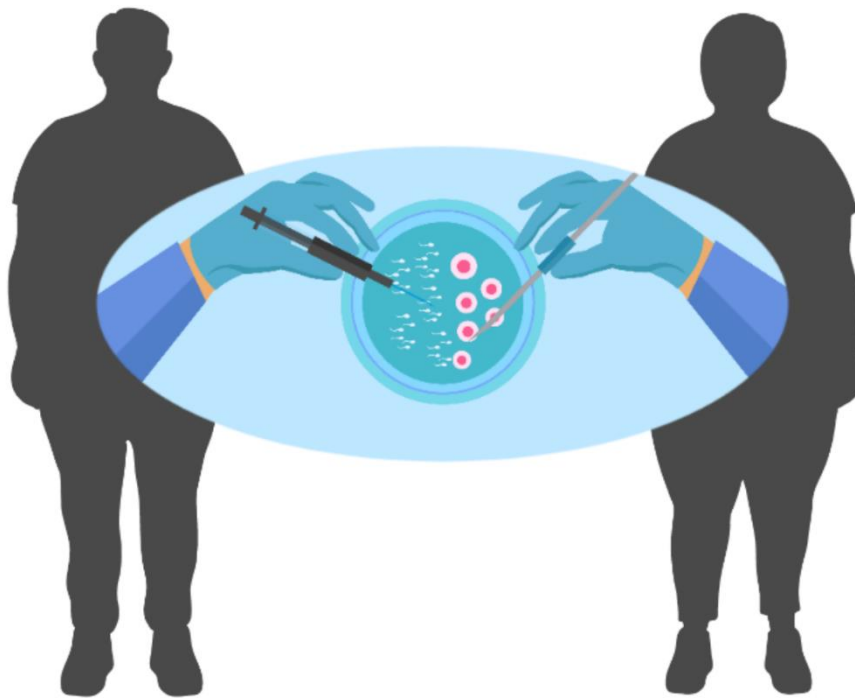# In Vitro Fertilization should not be the First Approach in Obesity-Related Infertility

Bachelor's Thesis Biomedical Sciences

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Summary

In vitro fertilization (IVF) is a method in which an egg is fertilized with a sperm cell outside the body. Couples that are unable to conceive within a year of regular unprotected sexual intercourse are considered infertile and are eligible for IVF. Where IVF used to be available mainly for obvious infertility problems such as fallopian tube obstruction, it is now increasingly possible to undergo IVF for no apparent medical reason, resulting in long waiting lists. Often there are also couples with obesity that have trouble in getting pregnant on these lists. This thesis shows that obesity, defined as a BMI greater than 30 kg/m^2, has a substantial effect on the fertility of a couple. Obesity is a result of energy disbalance, leading to a dysregulation of multiple hormones. High levels of the hormones insulin and leptin in obesity induce leptin and insulin resistance. This negatively affects the hypothalamic-pituitary-gonadal axis resulting in impaired sex hormone production. In obese women this can lead to irregular or absent menstrual cycles. In obese men the impaired sex hormone production could result in a low sperm count and motility dysfunction of the sperm cells. The hormonal dysregulation also has adverse effects on the IVF process and could lead to complications during the pregnancy. A change in lifestyle by losing weight results in increased fertility in both obese women and men. To reduce the waiting lists and to prevent complications during IVF and pregnancy, obese couples should therefore lose weight before initiating the IVF process.

Contents

Introduction

In vitro fertilization (IVF) is a fertilization technique in which an egg (oocyte) is combined with a sperm cell 'in glass' (in vitro), outside the body. The technique is used when there are problems in getting pregnant in a natural way. Prior to the IVF procedure there are multiple inquiries and physical examinations to find the (medical) cause of the infertility (Freya, 2017). Examples of medical causes are infrequent or absent ovulation, blockage of the fallopian tube in women, and dysfunctional sperm in men (Vander Borght & Wyns, 2018). If a couple has tried to conceive for at least 12 months and no medical causes are found, there is unexplained infertility. These couples and those with a clear medical cause for their infertility are eligible for IVF (Freya, 2017). The problem is that a lot of couples who already tried to conceive for a long time are placed on waiting lists. It can take at least 4 to 6 weeks to get an intake interview in the University Medical Centrum Groningen (UMCG, n.d.). The Maastricht University Medical Center has a waiting period of 3 months for the intake interview and another 3 months for the first treatment (MUMC+, 2021). The Amsterdam University Medical Center even has a waiting period of 5 months for the first visit and at least 4 months for the start of the IVF procedure (Amsterdam UMC, n.d.). Since the IVF procedure also takes at least 2 months, the whole IVF process could take several years (Freya, 2017). This time consuming process causes couples to experience stress and disappointments which can even result in depression.

The long waiting lists are a result of an increased number of couples that undergo IVF. For example, in the United States the number of IVF cycles in 2019 is doubled since 2005 (Centers for Disease Control and Prevention, 2019). The reason for this increase is not known, but it can be caused by increasing numbers of obese couples on the IVF waiting lists. Obesity is defined as having a Body Mass Index (BMI) greater or equal than 30 kg/m^2 (WHO, 2021). A BMI greater than 30 kg/m^2 is associated with a lot of disorders and diseases like diabetes mellitus (type 2), cardiovascular disease and high blood pressure (hypertension) (Meldrum et al., 2017). Importantly, obesity is also related to several fertility problems. This decrease in fertility caused by obesity is reported by multiple studies. For example, a study of Van Der Steeg et al. (2008) with 3029 ovulatory infertile women showed that a BMI higher than 29 kg/m^2 was associated with a decreased fecundity. Since obesity is the cause of the decreased fertility in these patients, IVF will also be substantially less successful. To reduce the long waiting lists, obese couples should therefore be excluded from these lists. In other words, IVF should not be the first approach in obesity-related infertility. However, is this a fair approach? Why is obesity related to infertility? What could be an alternative approach in obesity-related infertility? These topics will be addressed in this thesis.

1. IVF and fertility

In 1978, Louise Brown was the first baby born after a successful IVF procedure by surgeon Patrick Steptoe, physiologist Robert Edwards and nurse Jean Prudy at the Oldham General Hospital, in Lancashire, England. The mother of Louise, Lesley Brown, suffered from blocked fallopian tubes (Johnson, 2019). Nowadays IVF is also eligible for unexplained infertility. Worldwide it is estimated that 8-12% of reproductive-aged couples experience trouble with getting pregnant due to infertility (Vander Borght & Wyns, 2018). All these couples with or without (medical) reason are eligible for IVF. To understand the IVF procedure and the consequences of obesity on IVF, first an overview of fertility and endocrine functioning in reproduction is given.

1.1. Fertility and endocrine functioning in reproduction

For conception it is important that a man has enough motile sperm and that a woman has a regular menstrual cycle. The development of the reproductive system is regulated by the hypothalamus, the control centrum of the brain that also regulates the energy homeostasis which is discussed in chapter 3. The hypothalamus stimulates the pituitary, a gland at the base of the brain that produces

hormones. These hormones activate different glands in the body to secrete other hormones. At the moment of puberty, the hypothalamus starts to produce the gonadotropin-releasing hormone (GnRH), which stimulates the anterior lobe of the pituitary to secrete gonadotropins. Humans have two different gonadotropins called the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH). These hormones regulate the secretion of other sex hormones by the ovaries and the testes, the human gonads. The gonadal hormones oestrogen, progesterone and testosterone give feedback to the hypothalamus and to the anterior pituitary to regulate the hormone production. This hormonal pathway along the hypothalamus, pituitary and gonads including the feedback system is called the hypothalamic-pituitary-gonadal (HPG) axis (Herbison, 2016) (*Figure 1*).
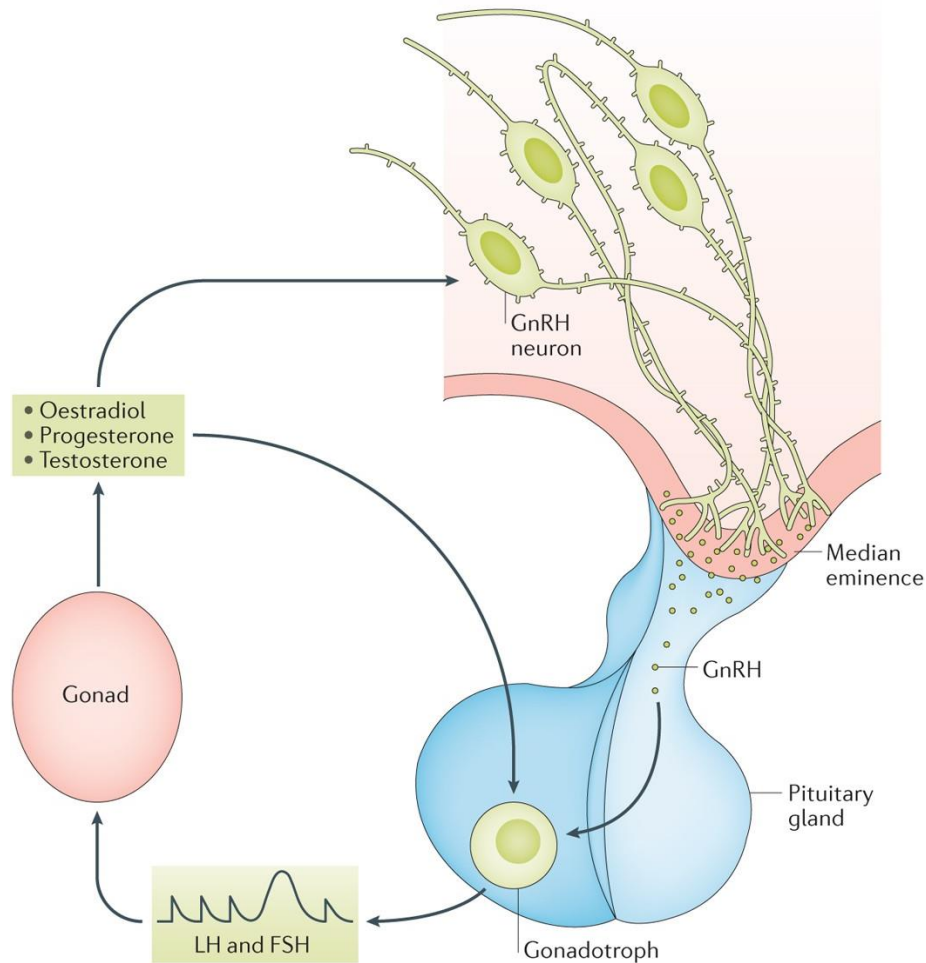

Figure 1: **The hypothalamic-pituitary-gonadal axis**. GnRH neurons in the hypothalamus produce the gonadotropin-releasing hormone (GnRH), which stimulates the production of the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) from the anterior pituitary. FSH and LH stimulate the production of oestradiol (an oestrogen), progesterone and testosterone by the gonads. These hormones exert feedback to the hypothalamus and the pituitary.
Derived from Herbison (2016).

The menstrual cycle is regulated by the HPG, lasts about 28 days and is divided into three phases. In the follicular phase the follicles start to grow. Granulosa and theca cells surround the oocyte in the follicle. FSH and LH stimulate the granulosa and theca cells to secrete hormones. Granulosa cells secrete Anti-Müllerian hormone (AMH), which makes follicles less sensitive to FSH to prevent the development of more than one follicle. Theca cells produce androgens that are converted to oestrogens by the enzyme aromatase. Oestrogens stimulate the growth of the endometrium, which is the inner layer of the uterus lining. The second phase of the menstrual cycle is the ovulation, in which the oocyte is released from the follicle on approximately the 14th day. Prior to the ovulation,

increasing oestrogen levels stimulate the secretion of GnRH (GnRH surge) leading to a peak of LH (LH surge) and FSH (FSH surge). The LH surge is greater than the FSH surge and stimulates the final maturation steps of the oocyte. Approximately 16-24 hours after the LH surge, the oocyte is released from the follicle and travels along the fallopian tube to be fertilized or to die. After ovulation, the follicle starts to transform into the corpus luteum in which the granulosa and theca cells develop into luteal cells. These cells start to produce progesterone, oestrogen and inhibin. Progesterone and oestrogen suppress the production of GnRH by the hypothalamus and the production of FSH and LH by the anterior pituitary. Inhibin also inhibits the secretion of FSH and LH. In this last phase of the menstrual cycle, called the luteal phase, progesterone stimulates the growth of the endometrium and the thickening of the cervical mucus. If the oocyte is not fertilized, the corpus luteum degenerates leading to a release of the inhibition of FSH and LH secretion. Since the luteal cells do not produce progesterone anymore, the endometrium starts to break down. Blood and cellular debris are discharged during this period called the menstruation. With the start of menstruation, the menstrual cycle begins again with the follicular phase (Silverthorn et al., 2015).

The HPG axis also regulates the male reproductive system. The male gonads, the testes, are present in the scrotum. The scrotum is distanced from the body for the ideal temperature at which spermatozoa are produced (spermatogenesis). Cells in the testes are stimulated by FSH and LH. FSH initiates the spermatogenesis supported by the Sertoli cells, which are located in the seminiferous tubules inside the testes. Between the seminiferous tubules, the Leydig cells are present in the interstitial tissue. LH stimulates the production of testosterone by the Leydig cells. Testosterone, the major androgen, is needed for spermatogenesis and plays an important role in the development of the male secondary sex characteristics like lowering of the voice and body hair growth. The production of GnRH and LH is inhibited by testosterone, because it exerts negative feedback on the hypothalamus and the anterior pituitary (Silverthorn et al., 2015).

Infertility can be caused by physical problems like fallopian tube obstruction or undescended testes. However, complications in the hormone production could also cause infertility. IVF was originally developed for patients with fallopian tube obstruction. The following chapter will explain why especially for these patients IVF is the most successful.

1.2. The IVF procedure
Before the actual initiation of the IVF procedure, there are multiple steps in the preliminary stage of IVF. When a couple has the wish to become pregnant but experiences trouble, they can make an appointment with the general practitioner (GP). The GP will query the couple about the period since they started trying to get pregnant. He or she will ask about the medical background, about the woman's menstrual cycle and about the frequency of unprotected coitus during the fertile period. If necessary, the GP provides the couple with the correct information about the fertile period and sexual intercourse. If the couple has tried to conceive for at least 12 months without success, a physical examination will be performed in which both the man and woman are examined including the sperm quality. If the chance of pregnancy is 30-40% and the woman is younger than 32 years old, it can be advised to try for some extra time to get pregnant. If the couple has tried to conceive for 18-24 months or if the pregnancy chance is lower than 30%, the couple is eligible for intra-uterine insemination (IUI) (Nederlands Huisartsen Genootschap (NHG), 2010). In IUI motile sperm cells (spermatozoa) are selected and concentrated in the laboratory and directly injected in the uterus. Before IUI, it is possible to stimulate the ovaria to increase the chance of success. Ovulation can be induced by the oral drug clomiphene citrate that diminishes the level of oestrogen. This stimulates the production of FSH by the pituitary leading to the development of the follicles. The ovaries can also be stimulated by subcutaneous FSH and LH injections. The downside of ovarian stimulation is

that it can lead to multiple pregnancies (Veltman-Verhulst et al., 2016). After 4 unsuccessful cycles of IUI with stimulation or after 6 cycles of IUI without stimulation, the couple is eligible for IVF. This is also the case if the woman is 38 years or older or if both fallopian tubes are damaged or blocked (NHG, 2010).

The IVF-procedure consists of several steps (*Figure 2*). First, the woman is treated with hormones to induce the production of multiple oocytes. A GnRH antagonist is used to suppress the natural production of hormones to prevent disturbances during the IVF-procedure. FSH treatment is used to stimulate the ovaries to start the development of multiple oocytes. Prior to the stimulation with FSH, an ultrasound scan is performed to look for cysts. The procedure is postponed if cysts are present or they are immediately removed. With regular ultrasound scans the development of the follicles is detected and when the follicles are large enough, human chorionic gonadotropin (hCG) is administered to stimulate the oocytes to detach from the follicles. Within 36 hours, the oocytes are retrieved from the follicles with a hollow needle that pierces through the vaginal wall. It is a painful procedure in which pain killers are administered before the puncture. The man is asked to deliver his sperm. After the puncture the oocytes and the spermatozoa are combined in a petri dish in the laboratory. Fertilization usually takes place in the petri dish, but it does not always occur. After two IVF attempts without fertilization it is possible to attempt Intracytoplasmic Sperm Injection (ICSI), in which one sperm cell is selected and immediately injected in the oocyte. After fertilization, one embryo selected on good quality is transferred into the uterus. When multiple embryos have arisen, they can be preserved in cryo (frozen) for later (Freya, 2017; UMCG, 2020).
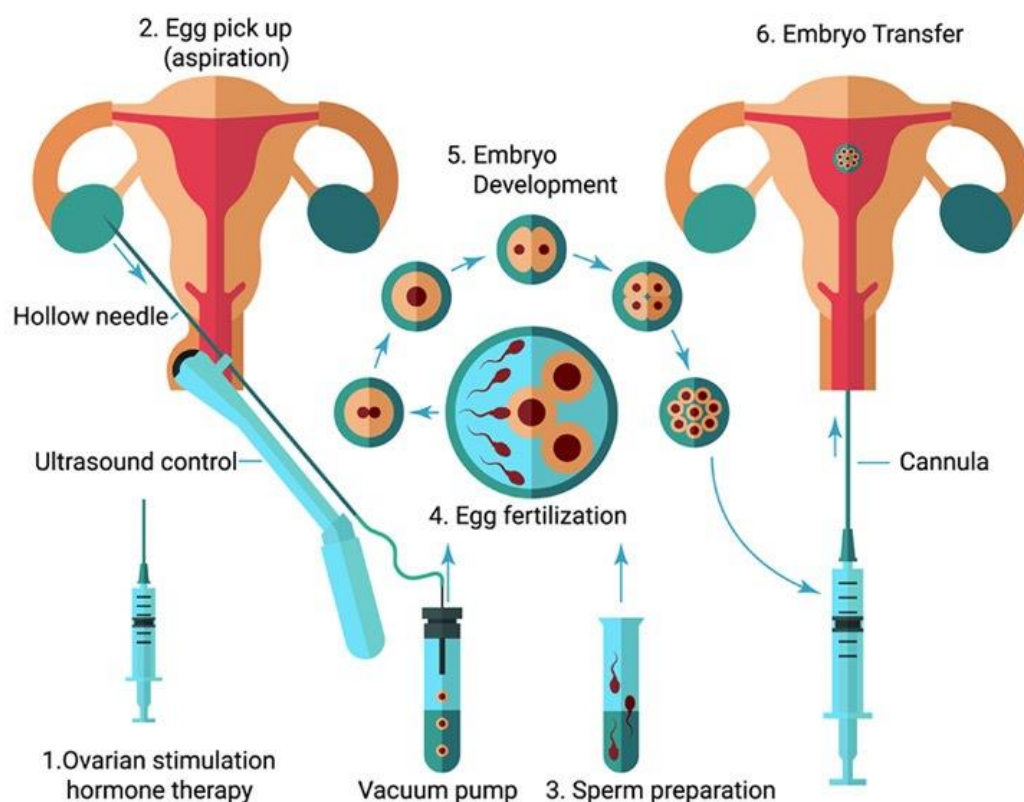

Figure 2: **Steps in the IVF procedure**. After ovarian stimulation with hormones, oocytes are retrieved from the ovaries using a hollow needles, a vacuum pump and an ultrasound scan. The sperm is prepared and combined with the oocytes resulting in egg fertilization. The embryos develop and one of them is transferred with a cannula into the uterus.
Image retrieved from http://holidez.com/in-vitro-fertilization-ivf/.

In patients with fallopian tube damage or obstruction conception cannot take place, because the oocyte cannot be fertilized by the sperm. If the patient is healthy and the quality of the sperm is good, IVF can be very successful. However, on the IVF waiting lists there are numerous patients with other fertility problems. A large group of these patients is obese. Since IVF for these patients is less successful, hospitals and fertility clinics have a maximum BMI for IVF/ICSI. In the Netherlands the maximum BMI varies between 30 kg/m² and 40 kg/m². There are also clinics without a maximum BMI (Freya, n.d.). Normally, three IVF cycles are reimbursed by health insurances. Nevertheless, long waiting lists or three unsuccessful IVF attempts induces people to go to commercial businesses (UMCG, 2020). However, IVF is a very expensive process and the problem is that it is doubtful whether more IVF attempts will lead to a successful pregnancy, considering that multiple complications can occur during the IVF process. Thereby, obesity is a major risk factor for these complications. This raises the question whether obese patients should even start IVF. This will be discussed in the following chapter.

2. Complications during the IVF procedure and pregnancy

The IVF procedure has a success rate of 40.2% in the Netherlands in 2019 (Stichting Landelijke Infertiliteit Registratie, 2019). However, there are a lot of complications that can occur during the IVF procedure. One of these complications is the ovarian hyperstimulation syndrome (OHSS), which can be caused by the administration of hCG. OHSS induces an increase of the vascular permeability leading to abdominal pain, nausea and vomiting, fluid leakage from the follicles, rupture of the follicles, thrombosis and acute renal failure. It is a dangerous and potentially even lethal disorder (Kumar et al., 2011). Moreover, IVF is associated with an increased risk for early pregnancy complications and ectopic pregnancy, in which the embryo attaches outside the uterus (Gelbaya, 2010). OHSS and ectopic pregnancy are risk factors of IVF and are less likely to happen in a normal pregnancy. Normal pregnancies are therefore always preferred.

There are also complications during the IVF procedure or pregnancy that are strongly associated with obesity. In a study of Pinborg et al. (2011) 487 couples were followed during several IVF/ICSI cycles. Clear effects of both maternal age and BMI were seen in the number of oocytes that were retrieved (*Figure 3A*). Less oocytes were retrieved from older and obese woman. Female BMI also had an effect on the number of developed embryos. A BMI of approximately 22 kg/m² gave the most optimal number of developed embryos (*Figure 3B*). BMI's greater than 22 kg/m² resulted in less embryos developed.
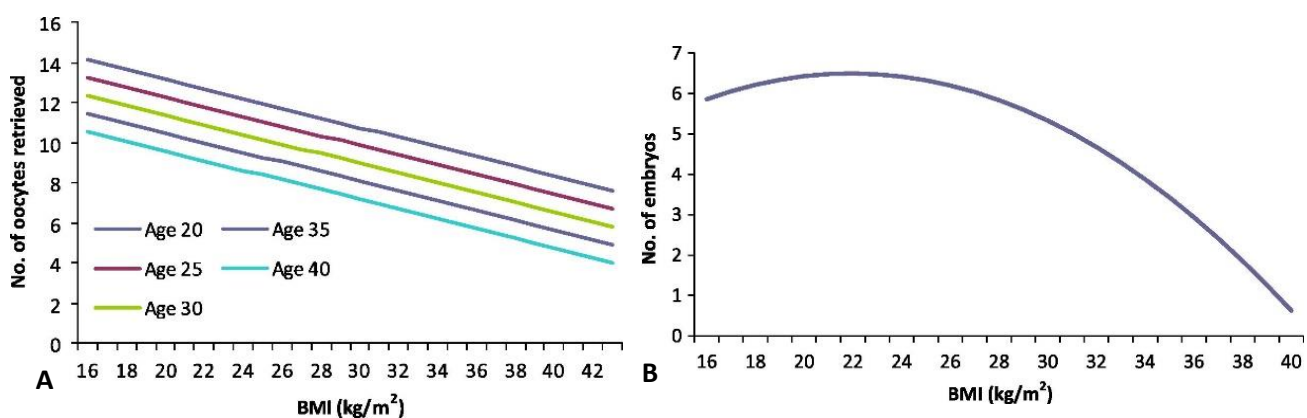

Figure 3: **The effect of maternal BMI on oocyte retrieval and embryos developed**. (A) The effect of maternal age and BMI on the number of oocytes retrieved. (B) The effect of maternal BMI on the number of embryos developed. Derived from Pinborg et al. (2011)

Pinborg et al. (2011) showed in the same study that the number of IVF/ICSI cycles that were cancelled was higher in the obese group than in the normal weight group. Moreover, the number of ongoing pregnancies was lower in the obese group compared to the normal weight group. The effects on the IVF/ICSI cycles of obese men were not included in this study. An obvious assumption could be that low sperm count and a decreased motility of the spermatozoa caused by obesity would lead to more failed IVF/ICSI cycles and less developed embryos.

Furthermore, it was found that women with overweight or obesity have an increased risk of hypertension, pre-eclampsia, gestational diabetes, Caesarean delivery and postpartum bleeding (Gaillard et al., 2013). Pre-eclampsia is a dangerous condition characterized by hypertension and proteins in the urine (Chen et al., 2009). Another risk of IVF is multiple pregnancies. Often only one embryo is transferred to the uterus. However, when a woman is older or when it is already the third attempt, sometimes more than one embryo is transferred. This increases the chance of twins, which can lead to preterm labor, low birth weight of the babies or postnatal depression of the mother (Gelbaya, 2010). The risk of very (early) preterm birth in twin pregnancies is also associated with overweight and obesity (Dickey et al., 2012). Another study found that obese maternal BMI increased the risk of second-trimester spontaneous pregnancy loss (Bressler et al., 2015).

Thus, because of the increased risk for these complications, obese patients should indeed be rejected from IVF waiting lists. If the complications during IVF and pregnancy are a result of impaired fertility, how can obesity lead to this impaired fertility?

3. Obesity and infertility

In order to be fertile, it is important that the hormones in the HPG axis are in balance. There is evidence that certain factors have an influence on the regulation of HPG axis. An important example of such a factor is the energy status, which will be discussed in chapter 3.2. However, to comprehend the impact of obesity on the fertility it is first important to understand the role of fat in energy homeostasis.

3.1. Obesity and the role of fat in energy homeostasis

Obesity has become a worldwide pandemic with 39% of adults aged 18 and older being overweight and 13% who are obese in 2016 (WHO, 2021). According to the Central Bureau of Statistics 44.4% of the Dutch population is overweight (BMI > 25 kg/m^2) of which 12.1% is obese (BMI > 30 kg/m^2) in 2020 (CBS, 2021). Obesity is a disbalance in the energy homeostasis, which is the balance between energy intake, energy expenditure and energy storage. Food intake determines the energy intake and energy expenditure is the energy that is used for physical activity or for the production of heat (Enriori et al., 2006). Carbohydrates are stored as glycogen mainly in the muscles and liver for short-term activities. Fat that is derived from glucose is stored in fat (adipose) tissue to use as a long-term energy storage (Pang et al., 2014). To maintain energy homeostasis several hormones need to inform the brain about the energy status. Based on these signals, the hypothalamus influences the eating behaviour and physical activity of the individual (Enriori et al., 2006). Examples of these hormones that stimulate appetite or satiety are ghrelin, insulin and leptin. Ghrelin, also called the hunger hormone, is secreted by the stomach and signals to the central nervous system where it stimulates appetite and food intake (Davis, 2018). In response to a rise in blood glucose after a meal, the pancreas secretes insulin which is transported throughout the body. Insulin plays an important role in the uptake of glucose from the blood by for instance skeletal muscles or adipose tissue. However, it also binds to receptors in the hypothalamus regulating the energy homeostasis. For example, insulin affects the energy expenditure by stimulating the hypothalamus to increase the body temperature (Dodd & Tiganis, 2017).

One of the most important hormones that has an effect on the energy homeostasis by stimulating satiety is leptin. Leptin is primarily secreted by white fat cells (Zhang et al., 1994). Leptin binds to leptin receptors (LEPRs), which are primarily expressed in the hypothalamus. The hypothalamus is divided in different groups of neurons called nuclei. In the arcuate hypothalamic nuclei LEPRs are expressed by pro-opiomelanocortin (POMC) neurons (Cheung et al., 1997) and neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons (Hahn et al., 1998). Leptin binds to the LEPRs of POMC neurons and stimulates the expression of the anorexigenic (appetite-suppressing) POMC (Cowley et al., 2001). POMC is a prohormone that is converted by enzymes to α-melanocyte stimulating hormone (αMSH). A-MSH activates the melanocortin receptors MC3R and MC4R. Activation of these receptors leads to a decrease in food intake and a decrease in body weight (Cummings & Schwartz, 2000). Leptin therefore has an indirect anorexigenic effect when it activates the LEPRs of POMC neurons in the arcuate hypothalamus. Leptin also suppresses the appetite by inhibiting NPY/AgRP neurons. NPY/AgRP neurons express neuropeptides that increase the food intake and decrease energy expenditure. The expression of these orexigenic (appetite-promoting) peptides is inhibited by leptin (Morton & Schwartz, 2001) (*Figure 4*).
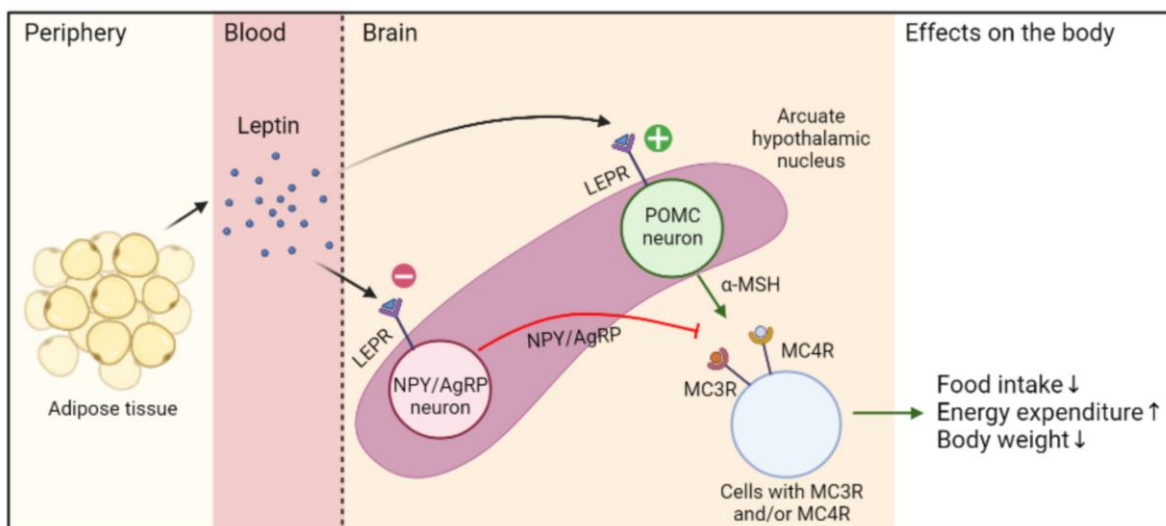

Figure 4: **Fat-derived leptin and its effects on the brain.** Leptin is secreted by adipose tissue and is transported via the blood to the brain. After crossing the blood-brain-barrier, leptin binds the leptin receptors of POMC and NPY/AgRP neurons, which are present in the arcuate hypothalamic nucleus. It stimulates the expression of POMC, which is converted to α-MSH by enzymes. Leptin also inhibits the expression of the neuropeptides NPY and AgRP. These neuropeptides normally inhibit the receptors MC3R and MC4R. A-MSH activates MC3R and MC4R which leads to a decreased food intake, an increased energy expenditure and a decreased body weight. Created with BioRender.com.

An excess of energy intake leads to an increased energy storage, which is often in fat tissue (Pang et al., 2014). Subsequently, this increase in body fat leads to an increased leptin concentration. Leptin levels in humans with severe obesity are therefore often not lowered, but extremely high (Considine et al., 1996). However, this high level of leptin does not result in a reduced food intake or weight loss. An explanation for this phenomenon is leptin resistance, in which the appetite is not suppressed and the energy expenditure is not increased despite the high concentration of leptin in the plasma (Enriori et al., 2006). There is not a clear explanation for how leptin resistance could occur. One explanation for this phenomenon is that the transport of leptin across the blood-brain-barrier is impaired in obese patients (Banks & Farrell, 2003). Another possibility is that there are complications in the LEPR signalling cascade induced by high levels of leptin (Münzberg & Myers, 2005). Since the concentration of leptin in the plasma of many obese patients is very high, leptin administration will not make a difference in the energy homeostasis of these patients.

Thus, fat and especially the secreted leptin, plays an extremely important role in the energy homeostasis. However, an extremely high fat level, which is the case in obesity, will result in leptin resistance. Leptin resistance has consequences on the energy homeostasis, but also on the HPG axis.

3.2. Energy status and the hypothalamic-pituitary-gonadal axis
In order to be fertile, it is important to have enough energy available for the development of the reproductive organs. Therefore, hormones that regulate the energy homeostasis inform the hypothalamus about the energy status. The fat-derived leptin is found to have an important function in the HPG axis in addition to its function in regulating the energy homeostasis. Studies in mice indicated that both leptin-deficient (*ob/ob*) mice and LEPR-deficient (*db/db*) mice showed infertility (Coleman, 1978; Robertson et al., 2010). Leptin administration in prepubertal female mice resulted in an earlier onset of puberty, accelerated reproduction and maturation of the reproductive tract (Ahima et al., 1997; Chehab et al., 1997). LEPRs are present on GnRH-producing neurons in the hypothalamus, so it could be hypothesized that leptin has a direct effect on the secretion of GnRH. Leptin indeed increases the secretion of GnRH, but the expression of the GnRH is not affected (Magni et al., 1999). A study in transgenic mice showed that deletion of the LEPR on GnRH neurons and not on other neurons resulted in normal fertility and only a slightly later onset of puberty in males. Meanwhile, deletion of the LEPRs in the whole forebrain caused infertility by preventing the onset of puberty (Quennell et al., 2009). This indicates that leptin only has an indirect effect on the secretion of GnRH. Multiple pathways have been found via which leptin can indirectly affect the GnRH secretion. As mentioned before, leptin stimulates the expression of POMC neurons and inhibits the expression of NPY/AgRP neurons (*Figure 4*). In addition to their effect on homeostasis, POMC and NPY/AgRP neurons have an effect on the reproductive system. The POMC-derived α-MSH was found to excite approximately 70% of the GnRH neurons via MCR3 and MCR4 in female mice (Roa & Herbison, 2012). However, deletion of the LEPR on POMC neurons did not result in infertility (Balthasar et al., 2004), suggesting that there are redundant mechanisms in which leptin and potential other hormones like insulin could inform the HPG axis about the energy status. NPY/AgRP-derived neuropeptides showed both excitatory and inhibitory effects on GnRH neurons (Roa & Herbison, 2012), indicating that neuropeptides derived from the same type of neuron could have different effects on the GnRH neurons. However, removal of the NPY/AgRP neurons in *ob/ob* mice resulted in both male and female fertile mice (Wu et al., 2012), suggesting that NPY/AgRP neurons strongly inhibit the HPG axis and that leptin prevents this strong inhibition.

In addition, leptin has an effect on the steroidogenesis, which is the production of the gonadal hormones oestradiol (a type of oestrogen), progesterone and testosterone. Studies in human granulosa cells, the cells that support the oocyte in the ovary, showed that leptin inhibits oestradiol and progesterone production in a concentration-dependent manner (Greisen et al., 2000; Lin et al., 2009) (*Figure 5*). This suggests that an abnormally high level of leptin (hyperleptinemia), which is the case in obesity, will result in an impaired steroidogenesis. Insulin also plays an important role in the steroidogenesis, since high levels of insulin (hyperinsulinemia) stimulate the production of testosterone by the human ovaries (Nestler et al., 1998). Hyperleptinemia, hyperinsulinemia, and leptin and insulin resistance are often present in obesity. The consequences on the fertility will be discussed in the next chapters.
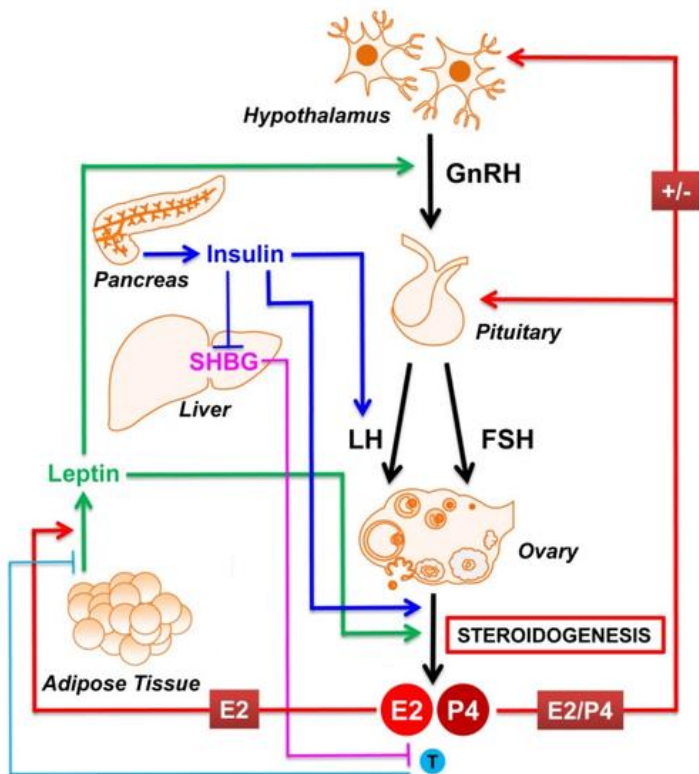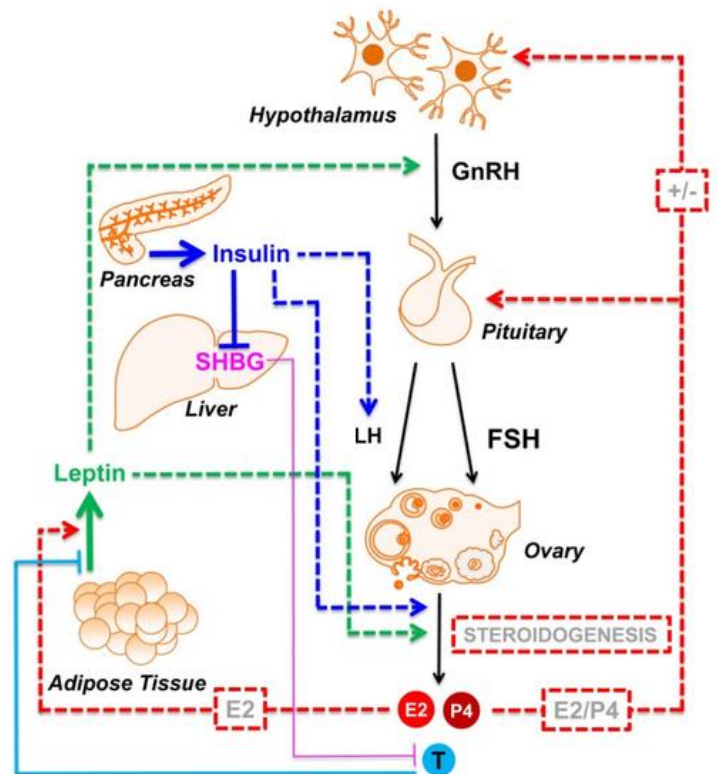
Figure 5: **The effects of leptin and insulin on the hypothalamic-pituitary-gonadal axis**. Insulin secreted by the pancreas has an effect on LH production, sex hormone-binding globulin (SHBG) production and steroidogenesis. The adipose-derived leptin affects the GnRH production and the steroidogenesis. Obesity-associated insulin and leptin resistance result in a dysregulation of the hormone production. E2 = oestradiol; P4 = progesterone; T = testosterone.
Derived from Fontana & Della Torre (2016).

3.3. Obesity-related infertility in women

As mentioned before, obesity is associated with leptin and insulin resistance. A potential cause of this resistance is a high level of triglycerides (hypertriglyceridemia), which is fat that is present in the blood. Calories that are not needed immediately after food intake are converted to triglycerides, which are stored in adipose tissue. During a period of fasting, the triglycerides are released for energy. Due to impairments in the lipid metabolism in obesity, obese patients suffer from hypertriglyceridemia (Subramanian & Chait, 2012). A study of Banks et al. (2018) found that triglycerides are present in the cerebrospinal fluid of humans. They also injected the triglyceride triolein with a radioactive marker in mice and showed that triglycerides are able to cross the blood-brain barrier. Another finding was that triglycerides inhibited leptin and insulin to activate their receptors, indicating that triglycerides induce leptin and insulin resistance (Banks et al., 2018). This suggests that the hypertriglyceridemia in obesity contributes to the leptin and insulin resistance.

High levels of leptin and insulin lead to a dysregulation of the HPG axis. Leptin resistance causes a decrease of GnRH production leading to a downregulation of the HPG axis. As discussed in chapter 3.2, hyperleptinemia and hyperinsulinemia result in impaired steroidogenesis. The synthesis of oestradiol and progesterone in the ovaries is impaired. Moreover, hyperinsulinemia directly and indirectly via the stimulation of LH production, increases the production of testosterone in the ovaries. Thereby, hyperinsulinemia results in a stronger inhibition of the synthesis of the sex hormone-binding globulin (SHBG) in the liver (*Figure 5*). SHBG inhibits oestradiol and testosterone by

binding to these hormones. High insulin levels result in low SHBG levels, which increases the exposure to oestradiol and testosterone. Increased levels of free testosterone (hyperandrogenism) can result in the polycystic ovary syndrome (PCOS) (Diamanti-Kandarakis & Dunaif, 2012). PCOS is a disorder characterized by multiple follicles that develop at the same time, but do not mature well. This prevents the ovulation and leads to an absence of menstruation, called amenorrhea. A lack or absence of ovulation (anovulation) and a dysregulation of the menstrual cycle substantially decreases the fertility in women (Patel, 2018).

3.4. Obesity-related infertility in men

Infertility of a couple does not only depend on the woman, but also on the man. Obesity also affects the HPG axis in men resulting in a disbalance of the male hormones. Due to insulin resistance, the levels of SHBG are decreased in obese men. This leads to an increased level of free testosterone. As mentioned before, testosterone inhibits the production of GnRH and LH (see chapter 1.1). Leptin resistance in the hypothalamus also causes a diminished stimulation of GnRH production. Moreover, the enzyme aromatase which converts testosterone into oestrogen is elevated in men with an obese BMI (Wake et al., 2007). This can result in hyperoestrogenemia, in which high levels of oestrogen inhibit the production of GnRH, LH and FSH. Obesity therefore can lead to a down-regulation of the HPG axis resulting in diminished levels of sex hormones (hypogonadism). Hypogonadism inhibits spermatogenesis resulting in a low sperm count (oligozoospermia) (Craig et al., 2017).

Furthermore, the temperature of the testes is affected, due to increased levels of adipose tissue in the abdomen and scrotum. An increased temperature will lead to an impaired spermatogenesis and a lower quality of the sperm (Katib, 2015). A study by Shafik & Olfat (1981) showed that removal of fat from the scrotum of 102 infertile men resulted in an increased sperm quality in 64.7% and pregnancy in 19.6%. This indicates that an excess of fat in that area leads to a reduced sperm quality. Another way in which obesity can lead to a reduced sperm quality is by oxidative stress. An elevated BMI is related to an increase in oxidative stress resulting in DNA damage, which leads to oligozoospermia and a decreased motility of the spermatozoa (Tunc et al., 2011).

4. A lifestyle change as a solution for obesity-related infertility

Up to now this thesis showed a lot of evidence with explanations how obesity affects fertility, IVF and pregnancy. To improve fertility, to reduce the waiting lists for IVF and to improve the success rate of IVF, obese couples that fail to conceive should therefore lose weight. Multiple studies showed improvements after losing weight. For example, a study by Becker et al. (2015) with 26 overweight or obese infertile women showed that a hypocaloric diet with carbohydrates that slowly raise the blood sugar induced a decrease in BMI, less body fat, and diminished leptin concentrations. This diet resulted in 85.4% more oocytes retrieved compared to the control group that did not follow the diet. Thereby, the pregnancy rate in the diet group was 21.4% compared to the control group where there were no spontaneous pregnancies. However, those were only short-term effects in a small group. Nevertheless, another study with 33 overweight, anovulatory patients with PCOS showed that at least 5% weight loss resulted in an improvement of the menstrual cycle and ovulations, making spontaneous pregnancies possible (Crosignani et al., 2003).

Weight loss also shows beneficial effects on the sperm concentration and quality. A study with 43 men with a BMI greater than 33 kg/m^2 showed that weight loss was associated with an increased total sperm count and increased SHBG and testosterone levels. However, it is not entirely clear whether these improved results were caused by simply the weight loss or also by other lifestyle factors such as smoking or alcohol consumption (Håkonsen et al., 2011). Nonetheless, other studies

also showed that weight loss resulted in improvements in the male sex hormones and sperm quality (Kaukua et al., 2003; Mir et al., 2018).

Conclusion

Obesity is a disbalance in the energy homeostasis inducing hyperleptinemia, hyperinsulinemia, and leptin and insulin resistance, resulting in a dysregulation of the HPG axis. Due to disturbances in the hormone production, the fertility of both obese women and men is affected. Moreover, obesity has adverse effects on the IVF process and pregnancy including risks for both the mother and the embryo. Since losing weight leads to a substantial improvement in the menstrual cycle of women and in the amount and quality of the sperm of men, a change in lifestyle should be the first approach in infertility related to obesity. This will diminish the waiting lists for other couples, for example those who have fallopian tube pathologies. For these couples IVF will very likely result in a successful pregnancy. A possible idea is to make three different waiting lists. In the first place, a list with patients that suffer from a clear medical condition for their infertility, for instance fallopian tube obstruction. These couples can be helped immediately. Next in order, a list with patients with unexplained infertility and a healthy lifestyle (BMI < 30 kg/m^2). Perhaps these patients should be allowed to have more than three IVF attempts. And lastly, a list with patients with obese patients that are encouraged to lose weight.

However, for some patients losing weight is not a possibility due to medical or other reasons like age. Therefore, IVF should not be forbidden for obese patients. It is important that the GP informs the couple about how a BMI greater than 30 kg/m^2 decreases the fertility and increases the risk for complications during the IVF process and pregnancy. Furthermore, there are also other environmental factors that can decrease the fertility such as smoking and alcohol consumption. Before initiating the IVF process, it is therefore important to advise the couple that is trying to conceive to acquire a healthy lifestyle.

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