Characteristics of the seminal fluid quality of infertile patients in the City of Quito

Guamán-Gualpa William
https://orcid.org/0000-0002-5972-7832
Faculty of Medical Sciences, Central University of Ecuador, Quito, Ecuador

Arias-Bustamante José
https://orcid.org/0009-0006-9307-2362
Quito Institute of Infertility, Quito, Ecuador

Villacis-Vaca Diego
https://orcid.org/0009-0007-4219-9723
Quito Institute of Infertility, Quito, Ecuador

Vasquez-Maya Carlos
https://orcid.org/0009-0004-8348-5548
Quito Institute of Infertility, Quito, Ecuador

Cuesta-Garzón Daniela
https://orcid.org/0009-0003-2729-8935
Quito Institute of Infertility, Quito, Ecuador

Luna-Prieto Maiella
https://orcid.org/0009-0002-4218-3493
Quito Institute of Infertility, Quito, Ecuador

Correspondencia:
William Guamán
wmguaman@uce.edu.ec

Recibido: 02 de febrero 2023
Aceptado: 21 de marzo 2023

Abstract

Introduction: Male infertility has a complex etiology and one of the determining factors is the quality of the semen. The evaluation of this factor is carried out using a spermogram; where the two components of semen are analyzed: the sperm; in number, mobility and morphology, as well as the seminal fluid produced by the accessory glands in charge of nourishing the sperm and interacting with the female genital tract.
Objective: To determine the characteristics of the semen, the most frequent alterations and the age effect on the seminal quality of patients who come to an infertility consultation in an assisted reproduction center in the city of Quito.
Material and Methods: An observational, descriptive, cross-sectional study of 55 spermograms from patients with infertility problems from the “Instituto Quiteño de Infertilidad” was carried out.
Results: 47.27% of the spermogram presented normozoospermia. Within the alterations, asthenozoospermia was 25.45% and teratozoospermia was 23.63%. The values of the sperm quality parameters and the number of alterations showed an age-related trend; we found that the highest number of affected patients with alterations in the number of sperm corresponds to those over 40 years.
Discussion: In our study, we have evidenced a high percentage of patients with at least one seminal alteration, especially in older patients, which indicates the importance of considering the age of males when evaluating infertile couples.

Keywords: male infertility, Semen Analysis, spermatozoa, andrology

Características de la calidad seminal de pacientes infértiles de la Ciudad de Quito

Resumen:

Introducción: La infertilidad masculina tiene una etiología compleja y uno de los factores determinantes es la calidad seminal, la evaluación de este factor se realiza mediante un espermograma; donde se analizan los dos componentes del semen: los espermatozoides; en número, movilidad y morfología, además del fluido seminal producido por las glándulas accesorias encargado de nutrir a los espermatozoides e interactuar con el aparato genital femenino.
Objetivo: Determinar las características del semen, las alteraciones más frecuentes y el efecto de la edad sobre la calidad seminal de los pacientes que acuden a consulta de infertilidad en un centro de reproducción asistida de la ciudad de Quito.
Material y Métodos: Se realizó un estudio observacional, descriptivo, transversal de 55 espermogramas de pacientes con problemas de infertilidad del "Instituto Quiteño de Infertilidad".
Resultados: El 47,27% de los espermogramas presentaron normozoospermia. Dentro de las alteraciones, la astenozoospermia fue del 25,45% y la teratozoospermia del 23,63%. Los valores de los parámetros de calidad espermática y el número de alteraciones mostraron una tendencia relacionada con la edad; encontramos que el mayor número de pacientes afectados por alteraciones espermáticas corresponde a los mayores de 40 años.
Discusión: En el presente estudio se evidenció un alto porcentaje de pacientes con al menos una alteración seminal, especialmente en pacientes de edad avanzada, lo que indica la importancia de considerar la edad del varón al evaluar parejas infértiles.

Palabras clave: infertilidad masculina, análisis de semen, espermatozoides, andrología

Cómo citar este artículo: Guamán W, Arias J, Villacis D, Vasquez C, Cuesta D, Luna M. Characteristics of the seminal fluid quality of infertile patients in the City of Quito. Rev Fac Cien Med [Internet]. 2023; 48(2): 26-35. Disponible en: https://doi.org/10.29166/rfcmq.v48i2.4377

Introduction

Infertility is a disease characterized by the inability of a sexually active couple, without the use of contraceptive methods, to achieve a pregnancy in 12 months, depending primarily on the age of the woman, or due to the deterioration of the ability to reproduce individually or with their partner. It is estimated that worldwide 8 to 15% of couples of reproductive ages suffer from infertility. The male factor alone is responsible for 20-30% of cases[1].

Male infertility has a complex etiology and one of the determining factors is the quality of the semen[2], its evaluation is carried out using a spermogram; where the two components of semen are studied: the sperm; in number, mobility, and morphology, as well as the seminal fluid produced by the accessory glands in charge of nourishing the sperm and interacting with the female tract [3]. In 2010, the World Health Organization (WHO) published new guidelines for the study of semen, to standardize the methods of analysis and establish the lower reference limits (LRI) of the seminal variables. Table 1 shows the newly established reference values [4].

The present work was performed based on the WHO Manual for the examination and processing of human semen fifth edition of 2010[5]. At the moment we have the WHO Manual for the examination and processing of human semen sixth edition published in July 2021[6] in which we have new parameters for sperm evaluation. However, the present work is still valid since the clinical implication for the management of infertile patients using the seminal evaluation parameters of the 2010 and 2021 WHO Manual does not vary.

Table 1 Lower reference limits established by the WHO 2010 for the study of semen characteristics.

Parameter	Lower limit of reference
Seminal volume (mL)	1.5
pH	≥7.2
Total number of sperm (millions in ejaculate)	39
Sperm concentration (millions per milliliter)	15
Total mobility (%)	40
Progressive mobility (%)	32
Sperm with normal shapes (%)	≥4
Leukocytes (millions per milliliter)	<1

The decrease in sperm motility is called asthenozoospermia. Oligozoospermia is the decrease in the concentration of sperm and the terminology. Teratozoospermia refers to the alteration of sperm morphology; a combination of these three parameters can be observed in the spermatograms.

The main objective when evaluating semen quality is to estimate the fertile potential of the male, that is, whether or not he can reproduce and the associated probabilities; however, there was a difficulty since the existence of sperm does not guarantee the capacity of paternity, although with low counts this is going to be low, while on the other hand the absence of sperm, since it is not known if it is permanent or not, does not allow ensure that it is impossible to conceive[7]. By itself, a spermogram is not predictive of natural conception, a limitation when estimating said potential is the female factor. Age affects fertility in women as well, due to decreased ovarian reserve, low oocyte quality, and decreased uterine receptivity[8]. Men with poor sperm quality could conceive when their relative sub-fertility is compensated by a young couple with a high probability of conceiving, the same man may experience conception problems if his partner is a woman over 40 years of age[9]. For this reason, the interpretation of the spermatogram must be carried out within the couple. Accordingly, predictive models of spontaneous pregnancy have been made, based on sperm motility, female age, duration of infertility, and type of infertility[10].

One of the tests that is commonly used to evaluate semen quality is motile sperm retrieval (REM), this consists of determining the number of sperm with progressive mobility in a final solution with culture medium, after treatment with sperm selection techniques, the most used at clinical evaluation are density gradients, swim up and seminal

lavage. According to the consensus of experts in Vienna 2017, the treatment recommendations in cases of male factor are based on the result of the recovery of motile sperm (REM)[11].

The implementation of the intracytoplasmic sperm injection (ICSI) technique makes it possible to achieve fertilization and pregnancy, with a very low number of sperm, regardless of the etiology of the seminal alteration. World reports indicate that until 2008, the proportion of ICSI cycles came to represent 66% of cases[12]. This generates interesting scenarios. First, in clinical practice, the study of the male factor in a comprehensive way is ignored, without investigating its causes. The origin of infertility can be a primary disease or secondary to, hormones, nutritional habits, inadequate environment, harmful working conditions, or viral infections, among others. Therefore, a deeper exploration could indicate if the situation is reversible, requires clinical treatment or surgical techniques, or is irreversible whose situation requires donor sperm.

On the other hand, the use of ICSI when choosing the sperm directly, avoiding the natural selection carried out by the oocyte, suggests looking for new diagnostic markers of the quality of the sperm at the cellular and molecular level. Currently, different complementary tests have been developed and gradually incorporated into the routine of the laboratories of assisted reproduction centers, one of them is the fragmentation of sperm DNA, which has become important, given the various studies that have shown that the integrity of DNA in sperm would affect the clinical results in assisted reproduction treatments, predictive models of its value have been built from seminal parameters studied in the spermogram, such as mobility and viability, in addition to the patient's age[13].

The seminal evaluation also takes into account other factors such as the couple's age and time of infertility, which provides a tool for adequate reproductive advice in cases of male factor; it could also be an indication for the exploration of male health or cellular factors, such as DNA fragmentation, which can affect sperm performance in reproductive success.

The general purpose is to determine the characteristics of the semen, the most frequent alterations, and the effect of age on the seminal quality, of patients who come to an infertility consultation in an assisted reproduction center in the city of Quito.

Subject and methods

Type of Study: An observational, descriptive, cross-sectional study was carried out using the spermatogram database of the Infertility and Assisted Reproduction consultation of the "Instituto Quiteño de Infertilidad" in the period from August 2019 to September 2020.

This research did not require approval of the Human Research Ethics Committees based on MINISTERIAL AGREEMENT No. 4889 – 2014, which was in force from July 1, 2014, until August 2, 2022, when the regulations were modified.

The universe consisted of 55 spermatograms taken from the database that included men from infertile couples in the entire province of Pichincha, who were between 19 and 60 years old. Patients with a history of genetic and oncological diseases affecting male fertility were excluded.

The basic spermogram included in the database was performed following the protocol established in the manual for the examination and processing of human semen, the semen samples being evaluated within the hour of collection. Sperm parameters of sperm concentration (106 per milliliter), total motility (progressive and non-progressive), and progressive motility (%) were evaluated with a Makler camera (Makler® counting chamber; Sefi Medical Instruments, Ltd.). The assessment of sperm morphology (%) was performed using the differential sperm staining technique (diff-quik rapid staining with the Stat III® Andrology Stain kit [ref: 85316-I; Mid-Atlantic Diagnostic, Inc.]), following their established protocol. Morphological defects were classified according to the strict Kruger criteria of the WHO manual, considering the affected area of the sperm (head, neck, and tail).

Results

Fifty-five couples who attended for infertility evaluation were included after an average time of 2.92 ± 2.85 years trying to conceive. The mean age of the men was 36.24 ± 7.47 years with a mean of 5.24

± 5.45 days of ejaculatory abstinence at the time of the spermatogram study. The seminal samples had a mean volume of 2.54 ± 1.50 mL, mean pH of 7.9 ± 0.2, leukocytes of 0.9 ± 0.4 x 106 / mL, sperm concentration of 80.06 ± 63.98 x 106 / mL, progressive mobility of 41.54 ± 19.57%, vitality of 67.26 ± 18.40% and mean of 7.43 ± 4.93% of morphologically normal sperm. The mean number of altered seminal parameters was 0.90 ± 1.10 with a minimum of 0 alterations to a maximum of 4. No individual was observed with all altered seminal parameters (Table 2).

Of the included patients 47.27% were normozoospermic and 52.72% had at least some altered seminal parameter. Asthenozoospermia was the most frequent seminal alteration 25.45% among the patients, either alone or combined with other alterations. Tables 3 and 4.

Table 2. Mean values of the quantitative seminal characteristics of the patients.

Parameters	**Media ± DE**
Age (years)	36.24 ± 7.47
Days of abstinence	5.24 ± 5.45
Volume (mL)	2.54 ±1.50
pH	7.9 ± 0.2
Leukocytes (x106)	0.9 ± 0.4
Sperm concentration (106 spz / mL)	80.06± 63.98
Progressive sperm (%)	41.54 ± 19.57
Viable (%)	67.26± 18.40
Total seminal alterations	0.90± 110

Table 3. Frequency of seminal alterations in patients

Seminal alteration	**Number of cases**	**%**
Asthenozoospermia	14	25.45
Hypospermia	13	23.63
Teratozoospermia	11	18.18
Necroozoospermia	6	10.90
Oligozoospermia	3	5.45
Hyperspermia	1	1.81
Azoospermia	2	3.62
Leukocytospermia	1	1.81

Table 4. Classification of the seminal quality of the evaluated patients

Classification	**Number of cases**	**%**
Normozoospermic	26	47.27
Asthenozoospermic	9	16.36
Asthenoteratozoospermic	3	5.45
Oligoasthenoteratozoospermic	1	1.8
Oligoastenozoospermic	1	1.81
Teratozoospermic	6	10.90
Oligozoospermic	1	1.81
Azoospermic	2	3.63
Cryptozoospermic	0	0
Necrozooaspermia	6	10.90

The total number of patients was divided into 3 groups by age, the group with the most patients was 30-39 years old (24) 43.63%, followed by over 40 years old (20) 36.36%, and then 19-29 years old (11) 20% of the patients.

The most affected patients with at least one seminal alteration correspond to those over 40 years of age at 60%, followed by the range of 30-39 years with 54.16%. The group of patient's least affected was 19 to 29 years old with only 27.7% (Figure 1).

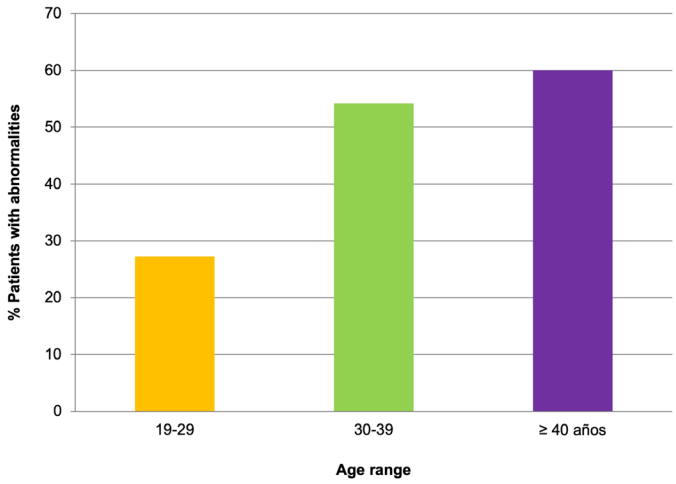

Figure 1. Frequency of patients with altered seminal parameters

The number of seminal alterations observed by age range also presents the same age-related trend, finding the highest number of sperm alterations per sample in the group over 40 years of age with a mean value of 1.35 ± 1.38 (Table 5).

Table 5. Mean values of seminal parameters by age group and motile sperm retrieval

	19-29	30-39	Over 40
Concentration (mill / mL)	97.98 ± 52.53	66.90 ± 58.04	
Progressive mobility (%)	49.06 ± 18.16	42.950 ± 15.92	85.33 ± 75.10
Normal sperm forms (%)	10.36 ± 4.54	7.080 ± 4.78	35.81 ± 23.07
Number of seminal alterations	0.27± 0.46	0.82± 0.88	6.15 ± 4.87
REM (millions)	40.05 ± 30.71	17.92 ± 12.01	1.35± 1.38
			18.02 ± 23.65

(REM).

According to the type of alteration, in our population young patients in the range 19-29 did not exhibit alterations related to oligozoospermia and teratozoospermia, only one case was related to asthenozoospermia and the rest of the cases with hypospermia. Similarly, oligozoospermia is not present in the cases of patients between 30-39 years of age, neither in isolation nor accompanied by another alteration, in this age range alterations such as teratozoospermia, astenozoospermia, and necrozoospermia predominated. In contrast, in the group over 40 years all the types of alterations that can be found in a seminal sample were present (Figure 2).

The recovery of motile spermatozoa (REM) average was 24.72 ± 24.95 x 106 spz, with the highest recovery in the group of younger patients (19-29 years) with 40.05 ± 30.71, in the group of older patients the sperm recovery The number of mobiles was up to 44% lower than that of the youngest group, being 17.92 ± 12.01 and 18.02 ± 23.65, in the 30-39 and older than 40 groups respectively.

Discussion

One of the key recommendations to couples in the fight against infertility is the timely consultation of a specialist, after a year of exposure, having frequent and unprotected sex. An earlier evaluation is indicated in women over 35 years of age, patients with a history of oligo or amenorrhea, known tubal or peritoneal pathology, such as endometriosis,

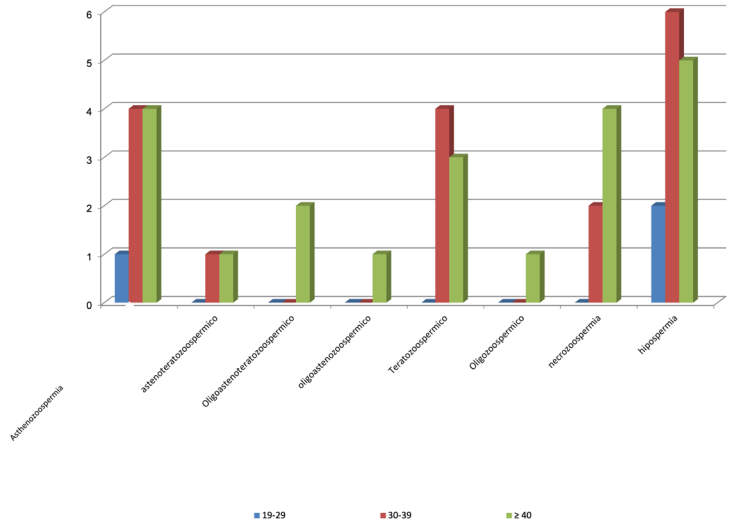

Figure 2. Frequency of the most common seminal disorders by age group.

and couples in which there is knowledge of a male infertility factor[14].

Couples who attended the consultation were, on average, 2.92 ± 2.85 years trying to conceive, which is longer than the recommended time of one year or immediately when the woman is older than 35 years[15,16]. The time of infertility is an important factor included in many predictive models of the success of assisted reproductive techniques, both in the pregnancy rate and in the live newborn rate[17]. The evaluation of male factors should be given concomitantly with the female evaluation since approximately 30% of the causes of infertility are pure female, 30% are pure male and in about 30% of the cases, there are shared causes of infertility. The remaining 10% is what is called idiopathic or unknown cause infertility[18].

From a general point of view, of the total number of patients who attended the clinic for fertility problems and who were included in this study, 47.27% were normozoospermic and 52.73% had at least one altered seminal parameter, that is, below the limit reference indicated by WHO.

Asthenozoospermia was the most frequent alteration (25.45%), followed by hypospermia (23.63%), while teratozoospermia was present in 18.18% of the evaluated patients.

Asthenozoospermia has been associated with structural abnormalities of the main part of the sperm tail such as malassembly of the axoneme[19,20] or the fibrous sheath[21], with subsequent failure of progressive sperm motility[22]. Likewise, asthenozoospermia has been associated with genetic defects of the flagellum structure. Sperm motility deficiency can originate from its testicular origin, in its passage through the epididymis or when it confluences with other elements of the seminal plasma[23].
Seminal quality is a concept that integrates all the characteristics of semen, however, asthenozoospermia is one of the main causes of male infertility because it reduces the ability of the sperm to reach the egg and fertilize it, its origin is influenced by cellular factors, such as the structure of the flagellum, molecular factors and also by lifestyle, such as diet, sexual activity, habits[24, 25].

According to the WHO indications, ejaculatory abstinence for the evaluation of semen should be from 2 to 5 days, however in some studies, the days of ejaculatory abstinence have been related to the quality of the semen, found that the increase in the period of abstinence decreases progressive and total mobility. Contrary to the duration of abstinence, it has a positive influence on the increase in sperm concentration[26, 27]. Some studies indicate that a period of less than

2 days of ejaculatory abstinence has been positively related to the indicators of success for IVF and ICSI[28,29]. This study did not adjust the days to a fixed period, but rather it kept the range established by the WHO.

Teratozoospermia was present in isolation, in 10.90%, and only in patients older than 30 years. Two patients did not undergo this evaluation because they did not present a sufficient number of spermatozoa to perform the corresponding staining, as in azoospermia (total absence of spermatozoa), cryptozoospermia or severe oligozoospermia (<2x106 / mL). The quantification of sperm morphology is one of the most common tests in the evaluation of male fertility[30]. The morphometric characteristics of the sperm head, midpiece and tail are appreciated.

Some studies have highlighted correlations between the percentage of normal forms and functional sperm abnormalities, as well as correlations with the ability to conceive in vivo and, in some situations, with the success of intrauterine insemination (IUI) or conventional IVF, however, the evaluation of sperm morphology has very little sensitivity and specificity in the diagnosis of infertility, revealing a great lack of analytical reliability of this test, mainly in the evaluation of the details of sperm abnormalities[31, 32]. Despite all the controversy about the clinical relevance of the sperm morphology test, it is not advisable to ignore its study, because in some cases specific sperm defects (easy to detect with 99 or 100% of affected sperm) would be related to genetic disorders (globozoospermia, macrocephaly, decapitated sperm syndrome, and fibrous sheath dysplasia)[33].

The alteration related to sperm concentration, oligozoospermia, was only present in 5.25% of the cases evaluated and only in patients over 40 years of age. The results of studies on the influence of age on human sperm concentration are contradictory; some describe a downward trend, while others observed an increase in concentration. Molina et al[34], report a significant decrease in sperm count related to age. There are many morphological and histological changes related to testicular aging: decrease in volume, arteriolar sclerosis, Leydig and Sertoli cell degeneration, germ cell depletion, and thickening of the testicular albuginea tunic. The participation in the decrease of testicular androgens in aging is related to the decrease in the number of Leydig cells associated with alterations in the functioning of the hypothalamic-pituitary axis. Sperm volume, concentration, total number, motility, and morphology decrease with male aging. However, there is no established age limit for these changes[35]. There is inter and intra-individual variability of sperm parameters and the selection of patients must be careful when interpreting the published results. Let us remember that this is a population of infertile patients and that in some way their sperm quality is affected. Sperm concentration shows considerable variation and at least two semen samples should be examined before concluding that the sperm concentration or total sperm count is below the reference range. This should be considered for future studies on sperm quality.

We must also consider that this age group, over 40 years old is the most economically active population in society, factors such as stress, a very demanding lifestyle due to work or social activities, could be influencing sperm production, this is pointed out by some authors as an important factor[36]. However, these factors were not evaluated in our study, so it is not possible to assert that the sperm quality of this population is reduced by socio-environmental factors.

In the present study, it was obtained that the presence of a single alteration is more frequent, with 51% than in the case of two or three, with 31% and 10.34% respectively, with very few cases where 4 alterations were reported, this it was 6.8% of the cases. The number of sperm alterations found in our patients also had a positive trend related to age, being higher in the group of patients older than 40 years[37].

There are other factors, such as genetics, that can influence the quality of semen and that affect sperm parts and functions, such as the constitution of the flagella and the mobility of the sperm.

Unlike the impact of female age on reproductive function, men do not experience a sudden cessation in their reproductive capacity. However, male aging may have a slightly negative effect on reproductive organs and tissues. Dammke et al[38] observed changes in testicular volume at an age above 80 years in

healthy men, with a reduction of 31% compared to the age group between 18 and 40 years. Otherwise, the effect of age on the variables analyzed in the spermatogram has been studied, without conclusive results. Apparently, the decrease in seminal volume is the variable most frequently associated with increasing male age, while other variables (concentration, motility, and morphology) did not decrease with age. In our study, hypospermia (low seminal volume) was observed in all age groups in 23.63% of the patients evaluated, with a higher proportion in the 30-39 years' group followed by the group over 40 years old, this may not only be associated with aging but it can also be related with high sexual activity, varicocele or infectious processes, such as papillomavirus (HPV)[39]. This invites to carry out more in-depth studies in the population, considering the multifactorial nature of semen quality.

Finally, only spermograms that are available in the database of the reproduction center were included to evaluate the male factor within the study of the infertile couple, where poor sperm quality plays an important role, therefore it cannot be extrapolated to the entire male population of the city and much less of Ecuador.

In this study, it is evidenced a high percentage of patients with at least one seminal alteration, especially in older patients, which indicates the importance of considering the age of the male when evaluating the infertile couple. Published studies have shown regional differences in the quality of semen, as well as a clear decrease in its quality, due to various factors. Therefore, it is necessary to develop studies that characterize the quality of the semen of the male population of fertile age in Ecuador and identify the causes that affect it to prevent future reproductive health problems. Additionally, an important goal would be to educate the population about reproductive health and its close relationship with age and lifestyle.

Approval and informed consent

This research did not require approval of the Human Research Ethics Committees based on MINISTERIAL AGREEMENT No. 4889 – 2014, which was in force from July 1, 2014, until August 2, 2022, when the regulations were modified.

Authors' contribution

The research protocol and its design, data collection, critical analysis, discussion, writing and approval of the final manuscript were prepared by all the authors who contributed equally to the entire process.

Interest conflict

The authors declare that they have no conflict of interest.

Funding

The authors declare that the financial resources for the preparation of this research (of the type of observation, data analysis) do not come from any fund, but from their self-management.

References

1. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril [Internet]. el 15 de enero de 2015; Available at: https://pubmed.ncbi.nlm.nih.gov/25597249/
2. Borght MV, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem [Internet]. el 3 de invierno de 2018; Available at: https://pubmed.ncbi.nlm.nih.gov/29555319/
3. Schjenken J, Robertson S. Seminal Fluid Signalling in the Female Reproductive Tract: Implications for Reproductive Success and Offspring Health. Adv Exp Med Biol [Internet]. 2015; Available at: https://pubmed.ncbi.nlm.nih.gov/26178848/
4. Cooper T, Noonan E, von Eckardstein S, Auger J, Baker G, Behre H, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update [Internet]. el 24 de noviembre de 2009; Available at: https://pubmed.ncbi.nlm.nih.gov/19934213/
5. Word Health Organization. Manual for the examination and processing of human semen fifth edition [Internet]. 2010. Available at: https://www.who.int/docs/default-source/reproductive-health/srhr-documents/infertility/examination-and-processing-of-human-semen-5ed-eng.pdf

6. World Health Organization. Manual for the examination and processing of human sixth edition [Internet]. 2021. Available at: https://www.who.int/publications-detail-redirect/9789240030787
7. Garrido N. Presente y futuro de la evaluación del semen con finalidad reproductiva en un centro de reproducción asistida. Revista Iberoamericana de Fertilidad [Internet]. el 9 de agosto de 2014; Available at: http://www.revistafertilidad.org/articulo/Presente-y-futuro-de-la-evaluacioacuten-del-semen-con-finalidad-reproductiva-en-un-centro-de-reproduccioacuten-asistida/173
8. Leushuis E, van der Steeg JW, Steures P, Repping S, Bossuyt P, Mol BW, et al. Semen analysis and prediction of natural conception. Human Reproduction [Internet]. el 2 de mayo de 2014;29(7):1360–7. Available at: https://academic.oup.com/humrep/article/29/7/1360/902833
9. Crawford NM, Steiner AZ. Age-related infertility. Obstet Gynecol Clin North Am [Internet]. marzo de 2015; Available at: https://pubmed.ncbi.nlm.nih.gov/25681837/
10. Hunault CC, Habbema JDF, Eijkemans MJC, Collins JA, Evers JLH, te Velde ER. Two new prediction rules for spontaneous pregnancy leading to live birth among subfertile couples, based on the synthesis of three previous models. Hum Reprod [Internet]. septiembre de 2004; Available at: https://pubmed.ncbi.nlm.nih.gov/15192070/
11. ESHRE Special Interest Group of Embryology, Alpha Scientists in Reproductive Medicine. The Vienna consensus: report of an expert meeting on the development of art laboratory performance indicators. Hum Reprod Open [Internet]. el 4 de agosto de 2017; Available at: https://pubmed.ncbi.nlm.nih.gov/31486806/
12. Dyer S, Chambers GM, de Mouzon J, Nygren KG, Zegers-Hochschild F, Mansour R, et al. International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010. Hum Reprod [Internet]. julio de 2016; Available at: https://pubmed.ncbi.nlm.nih.gov/27207175/
13. Jimmy P, Rosmary L, Luis N-H, Luis G. Modelo predictivo de fragmentación de ADN espermático usando parámetros evaluados en un espermatograma. Revista Peruana de Ginecología y Obstetricia [Internet]. 2014;60(1):21–8. Available at: http://www.scielo.org.pe/scielo.php?pid=S2304-51322014000100005&script=sci_abstract
14. Alpha Scientists in Reproductive Medicine, ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod [Internet]. mayo de 2011;26(6):1270–83. Available at: https://pubmed.ncbi.nlm.nih.gov/21502182/
15. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertility female: a committee opinion. Fertil Steril 2015;103:e44-e50.
16. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril 2015;103:e18-e25.
17. Masoli D. Diagnóstico de la infertilidad: estudio de la pareja infértil. Revista Médica Clínica Las Condes [Internet]. mayo de 2010;21(3):363–7. Available at: https://www.researchgate.net/publication/272641371_Diagnostico_de_la_infertilidad_estudio_de_la_pareja_infertil
18. Ratna MB, Bhattacharya S, Abdulrahim B, McLernon DJ. A systematic review of the quality of clinical prediction models in in vitro fertilisation. Hum Reprod [Internet]. el 1 de enero de 2020;35(1):100–16. Available at: https://pubmed.ncbi.nlm.nih.gov/31960915/
19. Afzelius BA, Eliasson R. Flagellar mutants in man: On the heterogeneity of the immotilecilia syndrome. J Ultrastruct Res. 1979;69:43-52.
20. Moretti E, Pascarelli NA, Federico MG, Renieri T, Collodel G. Abnormal elongation of midpiece, absence of axoneme and outer dense fibers at principal piece level, supernumerary microtubules: A sperm defect of possible genetic origin? Fertil Steril. 2008;90:1201.e1203–e1208.
21. Escalier D. New insights into the assembly of the periaxonemal structures in mammalian spermatozoa. Biol Reprod. 2003;69:373-378.
22. El-Taieb MA, Herwig R, Nada EA, Greilberger J, Marberger M. Oxidative stress and epididymal sperm transport, motility and morphological defects. Eur J Obstet Gynecol Reprod Biol. 2009;144(Suppl 1):199203.
23. Teppa GA, Palacios TA. Evaluación actual de la infertilidad masculina. Invest Clín. 2004;45:355-370.

24. Zegers-Hochschild F, Crosby JA, Musri C, Mdcb de S, Martinez AG, Silva AA, et al. Assisted reproductive technology in Latin America: the Latin American Registry, 2017. Reprod Biomed Online [Internet]. julio de 2020;41(1):44–54. Available at: https://pubmed.ncbi.nlm.nih.gov/32417198/
25. Verón GL, Tissera AD, Bello R, Beltramone F, Estofan G, Molina RI, et al. Impact of age, clinical conditions, and lifestyle on routine semen parameters and sperm kinematics. Fertil Steril [Internet]. el 1 de julio de 2018;110(1):68–75. Available at: https://pubmed.ncbi.nlm.nih.gov/29980266/
26. Shahrokhi SZ, Salehi P, Alyasin A, Taghiyar S, Deemeh MR. Asthenozoospermia: Cellular and molecular contributing factors and treatment strategies. Andrologia [Internet]. marzo de 2025;52(2). Available at: https://pubmed.ncbi.nlm.nih.gov/31680293/
27. Hanson BM, Aston KI, Jenkins TG, Carrell DT, Hotaling JM. The impact of ejaculatory abstinence on semen analysis parameters: a systematic review. J Assist Reprod Genet [Internet]. febrero de 2018;35(2):213–2020. Available at: https://pubmed.ncbi.nlm.nih.gov/29143943/
28. Comar VA, Petersen CG, Mauri AL, Mattila M, Vagnini LD, Renzi A, et al. Influence of the abstinence period on human sperm quality: analysis of 2,458 semen samples. JBRA Assist Reprod [Internet]. el 1 de diciembre de 2017;21(4):306–12. Available at: https://pubmed.ncbi.nlm.nih.gov/28985041/
29. Li J, Shi Q, Li X, Guo J, Zhang L, Quan Y, et al. The Effect of Male Sexual Abstinence Periods on the Clinical Outcomes of Fresh Embryo Transfer Cycles Following Assisted Reproductive Technology: A Meta-Analysis. Am J Mens Health [Internet]. julio de 2020;14(4). Available at: https://pubmed.ncbi.nlm.nih.gov/32804026/
30. Borges E Jr, Dpaf B, Zanetti BF, Iaconelli A Jr, Setti AS. 21. Borges E, Braga DPAF, Zanetti BF, Iaconelli A, Setti AS. Revisiting the impact of ejaculatory abstinence on semen quality and intracytoplasmic sperm injection outcomes. Andrology. 2019 Mar 1;7(2):213–9. Andrology [Internet]. marzo de 2019;7(2):213–9. Available at: https://pubmed.ncbi.nlm.nih.gov/30570220/
31. Auger J, Jouannet P, Eustache F. Another look at human sperm morphology. Hum Reprod [Internet]. enero de 2016;31(1):10–23. Available at: https://pubmed.ncbi.nlm.nih.gov/26472152/
32. Gatimel N, Moreau J, Parinaud J, Léandri RD. Sperm morphology: assessment, pathophysiology, clinical relevance, and state of the art in 2017. Andrology [Internet]. septiembre de 2017;5(5):845–62. Available at: https://pubmed.ncbi.nlm.nih.gov/28692759/
33. Kohn TP, Kohn JR, Lamb DJ. Role of Sperm Morphology in Deciding Between Various Assisted Reproduction Technologies. Eur Urol Focus [Internet]. el 22 de agosto de 2018;4(3):311–3. Available at: https://pubmed.ncbi.nlm.nih.gov/30143470/
34. Molina RI, Martini AC, Tissera A, Olmedo J, Senestrari D, de Cuneo MF, et al. Envejecimiento y calidad seminal: un análisis de 9.168 casos en Córdoba. Arch Españoles Urol [Internet]. 63(3):214–22. Available at: https://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S0004-06142010000300007
35. Sibert L, Lacarrière E, Safsaf A, Rives N. Fonctions testiculaires du sujet âgé. Presse Medicale [Internet]. el 20 de febrero de 2014;43(2):171–7. Available at: https://www.em-consulte.com/article/872179/fonctions-testiculaires-du-sujet-age
36. Juárez-Rojas L, Vigueras-Villaseñor RM, Casillas F, Retana-Márquez S. Gradual decrease in spermatogenesis caused by chronic stress. Acta Histochem [Internet]. abril de 2017;119(3):284–91. Available at: https://pubmed.ncbi.nlm.nih.gov/28236448/
37. Ilacqua A, Izzo G, Pietro Emerenziani G, Baldari C, Aversa A. Lifestyle and fertility: the influence of stress and quality of life on male fertility. Reprod Biol Endocrinol [Internet]. el 26 de noviembre de 2018;16(1):115. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6260894
38. Damke E, Kurscheidt FA, Balani VA, Takeda KI, Irie MMT, Gimenes F, et al. Male Partners of Infertile Couples with Seminal Infections of Human Papillomavirus Have Impaired Fertility Parameters. Biomed Res Int [Internet]. el 1 de agosto de 2017; Available at: https://pubmed.ncbi.nlm.nih.gov/28835893/
39. Kathrins M. Historical investigations into varicocele pathophysiology and sperm migration. Fertil Steril [Internet]. el 20 de diciembre de 2017;109(1):75–6. Available at: https://pubmed.ncbi.nlm.nih.gov/29274657/