smears stained by iron-hematoxylin, whose characteristics were depicted in drawings. He assumed that the absence of spermatozoa from the seminal fluid is a positive proof of sterility. The presence of moving spermatozoa in these ejaculates is usually considered a positive proof of creative power, but this belief is based on insufficient evidence. It should be highlighted that Martin was among the first to show that azoospermia was caused by spermatogenic failure or ductal obstruction.

In the lack of a standardized semen analysis, the microscopic analysis of the sample of semen collected through masturbation with a condom was mostly used for investigating the frequency of spermatozoa and the sperm motility.

Hühner provided a substantial step forward for extending this investigation.<sup>[53]</sup> He revived the postcoital test of Sims,<sup>[48]</sup> extending its reach of investigation. Apart from the assessment of the sperm motility, he included the assessment of the prevalence of spermatozoa by high-power fields in cervical mucus. Hühner performed exhaustive investigations seeking for collecting all information about male sterility, especially in men with oligozoospermia, azoospermia, and asthenospermia. He compared the prevalence of spermatozoa and their vitality and motility versus semen collected with condom. He concluded that the postcoital test was a valuable tool for investigating male sterility. Postcoital test was then assumed as a valuable tool for investigating barren marriage.

In 1914, John Adolph Detlefsen assessed the fertility potential from hybrid animals (wild cavy vs. ordinary guinea pig) based on the analysis of sperm motility in specimens aspirated from the epididymis.<sup>[54]</sup> He concluded: if there were no motile sperms, the animal was certainly sterile. The probability of fertility increases as the percentage of motile sperm increases. He assumed the importance of the analysis of the sperm motility for investigating sterility.

Reynolds also carried out an extensive study about male and female fertility and sterility and stated that the careful analysis of the sperm vitality in the vagina, cervix, and in the fundus of the uterus, aside from the sperm numerical frequency and motility, has also a positive relationship with men's reproductive capacity.<sup>[55]</sup> Reynolds also provided a rapid method for the assessment of the sperm motility, outlining five sorts of sperm motion: progressive vibratile, undulatory tactile, stationary bunting, rotatory swimming, and pendulum swimming. Reynolds assumed that the first three were successive normal phases of spermatozoic activity and the last two were abnormal. Seemingly, this was the first attempt for classifying patterns of sperm motility. Reynolds gave particular emphasis to the assessment of the sperm vitality based on the length (endurance) of the sperm motility; first, by the notation of the time in which motility persists in fresh semen; second, by the notation of the time in which motility persists in the natural medium (secretions of the female genital tract); and third, in various artificial media. He said that comparison of these results has seemed to give an accurate establishment of the vitality of semen, but the process involves a great amount of labor and is too cumbersome for general use. In fact, the procedure was really unfeasible to be used in the routine of the semen analysis.

In a study published in 1915, Zeleny and Faust reported a new methodology for the analysis of the sperm morphology based on the biometrical measurement of the sperm head lengths in high-magnification fields.<sup>[56]</sup> They examined semen from 15 species of animals, and the frequency distributions were plotted. The resultant curves were found to be distinctly two modal in 14 of the 15 species. In addition, they associated sperm size dimorphism with chromatin material, determining that the prevalence of either model could determine the gender of the fetus.

Long afterward, Williams also investigated sterility in bulls using the biometrical analysis of the sperm head in semen smears stained with carbol-fuchsin and methylene blue.<sup>[57]</sup> He concluded that sterility in bulls had a strong relationship with abnormal sperm morphology and head



length variations. On the other hand, Williams did not observe dimorphism in the head length as reported by Zeleny and Faust.<sup>[56]</sup>

Moench and Holt have also applied the biometrical analysis of the sperm head to investigate male sterility.<sup>[58]</sup> They determined a coefficient of variation (CV) based on the frequency distribution of the head length and found that CV in fertile men will seldom exceed 11. Values from 11.5 up to 12.5 were indicative of impaired fertility, whereas if CV exceeds 12.5, men might be considered sterile. Moench and Holt have also not observed the sperm dimorphism reported by Zeleny and Faust.<sup>[56]</sup>

In spite of having used during the 1920s and in the early 1930s, the biometrical analysis of spermatozoa has been progressively forgotten, because subsequently, there was a greater emphasis for investigating abnormalities in sperm head, midpiece, and tail. In addition, biometrical analysis of the sperm head was time consuming for the routine purpose. Likewise, some experts had also criticized this analysis<sup>[59,60]</sup> since it did not measure the head length, with no evaluation of the head width. Therefore, it did not measure the total volume of the sperm head, what it could define a closed relationship with sterility. Interestingly, the biometrical analysis of spermatozoa (now referred as morphometric analysis) has today a central role for the analysis of the sperm morphology using computer systems.<sup>[61,62]</sup>

Ultimately, from the earliest observations of Sims<sup>[48]</sup> up to 1915, the clinical interest in the semen analysis has been just for assessing the qualitative prevalence of sperm in the cervical mucus and its motility. Cary was the first one in reporting a standardization for semen analysis, which became a historic milestone.<sup>[63]</sup> In his article published in the American Journal of Obstetric Diseases in Women and Child, Cary reported a richly illustrated text, in which he depicted his experience in assessing semen specimens. The main highlights of his study are summarized as follows:

- A man is most reluctant to share any suspicion of responsibility for failure in barren marriage because the almost universal assumption is that, in the event of a childless marriage, the wife is wholly responsible. Thus, the examination and study of semen have been much neglected, although male responsibility has been estimated from 15% to 25%. In fact, the reluctance of men in assuming failure in barren marriages still remains today
- The specimens should be secure after 3 or 4 days of sexual rest. The patient provides himself the semen at home during intercourse using a condom. After the intercourse, the condom containing the semen is placed in a wide-mounted bottle that should

then be placed in a jar which contains water a few degrees warmer than the body temperature. The jar is immediately taken to the office of the physician. If this method is refused by the husband, the semen may be secured from the genital tract of the wife or using a condom, which is removed in the office. Both affect the condition of the specimens. Interestingly, there was a concern in maintaining the sample warmed, disregarding the harmful effect of the condom, as well as the contact with vaginal secretion, which is also harmful to the sperm. However, it should be taken into account the pioneering of the study, which greatly aided the evolution of the semen analysis in the following decades

- Upon delivery at the office, the bottle is removed of the jar and the semen is placed into a dry bottle or warm test tube. The following should be assessed: semen volume, reaction, the amount of sediment, gross appearance, temperature, and the time elapsed since coitus
- A more detailed examination must proceed if semen is defective. The sediment should be covered with a thinnest cover glass and must be examined with an oil immersion lens. The following must be assessed: spermatic crystals (occur exclusively in the prostatic secretion), azoospermia, immature germ cells (Cary illustrated the text with drawings, including the phases of sperm maturation on the spermiogenesis), and sperm morphology
- Cary showed a comprehensive portrayal of the sperm morphological characteristics, giving particular emphasis on some sperm abnormalities that were not previously reported, as follows: 1 – microcephalic (small head), a reduction in the size of the head; 2 – double-headed; 3 – crescent and irregular shapes; and 4 – multi-tailed cells. He also said that in some instances, the head is barely perceptible, appearing as simply a clubbed end of the tail (probably, he referred to pin-headed anomaly); tail joined to the head; sharp angles near the cephalic end; rudimentary or absent (Cary has assessed a specimen with many sperms bearing this anomaly, probably the tail-stump defect)
- While it is known that the testes furnish the fecundating elements of the semen, it is likewise important that we should recognize the complementary action of the seminal fluid. In addition to furnishing a vehicle for the spermatozoa, it contains properties that are essential to their vitality. Despite having not enough knowledge about the physical–chemical properties of the seminal plasma, Cary assumed its importance as a supporting environment for the spermatozoa after the ejaculation

and the protective effect after the deposition into the vagina

- Although Cary has used fresh semen in his study, he also hinted that sperm morphology analysis and the investigation of nonsperm cells (leukocytes, squamous epithelial cells, oval concretions [probably residual bodies], and red blood corpuscles) and another element in semen (lipoids and amyloid bodies and spermatid crystals) could also be made in semen smears stained. He said that chromatic dyes such as methylene blue, fuchsin, and gentian violet are best. When a slight preparation is desired, the specimen may be stained by hematoxylin and counterstained with eosin.

The current study gave a particular emphasis on Cary's study because it was the first in reporting a standardization for the semen analysis when this examination was not part of laboratory practice. Some hints reported by Cary a century ago are still part of the current semen analysis routine. Indeed, it is a milestone in the history of this examination.

While on the one hand, the article published by Cary in 1916 opened new pathways for the semen analysis to be assumed as a valuable tool for investigating men in barren marriage; on the other hand, it was also a target of criticism after its publication. In an article published in 1921, Hühner<sup>[64]</sup> made several criticisms against the standardization reported by Cary. He criticized the system of taking and transportation of semen specimens from home to the office and the recommendations in maintaining a stable temperature in all stages of the laboratory evaluation. He claimed that the tricky system of semen collection and the laboratory procedures discouraged physicians for the ultimate spreading of the semen analysis in offices (thereat, semen analysis was made in doctor's office), thereby restricting the examination of just few experts. Hühner said that this is wicked. Hühner has also commented about the presence of spermatid crystals in semen (Böttcher's crystals). He said that in semen containing many moving spermatozoa, these crystals, therefore, either do not form at all or do so slowly, whereas in semen in which there are no spermatozoa at all, or only dead spermatozoa, the crystals are formed very rapidly and are found in large numbers by the time the specimen reaches the office.

Aside from his criticism on the semen analysis, Hühner also defended the test he developed. He stressed that Hühner's test was most skillful for evaluating the sperm vitality and motility than semen analysis. To prove his claims, he made a lengthy explanation about the results of the Hühner's test and its value for the diagnosis. He advocated that semen analysis would

be necessary if only dead spermatozoa are found on the cervix. We then examined a condom specimen; if live ones, however, are found in condom, we diagnose at once that something about the genital secretions of the female has killed the spermatozoa. Based on this assumption, he concluded that I may perhaps be pardoned for enthusiasm concerning the test, but I really know of no other from which so much valuable information can be gained in so short a time. Finally, Hühner also criticized some physicians who advised the patients while in the doctor's office to masturbate for obtaining a specimen. He said that I have never made use of this method and consider it filthy and unjustifiable.

Williams was one of the pioneers in reporting the sperm morphology analysis in semen smears, which were stained with carbol-fuchsin and Loeffler's methylene blue.<sup>[65]</sup> Analyzing the semen of bulls, he classified the spermatozoon with normal appearance having four basic structures: head, neck, body, and tail. Williams also reported the characteristics of the nucleus, acrosome (which he called of cytoplasmic), midpiece, and the tail. On the other hand, he assumed that the motility of spermatozoa does not constitute a standard in the measurement of its vitality and powers of fecundation. Later, Williams and Savage published other observations on the assessment of morphological characteristics of spermatozoa, including the biometrical analysis, for the investigation of the fertility potential of bulls.<sup>[57,66]</sup>

In several experiments performed in human semen, Moench also depicted a wide picture of abnormal sperm morphology and attempted to establish a reference value for evaluating the capacity of fertilization of men.<sup>[67-69]</sup> According to Moench, semen with morphologically abnormal spermatozoa up to 20% would be normal. Semen between 20% and 23% would have decreased fertility and those with value >25% were compatible with sterility.

Nowadays, the cutoff points for sperm morphology have changed considerably, since it also takes into account other sperm characteristics such as the acrosomal region comprising 40%–70% of the head area, the length and width of the sperm head, and the presence of vacuoles, in addition to the morphological features of the neck, midpiece, and tail.<sup>[10]</sup> Hence, abnormal sperm morphology can affect up to 96% of spermatozoa, establishing a cutoff point of 4%. Although this cutoff point is smaller than those described by Moench, the sperm morphological characteristics and the association between abnormal sperm morphology and sterility as reported by Williams and Savage for bulls<sup>[57,66]</sup> and Moench and Holt for humans<sup>[67-69]</sup> were pivotal for



assessing the sperm morphology in the semen analysis routine after 1930.

A study published by Macomber and Sanders has been the starting point of the semen analysis that should be definitively introduced in clinical and laboratory practice.<sup>[70]</sup> They assessed the sperm concentration per cubic centimeter in semen samples using a blood-counting chamber. Earlier, this semen parameter was assessed by visual analysis of spermatozoa by high-power fields, in spite of the earlier efforts of Lode<sup>[49]</sup> and Benedict.<sup>[50]</sup>

Macomber and Sanders studied 294 men and established a relationship between pregnancy rates and sperm count higher than  $60 \times 10^6/\text{cc}$ . This was the first reference for sperm count in the routine of the semen analysis, which remained unchallenged for at least three decades. Since pregnancy could also be achieved by men with sperm count lower than  $60 \times 10^6/\text{cc}$  (Macomber and Sanders reported about four cases), this cutoff point had to be further refined.<sup>[71,72]</sup> However, one can be emphasized the great meaning of the manuscript published by Macomber and Sanders for the history of the semen analysis.

One year later, Vose<sup>[73]</sup> has reported his experience of using the technique reported by Macomber and Sanders using a direct dilution of semen in bicarbonate solution, instead of a white blood cell pipette. He concluded that if dilutions are made in the manner described here, the results are more uniform than when they made blood-counting pipette. Vose has found sperm counts in fertile men ranging from 75,000,000 to 200,000,000 spermatozoa per cc of semen. He said that it is probable that most men who are highly fertile have counts well over 100,000,000.

## STANDARDIZATION OF SEMEN ANALYSIS ROUTINE FOR LABORATORY PRACTICE

Once the basic parameters of the semen analysis have been exhaustively studied by careful and deliberate efforts of the physicians aforementioned, over the early decades of the 20<sup>th</sup> century, the focus for the first half of the 1930s was for establishing a routine for semen analysis that could be available in the office, for investigating men of childless marriage.

In this sense, Belding published a study in the American Journal of Obstetrics and Gynecology, in which he made many of the considerations on the challenges of implementing a semen analysis routine,<sup>[74]</sup> despite semen parameters such as concentration, vitality, motility, and morphology had indefeasibly consolidated their position for assessing infertile men. Belding reported the chief difficulties of the adaptation of a suitable technique and the establishment of satisfactory criteria for evaluating

the potency of human spermatozoa. In this regard, he said that it is necessary just to develop a practical technique for testing human spermatozoa, and second to establish standards based on normal variations of the fertile male. He also said that the several methods which have been suggested by various investigators possess not only limitations in technique, but also lack sufficient reliable data to establish their relative value.

The focus of the Belding's reports was to compare seminal variables in specimens collected through a condom, which was a common practice in the season versus semen specimens collected by masturbation. He found that the condom was seriously damaging the sperm vitality. Therefore, he still stressed the unsuitability of the condom as a vehicle for the collection of semen. Even aware of the dilemma to establish standards based on the normal variations of fertile males, he has firmly supported the need for developing a practical technique for assessing human spermatozoa, provided that male infertility had already a factual occurrence in barren marriage. Unlike his contemporaries, he did not agree that semen specimens needed to be placed at body temperature before the examination.

Finally, the essential protocol for the semen examination was defined by Cary and Hotchkiss.<sup>[59]</sup> They proposed the semen examination in three steps:

1. Collection of the specimen - They recommended (1) a self-produced specimen at the office in a quiet and suitable room; (2) collection outside the office by external emission using glass-graduated and wide-mouthed bottle. For collection in former, wait for the liquefaction and transfer it to the bottle; (3) in condom: patient is advised to wash and dry the condom before the collection to remove foreign bodies and various ingredients in these powders. At the three rules, Cary and Hotchkiss had shown a concern in maintaining the sample temperature like the body temperature. Interestingly, they recommended a sexual intercourse based on the ordinary habits of the couple with no deadline
2. Gross or macroscopic examination - They reported the analyses as follows: (1) amount – from men under 40 years old should exceed 3.5 ml; (2) color and appearance – extended period of sexual abstinence may impart a slightly yellow tinge to the normal grayish opaqueness; (3) viscosity – absence of viscosity with a lessening of opaqueness point to reduce cell content. Interestingly, they did not comment about hyperviscosity, a common finding usually detected in semen analysis; (4) pH – they reported values from 8.1 to 8.4 that can be considered too high today

3. Microscopic examination - They recommended performing two analyses; the first using one tick drop of fresh semen permitting a gross impression of density and motility, and second, a thin drop allowing preliminary study of individual cell morphology. Cary and Hotchkiss commented about the prevalence of progressive motility: If a specimen of normal quantity and rich cellular content (spermatozoa) shows 25% or more of these dynamic cells, a rate of relative fertility must be assumed. They also said that we agree with others' observations that counts above 70,000,000/cc are found in fertile specimens. However, they also said that our experience would make us hesitate in setting a medium arbitrary count below which fertility is impossible. Despite the existing well-defined standards for semen evaluation, the hindrance in establishing reference values to define the fertility capacity of men is noticeable.

In addition, Cary and Hotchkiss reported a technique for staining of semen smears based on the fixation with Schaudinn's solution and staining of the cells with eosin 5% and hematoxylin. They considered that semen with 25% of abnormal sperm morphology would indicate more serious impairment of the male fertility.

In a book, published in 1934,<sup>[75]</sup> Samuel Raynor Meaker also emphasized the role of the semen analysis for investigating male sterility. Beyond stressing the need of critical methods for the evaluation of semen, he concluded that when accurately evaluated, a noteworthy degree of infertility may be found and indicated that a large share of the blame for a childless mating devolves upon the male.

He highlighted the following as pivotal in assessing the semen:

- The arrangement of a practical schedule for our routine sterility study makes it necessary to see a seminal specimen 2 days after the postcoital test. Before the beginning of the study, however, continence for at least 1 week is ordered
- (1) Material from the postcoital vaginal pool is the least satisfactory. (2) Withdrawal and ejaculation into a clean wide-mouthed bottle constitute a technique perfect from the viewpoint of the examiner and preferred by us. (3) The condom is the means of collection most commonly used. It is generally satisfactory. In our experience, the talcum powder and the chemicals used in the manufacturing of different types of condoms have not proved to exert any harmful effect on the spermatozoa. The specimen is brought to the laboratory for examination within 2 h of ejaculation. In the laboratory, the end of the condom is cut off, the semen is emptied into a Petri

dish, and the examination is carried out as promptly as possible.

Meaker suggested four types of investigation in semen analysis, as follows:

- Physical and chemical tests - They encompass the volume (from 3 to 6 cc), turbidity, pH (from 8.0 to 8.4), and viscosity (he recommended the use of wooden applicators and toothpicks for the assessment of the viscosity)
- Study of formed elements other than spermatozoa: It includes the investigation and detection of bacteria, leukocytes, blood, epithelial cells, crystals, and the excess of mucus, in fresh semen and/or in stained smears stained
- Evaluation of spermatozoa: This evaluation included: (1) sperm count according to the procedure reported by Vose,<sup>[73]</sup> (2) sperm morphology analysis, which he made in smears stained as outlined above (the classification of normal and abnormal morphology followed the criteria reported by William and Savage<sup>[66]</sup> and Moench<sup>[67]</sup>); and (3) sperm motility, by simple observation of sperm progression in fresh semen. He also proposed the assessment of the sperm endurance, based on the measurement of the duration of the sperm vitality after the ejaculation. The sperm endurance measured the inherent vigor of spermatozoa and its survival.

In his article, Meaker made some interesting comments on his findings. In the sperm morphology analysis, he said that in highly fertile men, the incidence of abnormal forms, as we judge them, is well below 15%; in individuals of doubtful fertility, it may be as much as 40%, and occasionally even higher. Although these references are far below those in use today, it is praiseworthy the efforts of Meaker in setting them. He also said that the fact that abnormalities of the head are more significant than those of the body or tail, and that in normal specimen of semen, the head of all the spermatozoa will be found to show the comparative uniformity of size. If the measurement of 200 or 300 head lengths discloses much variations, then morphologic imperfection is demonstrated. Regarding the sperm motility, he said that the semen of exceptionally fertile men contains few, if any, dead spermatozoa. In the average normal case, 10%–15% may be nonmotile and about the same number may be sluggish, while the majority exhibit vigorous and lively motion. Deficiencies in motility are encountered in all degrees from slight subnormality down to complete necrospemia. Meaker also commented about the patterns of sperm motion reported by Reynolds.<sup>[55]</sup> He emphasized the utmost importance of classifying the patterns of the sperm motion and for identifying what must be normal

or abnormal. He concluded that the evidence of fertility is to be drawn from the quality as much as the quantity of activity.

Interestingly, Meaker also gave particular emphasis on the analysis of the endurance of the spermatozoa. He provided a technique for this assessment based on the analysis of single drops placed upon a slide, whose edges were sealed with Vaseline to prevent drying. Likewise, he also proposed this assessment through the insertion of fresh semen into capillary tubes, the ends of which are sealed by heat. He placed tubes in an icebox, in an incubator at 37°C, and at room temperature. After the incubation, the tubes were broken and the spermatozoa examined at intervals until motility are no longer present. He reported that motility is retained longer at room temperature than either in the icebox or in the incubator. Spermatozoa which have lost their motility in the cold often regain it, the reason why they are restored to a higher temperature; those that become inactive at body temperature are, as a rule, dead. He concluded that low temperature suppresses motility, but in so doing conserves the energy of the spermatozoa; higher energy of the cells is more rapidly dissipated. Meaker has found that spermatozoa from fertile men remain active for 12–24 h at room temperature. The longest lifetime observed was 146 h in the natural medium and 21 days in a buffered glucose solution. He reported that an abnormal sperm loses their motility in 8 h or fewer. Based on his skill in assessing endurance, he concluded that we recognize that motor energy and fertilizing power are not the same things. Meaker has found that never the pregnancy occurred in man, in which the spermatozoa could not survive at least 8 h after the ejaculation. Indeed, it is now known that the spermatozoa need to have a high strength capacity and enough energy to survive the adversities they face over their migration through the female reproductive tract.

Earlier, Reynolds also sought in performing a sperm endurance test, which he called of sperm vitality assessment.<sup>[55]</sup> However, he also acknowledged that it was too cumbersome for general use. In fact, the analysis of endurance was not a suitable essay to be included in semen analysis routine, because it was time consuming and of scant clinical value. It was not even recalled in studies afterward, except for Meaker.

In another study published by Hotchkiss,<sup>[76]</sup> he reported a definite standardization for the semen analysis to be used in laboratory practice, although there were still many unsolved issues, mainly about the reference values to be used in the examination. The main highlights of his standardization were as follows:

- Sample collection - Hotchkiss proposed a sexual intercourse of 3 days, but without giving a convincing

explanation about. Previously, he has considered this recommendation to be irrelevant<sup>[59]</sup>

- Semen specimens should be left at room temperature from 1 to 2 h. Although he has not featured a convincing reason, probably, he waited the seminal liquefaction, before running the proposed tests. He said that spermatozoa are able to exhibit motility, longer in low temperature than at body heat
- Hotchkiss recommended the semen collection in a wide-mouthed glass container. He pointedly refused the semen collection in condoms, claiming that the ingredients of the average condom are hostile to the survival of spermatozoa
- He also standardized the physical analysis of ejaculates. He suggested the analysis of volume using a standard reference from 3.0 to 4.0 mL, appearance, viscosity, and pH (standard reference from 7.7 to 8.5)
- For the analysis of the sperm motility, he said that it is extremely difficult to give a word picture of the description of the motility of spermatozoa. The type of activity, the number of activity, the number of crossing a microscope field, and the percentage of immature cells are all details of intent. How it can be noticed, Hotchkiss provides a sketch for investigating motility patterns and number of mobile sperm, although he did not report a methodology for this purpose
- He also recommended the assessment of the sperm vitality. However, he did not also provide a methodology for the analysis
- With no mention to Macomber and Sanders,<sup>[70]</sup> he urged in assessing the sperm count/mL and in the total ejaculate using a sodium bicarbonate-phenol solution and a blood-counting chamber. He said that an average fertile male will produce from 100,000,000 to 150,000,000 per cc as from 400,000,000 to 500,000,000 in the total ejaculate. Although they are reference values that can currently to be regarded as high, it should be acknowledged the efforts of Hotchkiss for standardizing the semen analysis, including reference values, even facing the difficulties of those times. Interestingly, he said that I believe that the more reliable and consistent cell counts have been on the basis of the cells present in the total ejaculate rather than the units by cubic centimeter. This is worthy of note, once it is also a current point of view of some experts in semen analysis<sup>[77]</sup> and of the last WHO manual for semen analysis,<sup>[10]</sup> although the sperm count per cubic millimeter is still the longest used reference standard. In this regard, Hotchkiss still said that the bulk of the semen undoubtedly originates in the prostate and the seminal vesicles, and variations in the amount of



these secretions will accordingly dilute or concentrate the sperm. In the former instances, an apparent deficiency may be inferred if the cell count is expressed in cubic centimeter, whereas the number of sperm in the total ejaculate may have to be normal. Undoubtedly, this is a very current comment

- Hotchkiss also commented on the reference value <20% of abnormal forms provided by Moench for the sperm morphology analysis.<sup>[69]</sup> Although he did not disagree, he argued: In an incomplete but rather large groups of cases of proved fertility now under study, I have yet to find an instance of normal pregnancy attributable to a seminal specimen with excessively a large number of abnormal sperm, yet this condition is not infrequently encountered in cases of disturbed fertility. In fact, it seems that he believed that a pregnancy might be achieved naturally, even for men who had many abnormal spermatozoa. He concluded that the test of times leaves the future to determine the actual value of this important theory, as information compiled to substitute or refute the current principles of sperm morphology. Moench assumed that more than 80% of normal sperm morphology is necessary for a man to achieve one pregnancy. In fact, this value is too high to the one currently recommended by the WHO manual for semen analysis.<sup>[10]</sup>

In that same study, Hotchkiss reported four cases he assisted at the New York Hospital of the Cornell University Medical College, from men who suffered from barren marriage. He commented on the diagnosis and treatment, focusing on the results of the semen analysis. Based on his experience in attending the patients, he concluded that the semen constitutes the chief index of male fertility. The proper evaluation of its fertilizing power is dependent on the complete analysis of the various factors and constituents of the specimen. He also commented that it is now generally agreed that the husband bears the chief or partial responsibility in approximately one-fourth of the involuntary barren marriage and accordingly his examination is now regarded to be as important as that of his wife. These remarks definitely strengthened the view that semen analysis was pivotal for investigating male fertility potential.

Seemingly, this is the first mention about the investigation and treatment of male sterility mainly based on the results of the semen analysis. However, it is important to highlight that Samuel Meaker, Charles Lawrence, Allan Rowe, and Samuel Vose composed an organized staff in the late 1920s, for handling barren marriage. The book published by Meaker<sup>[75]</sup> is a collection of the skill of those experts in handling sterility couples. However, in the

section of Urologic Treatment, they reported that treatment of sterility involves the correction, as far as possible, of all causative genital abnormalities in the male partner, such as testicular hypoplasia and injuries, varicocele, epididymal blockade, chronic prostatovesiculitis, and faults of delivery and reception of semen in the female reproductive tract with special emphasis on the clinical and surgical treatment of the disorders. There are little information about the use of the semen analysis in his handling. Therefore, to the best of knowledge of this author, Hotchkiss<sup>[76]</sup> was the first to report the treatment of male sterility based on the results of the semen analysis.

Ultimately, semen analysis was definitely introduced in laboratory practice in the mid-1930s, thousands of years after the earliest knowledge on human reproduction, about 250 years after the discovery of the spermatozoa, and about a century of the discovery of the ovum. In fact, the development of the semen analysis routine was fully dependent on the evolution of knowledge on reproduction over time.

## CONCLUDING REMARKS

As soon as it has been assumed the importance of the basic parameters of the semen analysis (concentration, motility, and morphology), a more detailed analysis of seminal samples of evaluating men's reproductive capacity became part of the routine investigation of sterility, chiefly in the early 1930s. Although considerable efforts have been made in the 1920s to evaluate spermatozoa in both animals and men, it was difficult for using suitable techniques for routine semen analysis, owed to the inability in establishing reliable reference values in both fertile and infertile men. Only after the standardizations reported on the early 1930s,<sup>[59,74-76]</sup> semen analysis could be introduced at long last in laboratory practice. Looking over time, we noticed that the role of semen analysis in investigating male sterility (now termed infertility) remains still controversial, regardless of the methodologies currently available. In fact, nowadays, there is a consensus that semen analysis is the gold standard for evaluating the sperm production and quality. However, it is a poor predictor of men's reproductive capacity, a disability that has not been overcome along more than 80 years of laboratory practice.

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