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NUTRITION AND FERTILITY

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1. SUMMARY AND KEY WORDS

Infertility is a disease that currently affects 1 out of 6 citizens worldwide in their fertile lifespan. The main strategy to treat it is the use of ART (Assisted Reproductive Technology), which has a huge impact and economic, emotional and psychologic costs on society. It is because of this that other approaches are being studied to prevent infertility and help improve the fertility taxes, with a special focus on promoting changes in both lifestyle and nutrition patterns. There is literature demonstrating that actions targeted to improve the overall diet quality help increase fertility, whereas unhealthy diets are detrimental for fertility in both men and women. There is evidence showing positive effects of nutrients such as n-3 Polyunsaturated Fatty Acids (PUFAs) and folic acid and a negative impact of *Trans* Fatty Acids. However, evidence regarding the benefits of other nutrients such as n-6 PUFAs, monounsaturated and saturated fats, vitamins (D, E, C, A) and minerals and trace elements (Fe, Zn, I, Se, Ca) is still limited. Further investigations are needed to clarify the topic and elaborate specific recommendations and clinical guidelines to boost fertility.

Key words: fertility, sterility, infertility, nutrition, diet.

RESUM I PARAULES CLAU

La infertilitat és una patologia que actualment afecta a nivell mundial a 1 de cada 6 ciutadans en la seva etapa fèrtil. La principal estratègia per tractar-la són les tècniques de reproducció assistida (en anglès ART- *Assisted Reproductive Techology*), les quals tenen un elevat impacte i cost en la societat, tant a nivell econòmic com a nivell emocional i psicològic. És per això que s'estan estudiant altres estratègies per prevenir la infertilitat i ajudar a millorar les taxes de fertilitat, amb un èmfasi especial en la promoció de canvis en l'estil de vida i en els patrons alimentaris i dietètics. Hi ha literatura que demostra que accions orientades a seguir una dieta més saludable ajuden a millorar la fertilitat, mentre que patrons dietètics poc saludables són perjudicials tant en l'home com en la dona. Hi ha evidència dels efectes positius de nutrients com els àcids grassos poliinsaturats n-3 i l'àcid fòlic i també de l'impacte negatiu dels àcids grassos *trans*. Tot i així, l'evidència sobre els beneficis d'altres nutrients com els àcids grassos poliinsaturats n-6, monoinsaturats i saturats, les vitamines (D, E, C, A) i els minerals i elements traça (Fe, Zn, I, Se, Ca) és molt limitada. En el futur calen més investigacions per clarificar aquest tema i per poder elaborar recomanacions específiques i guies clíniques per potenciar la fertilitat.

Paraules clau: fertilitat, esterilitat, infertilitat, nutrició, dieta.

2. INTRODUCTION

2.1. Physiology of the reproductive system

2.1.1. Female reproductive system

The feminine reproductive system includes two ovaries (female gonads), two Fallopian tubes (also called uterine tubes; they contain the infundibulum with fimbriae, the ampulla and the isthmus), the uterus (that includes fundus, the uterine body and the cervix- the lowest part of the uterus that connects the uterine cavity with the lumen of the vagina) and the vagina. It also has accessory glands called the Bartholin's glands (1,2). *(Figure 1)*

The functional structure of the ovaries are the ovary follicles - they contain a primary oocyte covered by follicular cells - which have endocrine functions and are also responsible for the gametogenesis process (2). Unlike masculine reproductive system, the feminine one undergoes cyclic changes, as it regularly prepares for fertilization and pregnancy. It is called the menstrual cycle and, on average, it lasts 28 days from the beginning of one cycle to the initiation of the next one (1).

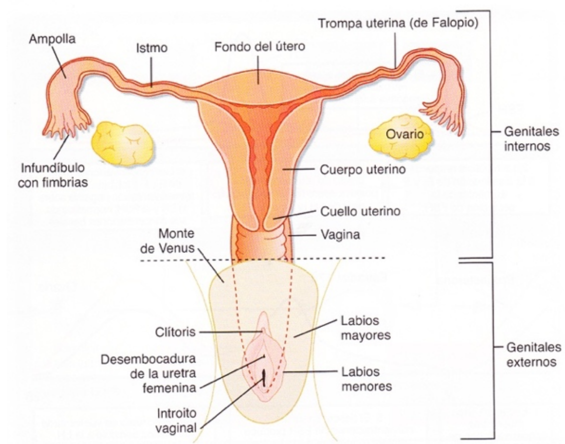

Figure 1. *Female reproductive system. Extracted from (2).*

If fertilization has not happened, menstruation occurs, which is a periodic vaginal hemorrhage that mainly contains blood, prostaglandins and endometrium tissue from the uterus. On average, the menstrual phase lasts 3 to 5 days, although it can go up to 8 days on healthy women. The amount of blood lost is approximately 30 mL, although up to 80 mL is considered normal (1). The menstrual cycle includes the ovary cycle and the uterine cycle. The ovary cycle is divided in three different stages - the follicular phase, ovulation and the luteal phase. At the same time, the uterus undergoes three different phases – the menstrual, the proliferative and the secretory ones (1). After birth, girls have around 7 million primordial follicles, which have a single layer of cells and contain the primary oocyte. In puberty, only around 300,000 primordial follicles are left (3).

During the entire fertile lifespan in women, at the beginning of every menstrual cycle, there is an increase on the FSH and LH levels, which leads to the development of 6 to 12 primordial follicles into primary follicles, which contain the primary oocyte and an outer layer of cells called granulosa cells. Afterwards, primary follicles evolve into secondary follicles through a process that involves the creation of the theca (a mass of cells that surrounds the granulosa cells), which is divided into the inner theca - secretes estrogens and progestogens – and the outer theca – a connective tissue layer that protects the follicle structure (2-4).

After this, the granulosa cells start to produce follicular liquid rich in nutrients and estrogens, which accumulates in a cavity called antrum and surrounds the primary oocyte. The secondary follicle also contains the zona pellucida, which is a glycoprotein layer surrounding the primary oocyte. Finally, around the zona pellucida there is an arrangement of granulosa cells that form what is called the Corona radiata (4).

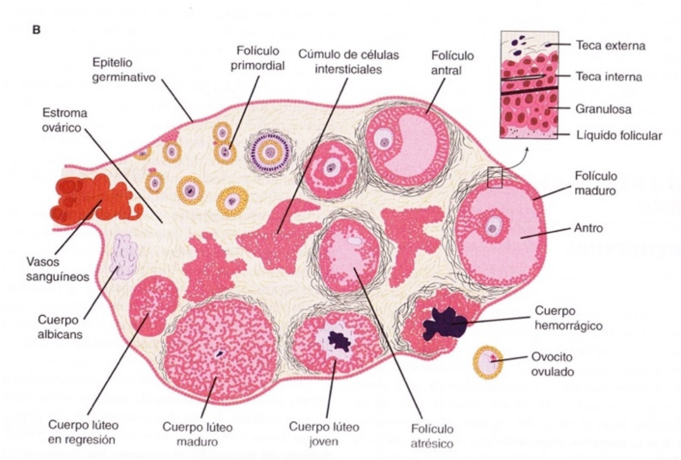

***Figure 2.** Follicle development in the ovary. Extracted from (1).*

Each menstrual cycle there is growth of multiple follicles but by day 6 or 7 only one – called the mature or dominant follicle - rapidly grows and it contains the secondary oocyte, which produces ovulation. The other 5 to 11 follicles degenerate in an apoptosis process called follicular atresia (1,3). *(Figure 2)*

During the ovary cycle, the follicular phase occurs from day 1 to day 13 and the development of the secondary follicles happens on the first 6 days. Afterwards, from day 7 to day 13, the secondary follicle evolves to Graafian follicle. The main source of estrogens are the granulosa cells, although the theca inner cells are also necessary for their production (1,4).

On day 14th ovulation happens: the secondary oocyte with its corona radiata is expelled out of the follicle to the Fallopian tubes. If fertilization happens, the fertilized egg will be implanted in the uterus. Otherwise, it will be discharged through the vagina during menstruation (1,4).

Finally, from day 15th to day 28th (end of the cycle) the luteal phase takes place, in which the broken follicle is filled with blood, both the granulosa and theca cells rapidly grow, and luteal cells proliferate, leading to the creation of the corpus luteum. Luteal cells, which are lipid rich and give a characteristic yellowish color, secrete both estrogens and progestogens. If ovum fertilization happens, the corpus luteum is maintained thanks to the hCG (human chorionic gonadotropin) until the end of the pregnancy. Otherwise, the corpus luteum degenerates into fibrous scar tissue called the corpus albicans, which is going to be discharged (1,4).

Regarding the uterine changes, during the menstrual phase (day 1 to 5 of the menstrual cycle) the endometrium thickness is quite small, as almost all the layers and tissue created have already been discharged through the vagina (1,2,4).

Afterwards, the proliferative phase (also called preovulatory phase) starts, as the estrogens produced by the ovary follicles promote the creation of a new endometrium layer, with a remarkable thickening during the 14 and 15 day. The ovulation usually happens around day 14th and, after it, the secretory phase starts and lasts until the 28th day. During this stage there is a huge increase on the vascularization thanks to both the estrogens and progestogens produced by the corpus luteum (1,2,4). *(Figure 3)*

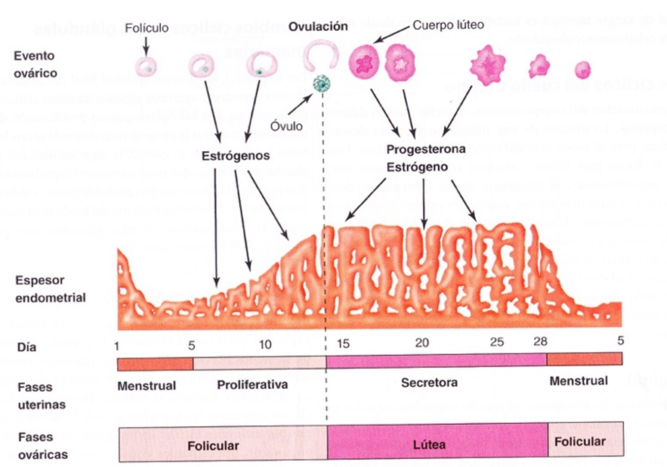

Figure 3. *Ovary and uterus changes during the menstruation cycle. Extracted from (1).*

Finally, if no fertilization occurs, the secretory phase takes place, in which the corpus luteum disappears and there is a decline on the estrogens and progestogens levels that leads to the endometrium layers' discharge. This is the starting point (day 1) of the new menstruation cycle (1,2,4).

Sometimes, there can be anovulatory cycles, in which no ovulation occurs and no corpus luteum develops. However, the estrogens would promote the endometrium growth and its posterior discharge (1).

Estrogens are sexual hormones produced by the ovary follicles, the corpus luteum and the placenta. They promote ovarian follicle growth and increase the motility of the uterine tubes and they also participate on the cyclic changes that occur in the endometrium, cervix and vagina during the menstrual cycle (1,4). Progestogens are also sexual hormones produced by the corpus luteum, the placenta and the follicles (but in smaller quantities in comparison to the estrogens). They promote endometrium changes and also participate in the cyclic changes that occur in the uterus and the vagina during the menstrual cycle (1,4). Cholesterol is the precursor of both the estrogens and progestogens (1).

Ovary function is regulated by the hypothalamus-hypophysis-ovary axis through a negative feedback. The hypothalamus produces GnRH, which stimulates the LH (luteinizing hormone) and FSH (follicle-stimulating hormone) production in the adenohypophysis. FSH stimulates the granulosa cells, whereas LH stimulates both the granulosa and the theca inner cells. Then, granulosa cells secrete inhibin B, a substance that inhibits FSH production whereas the theca cells produce estrogens, which will inhibit LH, FSH and GnRH production (1,2,4). *(Figure 4)*

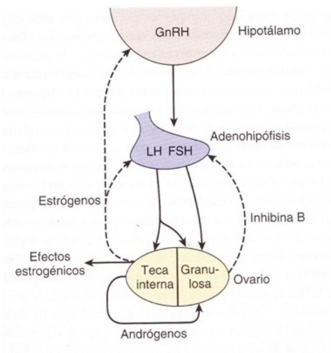

Figure 4. *Hypothalamus-hypophysis-ovary axis. Extracted from (1).*

2.1.2. Male reproductive system

The masculine reproductive system includes the testicles (male gonads), which are located inside the scrotum, and four ducts: the epididymis (head, body and tail), the vas deferens, the ejaculatory duct and the urethra. It also has three accessory glands: the seminal vesicles, the prostate gland and the Cowper's gland (1-4). *(Figure 5)*

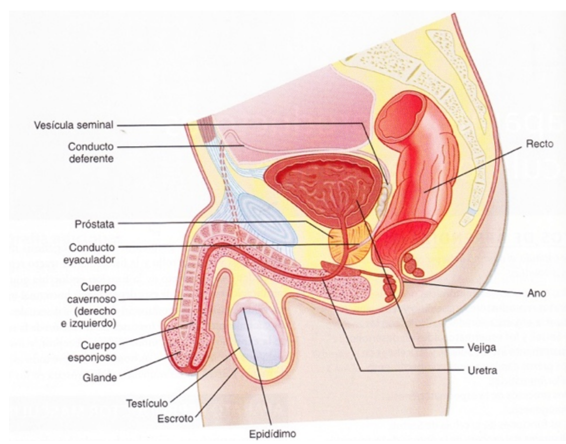

Figure 5. *Male reproductive system. Extracted from (2).*

The testicles have two main functions: the production of sexual cells in the process of spermatogenesis and the secretion of sexual hormones –big quantities of androgens, mainly testosterone, but also smaller amounts of estrogens (1).

The accessory glands secrete substances that mix with the spermatozoa during ejaculation to create the semen, which contains 10% of spermatozoa, 60% of the liquid from the seminal vesicles, 30% of the liquid from the prostate gland and small quantities of substances from the Cowper's gland (2,3).

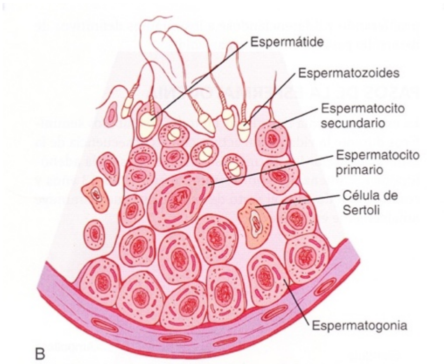

Figure 6. *Stages of the spermatozoa development. Extracted from (3).*

The spermatogenesis - process in which the spermatogonium (primitive germ cell) evolve into different stages (primary spermatocyte, secondary spermatocyte and spermatid) to end up developing the mature spermatozoa - occurs at the seminiferous tubules, which are little canaliculus located inside the testicles (1-4). This process duration is approximately 74 days (3). *(Figure 6)*

Once formed, the spermatozoa go through the rete testis to the efferent ducts to the epididymis head. Then, spermatozoa undergo a maturation process and become mobile once they arrive to the epididymis tail. Afterwards, they continue to the vas deferens and they may be stored more than one month in both the epididymis tail and the vas deferens lumen without losing their viability. Finally, they travel along the ejaculatory duct and arrive to the urethra and the prostate during ejaculation (1-4). *(Figure 7)*

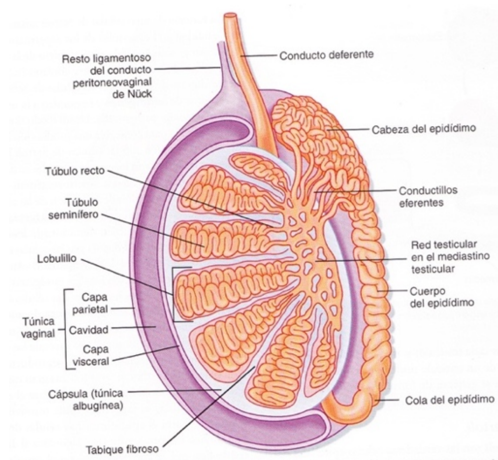

Figure 7. *Structure of the testicle. Extracted from (2).*

The seminiferous tubules contain two types of cells: the germ cells - which undergo maturation to produce the mature spermatozoon - and the Sertoli cells - which provide protection (through the tight junctions that form the blood-testis barrier) and nutrients (especially glycogen) for the spermatozoa formation. These cells also have an important endocrine function, as they produce the Anti-Müllerian hormone (AMH), which inhibits the development of the female reproductive system in the male body (1,2).

In between the seminiferous tubules Leydig cells are found, which produce testosterone (from cholesterol) to the blood. This sexual hormone participates in different processes in the reproductive system, including spermatogenesis maintenance, semen production and secondary sexual characters development (1,2). Testosterone also regulates the Sertoli cells function (2).

Each spermatozoon formed has some motility, but it is not until they go through the epididymis when they reach full ability to move. Spermatozoa contain a tail, which has a lot of mitochondria, and a head, that stores the DNA and is protected by the acrosome – a structure that contains big quantities of enzymatic substances (including the *hyaluronidase* – degrades proteoglycans, and the proteolytic enzymes – degrade proteins) that enable the acrosome reaction in the spermatozoa, which consists on the ovum outer layer's break down that will enable penetration and fertilization processes (1-3).

The endocrine function of the gonads is regulated by the hypothalamus-hypophysis-testicle axis through a negative feedback process (2,4). The hypothalamus produces GnRH, which stimulates the LH (luteinizing hormone) and FSH (follicle-stimulating hormone) production in the adenohypophysis. FSH stimulates the Sertoli cells, whereas LH stimulates the Leydig cells proliferation. Then, Sertoli cells secrete inhibin B, which selectively inhibits FSH production whereas the Leydig cells produce testosterone, which inhibits FSH, LH and GnRH production (1,2,4). *(Figure 8)*

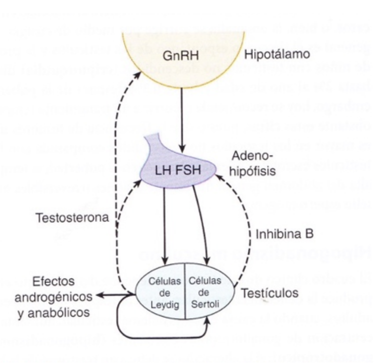

Figure 8. *Hypothalamus-hypophysis-testicles axis. Extracted from (1).*

2.2. Physiology of fertilization: ovum and spermatozoon role

During men ejaculation inside the women vagina during the sexual intercourse, there is a release of approximately 3,5 mL of semen. Although one single spermatozoon is necessary for fertilization, there are around 120 million spermatozoa/mL of semen, although this number can range from 35 to 200 million. Therefore, around 400 millions of spermatozoa are present in the quantity of semen ejaculated (3). The reduction of spermatozoa number in the semen is associated with infertility, as half of the men with 20-40 millions/mL and almost all of the men with less than 20 millions/mL of semen are sterile (1).

Sometimes, men with normal quantities of spermatozoa in the semen are sterile due to the presence of morphologic alterations in the spermatozoa, such as presence of two tails, two heads or abnormal shapes, as well as lack of motility (3).

After ejaculation, the spermatozoa go up through the uterus to the Fallopian tubes' ampulla thanks to the uterus' and Fallopian tubes' contractions, the prostaglandins of the semen and the oxytocin released by the feminine hypophysis (1,3). In the ampulla, their speed is reduced and they undergo *Capacitation*, which is the last step of the spermatozoa maturation. This process increases their motility and also promotes the acrosome reaction and, thus, gives them the ability to fertilize an egg cell (1,3,4).

During ovulation, the ovum and its Corona Radiata go out of the ovary, travel through the Fallopian tubes and finally to the uterus (3). Fertilization usually occurs at the Fallopian tubes' ampulla and, once the spermatozoon meets the egg cell in this region (thanks to the production of attractive chemical molecules), subsequent steps need to happen for success (2-4).

Firstly, the spermatozoon breaks down the extracellular matrix (*Corona radiata)* located around the ovum thanks to the *hyaluronidase* enzymes. Then, the acrosome reaction occurs, in which the acrosome is separated from the spermatozoon's head and all the enzymatic substances are freed so that they can help break down the zona pellucida (2-4). Once the spermatozoon is adhered to the ovum's cellular membrane, the spermatozoon nucleus is freed into the egg cell's cytoplasm and the calcium-mediated signal transduction starts. In the ovum, there is a huge calcium release that will participate in its activation (2). *(Figure 9)*

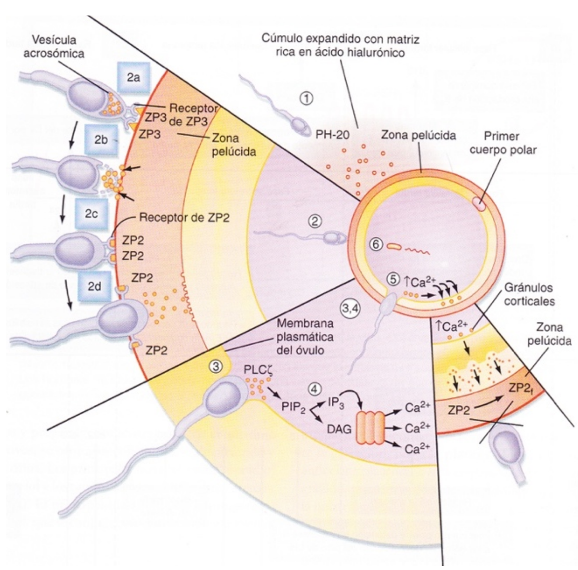

Figure 9. *Fertilization process. Extracted from (2)*

Inside the egg cell, both the spermatozoon and the ovum undergo specific changes to create the feminine and masculine pronucleus and, finally, they completely merge, so that the 23 feminine chromosomes and the 23 masculine chromosomes create a new structure with 46 chromosomes (23 pairs) that is called zygote (1,3,4).

Afterwards, the zygote will travel along the Fallopian tubes through the uterus, where blastocyte implantation in the endometrium will take place around day 5 to 7 after ovulation (3). During pregnancy, an organ called placenta develops, which is special because it is formed by two individuals (the mother and the fetus) and its functions include exchange of nutrients (glucose, amino acids, fatty acids…) and metabolic residues, hormone production to ensure pregnancy maintenance as well as protection against most of the microorganisms, although some viruses and substances (medicines and alcohol) can go through it (4).

2.3. Infertility:

2.3.1. Definition and prevalence

The World Health Organization (WHO) clinically defines infertility as "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse". Infertility is included in the International Classification of Diseases (ICD 10), which is a diagnostic tool that provides standardized basis for identifying and reporting health conditions and diseases globally (5). In the English language, infertility is considered a synonym of sterility.

Subfertility is a situation of reduced fertility with prolonged time without achieving conception. (6) Most of the pregnancies happen during the first six cycles of the fertile phase during the menstrual cycle (6), so that subfertility is usually described as the ability to become pregnant without medical help but in a period higher than a year, which is after 12 unsuccessful cycles (7). 50% of the couples with subfertility conceive spontaneously in the next 36 months after the first year, while the other half of the group that does not conceive is considered infertile (6).

Data from 2017 established that in the European Union (EU) more than 25 million individuals in a reproductive age have infertility (8). Worldwide, in the developed countries, about 1 out of 6 citizens suffer it during their reproductive lifespan (7,8).

The EU average total fertility rate is 1.58, while in Spain it is of 1.32. In our country, it is estimated that more than 800,000 couples suffer from infertility and the average age of women at the first childbirth is 31.8 years (8).

2.3.2. Causes

One of the main factors impacting the ability to have a child is the age of the progenitors. Although men account for about 25-35% of the infertility cases, women age is more crucial, as the maximum fecundity ages are between 20 and 30 years old and some epidemiologic studies have shown fertility hugely declines in women older than 38-40 years old, although at the age of 35 the reproductive capacity is already diminished. For men, the age factor is less clear and relevant, but some data establishes that the reproductive ability declines after the age of 40 (7).

Apart from advanced age, causes of infertility include toxic compounds exposure, diseases (such as Polycystic Ovary Syndrome (PCOS), endometriosis and hypothyroidism) and lifestyle habits (lack of physical exercise, chronic stress, unbalanced diets and drug consumption such a tobacco, alcohol, weeds and cocaine) (13).

There are factors that completely limit the reproductive ability, such as lack of sperm or obstruction of the Fallopian tube. There are others, though, that only reduce partially the likelihood of a pregnancy, as they affect the production of gametes (in men, reduced sperm motility or quantity; in women, anovulation related to PCOS or Premature Ovarian Failure), the interaction of gametes (alterations in the fertility process, in the sperm transport, in the ovum capacitation in the Fallopian tubes…) or the implantation of the fertilized egg (7).

The most common causes of infertility in developed countries are seminal alterations in men (25-35% of the cases) and ovulatory disorders (25% of the cases) and endometriosis (5-15% of the cases) in women. Finally, fertility from unknown causes accounts for up to 20% of the cases (7).

In men, erectile disfunction is also a cause of infertility and may be caused by different factors such as neurovascular diseases (for example, diabetes mellitus), insufficient androgen production, structural alterations or drug (including alcohol and tobacco) consumption (2).

In women, some cases of infertility are due to physical alterations on the feminine reproductive organs but other times they are related to either an altered physiological function of the reproductive tract or an abnormal development of the egg cell. The most frequent cause of infertility is the lack of ovulation (anovulation), which can be due to a reduced secretion of gonadotropins or it can be related to an abnormality on the ovaries, such as the presence of thick capsules that block the ovum expulsion (3). Another frequent cause of feminine sterility is endometriosis, in which the uterine endometrium grows out of it and surrounds the Fallopian tubes, ovaries and the pelvic cavity around the uterus. It leads to fibrosis and occlusion of the tubes, making it impossible for the egg cell to be freed from the ovary (3). Moreover, endometriosis can result in ovarian function impairment (7).

Other causes include Salpingitis – an inflammation of the Fallopian tubes usually caused by an infection; and the secretion of abnormally thick mucus – which makes it harder for sperm to move in the feminine reproductive tract (3).

PCOS is an heterogenous endocrine disorder characterized by abnormally high androgen levels (hyperandrogenism), presence of multiple cysts in the ovaries, ovulatory dysfunction and irregular menstruations (9,10). It is usually associated to obesity and insulin resistance (in 50 to 70% of the cases) (10, 11). Its prevalence varies depending on the diagnosis criteria and it is up to 15-20% of the population when using the *European Society for Human Reproduction and Embriology (ESHRE)/ American Society for Reproductive Medicine (ASRM)* criteria (Rotterdam criteria, 2004). Women are diagnosed if they meet two of the following criteria: clinical/biochemical hyperandrogenism; oligo or anovulation; polycystic ovaries (10). Clinically, women present hirsutism, oligomenorrhea (abnormally infrequent menstrual flow) or amenorrhea (abnormal absence of menstruation) and, frequently, infertility (10). PCOS is the most common cause of anovulation in women (12).

It has been stablished that extremes on body weight may be one of the causes of infertility, as women with IMC lower than 20 have higher risk of anovulation, whereas both women and men presenting overweight have higher rates of subfertility (14). As some cases of infertility or subfertility are related to excessive body weight, weight loss and practice of physical activity may help increase likelihood of pregnancies (14).

2.3.3. Approaches to treat infertility

There is a wide range of Assisted Reproductive Techniques (ART) treatments, which have different levels of complexity. In Spain, the available methods include Intrauterine Insemination (IUI); In Vitro Fertilization (IVF); Intracytoplasmic Sperm Injection (ICSI) and pre-embryo transfer; Embryo Freezing and Frozen Embryo Transfer (FET); Preimplantation Genetic Diagnosis (PGD); Preimplantation Genetic Screening (PGS); Gamete, Double Gamete and anonymous Embryo Donation. Surrogacy is not available. Currently, ICSI is the most widely used (8).

However, ART techniques have high emotional and economic costs, and this is why emerging studies are focusing on identifying modifiable lifestyle factors, including dietary patterns, that may promote fertility on specific disorders and diseases (15).

In women suffering PCOS, treatment includes usage of oral contraceptives (they reduce hirsutism and menstrual cycle alterations) and anti-diabetic drugs such as metformin (it improves insulin sensitivity). Furthermore, lifestyle changes such as weight loss have shown to improve menstrual irregularities, hyperandrogenism-related symptoms, insulin sensitivity and infertility (10,11).

2.4. Importance of nutrients on fertility

Both macronutrients (carbohydrates, proteins and fats) and micronutrients are needed to maintain normal energetic, structural and regulatory functions in the body. Vitamins and minerals are essential molecules, as humans cannot synthetize them – or at least in adequate quantities – so that they need to obtain these micronutrients from the diet. Furthermore, some foods contain phytochemicals, which also participate on many human body processes.

Emerging studies are focusing on the importance of an overall optimal nutritional status and an adequate body weight and composition for fertility. More specifically, nutrients such as vit D, E, C, A, Ca, Fe, Zn, Se, I, folic acid, vit B12 and omega-3 fatty acids are thought to be necessary to boost fertility and ensure a successful pregnancy, as they are involved in the fertilization process (13). It has been demonstrated that specific dietary changes help decrease the frequency of ovulatory disorders and, thus, improve fertility (14).

3. OBJECTIVES

Based on the fact that infertility affects a significant part of the population worldwide and the promising positive impact diet and specific nutrients may have on it, the main objective of this project is to understand if diet modifications can help improve the fertility on both female and male.

The secondary objectives of this bibliographic research are:

- Understand the role and function of specific nutrients on the fertilization process.

- Establish foods or nutrients that may be positively and negatively affecting fertility.

- Search for possible nutritional approaches and dietary changes to improve fertility in both male and female.

- Provide recommendations for the general population to improve fertility and pregnancy likelihood.

4. MATERIAL AND METHODS

This work has been completed by using the Information from both bibliographic databases and physical books.

The bibliographic database used for the search was Pubmed, accessed through the website of the *"Centre de Recursos per a l'Aprenentatge i la Investigació" (CRAI)* from the *Universitat de Barcelona (UB).* The information extracted comes from articles and their selection has been done based on the most relevant ones for the topic, trying to prioritize the most recent ones.

The search was conducted in English to ensure the finding of all the important articles, as literature and studies are usually in this language and the terms introduced include (nutrition OR nutrient) AND (fertility OR fecundity), food AND (fertility OR fecundity), fertility AND (causes OR factors), Zinc AND (fertility OR fecundity), vit D AND (fertility OR fecundity), (omega-3 OR PUFAs) AND (fertility OR fecundity), (folic acid OR B9) AND (fertility OR fecundity), B12 AND (fertility OR fecundity), (Se OR Selenium) AND (fertility OR fecundity), vit E AND (fertility OR fecundity), etc.

The filters applied during the search were the data of publication (articles published from 1999 - during the last 20 years) and the species of the studies (studies only in humans).

The search was first restricted to reviews and metanalyses to get an overall understanding of the topic and, then, the search was focused on finding primary articles, giving priority to the best quality studies - randomized clinical trials and prospective cohort studies, although evidence was limited and other studies - such as case-control - were considered as well.

The physical books used were borrowed from the Library of the *Universitat de Farmàcia i Ciències de l'Alimentació - Campus Torribera*, *Universitat de Barcelona (UB).* The sections used for the elaboration of this project were "Nutrition and Dietetics" and "Physiology and Physiopathology".

This project includes three main sections: the introduction, with the objective of determining basic physiology and physiopathology of the human reproductive tract to later understand the results; the results and discussion, which provides specific data of the most relevant and recent studies, providing the currently available scientific evidence of the topic; and the author's contribution and suggestions for the topic, focusing on the elaboration of a leaflet summarizing the most important findings that could be provided to the general population to promote specific dietary patterns and lifestyle habits to improve fertility.

5. DETAILED DESCRIPTION OF THE ACTUAL KNOWLEDGE OF THE TOPIC

5.1. Macronutrients

5.1.1. Carbohydrates

It has been established that the quantity and quality of dietary carbohydrates may have an impact on fertility (15).

The *NHS-II (Nurses' Health Study-II)* - a prospective cohort study – found an association between glycemic load and the risk of anovulation. It also found a positive relationship between total carbohydrate intake and ovulatory infertility when this macronutrient was eaten in higher amounts in substitution for naturally occurring fats: after adjustments were done, women in the highest quintile of carbohydrate intake had a 78% higher risk of ovulatory infertility in comparison to those women on the lowest quintile. Statistically significant data showed that, the higher intake of total carbohydrate, the higher risk of anovulation (12).

However no statistically significant association was found regarding total fiber intake, fiber intake from different foods or overall high glycemic index foods intake with ovulatory infertility (12). Also, the association between fiber-rich diets and anovulation is not clear, as different studies have shown opposed effects (15).

It has been demonstrated that women suffering PCOS have superior high-glycemic index foods' intake in comparison to healthy women (15). A controlled intervention study showed that a dietary pattern based on reducing dietary carbohydrates in a weight-maintaining diet was associated with an improvement on the metabolic profile, including reduced fasting glucose and fasting insulin levels and increased insulin sensitivity. Reduced fasting insulin was statistically associated with a reduction on testosterone levels, potentially enhancing ovary function (9). Another controlled intervention study that gave participants an eucaloric low-carbohydrate diet also showed an improvement on insulin sensitivity (11). However, other studies showed that total carbohydrate intake did not seem to have any effect on the hormone levels in healthy women (15).

Although these studies consisted on controlled interventions, some limitations were noticed – such as small sample size and restrictive inclusion criteria, which limits the generalizability of the results.

Regarding men fertility, lower high-glycemic index foods consumption has also been associated with better semen quality in comparison to diets rich in refined and high glycemic index carbohydrates (13).

5.1.2. Fats

Fats play an important role on the reproductive function. Omega-3 (*n-3*) PUFAs *(polyunsaturated fatty acids)* are necessary for female fertility, as they are involved in oocyte maturation and embryo development and are also needed for the synthesis of substances involved in the implantation and pregnancy maintenance. Conversely, TFAs *(trans fatty acids)* are detrimental for fertility purposes, as they promote insulin resistance, which can lead to alterations on the ovary function and to ovulatory infertility (15,16).

Therefore, the type of fats greatly influence fertility, as a diet high in PUFAs from the omega-3 series combined with a diet low in TFAs has been associated with better fertility. There is still little evidence, though, regarding the impact of SFAs (*Saturated Fatty Acids*), MUFAs *(monounsaturated fatty acids)* and omega-6 PUFAs on female fertility (15).

The *NHS-II* study showed that ovulatory infertility was not associated with total fat, cholesterol and most types of fatty acid consumption, but was associated with TFAs, as each 2% of the daily energy obtained from this type of fat rather than from carbohydrates was correlated with a 73% higher risk (Relative risk (RR) = 1.73; 95% CI: 1.09, 2.73) of suffering ovulatory infertility after adjusting for confounding factors. The relative risk for ovulatory infertility when TFAs was consumed instead of omega-6 fatty acids was similar to the previous one (17).

Furthermore, when obtaining the 2% of the daily energy from TFAs instead of MUFAs, the risk of ovulatory infertility doubled (RR=2.31; 95% CI: 1.09, 4.87). The TFAs substitutions were made in an isocaloric diet, to avoid the diet being a confounding variable (17).

This same cohort study showed women consuming higher amounts of TFAs had 48% higher risk of suffering endometriosis. Conversely, an inverse association between PUFAs and endometriosis risk was observed, as women in the highest fifth of long-term PUFAs consumption had 22% less likelihood of suffering endometriosis in comparison to women in the lowest fifth of intake (18).

Moreover, statistically significant data showed that each 1% of energy coming from TFAs instead of any other fat source was associated with higher risk of endometriosis, whereas each 1% of energy coming from n-3 PUFAs consumption rather than TFAs correlated with almost 50% lower risk of endometriosis. No significant associations where found when each 1% of additional energy came from omega-3 PUFAs rather than MUFAs, n-6 PUFAs or SFAs.

Another large prospective cohort study also associated high TFAs consumption and low n-3 PUFAs with reduced fertility (19).

A cohort study showed that the higher tertile of DHA (*Docosapentaenoic acid*) consumption, which is a type of n-3 PUFA, was associated with reduced risk of anovulation in comparison to the lowest tertile (RR = 0.42; 95% CI:0.18-0.95). (20) Another US and Canada cohort study showed increased fecundability with higher n-3 consumption, although this association was not found in a Denmark cohort that already had much higher n-3 PUFAs baseline intake (19).

TFAs are associated with negative fertility outcomes - reduced sperm quantity and increased insulin resistance, which also negatively impact ovulation and sperm quality (13).

In men, diets high in saturated fat have been associated with lower sperm counts and overall sperm concentration. One study found that the highest quartile for saturated fat consumption had a 41% reduction on sperm count. Another study found that a higher saturated fat consumption was associated with 43% lower sperm count and 38% lower sperm concentration. Moreover, men with higher TFAs levels in seminal fluid showed reduced sperm count as well (21). Regarding omega-3 fatty acids, those who had a higher intake of them showed a mild improvement in sperm morphology (21).

Supplementation with omega-3 has shown to help improve the insulin sensitivity in women suffering PCOS (13) and to improve the sperm motility in men (22).

5.1.3. Protein

Currently there is a lot of controversy about the effects dairy, animal protein and soy products may have on fertility, as they have been linked with an increased intake of pesticides, endocrine-disrupting substances as well as steroid hormones and growth factors, which can all lead to alterations in the reproductive function and the hypothalamus-hypophysis-gonad axis (15).

5.1.3.1. Dairy

The detrimental impact dairy may have on fertility is still controversial, but the most recent studies have not found conclusive evidence of a negative impact on overall fertility nor ovulatory infertility (15,16).

For instance, the *NHS-II* study showed no correlation between total dairy intake and risk of anovulatory infertility, but did show that low-fat dairy intake increased the risk of anovulatory infertility whereas high-fat dairy intake reduced this risk (23). Conversely, a study using two different cohorts (one in Denmark and one in North America) did not support this hypothesis, as it did not show any clear effect of low-fat or high-fat dairy consumption on fecundability in either cohorts. Only in women younger than 30 years old, cheese and high dairy intake were associated with enhanced fertility (24).

Another cohort study observed associations between intakes of yogurt (RR: 2.1; 95% CI: 1.2, 3.7) and cream (RR: 1.8; 95% CI: 1.0, 3.2) and sporadic anovulation when compared with no intake, but did not find association of dairy fat consumption with ovulatory function alterations in women with regular menstrual cycles (25).

In a case-control study in Wisconsin, high consumption of milk (≥3 glasses/day) showed protection for female fertility (OR=0.3; CI:0.1-0.7) (26).

5.1.3.2. Animal protein

Although there is also controversy on the effect meat may have on fertility, current evidence potentially suggests that red meat intake is associated with an increased risk of infertility (16), whereas replacing animal protein by vegetable protein may improve fertility and ovulation (13).

Among the *NHS-II* participants, increased meat consumption was positively associated with ovulatory infertility, as adding one serving of meat daily significantly increased the risk by 32%. Also, higher intake of protein from vegetable sources was associated with a 50% less risk of suffering ovulatory infertility and increasing the protein intake from vegetable foods by 5% instead of protein from animal foods, showed a RR = 0.42 (95%; CI:0.24-0.76) of ovulatory infertility. This study concluded that risk of anovulation may be reduced by replacing animal sources of protein, especially chicken and red meats, with vegetable sources (27).

Contrarily to the results for meat consumption, fish consumption has been associated with higher likelihood of pregnancy (16). For instance, a cohort study found that high seafood consumption (8 servings/cycle) was strongly associated with reduced time to pregnancy – improved fecundity – in comparison to couples with low intake (28).

Although presence of methylmercury – a toxicant found in big predator fishes- on the blood has been associated with infertility, currently there are no strong conclusions on the negative impact mercury and other contaminants present on the fish may have on fertility (16). Furthermore, recent recommendations from the *American College of Obstetricians and Gynecologists (ACOG)* for women who may become pregnant, pregnant women and breastfeeding mothers promoted an intake of 2-3 times/week of a variety of low-contaminated fish, as this animal product is one of the main sources of n-3 PUFAs. However, they did highlight the need to restrict to one serving fish such as albacore tuna, halibut and carp and to completely avoid fishes such as bigeye tuna, swordfish, king mackerel and tile fish due to their big mercury concentration (29). *(Figure 10)*

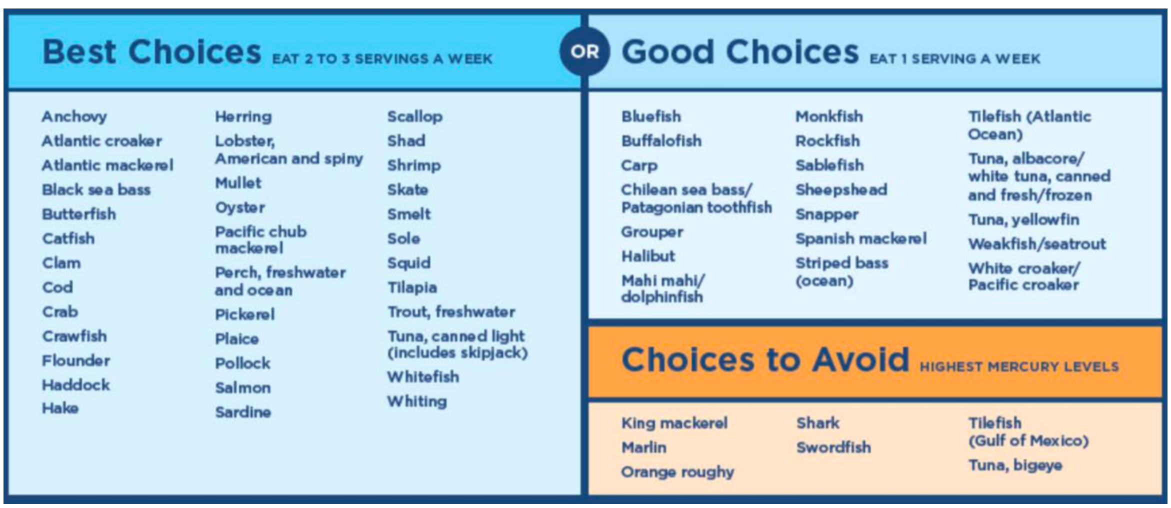

Figure 10. *ACOG's seafood consumption guide. Extracted from (29)*

5.1.3.3. Soy

Although soy and phytoestrogens supplements were thought to be detrimental for endocrine pathways, ovarian reserve and fertility, no conclusive studies have shown a negative impact on it (16). Available studies are small and heterogenous and are lacking evaluation of specific ovarian reserve markers, leading to the impossibility of drawing conclusive results (15).

From current data, no association has been found between soy isoflavones' consumption and acceleration of age at menopause nor fecundity (15).

Some evidence has shown to provide positive effects on fertility in couples undergoing infertility treatments, as the supplementation improved the likelihood of becoming pregnant, the live birth rates and the endometrial thickness (16).

5.2. Micronutrients

5.2.1. Folic acid

Folic acid rich foods include vegetables (especially the dark green leafy ones), fruits, nuts, beans, peas, grains, seafood, eggs, dairy, meat and poultry. Liver, spinach, asparagus and Brussel sprouts are among the foods with a higher content of it (36).

This nutrient is crucial for DNA synthesis, gametogenesis, fertilization and pregnancy (15) and its lack is related to alterations of the ovulation in women and reduction of the quantity and quality of sperm in men (13). Moreover, the folic acid deficiency during pregnancy is associated with neural tube defects, premature birth and reduced intrauterine growth (13).

Its supplementation is essential to prevent neural tube defects (NTD) in the fetus but different studies -including RCTs *(Randomized Control Trial)*- have shown that, beyond this function, this B-vitamin may also be beneficial for female fertility, as supplementation with higher doses (>800 µg/day) than those taken only to prevent NTD (usually 400 µg/day) have been associated with improvements on fertilization (15,16). Some prospective cohort studies found lower risk of ovulatory infertility (30), decreased frequency of anovulation periods (31) and shorter time to pregnancy (32) in women taking a folic acid supplement.

Also, higher reproductive success (increased implantation, clinical pregnancy and live births) in ART was observed in women in the higher quartile of folate intake and of serum folates (33,34).

Although some studies established a higher risk of fetal loss with high folic acid doses (>800 µg/day), the latest Cochrane evidence concluded that there is not higher risk of miscarriage (15,16). Furthermore, later observational studies even showed a decreased risk for spontaneous abortion (35,37,38). For instance, the *NHS-II* study, which included more than 11,000 women in the cohort, established women taking a high-dose (700-800 µg/day) folic acid supplement had a relative risk of 0.80 (95%; CI 0.71-0.90) of spontaneous abortion in comparison to women without supplemental folate intake (35).

5.2.2. Vitamin D

The main source of vit D for humans is sun exposure and there are only few foods with significant amounts of Vit D. The best dietary sources are fatty fish (salmon, tuna and mackerel) and fish liver oils and other foods with smaller amounts include beef liver, cheese and egg yolks. There are also mushrooms with enhanced vit D2 levels thanks to ultraviolet light exposure (36).

This vitamin has been thought to be important for fertility, as vitamin D receptors (VDR) have been found in the ovary, uterus, endometrium and placenta (14,15) and it promotes the synthesis of the Anti-Müllerian hormone (AMH) (13). Also, this liposoluble vitamin stimulates follicular maturation in the ovary and regulates the implantation process through gene regulation (15). Vit D may be related with infertility in both men and women, as its deficiency is associated with the pathogenesis (including metabolic syndrome and insulin resistance) of PCOS and endometriosis in women and with lower testosterone levels and decreased sperm quality in men (39).

It was thought that vit D may be a key nutrient to improve human fertility due to its participation on reproductive functions and also in relation to some promising animal studies (15). However, currently there is a lot of controversy around the possible positive impact of vit D on human fertility, as most recent studies have heterogenous outcomes and have not showed a strong correlation of vit D supplementation and the likelihood to become pregnant, especially on women who have sufficient vit D serum levels (16).

For instance, the *NHS-II study* could not establish any relationship between vit D intake and anovulatory infertility (15). The *Danish cohort* study did not find correlations between vit D serum levels and overall conception rates as well, although it showed that low 25-OH-vitD levels may increase the risk of late miscarriage (40).

Supplementation may be indicated and improve the conditions during severe vit D deficiency, obesity, insulin resistance, low AMH concentrations and oligospermia (13), but the current available evidence has not established any causality – only correlations- nor the ability to treat infertility through vit D (13).

Although vitamin D deficiency may be detrimental for fertility purposes, more evidence, especially from RCT is needed to clear the topic and establish causality, if existing (16).

5.2.3. Vitamin A

Vit A richest foods are liver and fish oil and other sources include eggs and milk. Provitamin A is found in vegetables such as leafy greens, orange, yellow and red vegetables, tomatoes and fruits (36).

It has many functions associated with the reproduction and it is necessary to ensure adequate sexual hormones' synthesis and the spermatogenesis process, to protect the ovum and sperm from oxidative stress as well as to promote the successful implantation of the fertilized egg cell. Moreover, it promotes the correct development of the placenta and also participates in the embryogenesis and organogenesis. The use of β-carotenes (provitamin-A) supplementation has been associated with better sperm motility and quality (13).

5.2.4. Vitamins E and C

The best sources of vit E include nuts, seeds and vegetable oils but it can also be found in some green leafy vegetables and fortified cereals (36). Vit C is found vastly in fruits and vegetables, including citrus fruits, berries, peppers, kiwis, broccoli, Brussels sprouts, tomatoes and potatoes (36).

They are antioxidant vitamins that protect the ovum and sperm from oxidative stress. Vit E, which is a liposoluble vitamin, participates in the fertilized egg cell implantation and the placenta development (13).

Vit C is essential for collagen biosynthesis, which is extremely important for adequate ovarian follicle growth and also for the ovulation and luteal phase due to the high amounts of collagen present on the ovarian tissue (22). A few studies associated high doses of vit C (ranging from 500 to 750 mg/day) with improved fertility and higher pregnancy rates (22) and Vit E and C supplementation may help increase sperm quality and quantity (13).

5.2.5. Calcium

Dairy products (milk, yogurt and cheese) are rich natural sources of calcium, but it can also be found in non-dairy products such as Chinese cabbage, kale, broccoli, almonds, tofu and sardines with bones (36).

Calcium is a mineral that plays a key role in the hyperactivation and the acrosome reaction on the sperm, processes that are essential for fertilization success. Moreover, it also participates on the spermatogenesis process and the sperm motility (13,14).

5.2.6. Iron

Heme iron is found in meat, fish and seafood, whereas non-hem iron is found in eggs, milk, nuts, beans, vegetables and specific fortified products such as cereals and breads (36).

Iron is a crucial mineral for fertility, as it helps the fertilized ovum implantation process. Also, it reduces pregnancy complications and is essential for the fetus nervous system development (13).

The *NHS-II* studied the effect of iron on fertility and found that women with a higher iron intake either from supplementation or from greater dietary intakes had improved fertility. The authors established this could be due to the fact that the ovary uses iron for its functions (22).

5.2.7. Zinc

A wide variety of foods contain Zinc (Zn), including oysters, eggs, red meat, poultry, seafood (crab and lobster), beans, nuts, whole grains and dairy products (36).

Zn is a trace element that plays a key role in fertility for both female and male, but has a greater importance for men, as it promotes testicle development, testosterone synthesis and sperm viability - motility, maturity, structure, function, quality and quantity (14,41). It is also involved in the interactions that happen in the female reproductive tract - including capacitation and fertilization (41).

Moreover, it protects both the sperm and the egg cell from oxidative stress, participates in the embryogenesis process and the placenta formation. Finally, it is also crucial for the fetus' nervous system growth and development (13). Supplementation with Zn has shown benefits for the fertility in men (41).

5.2.8. Selenium

Foods rich in Selenium (Se) include Brazil nuts, seafood and fish (tuna, halibut, sardines, shrimp), muscle meats (beef, ham), cereals and other grains and dairy products (36).

Se is a trace element that has many potential implications in reproductive functions, as selenoproteins play an important role in both female and male fertility (42,43). In men, Se helps maintain the spermatozoa integrity and viability by protecting them from oxidative damage (43). It also participates in testosterone biosynthesis and in spermatozoa development and maturation (42,43). In female, Se is necessary for follicle growth, maturation and dominance, for embryonic development and for protecting against oxidative stress both the dominant follicle and the endometrial remodeling for later implantation (42). It also helps in the placenta development and is necessary for the adequate development of the fetus' nervous system (13).

Some evidence suggests that a higher Se dietary intake improves the antioxidant activity and, thus, male fertility. There are some studies carried out in men showing a relationship between Se supplementation and increased fertility, sperm motility, semen quality and spermatozoa motility and morphology. Conversely, some other studies showed Se supplementation did not have any benefit and some even showed a decrease on sperm motility (43).

In women, evidence is even more scarce, as most of the studies are centered on the importance of Se during pregnancy and only few focus on its impact on fertility and, more specifically, on oocyte development and ovarian physiology (42).

Although evidence is growing, most of the current available studies are done under in vitro conditions and there is still a lack of high-quality studies on humans to draw solid conclusions to do clinical and guideline recommendations (42). Studies regarding Se supplementation show that it is beneficial for Se-deficient individuals, but it can be harmful if excessive doses are given. Therefore, health care providers should be careful when recommending its supplementation, especially on those individuals who may already have sufficient or high levels (42). Moreover, other two crucial factors that should be taken into account are the dosages and the Se status of the population, as Se content of specific food hugely varies depending on the soil (for instance, in the US soils are richer in Se, whereas Europe soils provide a poorer source of Se) (36,43).

5.2.9. Iodine

Iodine can be found in seaweed, seafood and dairy products. Fruits and vegetables also contain iodine, but their concentration varies depending on the soil content (36). Generalized use of iodized table salt is recommended to avoid deficiencies (22).

It is necessary for maintaining fertility and developing the placenta. It is also needed for the growth and development of the fetus' nervous system (13). A study showed that hypothyroidism was related to an increased risk of subfertility and established that adequate iodine intake is really important not only for pregnancy and child development, but also for conception (22).

5.2.10. Vitamin B12

Vitamin B12 is only found in animal products, including fish, meat, poultry, eggs, milk and milk products (36).

This vitamin participates on DNA synthesis and is necessary for the development and functionality of the placenta. A good nutritional status thanks to its supplementation has shown to improve the quality of sperm and to prevent spontaneous abortion (13).

5.2.11. Antioxidants

Although there is an association between oxidative stress and reduced spermatogenesis, evidence regarding the positive benefits of antioxidants on fertility is weak and inconclusive (14). There are only limited studies about the impact of antioxidants in fertility and the existing ones have many limitations and low quality. Furthermore, the term "antioxidant" is so wide that every study made used a different compound and dosage, making it really difficult to draw conclusions (16).

Some studies have established that oral supplementation with antioxidants may provide benefits on male fertility. For instance, a double-blinded RCT showed that the supplementation with 66 mg of Zinc and 5mg of folic acid together produced a significant 74% increase on the total normal sperm count in subfertile men after the 26 weeks intervention (44). A Spanish case-control study established that a reduced intake of antioxidants such as lycopene, folates and vitamin C was associated with a poorer semen quality (45).

A review study including 35 articles indicated that diets high in nutrients such as omega-3 fatty acids, vit D, folate, antioxidants (such as vit E, C, Se, Zn, β-carotene, lycopene and cryptoxanthin) and low in SFAs and TFAs had an inverse association with low semen quality parameters (46).

From the current evidence available, it can be established that supplementation may be beneficial in improving male fertility, but it does not provide improvements on females undergoing ART. However, even if the supplementation may be positive for the male partner, there is still lack of evidence regarding the dosage and the specific antioxidants associated with the benefits (13,16). Also, it is still unknown if supplementation is as effective as receiving them from an antioxidant rich diet (13).

It is important to determine that, although semen quality is evaluated and associated with specific nutrient consumption, there is no strong association between semen quality and fertility or vice versa. Therefore, producing changes on semen quality through nutrition does not strongly imply producing an effect on the couple fertility (16).

5.3. Toxic substances

It has been well stablished that alcohol and caffeine consumption during pregnancy time has many risks associated, including miscarriage, growth impairment and development of fetal alcohol syndrome. However, surprisingly to what is generally thought, the evidence demonstrating negative effects of caffeine and alcohol on fertility is inconsistent, probably due to the low quality and bias of many of the studies, making it difficult to draw a conclusion regarding the caffeine and alcohol harm for fertility (16).

Some evidence has shown detrimental effects, such as a review study which found that a high male intake of alcohol and caffeine had a detrimental impact on the chances of pregnancy in their female partner (46). Conversely, others such as the *Environment and Reproductive Health Study*, which is an ongoing prospective cohort study, have not found any association between male alcohol and caffeine consumption and semen quality among fertility patients (47).

Evidence regarding to what extent alcohol affects male reproductive health is less clear than the one regarding tobacco. Specifically, a meta-analysis showed a clinical association between cigarette smoking and infertility, as it reduced sperm count and motility and increased abnormal sperm morphology (21).

Although there is a lack of interventional studies and evidence is not solid enough due to the presence of multiple confounding factors, there is an existing association between all these toxic substances consumption and impaired male fertility, including reduced spermatogenesis and sperm parameters as well as increased DNA oxidative stress. Therefore, it would be safe to assume that recommendations of tobacco smoking cessation and avoidance of alcohol and recreational drug intake in male would be the best course of action for couples trying to achieve pregnancy (48).

Moreover, recent studies are starting to show that environmental toxicants may interact with food nutrients and may impact the human health and reproductive success. For instance, it has been stablished that high pesticide levels on fruits and vegetables may negatively impact reproductive success (pregnancy events and live births) in ART (49). Thus, human exposure to environmental contaminants such as methylmercury on fish, hormones and antibiotics in animal products and pesticides in produce should be considered in the future when studying the impact of foods and nutrients on human fertility (15).

5.4. Body weight: overweight and underweight

Maintaining an adequate body weight is extremely important for fertility purposes, as both underweight and overweight have shown to be detrimental for pregnancy success (14,50).

In obese or overweight individuals, the excessive adipose tissue produces higher amounts of leptin, a hormone that alters the hypothalamus-hypophysis-ovary/testicle axis and leads to higher testosterone concentrations and lower estrogen concentration in women and to higher estrogen concentrations and lower testosterone concentration in men, producing an inversion of the normal amounts of sexual hormones (13). Moreover, the excess of free fatty acids in the blood leads to insulin resistance, which negatively impacts the ovulation and the sperm quality (13). Obesity negatively affects the oocyte quality and uterine receptivity (50). Also, obesity clearly increases infertility risk and is associated with menstrual disorders such as PCOS (50).

Some studies established that in ovulatory women suffering subfertility and with a BMI > 29 Kg/m^2, the chances of conception were decreased by 5% for each increased unit of BMI (50).

In this regard, weight loss is a strategy recommended in order to enhance fertility status in overweight or obese women and moderate physical activity practice has shown to improve metabolic function and hormonal profiles (50).

On the other hand, underweight status is associated with anovulation, amenorrhea and reduced luteal phases in women and with decreased production, motility and viability of sperm in men (13). Also, the initiation of the menses requires a minimum of fat mass in order to maintain ovulatory function. Eating disorders, malnutrition as well as practicing exercise to exhaustion have been associated with subfecundity and infertility (50). One study determined that underweight women (considering a BMI < 19 $\mathrm{Kg/m^2}$) had an average 29-month long time to pregnancy, which is considerably longer than the 6.8 months needed for those women with normal weight (50).

Regarding men, a meta-analysis from 2012 that included 13,077 patients and 21 studies found that obese or overweight men had a higher risk of suffering infertility. Also, another cohort study including more than 10,000 men found that as BMI increased, the concentration and number of sperm as well as the semen volume decreased. Finally, another meta-analysis including over 115,000 patients concluded that obesity was related to increased infertility rates (OR = 1.66) (21).

5.5. Dietary patterns and overall diet quality

Different studies have investigated the positive effects of an overall "healthy" diet on fertility. For instance, the *NHS-II* study showed that women consuming a "fertility" diet had significant lower risk (RR=0.35; 95% CI:0.23-0.48) of suffering ovulatory infertility; a Spanish case-control study showed higher adherence to the Mediterranean diet decreased the likelihood of having difficulties becoming pregnant (OR=0.56; 95% CI: 0.35-0.95) in comparison to low adherence to this diet (51,52).

A review that included 23 observational studies concluded that dietary patterns including fruits and vegetables, fish and whole grains, were associated with improved semen quality in men (53). Another review including 35 articles indicated that intake of foods such as shellfish, seafood, poultry, cereals, vegetables, fruits, low-fat dairy and skimmed milk were associated with a benefit on different semen quality parameters, whereas diets rich in processed meats, soy foods, full-fat dairy, cheese, sweets and sugar-sweetened beverages were correlated with negative effects on semen quality (46).

Although studies slightly differed on what was defined as a "healthy diet", they all had in common a higher intake of protein from vegetable sources, a higher intake of animal protein sources from fish and poultry rather than meats and processed meats, a higher intake of MUFAs and n-3 PUFAs in comparison to TFAs, a higher intake of whole grains, fruits and vegetables and a lower intake of processed foods. They all showed benefits for fertility in women and for semen quality in men as well as increased odds of pregnancy events (15,16).

On the other hand, "unhealthy diets", which may be defined as the ones high in processed meat and processed foods, fried foods and sugar-sweetened beverages, are thought to negatively impact women fertility and are associated with lower semen quality and poor testicular function (16). This was shown in a multi-center retrospective study in which higher fast food intake frequency as well as reduced fruit intake was associated to longer time to pregnancy and infertility (54).

Proposed dietary recommendations for improving male fertility include providing high fiber and low glycemic index carbohydrates, promoting MUFAs and avoiding TFAs, reducing animal protein intake and increasing the one from vegetable sources. Being moderately physically active may help as well (13).

6. DISCUSSION

Evidence shows many nutrients (both macronutrients – carbohydrates, fats and protein - and micronutrients – vitamins, minerals and trace elements) regulate and participate on the different stages of the fertilization process and that dietary factors impact human fertility. However, there is not the same level of evidence for all the nutrients. Furthermore, differences regarding their impact on male and female fertility have been found.

Currently there is more evidence on the impact different nutrients have on women fertility, whereas fewer studies have investigated the impact of a variety of nutrients on the male population. For men, most of the available literature focuses on the impact and possible benefits of antioxidants, but studies are extremely heterogenous, as they use a wide range of supplements (16). Other nutrients that should be considered when tackling male fertility include Se and Zn, as they both have shown to be especially important for male fertility processes (13,14,41-43) in comparison to other micronutrients that are less studied. However, it is worth highlighting that most of the studies focus on semen quality and parameters improvement, which have very limited predictive value for spontaneous pregnancy events, so that further studies need to be conducted to assess how diet may impact overall fecundity on the male partner.

On the one hand, for the general population it is frequently recommended to follow an overall healthy diet, which promotes a higher intake of whole grains, legumes, nuts, fruits, vegetables and fish and a reduced consumption of trans fatty acids and red meat to improve fertility (15,16). There is evidence supporting it, as the positive outcomes on fertility have been established on the literature - different studies show associations between this nutritional approach and a reduction of ovulatory infertility (51,52) in women and an improvement on semen quality parameters on men (53).

On the other hand, conversely to the results found for a healthy diet, an unhealthy diet that includes high amounts of processed foods and sugar-sweetened beverages and is high in TFAs and SFAs has been associated with detrimental effects on semen quality (16,46) and worse fertility outcomes, including longer time to pregnancy (54).

It has also been shown that BMI is a factor that should be taken into account for both men and women when considering fertility, as both under and overweight are detrimental (14,21,50). Therefore, promoting a healthy weight should be another strategy to boost fertility and the regular practice of physical activity targeted to weight loss should be encouraged on patients suffering obesity, insulin resistance or PCOS (in female patients) (50).

Although causality cannot be established, as an overall healthier diet has shown to be beneficial not only for fertility purposes but for the prevention of many other chronic diseases – including certain cancers, diabetes mellitus type II and cardiovascular diseases – it would be wise to promote a change of dietary patterns on the general population and, more specifically, on the adults in their fertile lifespan, to promote better health status, prevent specific diseases and, likely, boost their fertility. Apart from potentiating healthier nutrition habits, promoting lifestyle changes - such as the practice of physical activity - focused at maintaining a normal BMI should also be considered based on the current evidence (13,50).

Evidence is more solid on the benefits of an overall nutritional approach, whereas the possible benefits of specific nutrients on fertility are still more unknown and less established. There is literature showing their participation on fertility processes (such as spermatogenesis, follicle maturation, implantation…) but the clinical significance and impact of these nutrients on fertility has not been well established yet. Currently there is more information regarding the benefits of n-3 PUFAs (18,19,21,22) and the detrimental effects of TFAs (13,15,16,19,21). There is also strong evidence showing the benefits of taking a prenatal folic acid supplementation to prevent neural tube defects, as well as to promote fertility and maintain a healthy pregnancy (15,16,30,31-34).

However, there are still few evidence-based and high-quality studies showing an impact of some macronutrients on fertility. For example, there is inconclusive evidence about whether there are different outcomes with high and low-glycemic diets or not (12,15). Also, currently there is controversy about the positive, negative or neutral effect of proteins from dairy, animal sources and soy products on fertility (15,16, 23-25). Finally, little literature is available for fat types such as n-6 PUFAs, MUFAs and SFAs (15).

Similarly, little literature focuses on the benefits of most vitamins (including vitamin A, D, E, C and B12) and minerals (including Fe, I, Ca) on fertility. For instance, although vitamin D was thought to be a nutrient with potential therapeutic effects, most recent evidence is inconclusive and has not shown as many benefits as expected (13,16), especially considering that vitamin D is involved in many stages of the fertilization process (14,15). Calcium is another nutrient thought to be necessary for fertility (13,14), as it participates on crucial steps, but evidence about the impact on clinical outcomes is extremely limited.

Regarding toxic substances, some studies have shown opposite results, especially for alcohol and caffeine, so that a strong conclusion cannot be made for now (46-47). However, in terms of public health promotion it would be accepted to promote a reduction of alcohol and tobacco intake in the general population (48).

Finally, it is needed to highlight that most of the studies are observational, so that it should be taken into account that confounding factors may influence the results and no causality can be established. It is because of this that there is a need to conduct future interventional studies to establish causality, especially for those nutrients which may have more promising results. If a positive benefit can be demonstrated and a relationship dose-response can be established, more specific recommendations could be given to patients to improve their fertility outcomes.

7. CONTRIBUTIONS AND SUGGESTIONS TO THE TOPIC: ELABORATION OF A LEAFLET

7.1. Rationale for the leaflet elaboration

Currently, there are guidelines for pregnancy, including the *"Guia per embarassades",* published on 2018 by the *Generalitat de Catalunya*. However, there are no guidelines or information available at the state (Catalonia, *Generalitat de Catalunya*) nor national (Spain, *Sociedad Española de Nutrición*) level to provide specific and useful information to the general public regarding the usefulness of nutrition to improve fecundity and the importance of diet for fertility purposes.

By this time there are still a lot of gaps regarding the importance of specific nutrients and their dosages on fertility and more studies need to be conducted to be able to make specific recommendations for the population. Nowadays public health recommendations for the periconceptional period are mainly based on folic acid supplementation but there is evidence showing that many other nutrients are important in this period of the lifecycle (14).

It has been stablished that many women entering pregnancy have a suboptimal nutritional status related to low intake of nutrients such as Fe, Zn and PUFAs (13), which leads me to consider that periconceptional guidance should be broadened and the importance of having an adequate diet to ensure adequate intake of all the needed nutrients and not only folic acid should be highlighted. Therefore, although the evidence is inconclusive about whether specific nutrients may improve infertility, the importance of having an overall optimal nutritional status before pregnancy is clear. Therefore, I have thought it would be useful to elaborate specific information for the general population to promote appropriate lifestyle and dietary habits.

7.2. Leaflet design

In this part of my work I have elaborated a leaflet *(Annex)* containing the most essential and crucial messages to be provided to the public in an easy and simple way to be understood. It is mainly focused on raising awareness of the importance of switching towards an overall healthier diet for fertility purposes and it provides some examples of specific foods that should be included to the diet to provide ideas to make it easier for the individuals to make small changes in their diet. It also presents groups of foods whose intake should be limited in the daily diet. Furthermore, it includes advice on reducing alcohol, tobacco, drugs and caffeine consumption because, although evidence on their negative impact on male fertility is inconclusive, I consider it is cautious to remind the population about it as a general public health recommendation.

This piece of information could be displayed in medical centers, hospitals as well as other public spaces to reach the target population, which are both adult female and male in their fertile lifespan. The title is called “Nutrition & fertility”, the subtitle establishes “Small changes to boost your fecundity” and it includes a section of “Include more of..” and a section of “reduce the intake of…”. This design has been inspired by the newly published “*Petits canvis per menjar millor*” from the *Generalitat de Catalunya* (55).

I have elaborated an English version, as this work has been elaborated entirely in English, but also a version in Catalan, as the information would be handed in centers of Catalonia, so that providing it in this language would facilitate the understanding for the target population. Both versions can be found in the *Annex* of this project.

8. CONCLUSIONS

- Many nutrients participate in the fertilization process and are necessary for achieving a healthy pregnancy. These include folic acid, vitamins D, A, E and C, Calcium, Iron, Zinc, Selenium, Iodine and vitamin B12. They play important roles in a wide range of processes, such as the sexual hormones synthesis, gametogenesis, follicle maturation, ovulation, acrosome reaction, fertilized egg implantation and protection of the sperm and the ovum from oxidative stress.

- There is strong evidence on the impact on fertility only for few nutrients. Folic acid and n-3 PUFAs have proved to be beneficial, whereas TFAs have shown to be detrimental for fertility purposes. Regarding the effect of many other macronutrients (including fat types such as n-6 PUFAs, MUFAs and SFAs and also protein from dairy, animals and soy products) and micronutrients (including vitamins D, A, C, E and Se, Fe, I), literature is still very limited. Furthermore, toxic substances such as alcohol and caffeine do not have strong and convincing evidence as there are only few studies and some are contradictory.

- An overall "healthy" diet - based on a higher intake of protein from vegetable sources, that prioritizes fish and poultry from animal protein sources and promotes a higher intake of fruits, vegetables, whole grains, MUFAs and n-3 PUFAs - has shown to generally have a positive impact on fertility and, therefore, should be recommended to the general population. Conversely, an "unhealthy" diet, in which there is a high intake of sugar-sweetened beverages, processed foods, red and processed meats, SFAs and TFAs, has shown the opposite effect, as it has proved to be detrimental for fertility purposes.

- Promoting a switch towards an overall healthier diet as well as the practice of physical activity regularly should be recommended for both male and female in reproductive age to help improve their fertility. Health professionals should be encouraged to provide advice on the importance of an adequate nutritional status before conception beyond the folic acid supplementation. Presenting the information in a leaflet targeted to couples wanting to achieve pregnancy could be a useful strategy to increase awareness about the topic.

All in all, evidence shows dietary patterns as well as lifestyle habits have an impact on fertility. However, more studies need to be conducted to have stronger evidence, especially double-blinded RCT when possible, to establish causality on the effects of specific nutrients on fertility as well as the benefits of supplementation and the adequate doses.

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10. ANNEX

Leaflet – english version

Image 1. Front part of the leaflet

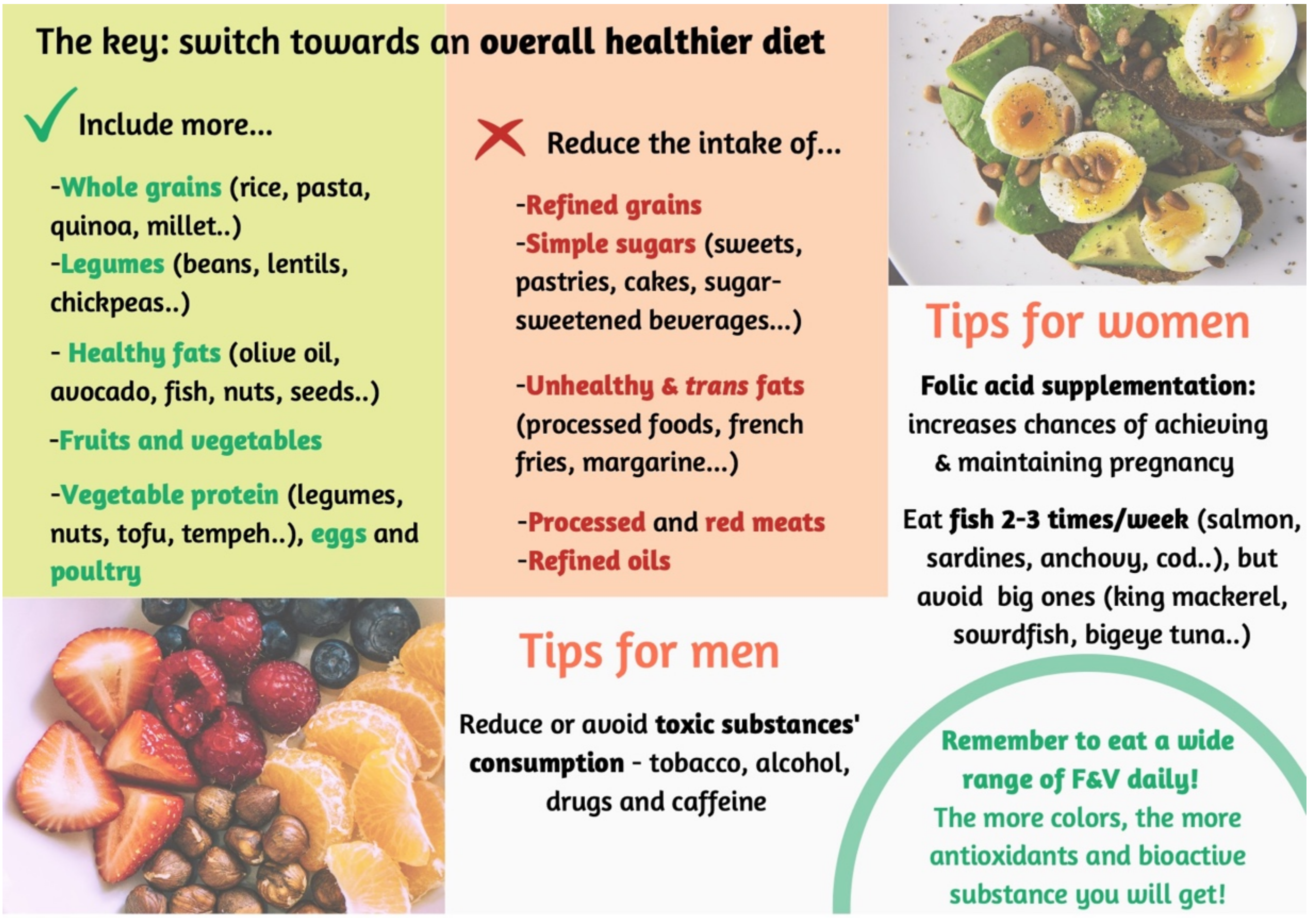

Image 2. Back part of the leaflet

Tríptic informatiu – versió en català

Image 3. Part frontal del tríptic informatiu

Image 4. Part posterior del tríptic informatiu