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EFFECTS OF CHRONIC TOBACCO CONSUMPTION ON MALE FERTILITY

Léo Pedro Rufino
Acadêmico do Curso de Medicina da Universidade José do Rosário Vellano - Unifenas - Campus Alfenas MG
https://orcid.org/0000-0002-4433-4593
http://lattes.cnpq.br/3244511411481461

Alessandra dos Santos Danziger Silvério
Docente do Curso de Medicina da Universidade José do Rosário Vellano - Unifenas - Campus Alfenas Mg
https://orcid.org/0000-0002-9513-1331
http://lattes.cnpq.br/3602445800288167

Abstract: RUFINO, Léo Pedro. **Effects of chronic tobacco consumption on male fertility**. Advisor: Professor: Alessandra dos Santos Danziger Silvério. Alfenas: UNIFENAS, 2022. Scientific Initiation Work. Support: FAPEMIG. **Introduction**: smoking is one of the biggest challenges in the health sector, due to its high prevalence of use, as well as the harmful effects it causes on the human body. As its use is progressing, it is important to understand its impact on human reproduction and the health risks. Objectives: the present study aimed to evaluate the qualitative and quantitative effect of tobacco on seminal parameters in a given population, guiding the individual regarding the harm to male fertility resulting from tobacco use. Material and methods: the study included the analysis of the spermogram of 24 volunteer individuals, 12 of whom were heavy smokers and 12 were non-smokers. All participants signed an Informed Consent Form before semen collection, which was carried out at home to avoid embarrassment, and also completed a questionnaire containing information such as period of sexual abstinence, date and time of sample collection and use of medications that could interfere with the results, each of which received collection instructions as recommended by the World Health Organization. Number of the approval opinion by the research ethics committee of the José do Rosário Vellano University: 235,429. Results: smoker volunteers had changes in the sample in parameters such as vitality, viscosity, liquefaction, concentration and motility, important components for normal male reproductive functioning. Conclusion: the study proved that these changes caused by smoking can interfere with a man's reproductive capacity, becoming a more significant situation when applied to the universe of infertile couples.
Keywords: Spermogram. Infertility. Tobacco

INTRODUCTION

Infertility is a problem with major emotional, social and economic implications, affecting around 10-15% of couples. The demand for infertility consultations has been increasing in recent decades.

A couple's infertility may be due to female, male or mixed factors. In 15 to 20% of cases it is not possible to identify a cause. The identification of male factor occurs in around 25 to 40% of cases, with the majority of these cases involving testicular pathology.

After the clinical history and objective examination, the initial assessment of the male factor is carried out using the spermogram. When a change is identified, it must be confirmed in a second assessment. Deleterious effects of lifestyle on sperm quality have been reported.

The effect of tobacco smoke represents one of the greatest global health challenges, due to its high prevalence worldwide and the high morbidity and mortality resulting from its numerous complications. As the number of smokers is increasing worldwide, it is important to fully understand the impact on human reproduction and the risks associated with cigarette smoking. Tobacco consumption is a relatively common habit throughout the world, which, combined with the fact that it contains several mutagenic and carcinogenic substances, has worried the scientific community in several aspects, particularly with regard to its effect on fertility.

Recent studies have shown the passage of several components across the blood-testicular barrier. The presence of these components in semen can induce the degradation of seminal parameters, as well as nuclear changes in sperm with possible impairment of fertility.

GOALS

GENERAL GOAL

The present work aimed to evaluate the effect of tobacco on seminal parameters in a given population.

SPECIFIC GOALS

Associate smoking with likely changes in semen quality, such as density, mobility and presence of anomalous forms.

Advise the individual regarding the harm to male fertility resulting from tobacco use.

JUSTIFICATION

Tobacco consumption is a relatively common habit throughout the world nowadays, which, combined with the fact that it contains several mutagenic and carcinogenic substances, has worried the scientific community in several aspects, particularly with regard to its effect on fertility. Recent studies have shown the passage of several components across the blood-testicular barrier.

The presence of these components in semen can induce the degradation of seminal parameters, as well as nuclear changes in sperm with possible impairment of fertility. Proving that the effects of tobacco negatively affect male fertility is extremely important for educating and encouraging smokers to help them stop the habit.

With the knowledge that tobacco affects semen in a harmful way, it may be possible to change habits, thus reducing adverse effects on fertility and increasing the chances of successful fertilization.

THEORETICAL REFERENCE

ANATOMO-PHYSIOLOGICAL BASES OF MALE FERTILITY

ANATOMICAL DESCRIPTION OF THE TESTICLE

According to Pina, 2004, the testicles are secretory and excretory organs designed to produce the main element of spermatic fluid or sperm, spermatozoa – male germ cells. They also produce hormones, including testosterone, which play an important role in determining secondary sexual characteristics.

The testicles are ovoid in shape and are suspended inside the scrotum by means of the spermatic cords. They are found below the penis, between the thighs, in the anterior part of the perineum (Figure 1.1). They are inside bags made up of several layers called scrotal bags (Figure 1.2). The left testicle is slightly lower than the one on the right. They are quite mobile in every way. They measure between 40-45mm wide and 80mm high. They have an elastic, soft and flaccid consistency. Two faces (external and internal face), two edges (antero-inferior and postero-superior) and two ends (superior and inferior) can be considered (PINA, 2004).

Regarding the structure of the testicle (Figure 1.3), we can distinguish the tunica albuginea, the sperm-producing ducts and the sperm-excreting ducts. The albuginea is a fibrous tunic that circumscribes the testicular tissue. The sperm-producing ducts are also called seminiferous or seminal tubules. The seminiferous tubules are organized into spermatic lobes, with a variable number of 220-230, which are separated by fibrous albuginea septa. In relation to the excretory ducts we have: the Haller network and the efferent vessels. (PINA, 2004).

According to Pina, 2004 efferent channels

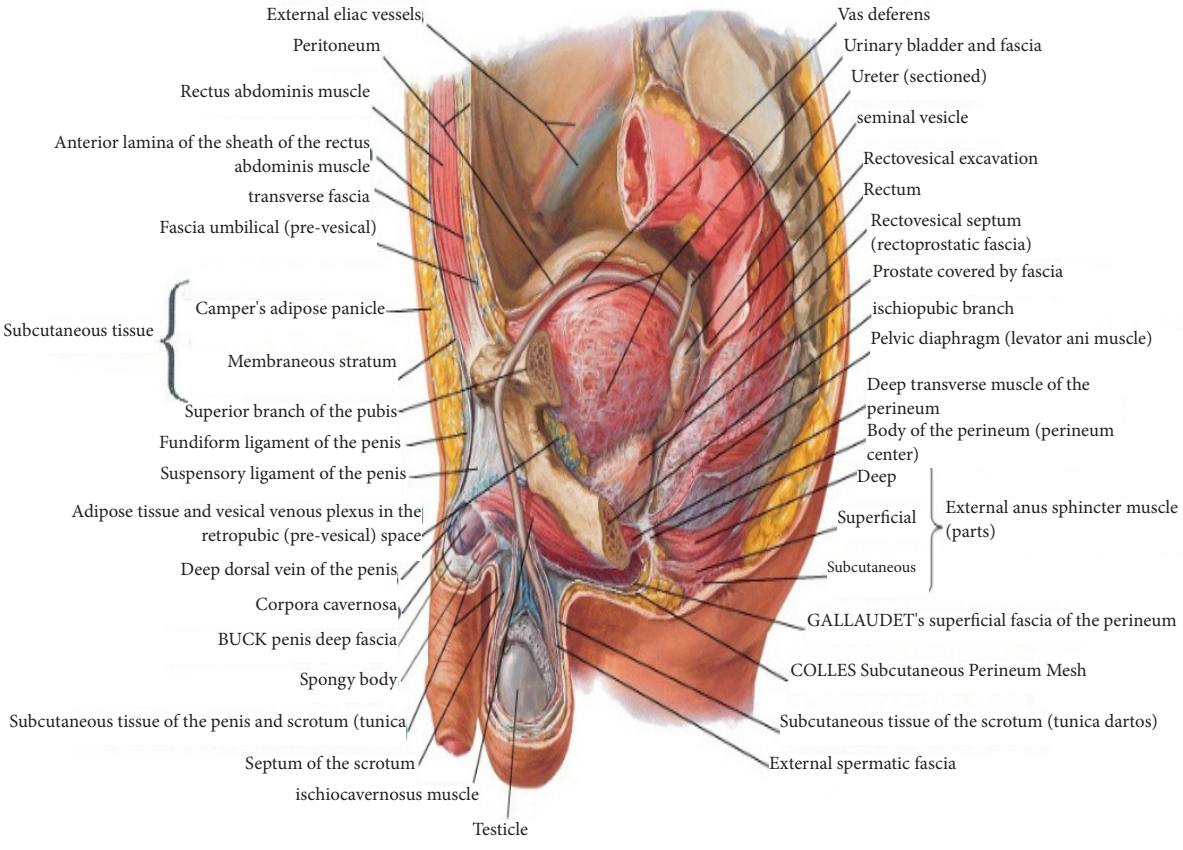

FIGURE 1: Anatomical relationships of the testicle with the organs of the male genital system (OLIVEIRA, 2010).

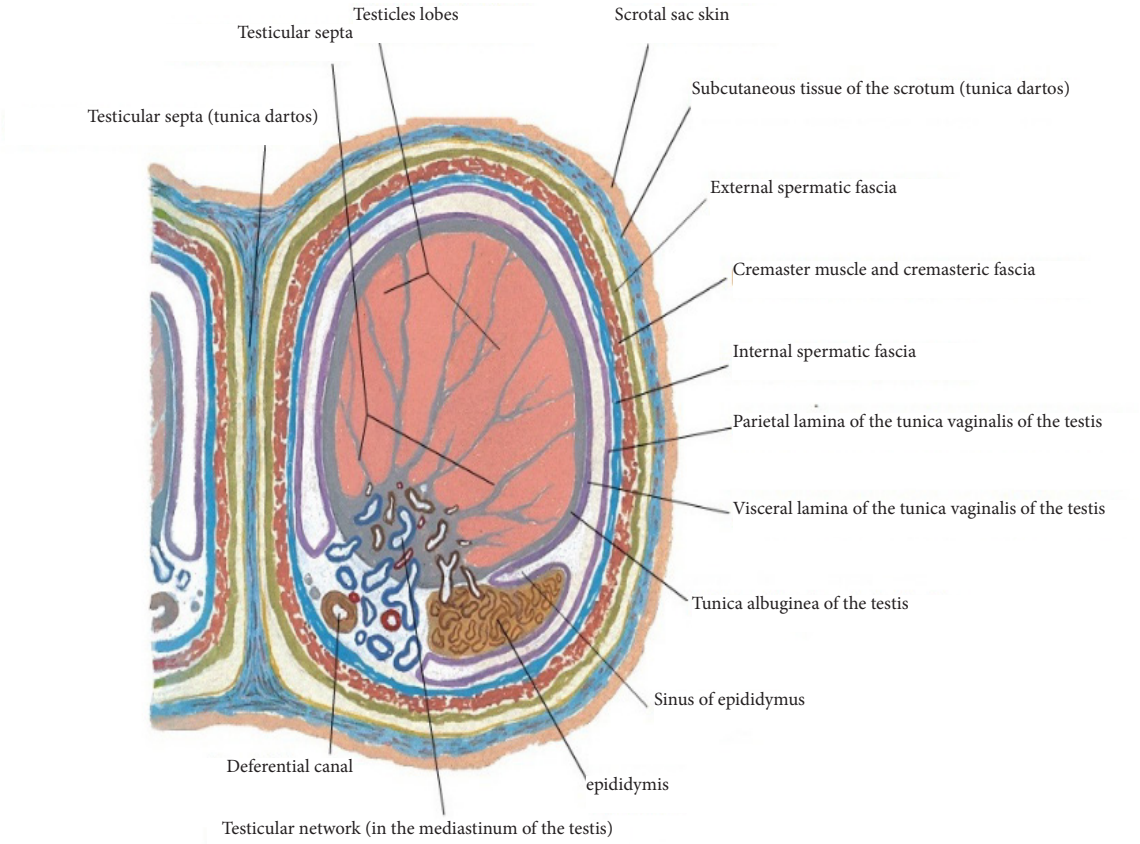

FIGURE 2: Cross section of the scrotum and testicle showing layers that make up the sacs (OLIVEIRA, 2010).

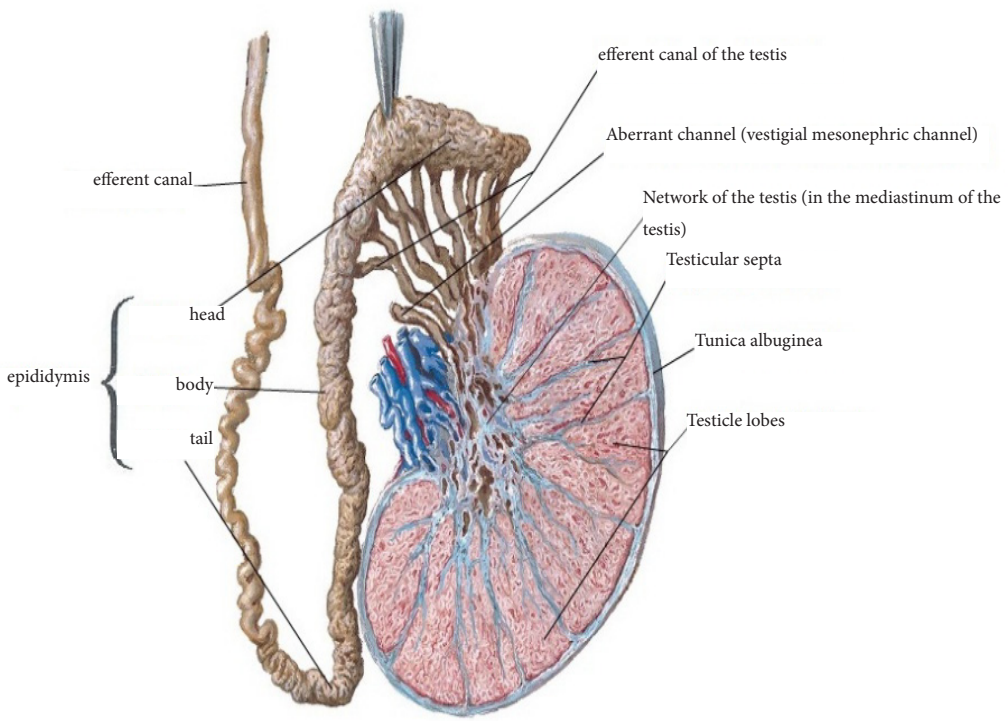

FIGURE 3: Representative diagram of testicular organization in a frontal section (OLIVEIRA, 2010).

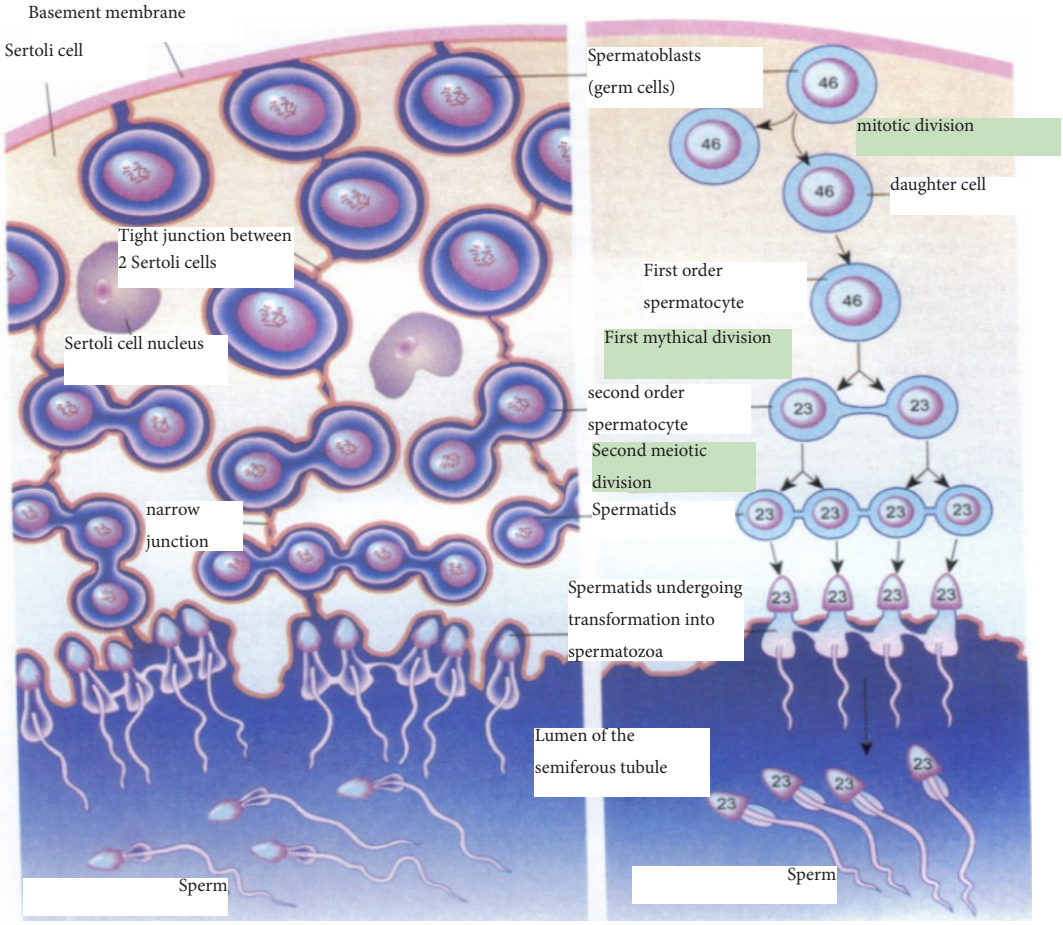

FIGURE 4: Illustrative diagram of the stages of spermatogenesis (OLIVEIRA, 2010).

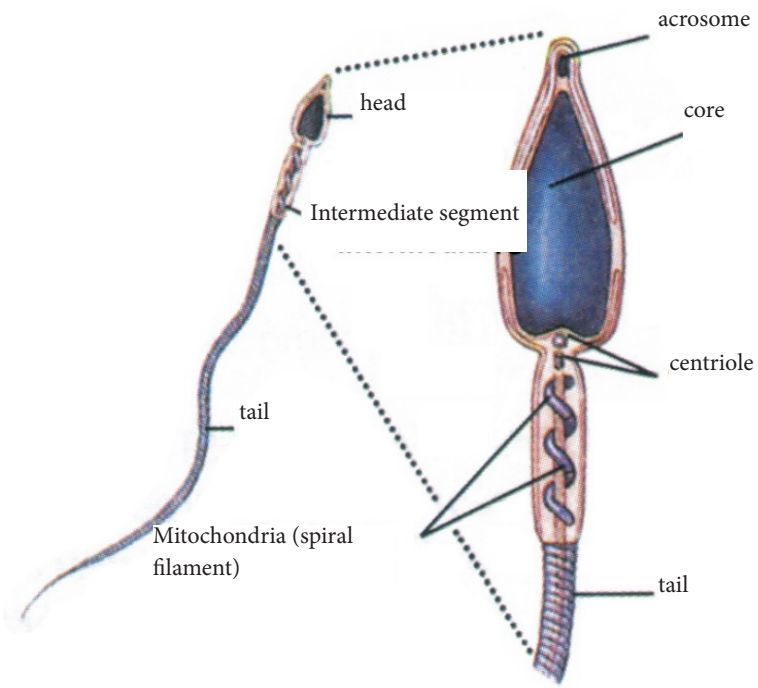

FIGURE 5: Structure of a mature human sperm (OLIVEIRA, 2010)

transport newly formed sperm from the testicular network to the epididymis, where they are stored for short periods until they mature. The tail of the epididymis is continuous with the vas deferens, which transports sperm from the epididymis to the ejaculatory duct for expulsion into the prostatic part of the urethra.

SPERMATOGENESIS: CELLULAR ASPECTS

Spermatogenesis corresponds to the process of sperm formation and occurs in all seminiferous tubules during active sexual life, by stimulation of gonadotropic hormones from the anterior pituitary gland. On average, it begins at 13 years of age and continues throughout most of life, however, it decreases markedly in old age (GUYTON, 2002).

Figure 1.3 shows the stages of spermatogenesis. The seminiferous tubules are lined by germinal epithelial cells, the spermatogonia, which are located in two or three layers along the inner tubular surface. During the first stage, spermatogonia migrate between the Sertoli cells, to the central lumen of the seminiferous tubule. Sertoli cells have cytoplasmic barriers that surround the developing spermatogonia all the way to the central lumen of the tubule.

During a period of 24 hours, on average, each spermatogonia that crosses the Sertoli cell barrier changes and increases in size, forming the primary spermatocyte. This, in turn, divides into two secondary spermatocytes. After a few days, secondary spermatocytes divide to give rise to spermatids that will later transform into spermatozoa, which will be released into the lumen of the seminiferous tubules (GUYTON, 2002).

During the transition from spermatocyte to spermatid, the 46 chromosomes (23 pairs of chromosomes) of the spermatocyte are divided by the process of meiosis. Thus, 23 chromosomes go to one spermatid and the other 23 to the second spermatid. This is why only half of the characteristics of the future fetus come from the father, while the other half come from the mother's oocyte (GUYTON, 2002).

According to Guyton, 2002, this entire period of spermatogenesis, from the germ cell to the sperm, lasts about 64 days.

The tail of the sperm is responsible for its mobility and is made up of the neck area, main part and end area. This tail is made up of nine pairs of filaments or microtubules arranged radially around two central ones. These filaments in turn, they are surrounded externally by nine thick fibers, which appear to be associated with the nine pairs of filaments. In addition to these filaments, the entire main area of the tail is covered by mitochondria that provide the energy necessary for sperm mobility (OLIVEIRA, 2010).

HORMONAL REGULATION OF SPERMATOGENESIS

According to Guyton, 2002, at puberty the hypothalamus begins to produce the GnRH-releasing hormone, which is responsible for initiating much of the control of male sexual functions. GnRH stimulates the anterior pituitary gland to secrete two other hormones, called gonadotropic hormones: Luteinizing Hormone and follicle-stimulating hormone. Hypothalamic neurons secrete GnRH, which is released into the hypothalamic-pituitary portal vascular system and transported to the anterior pituitary, stimulating the release of LH and FSH.

GnRH is secreted intermittently for a few minutes, once every 1 to 3 hours. The intensity of this stimulus depends on two criteria: frequency of these secretion cycles and amount of GnRH released in each cycle (GUYTON, 2002).

LH secretion by the anterior pituitary is

also cyclical, following the pulsatile release of GnRH. On the other hand, FSH secretion increases, and decreases, only slightly with each fluctuation in GnRH secretion. Due to the much closer relationship between GnRH and LH secretion, GnRH is also known as LH-releasing hormone (GUYTON, 2002).

LH is responsible for the primary stimulus for T secretion by the interstitial cells of the testis, which are also called Leydig cells. FSH stimulates spermatogenesis through its action on Sertoli cells (GUYTON, 2002).

SPERM ANALYSIS

According to the WHO, for a sperm analysis to be considered abnormal it must meet at least one of the following conditions: volume less than 2.0 ml, sperm concentrations less than 20x106/ml, total sperm count less than 40x106 per ejaculation, motility less than 50% of cells with anterograde progression with quality less than 2 (on a scale of 0 to 4) within a 60-minute ejaculation period and morphology with less than 30% of normal forms. The terms oligospermia, asthenospermia, teratospermia, or a combination thereof refer to individual sperm samples with abnormalities in number, motility, morphology, or a combination thereof, respectively. Besides, according to the WHO, sperm morphology can be classified as: normal (oval), amorphous (large or small, or any defect), duplicated, or immature (WHO, 2002).

VOLUME

Under normal conditions it varies between 2 and 5 mL. Since the volume coming from Cowper's glands, epididymis and testicles is extremely reduced, the final seminal volume is directly proportional to the amount of secretion from the prostate and seminal vesicles (PEREIRA & JANINI, 2001).

Volumes below 2 mL constitute hypospermia, while the absence of sperm is called aspermia. The volume also varies in the same individual, decreasing with repeated ejaculations in a short space of time and increasing with longer abstinence. It is common to find high seminal volumes in azoospermics, except for those who present agenesis of the vas deferens, when the volume is quite reduced, since, as they have the same embryological origin, seminal vesicles also do not exist (PEREIRA & JANINI, 2001).

VISCOSITY

It can be estimated using a pipette or stick, observing the semen fillet, which must not exceed 2 cm. A normal consistency, for example, due to a high amount of mucus can interfere with the determinations of various sperm characteristics, such as motility and gamete concentration, as well as in tests to identify the presence of sperm coat antibodies (PEREIRA & JANINI, 2001).

Semi-quantitative methods are also used. Using a 0.1 mL pipette with an 11 cm sperm column, start the timer and let 3 drops drip (PEREIRA & JANINI, 2001).

- 4.8 to 5.2 seconds – Normal
- Greater than 5.2 seconds – High viscosity
- Less than 4.8 seconds – Low viscosity

COLOR

Normally semen is light gray, becoming translucent after liquefaction. The presence of pyocytes in large quantities gives the sperm a yellowish color, while the presence of red blood cells gives it a reddish color (PEREIRA & JANINI, 2001).

ASPECT

A normal sample has a homogeneous appearance of grayish opalescence. This appearance is less opaque if the sperm concentration is very low (PEREIRA & JANINI, 2001).

ODOR

Recently omitted sperm has a sui generis odor that can be compared to that of chestnut juice; This is due to substances produced in the prostate (spermine, spermidine and putrecine), such that in prostate atrophy and prostatitis this characteristic odor may be missing (PEREIRA & JANINI, 2001).

PH REACTION

It must be determined at a standard time within one hour after ejaculation and must range from 7.2 to 8.0. Kept in a closed container, which prevents CO2 from escaping into the environment, sperm has a pH that remains at 7.2 to 7.4. In contact with air, the pH increases and reaches 7.8 to 8.2. A pH greater than 8.0 from the moment of emission almost always indicates a prostate deficiency. If the pH is lower than 7.0 in a sample with azoospermia, it may be a case of dysgenesis of the vas deferens, seminal vesicles or epididymis. If the pH is lower than 6.8 or higher than 9.0, total sperm inactivation will occur (PEREIRA & JANINI, 2001).

LIQUEFACTION

A seminal sample liquefies within thirty minutes at room temperature. The presence of mucous lumps, a sign of incomplete liquefaction. It can interfere with sperm count. Normal seminal samples may contain gelatinoid or non-liquefying granulations. The meaning of this fact is unknown (PEREIRA & JANINI, 2001).

Liquefaction can be:

- Primary: semen is completely liquefied after ejaculation due to the absence or insufficiency of seminal vesicles
- Partial: semen partially liquefied immediately after ejaculation due to spermolysin deficiency.
- Secondary: normal liquefaction, occurring up to 30 minutes after ejaculation

COAGULATION

Immediately after emission, the sperm transforms into a gel through an enzymatic process that is not well defined. It then acquires a heterogeneous appearance, forming clots, probably to protect sperm from contact with hostile vaginal contents. At room temperature, the gel transforms into a solution within 15 to 30 minutes of emission. This second process is controlled by fibrinolysin, an enzyme contained in prostate secretion. Changes in liquefaction time or the absence of liquefaction are therefore due to prostatic dysfunction (PEREIRA & JANINI, 2001).

GELLING

The sample must be well homogenized in the original vial. Incomplete homogenization is probably one of the biggest causes of errors in determining sperm concentration. The continuous homogenization by rotating the bottle during liquefaction reduces this error (PEREIRA & JANINI, 2001).

EFFECTS OF TOBACCO ON HUMAN SPERMATOGENESIS

Spermatogenesis is a process that is particularly sensitive to changes caused by cigarette smoking, as it is a process that involves multiple cell divisions. When germ cells enter meiosis (I and II), they become vulnerable to exposure to environmental toxins, such as those from cigarette smoke (OLIVEIRA, 2010).

Spermatogonia and spermatocytes have some capacity to repair DNA, and there are also proofreading mechanisms to eliminate cells with structural errors of reduced viability. However, during the last stage of cellular differentiation of spermatogenesis, spermatids have little or no cellular repair capacity and, when chromatin is completely condensed, DNA repair is impossible. Having no repair capacity, ejaculated sperm run the risk of

transmitting genetic changes to offspring. This transmission can result in failure of uterine implantation, can compromise the uterine development of the embryo, cause spontaneous abortion or post-neonatal development disorders (OLIVEIRA, 2010).

According to Oliveira, 2010, many of the known toxic components of cigarettes are considered mutagenic, carcinogenic and can adversely affect rapidly dividing cells, including male germ cells. They can induce defects in sperm quality and sperm DNA, thus compromising pregnancy. Some of these toxic components are found to be higher in spermatic fluid compared to blood, a fact that alone can warn about the harmful effects of cigarette smoke on human spermatogenesis. The smoking population presents a significant decrease in the volume, density and mobility of sperm, as well as an increase in significant morphological changes compared to the non-smoking population.

The viability of sperm depends on all these parameters to fulfill its main objective, which is to cross the female genital canal and penetrate the egg. During the last decades the number of sperms in men has universally decreased. In addition to environmental factors, tobacco smoke is also responsible for this change. (OLIVEIRA, 2010)

It is known that the harmful aspects caused by cigarette smoke on sperm quality are directly proportional to the dose of inhaled cigarette smoke. Thus, the greater the amount of smoke inhaled, the greater the decrease in sperm quality. This fact may be related to the number of toxic substances contained in cigarette smoke, which cause an increase in free radicals and oxidative stress in sperm. In relation to cotinine (nicotine metabolite), for example, it is known that the greater the number of cigarettes smoked, the higher its concentration in the blood and seminal fluid and that if you stop smoking this value drops (OLIVEIRA, 2010) .

CHROMOSOMAL AND SPERM DNA CHANGES

Several studies have been carried out to check whether there is any association between sperm aneuploidy and tobacco smoking, using the technique of chromosome preparation and FISH in situ hybridization. This technique, using strips of DNA from specific chromosomes, has been an increasingly used way to verify aneuploidy in male germ cells. Only in one of these studies did sperm donors have the same lifestyle, the same demographic characteristics, differing only in terms of being smokers, that is, the relationship between aneuploidy and cigarette smoking was more precise. It was concluded that cigarette smoking increases the risk of aneuploidy only in some chromosomes (for example, in chromosomes 1 and 13), although the exact mechanism that induces aneuploidy in the chromosomes of male germ cells is still unknown (SHI, 2001).

Some of the metabolites of cigarette smoke can increase the levels of reactive oxygen species in sperm, thus leading to high levels of these particles in men who smoke (TRUMMER et al., 2002). ROS (reactive oxygen species), when produced at physiological levels, are important for sperm, as they begin the capacitation process. However, when produced in excess they can cause sperm damage. We know that sperm cells are highly susceptible to damage caused by ROS because their plasma membrane contains many polyunsaturated acids and a low concentration of peroxidase enzymes in the cytoplasm. Furthermore, it is known that there is a direct correlation between smoking and a high level of leukocytes in sperm and that these are cells that generate ROS in ejaculate. Thus, cigarette smoke can cause oxidative changes in the symmetry

of the plasma membrane and also damage to the DNA of sperm cells. It is also known that vital cells with an intact cell membrane contain a negatively charged phospholipid component, called phosphatidylserine, which is normally found on the cytosolic side of the plasma membrane. One of the disorders that can be caused by ROS is the passage of FSH to the external side of the plasma membrane, thus exposing it to the outside of the cell. This externalization marks the cell as apoptotic and can be phagocytosed by macrophages. Annexin V is a protein with the ability to strongly bind to negatively charged phospholipids on the cell surface (as long as there are calcium ions) with great affinity for FS. This union makes it possible to identify cells in the sperm with deteriorated cell membranes, which is the first sign for cell apoptosis to occur. The identification of these apoptotic cells linked to Annexin V is done with very high sensitivity. Therefore, we can state that there is a relationship between smokers and sperm cell apoptosis, that is, smokers have a significant increase in apoptotic sperm compared to non-smokers (BELCHEVA et al., 2004).

Sperm DNA is structured in a special way that keeps the chromosome very stable and compact within the cell nucleus. The integrity of sperm cell DNA is essential for the precise transmission of genetic information to offspring (ESHAL, et. al, 2009). ROS are considered mutagenic particles that are involved in many pathophysiological processes, which is why smokers are at greater risk of having sperm cell DNA defects than non-smokers (BELCHEVA et al., 2004). The status of sperm DNA can be determined using the gel electrophoresis technique in an alkaline medium. This method is capable of measuring breaks in the DNA of sperm cells. It is known that smokers have a greater degree of DNA fragmentation in their sperm cells compared to non-smokers and that this DNA fragmentation is related to high levels of ROS. We can conclude that cigarette smoke causes errors in the DNA or chromosomes of human germ cells (OLIVEIRA, 2010).

CHANGES IN SPERM MORPHOLOGY AND MOBILITY

MORPHOLOGY

The effect of cigarette consumption causes damage to the morphology of sperm, especially the shape of the head. An abnormal sperm morphology is associated with a reduction in human fertility. Abnormalities in sperm morphology are responsible for increasing the time it takes for a couple to get pregnant, as well as reducing the success of in vitro fertilization. Normally, when changes in sperm shape increase, fertility decreases (OLIVEIRA, 2010).

Studies have shown that there is a significant relationship between the concentration of cotinine in seminal fluid and a defect in sperm morphology. Other studies have demonstrated an increase in the fraction of "round-headed sperm", as well as an increase in the fraction of sperm with disomy (aneuploidy) in the smoking population, compared to non-smokers (WONG, 2000).

One of the changes in the normal morphology of sperm in men subjected to tobacco smoke is the complete disappearance of one or more of the nine pairs of filaments or microtubules in the center of the tail, thus causing a serious morphological deficiency (OLIVEIRA, 2010).

MOTILITY

Several studies indicate that this is the first defect to appear in a smoker's sperm and that it appears before the defect in sperm concentration, volume and density. Tobacco smoke causes changes in the normal constitution of the sperm tail, causing a

reduction in sperm mobility and consequently a reduction in the fertility rate, as is observed in people with asthenospermia (GAUR, TALEKAR, PATHAK, 2007).

The damage caused by oxidative stress, more particularly by ROS, in the sperm membrane is responsible for the permanent loss of normal sperm mobility (COLAGAR, JORSARAEE, MARZONY, 2007). There is an 18.7% decrease in total motility in sperm from smokers compared to non-smokers. The fact that smokers have a high level of cadmium in their sperm can cause toxic effects in such a way that in themselves they can cause asthenospermia (KUMOSANI et al., 2008).

A cotinine concentration in seminal fluid of 400-800 ng/mL impairs sperm mobility as well as cell membrane function. By damaging the cell membrane, high levels of cotinine contribute to a defect in the cascade of events that constitute sperm activation and consequently to its ability to fertilize the egg, as well as to a defect in its mobility. Considering that the concentration of cotinine in the sperm of almost all smokers is greater than 400ng/ml, it can be stated that this contributes to a loss of fertilization capacity in smokers (SOFIKITIS, 2000).

CONSEQUENCES ON SPERM DENSITY AND VOLUME

Several studies indicate that the first defect to appear in smokers' sperm is a decrease in volume, which must appear before the defect in sperm concentration, mobility and morphology (OLIVEIRA, 2010).

There is evidence that smokers may have a more active sex life than non-smokers, possibly due to the fact that they have slightly higher T levels. Thus, the frequency of ejaculations is greater. This fact alone can reduce the density and volume of sperm in smokers (PASQUALOTTO et. al, 2006).

Cigarette smoking is associated with low sperm density and a decrease in sperm volume compared to non-smokers of the same age. It has been shown that there is a 13-17% decrease in sperm density in smokers, although it is not known for sure what daily dose of cigarettes is necessary to smoke for this decrease in density to occur (OLIVEIRA, 2010).

It was also demonstrated that there is a 29% decrease in the total number of sperm and a 19% decrease in sperm concentration in men who smoke more than 20 cigarettes per day compared to non-smokers (RAMLAU-HANSEN et. al, 2007).

It is known that the damage caused by excess ROS in sperm can cause apoptosis in germ cells, which will lead to a decrease in the number of sperms produced and consequently to a decrease in sperm volume (SALEH et. al, 2002).

EFFECT OF TOBACCO ON SEMINAL FLUID CONSTITUENTS

One of the notable particularities of human seminal fluid is its high concentration of some organic substances and enzymes, such as fructose, citric acid, glycerophosphocholine, acid phosphatase, 5'-nucleotidase and vitamin C (OLIVEIRA, 2010).

VITAMIN C

Cigarette smoking is associated with a 107% increase in ROS levels, and a 10-point decrease in the antioxidant capacity of ROS (SALEH et. al, 2002), which together with the addition of ROS through exogenous sources creates a corresponding requirement for an increase in Vitamin C, since this is an essential antioxidant element in seminal fluid. Studies have shown that in men, tobacco smoke is associated with a 20-40% decrease in Vitamin C in the blood, a fact that leads us to conclude that antioxidant compensation by Vitamin C is not carried out, thus giving rise to a greater number of changes in sperm quality.

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Other studies have suggested that Vitamin C supplements in men who smoke vigorously improved sperm quality, supporting the previous finding (MOSTAFA et. al, 2006).

ROS AND LEUKOCYTES

Cigarette smoking is correlated with increased levels of sperm oxidative stress, due to a decrease in the total antioxidant capacity of ROS. Recent studies indicate that the total antioxidant capacity of ROS decreases as a result of an imbalance between ROS levels and the antioxidant species present in sperm. The significant reduction in total antioxidant capacity associated with cigarette smoking can be attributed to the increase in ROS in seminal fluid (SALEH et. al, 2002).

An additional factor that may explain why the sperm of men exposed to cigarette smoke have high levels of ROS may be the fact that tobacco itself contains high values of ROS, such as superoxide anion, hydrogen and hydrogen radicals. The fact that oxidative stress levels are increased due to cigarette smoking has significance and may have important implications for the fertilization potential of infertile men (SALEH et. al, 2002).

The link between cigarette smoking and increased seminal ROS levels may be related to the significant increase in the concentration of leukocytes in the sperm of infertile men. One possible explanation is that the metabolites present in cigarette smoke can induce an inflammatory reaction in the male genital canal, with a subsequent release of chemical mediators of inflammation. Inflammatory mediators, such as IL-6 and IL-8, can recruit and activate leukocytes. In turn, active leukocytes can generate high concentrations of ROS in seminal fluid, which can exceed the antioxidant capacity, resulting in oxidative stress. Another possible explanation is the fact that the toxic components of cigarette smoke can disrupt spermatogenesis, resulting in the production of defective sperm. In this case, leukocytes infiltrate the male reproductive system to eliminate defective sperm by phagocytosis (SALEH et. al, 2002).

MATERIAL AND METHODS

The work was submitted for approval by the Research Ethics Committee of the José do Rosário Vellano Câmpus/Alfenas University, with protocol number 253.429. The ethical principles complied with Resolution No. 196/96 of the National Health Council. The volunteers agreed to the Free and Informed Consent Form, presented and signed prior to the collection.

SAMPLE COLLECTION

To carry out this quantitative and qualitative experimental study, 24 semen samples were collected, 12 samples from heavy smoker volunteers and 12 samples from non-smoking volunteers.

The volunteer received a bottle suitable for collecting semen. He was informed about the appropriate method and recommendations for collection, such as avoiding urinating 1 hour before semen collection, washing hands and penis before collection, collecting material from a single ejaculation, avoiding loss of material, capping the bottle immediately after collection. obtaining the material, do not refrigerate the semen and send the material to the analysis site immediately after collection. The semen samples were collected in a laboratory environment through self-masturbation. Samples were kept at room temperature and processed within a maximum of 30 minutes after collection.

SAMPLE ANALYSIS

The analysis of seminal parameters was carried out at the Cytology and Clinical Hematology Laboratory of UNIFENAS in accordance with the WHO normality criteria. Relevant data such as the period of abstinence, date and time of collection, as well as the period of interval between collection and examination, and medications in use were recorded through a specific questionnaire, which is extremely important given that it can significantly influence the sperm count.

Semen characteristics were analyzed regarding macroscopic aspects (volume, appearance, color, odor, coagulation, pH, viscosity, liquefaction) and microscopic aspects (sperm concentration, mobility and morphology).

DATA ANALYSIS

The analysis of seminal parameters was carried out according to the normality criteria of the (WHO) World Health Organization: Volume - normal 2 and 6 ml, abnormal < 2; concentration - < 20 (x106/ml), abnormal < 20 (x106/ml); Mobility - grade 2+3; Morphology - > 15% normal spermatozoa; Abnormal < 15% abnormal.

As it is considered relevant for the interpretation of results, the following subgroups will be considered when evaluating changes in concentration:

- Moderate to severe change if concentration is less than 10 x106/ml
- Slight change if concentration is equal to or greater than 10x106/ml and less than 20x106/ml

Two spermograms will be performed for subjects who show any change in the semen, collections will be at an interval of at least 7 days and a maximum of 90 days. The arithmetic mean will be calculated for each of the seminal parameters evaluated.

For the study, the population was divided into two groups: smokers (consumption equal to or greater than 20 cigarettes/day, have been consuming tobacco for at least 2 years) and non-smokers (never used tobacco). Data will be obtained through a questionnaire that will be applied prior to sample collection.

RISKS

Volunteers may feel slight discomfort due to the way the sample is obtained and when filling out the questionnaire. There are no health risks, obtaining a semen sample is not an invasive act.

Study participants will provide written consent and answer a questionnaire that will be read and completed in a restricted area to avoid further embarrassment and/or discomfort.

During the entire procedure, from receiving the sample to issuing the results, researchers will follow biosafety standards recommended by ANVISA (National Health Surveillance Agency), including the use of personal protective equipment -PPE, technical procedures, biosafety precautions and conduct to be adopted in accidents.

INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA

Heavy smokers will be included in this work, that is, subjects who have been chronically consuming tobacco for more than 2 years, with an average of 20 cigarettes per day. Age range between 18 and 40 years old and sexual abstinence of 5 days.

EXCLUSION CRITERIA

Samples will be excluded from:

- Non-smoking subjects.
- Subjects who smoke sporadically.
- Light smokers (if tobacco consumption is less than 20 cigarettes/day).
- The existence of a personal history

of drug use, surgery or previous genital trauma and organic pathologies (for example, endocrine or infectious pathology or causes of obstructive azoospermia) that could cause changes in seminal parameters.

- Subjects who are not undergoing sexual abstinence for 5 days.

BENEFÍTS

To carry out this work aims to contribute to highlighting the harmful effects of tobacco and its relationship with male infertility. Participants also enjoy the results of their personal diagnosis and will be advised on the harm caused by tobacco use in relation to their fertility.

RESULTS AND DISCUSSIONS

The present study found support for the importance of the high incidence of smoking among the population. The importance of evaluating the semen quality of smokers is due to the fact that it is related to the high rate of infertility in chronic smokers. This is combined with the fact that in recent years the quality of human sperm and their potential fertility have undergone changes. Thus, infertility stands out as a major problem for society, as there is important evidence suggesting changes in semen quality, partly as a result of environmental ecotoxicity.

In this research it was observed that the presence of normal viscosity in the semen occurs in 100% of the Control Group, while in the Smokers Group it occurs in 83.50%. According to Marinelli (2007), viscosity corresponds to the formation and fall of a drop of semen, equivalent to 20 seconds, but does not present a relevant clinical correlation.

Observa-se que o Odor e Aspecto de ambos os Grupos foram de acordo com os valores de referência estabelecidos pela OMS, *Sui generis* e Homogêneo, respectivamente.

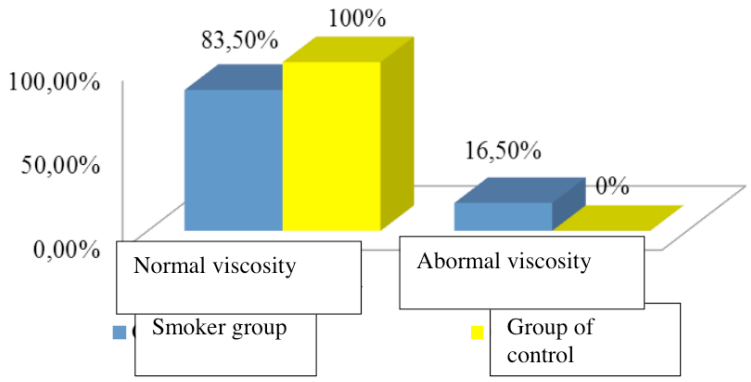
Through macroscopic analysis it can be observed that 100% of the Smoking Group had Opalescent White sperm, according to the reference parameters established by the WHO. However, 16.50% of the Control Group showed a yellowish color, which was due to the ingestion of the medication Annita. According to its leaflet, Annita can produce a change in the color of physiological fluids to greenish-yellow, without any clinical significance. This is due to the coloring of some of the components of the formula.

The pH of both groups is within the reference values established by the WHO. The Smokers Group had a slightly alkaline pH compared to the Control Group, but this did not cause any clinical changes.

It is observed, through macroscopic analysis, that only 16.50% of the Smokers Group presented Secondary Liquefaction, while 100% of the Control Group presented Liquefaction according to the parameters. In the Smokers Group, 67% achieved Primary Liquefaction and 16.50% Secondary Liquefaction. Analyzing semen coagulation, it was found that 100% of the Control Group had normal coagulation, compared to only 33.50% of the Smoking Group.

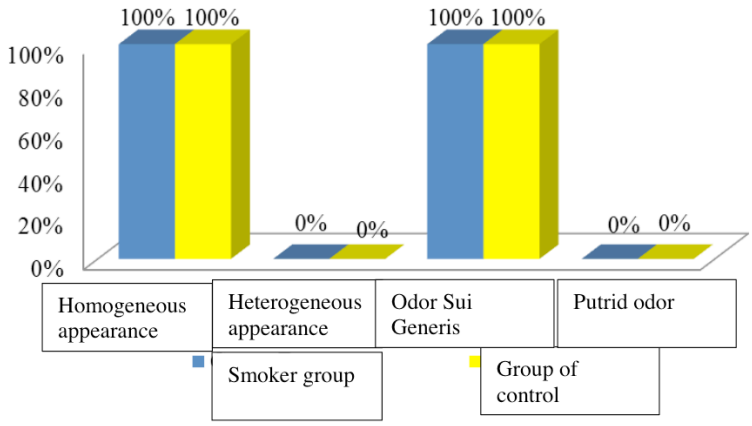
According to Marinelli (2007), after ejaculation, through the action of enzymes secreted by the seminal vesicles, the semen coagulates. The absence of clotting may indicate the absence or deficiency of these glands. On average, after approximately 20 to 25 minutes, sperm liquefaction occurs, which depends on proteolytic enzymes (proteases) secreted by the prostate. Alterations in both coagulation and liquefaction can determine changes in sperm motility and density.

Through microscopic analysis it was concluded that only 16% of the Smokers Group presented motility A, compared to 32% of the Control Group. In Motility B, both Groups presented the same value, 32%.



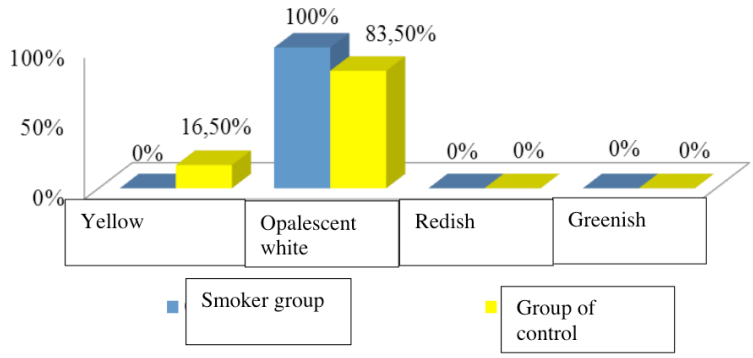
GRAPHIC 1 - Assessment of macroscopic aspects regarding the viscosity of human semen in a population in the south of Minas Gerais.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.



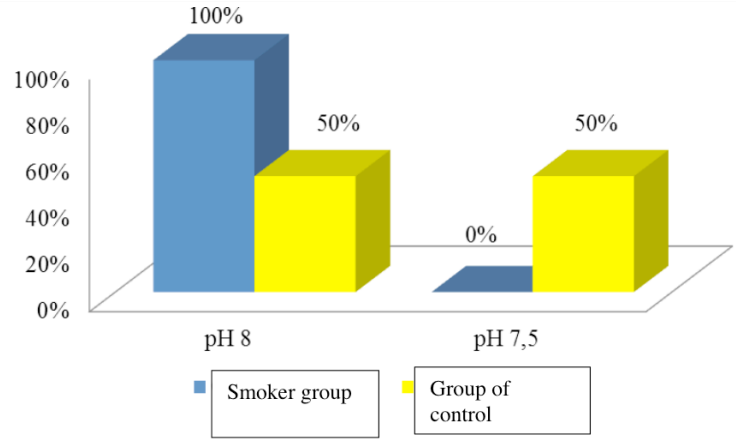
GRAPHIC 2 - Assessment of macroscopic aspects regarding the appearance and odor of human semen in a population in the south of Minas Gerais.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.



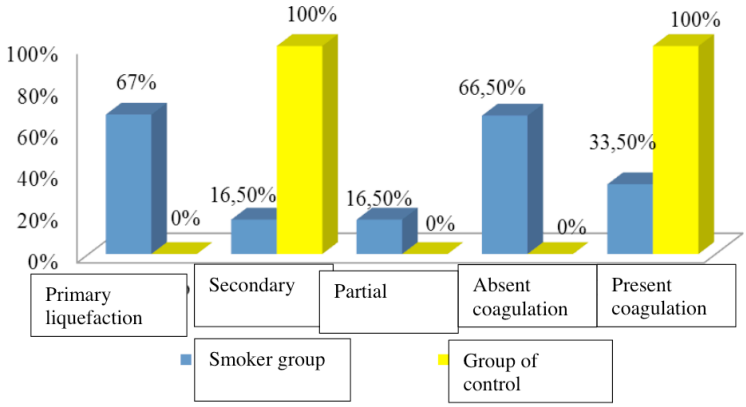
GRAPHIC 3 - Assessment of macroscopic aspects regarding the color of human semen in a population in the south of Minas Gerais.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.



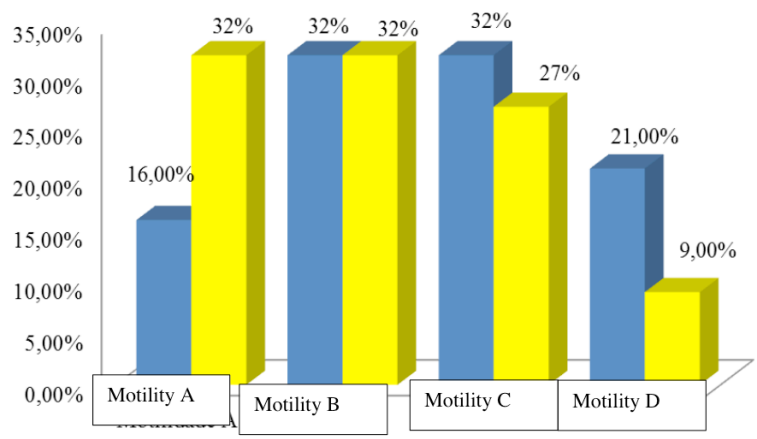
GRAPHIC 4 - Assessment of macroscopic aspects regarding the pH of human semen in a population from the south of Minas Gerais.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.



GRAPHIC 5 - Assessment of macroscopic aspects relating to liquefaction and coagulation of human semen in a population in the south of Minas Gerais.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.



GRAPHIC 6 - Assessment of microscopic aspects relating to motility and agglutination of human semen in a population in the south of Minas Gerais.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.

The Smoker Group had a higher number of C and D motilities, 32% and 21% respectively. Against 27% and 9%, respectively, of the Control Group.

Several studies indicate that the defect in motility is the first to appear in a smoker's sperm and that it appears before the defect in sperm concentration, volume and density (GAUR; TALEKAR; PATHAK, 2007). According to Oliveira, 2010 deficiencies in the shape of sperm can cause a decrease in mobility, meaning that the time it takes for them to travel through the female reproductive system to the fertilization area is longer. Studies indicate that tobacco smoke causes changes in the normal constitution of the sperm tail, causing a reduction in sperm mobility and consequently a reduction in the fertility rate, as observed in people with asthenospermia.

The damage caused by oxidative stress, more particularly by ROS, in the sperm membrane is responsible for the permanent loss of normal sperm mobility (COLAGAR; JORSARAEE; MARZONY, 2007). According to Kumosani et. Al, 2008, the fact that smokers have a high level of cadmium in their sperm can cause toxic effects in such a way that in themselves they can cause asthenospermia.

It was observed that 100% of the individuals participating in the Control Group had 100%, 95% and 90% sperm vitality, compared to 0% in the Smokers Group.

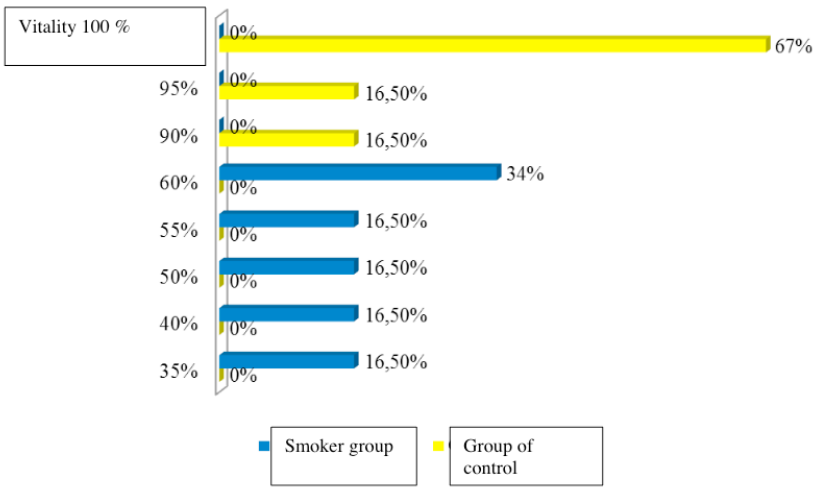
The Smokers Group presented values well below the sperm vitality scale when compared to the Control Group. Obtaining 60% vitality 34% of participants, 55% vitality 16.50% of individuals, 50% vitality 16.50% of smokers, and 40% and 35% vitality 33% of participants. The latter presented abnormal values according to the WHO.

Through the analysis of seminal volume, it can be observed that both groups are in accordance with the reference values established by the WHO.

CONCLUSION

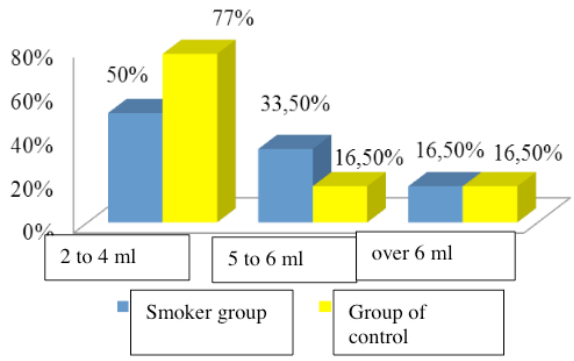
It is concluded from this work that smoking affects male fertility, as there was a higher incidence of changes in smokers' samples: viscosity, coagulation, liquefaction, motility, vitality and concentration.

If in the general population the changes caused by smoking in fertility may have little significance for overall fertility, these same changes may have a much more significant weight when applied to the universe of infertile couples. Furthermore, the couple's motivation in this situation can be a unique opportunity to acquire a healthier lifestyle whose benefits go far beyond the eventual improvement in fertility.



GRAPHIC 7 - Evaluation of microscopic aspects regarding the vitality of human semen in a population in the south of Minas Gerais.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.



GRAPH 8 - Assessment of macroscopic aspects regarding the volume of human semen in a population in the south of Minas Gerais.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.

SMOKER GROUP		**GROUP OF CONTROLS**	
PZ/m²	**%**	**SPZ/m²**	**%**
5 to 20x10⁶	50%	30 to 40x10⁶	50%
25 to 55x10⁶	33,50%	40 to 50x10⁶	33,50%
55 to 100x10⁶	16,50%	50 to 60x10⁶	16,50%
SPZ in Ejaculate		**SPZ in Ejaculate**	
10 to 150x10⁶	50%	100 to 200x10⁶	83,50%
200 to 350x10⁶	33,50%	200 to 300x10⁶	16,50%
350 to 500x10⁶	16,50%		

TABLE 1: Assessment of microscopic aspects regarding the concentration of human semen in a population in the south of Minas Gerais.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.

SMOKER GROUP					
Has the person ever had a spermogram?	YES	0%	NO	**100%**	
Is the person in sexual abstinence?	Less than 5 days	67%	5 days	**16,5%**	Over 5 Days **16,5%**
The person has an STD	YES	0%	NO	**100%**	
There is a history of infertility in the family	YES	0%	NO	**100%**	
The person currently presents any symptoms of genital infection	YES	0%	NO	**100%**	
The person has a pathology	YES	16,5%	NO	**83,5%**	
The person uses medication	YES	33%	NO	**67%**	
The person has an active sex life	YES	83,5%	NO	**16,5%**	
Fixed partners	YES	33%	NO	**67%**	
The person is a tobacco cigarette smoker	YES		NO	**0%**	
	100%				
A quanto tempo faz uso de cigarro de tabaco	Less than	**0%**	Over 1 year	**100%**	
How many cigarettes are consumed	Over 20	**50%**	Less than 20	**50%**	
The person has no children	YES	**0%**	NO	**100%**	

Note: Medications used: Valdoxan, Patz SL and Hydroxine

TABLE 2: Source of information and adequacy on human semen from a smoking population in the south of Minas Gerais, 2013. N =

CONTROL GROUP					
Has the person ever had a spermogram?	YES	**0%**	NO	100%	
Has the person been sexually abstinent?	Less than 5 days	**67%**	5 days	16,5%	Over 5 Days **16,5%**
The person has an STD	YES	**0**%	NO	100%	
There is a history of infertility in the family	YES	**0**%	NO	100%	
The person currently presents genital infection symptom	YES	**0**%	NO	100%	
Holder of some pathology	YES	**16,5**%	NO	83,5%	
The person uses medication	YES	**16,5**%	NO	83,5%	
The person has an active sex life	YES	**83,5**%	NO	16,5%	
Fixed partners	YES	**33**%	NO	67%	

Note: Medicines used Seretide, Flixionase and Annita

TABLE 3: Source of information and adequacy on human semen from a control group population in the south of Minas Gerais, 2022. N = 24.

Source: Clinical Cytology and Hematology Laboratory (UNIFENAS), 2022.

REFERENCES

BELCHEVA, A; IVANOVA-KICHEVA, M; TZVETKOVA, P; MARINOV, M. Effects of cigarette smoking on sperm plasma membrane integrity and DNA fragmentation. **Int J Androl**. 2004;27(5):296-300.

BRUGO, O. S.; CHILLIK, C.; KOPELMAN, S. Definition and causes of infertility. **Reprod; Biomed Online**. 2001;2(1):41-53.

COELHO, C.; JÚLIO C.; SILVA G,; NEVES A. Tabaco e infertilidade masculina. Estudo retrospectivo em casos inférteis. **Acta Med Port**. 2009; 22: 753-758.

COLAGAR, A.H; JORSARAEE, G.A.; MARZONY , E.T. Cigarette smoking and the risk if male infertility. **Pak J Biol Sci**. 2007;10(21):3870-4.

Esperança Pina JA. Anatomia Humana dos Órgãos. Lisboa (Portugal). Lidel; 2004.

GAUR, D.S; TALEKAR, M.; PATHAK, V.P. Effect of cigarette smoking on semen quality of infertilemen. **Singapore Med J**. 2007;48(2):119-23.

GUYTON, A.C; HALL, J.E. **Tratado de Fisiologia Médica**. 10ª ed. Rio de Janeiro (RJ). Guanabara Koogan SA; 2002.

KUMOSANI TA, E.M.F; JONAID, A.A; ABDULJABAR, H.S. The influence of smoking on semen quality, seminal microelements and Ca2+-ATPase activity among infertile and fertile men. **Clin Biochem** 2008;41(14-15):1199-203.

MOSTAFA, T.; TAWADROUS, G.; ROAIA, M.M.; AMER, M.K.; KADER, R.A.; AZIZ, A. Effect of smoking on seminal plasma ascorbic acid in fertile and fertile males. Andrologia 2006;38(6):221-4.

OLIVEIRA, ÁLVARO ANDRÉ VILELA . Efeito do Consumo do Tabaco na Fertilidade Masculina. Faculdade de ciências da saúde. Universidade da Beira interior Covilhã/Portugal. [Dissertação de Mestrado]. Covilhã, 2010.

PASQUALOTTO, F.F.; SOBREIRO, B.P.; HALLAK, J.; PASQUALOTTO, E.B.; LUCON, A.M. Cigarette smoking is related to a decrease in semen volume in a population of fertile men. **BJU Int**. 2006;97(2):324-6.

PASQUALOTTO, F.F; LUCON, A.M.; SOBREIRO, B.P.; PASQUALOTTO, E.B.; ARAP S. Effects of medical therapy, alcohol, smoking, and endocrine disruptors on male infertility. **Rev Hosp Clin Fac Med**. São Paulo 2006;59(6):375-82.

SALEH RA, A. A.; SHARMA, R.K.; NELSON, D.R.; THOMAS, A.J.J. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. **Fertil Steril**. 2002;78(3):491-9. SHEFI, S.; TUREK, P.J. Definition and current evaluation of subfertile men. **Int Braz J Urol** 2006;32(4):385-97

SHI Q, K. E.; BARCLAY, L.; HOANG, T.; RADEMAKER, A.; MARTIN, R. Cigarette smoking and aneuploidy in human sperm. **Mol Reprod Dev** 2001;59(4):417-21.

SOFIKITIS, N.; TAKENAKA, M.; KANAKAS, N.; PAPADOPOULOS, H.; YAMAMOTO, Y.; DRAKAKIS, P., MIYAGAWA, I. Effects of cotinine on sperm motility, membrane function, and fertilizing capacity in vitro. **Urol Res** 2000;28(6):370-5.

RAMLAU, H.C.H; THULSTRUP, A.M.; AGGERHOLM, A.S; JENSEN MS, T.G.; BONDE, J.P. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. Hum Reprod 2007;22(1):188-96.

TRUMMER, H.; HABERMANN, H.; HAAS, J.; PUMMER, K. The impact of cigarette smoking on human semen parameters and hormones. **Hum Reprod** 2002;17(6):1554-9.

Whorld Health Organization, 2004; Acessado em 17 de julho de 2013: <URL:http://www.who.int/mediacentre/factsheets/fs310/en/index.html>

Whorld Health Organization, 2012; Acessado em 17 de julho de 2013: <URL:http://www.who.int/topics/tobacco/facts/en/index.html>

WONG WY, T.C.M.; MERKUS, H.M.; ZIELHUIS, G.A.; DOESBURG, W.H.; STEEGERS, T.R.P. Cigarette smoking and the risk of male factor subfertility: minor association between cotinine in seminal plasma and semen morphology. Fertil Steril. 2000;74(5):930-5.

ANNEX

UNIVERSIDADE JOSÉ DO ROSÁRIO VELLANO UNIFENAS- ALFENAS
FREE AND INFORMED CONSENT TERMS

INVITATION TO PARTICIPATE IN THE RESEARCH PROJECT: "EFFECTS OF CHRONIC TOBACCO CONSUMPTION ON MALE FERTILITY"

Responsible Researcher:

Léo Pedro Rufino, sob orientação da Professora Alessandra dos Santos Danziger Silvério

Institution to which the Principal Researcher belongs:

Universidade José do Rosário Vellano – UNIFENAS, Alfenas-MG

Telephone: (035) 98472-5606

Volunteer name:

....
.....

Age years.
Id card, number.....

You are being invited to participate in the research project "EFFECTS OF CHRONIC TOBACCO CONSUMPTION ON MALE FERTILITY" by

responsibility of researcher Léo Pedro Rufino, under the guidance of Professor Alessandra dos Santos Danziger Silvério.

The main objective of the work is to evaluate the effect of tobacco on seminal parameters. Associate smoking with likely changes in semen quality, such as density, mobility and the presence of anomalous forms. Advise the subject regarding the harm caused by chronic tobacco use in relation to their health, especially the fertility mechanism.

It will be necessary to collect a SEMEN sample obtained by SELF-MASTURBATION, collected in a laboratory environment, in sexual abstinence (not having sexual intercourse) of 5

days. The sample will be kept at room temperature and must be analyzed within a maximum of 30 minutes after collection.

Your participation is voluntary and may be withdrawn at any time, without harm to the treatment community. We guarantee the confidentiality of the information generated and the privacy of the research subject. You will not incur any type of expense while participating in the research.

Procedures to be used:

If you agree to participate in the research, the only procedure carried out will be collection of a SEMEN sample obtained by SELF-MASTURBATION, in the laboratory, you must not have sexual intercourse 5 days before semen collection. Collecting this semen is important so that the necessary tests can be carried out. There is no risk to your health associated with collecting this sample, what may occur is psychological discomfort regarding the type of collection.

Alfenas,.....de 2013.

.....

Léo Pedro Rufino; Alessandra dos Santos Danziger Silvério

I,.....
...,ID card, ...number:..... I declare that I have been informed and agree to participate, as a volunteer, in the research project described above.

.....

.....

Patient's name and signature

QUIZ

PARTICIPATION IN THE RESEARCH PROJECT: "EFFECTS OF CHRONIC TOBACCO CONSUMPTION ON MALE FERTILITY"

Age:_____years DATE:_/_____/_____

1) Have you ever done a SPERMOGRAM? () Yes No
If yes, how long? () less than 6 months ago () 1 year ago () More than 1 year ago

2) Have been sexually abstinent for: () less than 5 days () 5 days () more than 5 days

3) Have you ever had a genital infection (STD – Sexually transmitted disease)? () Yes No
If so, did you seek medical advice or undergo any treatment? () Yes () No Was a diagnosis made (do you know what the disease is)?

4) Is there a history of infertility in the family? () Yes No
What type?

5) Are you currently experiencing any symptoms suggestive of a genital infection? () Yes No
Quote:

6) Have you experienced significant weight loss in recent months? () Yes No

7) Do you have any chronic pathology? () Yes No
Quote:

8) Do you use any ongoing medication? () Yes No
Which one(s)?

9) Do you have an active sex life? ( ) Yes No

10) Sexual partners: () Permanent () More than one

11) Sexual partners: ( ) Permanent ( ) More than one

12) Are you a tobacco cigarette smoker? ( ) Yes No

13) How long have you been using tobacco cigarettes? () Less than 1 year ( ) More than 2 years

14) How many tobacco cigarettes are consumed per day? () More than twenty cigarettes ( ) Less than 20 cigarettes

15) Do you have children? () Yes () No ( ) 1 child ( ) More than 1 child