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Evaluating the effect of female and male weight and age on Assisted Reproductive Technologies outcomes

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Resumo

Introdução: A idade avançada e o excesso de peso são comuns em casais inférteis, surgindo o dilema de adiar o tratamento (promovendo primeiro a perda de peso) ou iniciá-lo imediatamente (evitando o envelhecimento). Apesar do reconhecido impacto destas variáveis na fertilidade, os escassos estudos que avaliam o seu efeito na taxa de nascimentos e nos desfechos neonatais na procriação medicamente assistida (PMA) são pouco esclarecedores.

Objetivos: Pretendeu-se avaliar o efeito combinado do índice de massa corporal (IMC) e idade nos desfechos da PMA, e averiguar a existência de limites acima dos quais atrasar o tratamento para otimizar o peso pode não ser a melhor abordagem.

Métodos: Estudo retrospetivo que incluiu doentes submetidos ao primeiro ciclo de PMA entre Janeiro de 2013 e Fevereiro de 2018, numa clínica do grupo *Instituto Valenciano de Infertilidad*, num total de 14260 ciclos. O IMC foi subdividido em normal (18,5-25 kg/m^2), baixo peso ($\leq$18,5 kg/m^2), sobrepeso (25-30 kg/m^2) e obesidade ($\geq$30 kg/m^2). Analisou-se a taxa cumulativa de nados vivos como desfecho primário, sendo os desfechos secundários o tempo para engravidar, o peso ao nascer e a prematuridade. Utilizaram-se modelos de regressão multivariadas apresentando razões de probabilidade ajustadas (aOR) e com o respetivo intervalo de confiança a 95% (IC 95%).

Resultados: A taxa cumulativa de nados vivos foi inferior em mulheres com sobrepeso (aOR 0,83, IC 95% 0,75-0,92) e obesas (aOR 0,69, IC 95% 0,58-0,81) e não teve diferença estatisticamente significativa entre os valores de IMC masculino, diminuindo 1.09% por ano de idade masculina (IC 95% entre 0,26% e 1,92%) e acentuadamente acima dos 35 anos na mulher, idade a partir da qual a perda de peso durante o ano seguinte não resultou em benefício suficiente para compensar o efeito pejorativo do envelhecimento.

Conclusão: Existe potencial benefício em adiar o tratamento em até um ano para perda de peso prévia em mulheres com IMC $\geq$ 25 kg/m^2 até aos 35 anos, idade após a qual pode ser mais benéfico o início imediato. A idade e o peso masculinos parecem influenciar a taxa cumulativa de nados vivos em muito menor dimensão.

Palavras-chave: infertilidade, obesidade, idade, procriação medicamente assistida, taxa cumulativa de nados vivos

Abstract

Background: Body weight excess and advanced age are both common findings in infertile patients, originating the dilemma to either postpone treatment (to first promote weight loss) or to start treatment immediately (to avoid ageing). Despite their known impact on fertility, studies assessing their effect on live-birth and neonatal outcomes are still scarce and conflicting.

Objective: We aimed to assess the combined effect of the combined effect of body mass index (BMI) and age on assisted reproduction outcomes and to evaluate whether there are thresholds above which delaying treatment to optimize female/male weight may not be the best approach.

Methodology: This retrospective study included patients undergoing their first cycle between January 2013 and February 2018 in a clinic belonging to the Instituto Valenciano de Infertilidad group, in a total of 14260 cycles. BMI was subdivided into normal weight (18.5-25 kg/m^2), underweight (≤18.5 kg/m^2), overweight (25-30 kg/m^2) and obesity (≥30.0 kg/m^2). The primary outcome was cumulative live-birth rate and the secondary outcomes included time-to-pregnancy, birth weight and prematurity. Multivariate regression models were used, presenting adjusted odds ratios (aOR) with the respective 95% confidence interval (95% CI).

Results: Cumulative live-birth rates (CLBR) were lower in overweight (aOR 0.83, 95% CI 0.75-0.92) and obese (aOR 0.69, 95% CI 0.58-0.81) women and there was no statistically significant difference between male BMI categories, decreasing 1.09% per year of male age (95% CI between 0.26% and 1.92%) and markedly above 35 years in women, age from which weight loss during the following year did not result in sufficient benefit to compensate for the pejorative effect of aging.

Conclusions: There is a potential benefit of weight loss strategies prior to treatments in women under 35 years with BMI ≥ 25 kg/m^2, while those above this age may benefit from immediate treatment. Male age and BMI seem to influence CLBR to a lesser extent.

Keywords: infertility, obesity, age, assisted reproductive technology, cumulative live-birth rate

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Introduction

Parenthood is a lifegoal in most societies (Boivin et al., 2018), regardless of whether it is a family constituted by a married couple or a lone parent. However, despite the desire to eventually exercise parenting, many have elected to postpone pregnancy to as late as possible in their lives (Virtala et al., 2006). Parenting has increasingly become a matter of personal preference rather than biology, which constitutes an actual problem, given that the most significant negative predictive factor of fertility is advanced female age at conception (Vander Borght & Wyns, 2018). Besides the postponement of parenthood, other factors including genetic, environmental and lifestyle factors also play a role on the probability of having a child (Vander Borght & Wyns, 2018) and because of that, not all couples who wish to become parents will achieve this goal without medical intervention (Boivin et al., 2009). Specifically, in the general population, the probability of a natural conception in each menstrual cycle is between 20% and 25%, a figure which accrues over time with the number of attempts to conceive, reaching 45% after 3 months, 65% after 6 months (Luke et al., 2012) and, cumulatively after one year, will result in approximately 80-85% of couples eventually succeeding (Frey & Patel, 2004). For those who end on the ill-fated side of these numbers, the failure in conceiving has a considerable impact on their well-being (Tavares et al., 2016). Specifically, subfertility is a common problem that is often associated with emotional and financial burden, such as anxiety, depression, anger and personal devaluation, which in turn can enhance pre-existing infertility factors (Jones & Toner, 1993) (Frey & Patel, 2004). In the last years, with the increase in the availability of treatments, there is an evidence of increased reporting of both fertility problems and the number of those seeking Assisted Reproductive Technologies (ART) (Datta et al., 2016). According to the latest available data, in 2016 the number of children originating from ART represented around 3% of the total children born in Portugal (Conselho Nacional de Procriação Medicamente Assistida, 2017). These figures can lead couples to have unrealistically high expectations of ART, a procedure which is known to have a wide interval of success of varying from 2% to 50% per attempt, a success rate which is strongly dependent on factors such as female age and body weight (Kamel, 2010).

Definition and prevalence of infertility

Infertility is defined as the inability to attain a clinical pregnancy after at least 12 months of regular sexual intercourse without any contraception (World Health Organization, 2020), (Boedt et al., 2019), or owing to an impairment of a capacity to reproduce either as an individual or with his/her partner (Zegers-Hochschild et al., 2017). According to the World Health Organization (WHO), infertility is a global public health problem that affects approximately one in seven couples in developed countries (Eisenberg et al., 2014) (Duval et al., 2015) (Sarais, Reschini, et al., 2016) and one in four couples in developing countries (Vander Borght & Wyns, 2018). Such a discrepancy is due to limited resources for diagnosis, investigation and treatment in the latter group of countries (Kamel, 2010). Around half of these couples will eventually seek treatment which may include either intrauterine insemination or in-vitro fertilization (IVF) (Boedt et al., 2019). Nonetheless, the prevalence estimates of infertility are very difficult to assess, with studies mentioning the possibility that one in four women may have difficulties in becoming pregnant throughout their lives, totaling more than 70 millions of couples worldwide (Ma et al., 2019).

Primary female infertility is established when a woman has never been diagnosed with a clinical pregnancy and complies with the criteria of being infertile, while primary male infertility is established when a man has never initiated a clinical pregnancy and meets the criteria of being infertile (ICMART, 2017). On the other hand, secondary infertility is diagnosed when the patients are unable to initiate or establish a new clinical pregnancy but have already had a pregnancy at least once (ICMART, 2017).

There is a consensus that the process of diagnostic testing of infertility and evaluation of possible factors involved should not be delayed in a patient with either medical or physical findings suggestive of an impairment of reproductive function, taking especially into account the age of patients (Practice Committee of the American Society for Reproductive Medicine, 2020). Moreover, Fields et al. (2013) suggested that it may be necessary to inform couples that the effectiveness of complementary therapies for infertility requires further research and that IVF treatment could be offered to patients who have not achieved pregnancy after two years of regular unprotected sexual intercourse (Ella Fields et al., 2013). In the same line, more recently, other researchers also argued that fertility interventions for evaluation may be considered even prior to one year (Zegers-Hochschild et al., 2017) and that treatment can be offered after one year in women aged less than 35 years, with the possibility to start as soon as after 6 months of subfertility in women above this age. Furthermore, patients over 40 must be provided with early, possibly immediate, evaluation and treatment (Practice Committee of the American Society for Reproductive Medicine, 2020).

The causes of infertility are multiple and may or may not be associated with abnormalities of the male or female reproductive system. This investigation must simultaneously cover the two elements of the couple, since in around 30% of cases, both will potentially contribute to the problem (World Health Organization). The main causes of infertility are ovulatory disorders, tubal damage, uterine disorders and male factors. Besides these, up to 30% of all cases are otherwise unexplained (National Institute for Health and Care Excellence et al., 2013). Furthermore, according to several studies, it has been thought that differences in individual and life-style characteristics play an important role in the cause of infertility and in the success of subsequent treatment (Hassan & Killick, 2003).

Relation between age and fertility/infertility

In the past years, with the rise of life-expectancy and emphasis on professional careers in industrialized countries, there has been a widespread trend to delay childbearing (Kühnert & Nieschlag, 2004) (Ferreira et al., 2010) and transfer family planning to more advanced ages. For example, the average female age at first delivery increased from 22.7 years in the 80's, to 28 years in 2013 (Steiner & Jukic, 2016). One of the reasons why women seem to no longer be afraid of postponing motherhood may be a false sense of security associated with the existence of increasingly advanced medically assisted reproduction techniques, including oocyte cryopreservation and donation (Yogev et al., 2010). Many fertility centers are actually offering “social oocyte cryopreservation”, which consists in an emerging solution for young healthy women who intend to cryopreserve oocytes at a younger age for later use in their lifespan, despite the lack of an actual medical indication for such (Stoop, 2010). Although embryo vitrification (an ultra-rapid cryopreservation procedure which prevents ice formation within a cell) was previously considered the standard practice of fertility preservation, oocyte cryopreservation has the advantage of not needing sperm at the time of retrieval for fertility preservation (Stoop, 2010).

As reported by Steiner and Jukic (2016), the average time to achieve pregnancy is 3 months for women under 38 years, 8 months for women over 40 years, and more than 12 months for women over 42 years. Additionally, women without a previous conception have a higher risk of infertility, regardless of age (Steiner & Jukic, 2016).

Several studies have been performed in order to evaluate how age may affect pregnancy outcomes and clarify the risks of postponing maternity (Hart, 2016; Klein, 1964; Steiner & Jukic, 2016), with reports showing a linear decline in fertility beyond the age of 35 (Steiner & Jukic, 2016). The cause of this relationship between increasing age and subfertility is multifactorial, including an increase in ovulatory disorders, a decrease in the quality of oocytes through the years and a reduction in the frequency of ovulation (Hart, 2016).

One of the most supported explanations for this downward trend is the relationship between maternal age and aneuploidy, especially in women older than 35 years (Goldman et al., 2019). The best reported reasons for the increased number of cases of aneuploidy with older oocytes are meiotic segregation errors, most commonly the premature separation of sister chromatids during meiosis I, a slow meiotic spindle formation with predisposition to instability and a progressive decline in mitochondrial function (Cimadomo et al., 2018; Hart, 2016). Moreover, epigenetic alterations are also an additional possible explanation (Hart, 2016). In a landmark large retrospective study, the lowest level of aneuploidy was shown to occur between 26 and 30 years of age, with a steady increase until 43 years, after which a plateau of 85% was noted (Franasiak et al., 2014).

The reduction in ovarian reserve is also an important factor to consider, despite its limited value in natural fertility, since women are born with a finite number of oocytes and this follicular pool declines with ageing (Crawford & Steiner, 2015) (Steiner et al., 2017). As consequence of this reduction, given the depletion of follicles to recruit, a decline in ovulatory frequency and an increase in the frequency of anovulatory cycles also occurs (Hart, 2016). Furthermore, a reduced function of granulosa cells leads to a decreased secretion of inhibin B which leads to an increase in early follicular phase follicle-stimulating hormone FSH secretion that in turn leads to premature initiation of follicular recruitment, early ovulation, and a shorter follicular phase. Ovarian reserve tests, such as early follicular-phase serum FSH and serum Antimüllerian hormone (AMH) levels, can predict response to ovarian stimulation in women, although caution is needed when interpreting these results since they do not assess oocyte quality (Crawford & Steiner, 2015). Thus, abnormal hormone results do not necessarily imply a diagnosis of infertility and there is no evidence of any benefit in assessing these biomarkers to gauge natural fertility potential (Steiner et al., 2017).

Regarding ART, in a study published in 2015, the cumulative live-birth rates decreased on par with increased age, from 42% in women $<$ 35 years to 21.9% in women of 38-40 years of age (Goldman et al., 2019). Another argument supporting a strong relationship between oocyte age and ART fertility outcomes are studies assessing cycles using donated oocytes, which demonstrated systematically higher live-birth rates when compared to cycles using autologous oocytes, especially as the age of the recipient women advances (Luke et al., 2012).

Besides the implications in fertility, the possible complications of aging on pregnancy and offspring outcome should not be overlooked. Both maternal diseases, such as chronic hypertension and pregestational diabetes mellitus, and medical pregnancy complications, including gestational diabetes and preeclampsia, are increased in the advanced maternal age groups (Cavazos-rehg et al., 2015) (Poston et al., 2016). Likewise, the probability of preterm delivery, cesarean delivery, low birth-weight, puerperal complications and time of hospitalization are also larger in this population (Yogev et al., 2010).

The decreasing fertility and increasing risks during pregnancy associated with maternal age should also be taken into consideration together with the potential role that concomitant advanced paternal age may have (Kühnert & Nieschlag, 2004). The literature, however, is controversial regarding this topic, because standard sperm and endocrine parameters are not precise indicators of fertility and a failure to demonstrate alterations in these parameters with increasing age is not a synonym of a lack of relationship between aging and fertility. For example, some important alterations in reproductive cells, such as degenerative forms, can occur despite a so-called "normal production" of semen in men of advanced age (Hassan & Killick, 2003). On the other hand, when using other parameters such as *time-to-achieve-pregnancy*, a significant fivefold increase was observed in men with ages over 45 years (Hassan & Killick, 2003). All in all, the reproductive function of men does not seem to decrease in such an abrupt rhythm as in women over their thirties, despite some alterations in seminal parameters which may be observed in terms of the volume, motility, morphology and concentration (Ferreira et al., 2010), possibly contributing to a decline in fertility, particularly if it is coupled with advanced maternal age (Kühnert & Nieschlag, 2004).

Concerning ART, there is also some controversy regarding the influence of paternal aging on IVF outcomes and the evidence is unclear when compared with studies evaluating maternal aging. While Spandorfer et al. (1998) stated that paternal age did not affect semen parameters when comparing young and old men undergoing intracytoplasmic sperm injection (ICSI), more recent studies have reported a decrease in pregnancy rates in couples with a male partner of advanced age, even after accounting for the simultaneous existence of advanced maternal age. Specifically, while Ferreira et al. (2010) observed no correlation between paternal age and sperm concentration, sperm motility, embryo quality or miscarriage rate, they noticed a negative effect on implantation rate, with a 5% decrease per year of aging in oligozoospermic men who underwent ICSI. Additionally, Horta et al. (2019) found in their study that in couples with idiopathic infertility, the increase of age in the male partner was associated with a decline of 4.1% in the odds of live-birth for each extra year of advancing male age, in addition to the decline in clinical pregnancy and embryo implantation, while miscarriage rates increased.

Definition of cumulative live-birth rate

Medically assisted reproduction, as mentioned above, has come a long way in recent years. Because of this increase in demand, numerous trials have been developed in an attempt to predict the influence of several variables on ART outcomes. However, these clinical trials frequently do not report essential issues of the interest to health professionals or the public in general and, mainly, for patients undergoing treatment. Moreover, following treatment, there is generally a long interval of time needed to achieve a live-birth, making it more likely to encounter loss to follow-up, which contributes to the inadequate reporting of outcomes and complications. For this reason, it has been thus far difficult to define the optimal primary outcome of ART trials and include issues that match to major outcomes, such as live-birth, maternal risks and fetal complications.

The quality of retrieved oocytes, quality of embryos, implantation and miscarriage rates are common outcomes that has been frequently reported in ART trials (Sneed et al., 2008) (Rehman et al., 2013) (Di Gregorio et al., 2019) (Matorras et al., 2020). Conversely, many studies and national registries of ART around the world have opted to report the main outcomes as live-birth rates per cycle. Nevertheless, because most patients will require more than one treatment cycle, live-birth rate per cycle is still insufficient to estimate the overall rate of success for an individual patient (Luke et al., 2012). Thus, cumulative birth rates are a more useful measure that should be reported in outcomes of women who receive continued interventions (Luke et al., 2012) (Legro et al., 2014).

Cumulative live-birth rate (CLBR) is defined as at least one live-birth after continued infertility treatments or spontaneous pregnancies (Kluge et al., 2019). Previous studies using this measure as a primary outcome have reported three types of results, which are the live-birth rate of a specific complete cycle (including both fresh and eventual subsequent transfers of remaining cryopreserved embryos), a conservative estimate of the cumulative live-birth rate overtime and an optimal estimate of the cumulative live-birth rate (Luke et al., 2012) (Li et al., 2019). A conservative estimate was based on the theory that women who did not return for subsequent cycles would not have a live-birth. An optimal estimate assumed that women who did not return would have equal live-birth rates as women who returned.

Impact of obesity on society

Obesity is a worrying epidemic in our society, which currently affects all age groups, constituting a public health problem around the world (Marques et al., 2018) with over one billion people being affected (Petersen et al., 2013). The consequences of obesity are extremely significant, since practically all organ systems are negatively affected (Matorras et al., 2020), increasing the chance of severe comorbidities such as cardiovascular diseases, type II diabetes (Goldman et al., 2019), cancer, osteoarthritis, asthma and depression, leading to considerable loss of quality of life, morbidity and mortality (Marques et al., 2018).

Worldwide, 13% of men and 21% of women are classified as having obesity (Vander Borght & Wyns, 2018). Specifically, according to the American National Center for Health Statistics, in 2009-2010 the prevalence of obesity was 35.5% among adult men and 35.8% among adult women, including 17% with a body mass index (BMI) between 35 and 39 and 8% with a BMI ≥40 Kg/m^2 (Flegal et al., 2012), constituting the most common chronic disease and one of the 10 diseases contributing the most to the global health burden (Arabipoor et al., 2019). On the other hand, in Europe, the prevalence of overweight and obesity in adults was 53.1% in 2014 (Marques et al., 2018).

The obesity epidemic encompasses all subpopulations and has a significant burden on reproductive ages. For example, between 1971-1974 and 2005-2006, the fraction of obese young adults, with ages between 18 and 29 years old, went from 8 to 24%, doubling in the majority of all other adult age groups in that same period (Luke et al., 2011). According to the National Center for Health Statistics (NCHS), in 2002 the percentage of overweight was 24.5% and the percentage of obesity was 23%, in women between 20 and 44 years old, with an increase being observed as age advanced (Vahratian, 2009) (Broughton & Moley, 2017). This prevalence varies according to countries and its degree of development (Poston et al., 2016). The increase in obesity rates among European countries was fast, with UK women having the highest prevalence. In 2013, in England, 18% of women aged between 25 and 34 years were classified as having obesity (Poston et al., 2016).

As it is such a significant medical and economic burden, health professionals have attempted to develop concrete strategies to monitor the incidence and prevalence of obesity, while improving the counselling around the risks associated with being overweight (American College of Obstetricians and Gynecologists, 2019). Taking into account that even a slight percentage of weight loss is associated with important improvements in public health and in health care system economy, the counselling and the development and enhancement of effective lifestyle programs are fundamental (Marques et al., 2018). For example, the development of trials of intensive lifestyle modification in at-risk population, that includes the strategy of weight loss have demonstrated benefits in the prevention and control of chronic diseases such as diabetes (Rothberg et al., 2016). In a clinical trial that involved 27 centers with individuals who were at high risk for diabetes, type 2 diabetes could be prevented or delayed, with a reduction of the incidence by 58% after lifestyle interventions and 31% with metformin, when compared with placebo (Knowler et al., 2002). Moreover, weight loss interventions in obese patients can prevent or even improve other cardiovascular risk factors and obesity-related risk factors for coronary heart disease (Rothberg et al., 2016), namely the reduction of left ventricular mass by decreasing the sympathetic activity, plasma norepinephrine levels, and subsequently reducing blood pressure (Poirier et al., 2006). Furthermore, Rothberg et al. (2016) found that weight loss of at least 5% in obese women can result in the increase of insulin sensitivity and a trend towards the normalization of reproductive hormonal patterns favoring restoration of menstrual cyclicity.

Impact of obesity in the reproductive age on fertility/infertility

Effect on female fertility

The rising prevalence of obesity has become critical for women of childbearing age, owing to its association with short and long-term consequences for their fertility and for the health of their offspring (Vahratian, 2009). Specifically, approximately 50% of women of childbearing age are overweight when they conceive (Zera et al., 2011), which means that they are a special at-risk population for obesity and its known complications, and the need for further investigation on the impact on reproductive function is urgent (Eisenberg et al., 2014).

Obese women are more prone to menstrual cycle irregularities and both endometrial and breast cancer (Matorras et al., 2020). The detrimental consequences of obesity on time to pregnancy and subfertility (Goldman et al., 2019) are well acknowledged and challenging concerns during both the short and long-term management of this population (Sarais, Pagliardini, et al., 2016). Moreover, the morbi-mortality associated to pregnancy are greater when compared to adverse pregnancy outcomes in normal-weight women (Zera et al., 2011), such as gestational diabetes, preeclampsia, cesarean delivery (Sneed et al., 2008), shoulder dystocia, fetal distress, small or large for gestational age newborns (Sarais, Pagliardini, et al., 2016) and stillbirth (Zera et al., 2011). It is also more likely for women with body weight excess to have hypertension and thromboembolism in the postpartum period (Duval et al., 2015). Moreover, as maternal weight increases, higher perinatal complications and congenital defects in their descendants have also been reported (Matorras et al., 2020). Finally, chronic metabolic diseases are more likely in offspring of overweight women and this fetal epigenetic reprogramming may have adverse lifelong consequences (Rothberg et al., 2016).

Pathophysiological mechanisms

The overall mechanism in which obesity may affect the female reproductive system is complex. Obesity is correlated with hyperinsulinemia and consequent insulin resistance, which causes an increased hormonal production in the ovaries and a reduced hepatic production of sex hormone-binding globulin (SHBG). This mechanism increases peripheral conversion of ovarian and adrenal androgens, which decreases gonadotropin-releasing hormone (GnRH) and causes a rise of the androgen/estrogen ratio, with a consequent hyperandrogenic state that may increase the incidence of anovulatory cycles, oligomenorrhea or amenorrhea (Di Gregorio et al., 2019). In fact, ovulatory disfunction is a frequently advanced hypothesis in the literature, with prospective studies reporting that anovulation is the main cause of infertility in obese women (Sathya et al., 2010), not only due to hyperandrogenism but also because of the higher prevalence of polycystic ovary syndrome (PCOS) (Sarais, Pagliardini, et al., 2016). Besides the known relationship between obesity and ovulation, obesity is also associated with difficulties to conceive even in ovulatory women (Duval et al., 2015), and an increased BMI may deleteriously affect oocyte and/or embryo quality (Petersen et al., 2013). One of the theories supporting this conclusion is that oocyte development may be hindered by disrupted meiotic spindle formation or an altered mitochondrial architecture, predisposing to aneuploidy (Qiu et al., 2019). Another theory is lipotoxicity, suggesting that oocytes are induced to present a different kinetic pattern, while blastocysts also present a specific metabolic pattern (Matorras et al., 2020). The percentage of embryos with normal morphological characteristics decrease inversely with increasing BMI while the percentage of embryos with morphological anomalies increase proportionally with BMI (Di Gregorio et al., 2019).

Finally, female obesity also seems to contribute to lower levels of circulating AMH, a result that is somewhat difficult to interpret since a) body mass has only a minor impact in women with previously low or undetectable AMH levels, b) the number of antral follicles does not differ between normal weight and c) obesity is not associated with early menopause (Vitek et al., 2019). These findings, associated with the fact that circulating AMH is not a direct product of primordial follicles despite its correlation with them to estimate the fertile lifetime (Donnez & Dolmans, 2017), raise the question whether the lower level of AMH may be due to physiologic alterations present in the obese instead of an actual decrease in ovarian reserve (Vitek et al., 2019).

Effects on ART

The relationship between female obesity and ART outcomes continues unclear (Shah et al., 2011), due to the disparity in the design of the studies carried out in this area, the lack of standardized criteria (Espinós et al., 2017), the small sample sizes, varying BMI classification systems and inconsistency in the defined outcome measures (Legge et al., 2014).

Obesity is considered a chronic inflammatory status and assisted conception may further potentiate this inflammatory response with possible consequences on endocrine and biochemical factors, affecting the function of the corpus luteum, endometrial receptivity and early embryo development (Sneed et al., 2008). Alterations in the local insulin-like growth factors, cytokines, and leptin levels can also play a role in the detrimental effects of an increased BMI on ART outcomes (Arabipoor et al., 2019) and excess adiposity is also associated with an inflammatory status which may lead to abnormalities in coagulation, fibrinolysis and metabolic syndrome (Sarais, Pagliardini, et al., 2016).

Follicular growth stimulation and ovulation induction are also less effective in obese women (Rothberg et al., 2016), with a reported increase in gonadotrophin consumption, prolonged treatment and superior rates of cycle cancelation due to poor response as well as a lower number of mature eggs and worse-quality embryos (Espinós et al., 2017). According to a retrospective study which compared women with a normal BMI and class I, II, and III obese women, lower estradiol levels were observed on the day of human chorionic gonadotropin (hCG) administration in the latter group (Shah et al., 2011). Several multicenter studies have suggested that the quality of oocytes may be an essential factor influencing pregnancy outcomes in overweight women (Qiu et al., 2019). Specifically, an analysis of 45,163 cycles from the database of the Society for Assisted Reproductive Technologies (SART) described an inverse relationship between obesity and pregnancy rates after the use of autologous eggs, an issue which was overcome with the use of donor oocytes (Shah et al., 2011), suggesting that obesity may have a role on the quality of obese women's own oocytes (Sarais, Pagliardini, et al., 2016). Conversely, a potential decrease in endometrial receptivity was also posited in a later study by the observation of a decrease in pregnancy rates also in obese women who underwent treatment with donated oocytes (Espinós et al., 2017).

The effect on rates of miscarriage is also well recognized (Luke et al., 2011), regardless of the method of conception (spontaneous pregnancy or ART), with an evident increase in women with BMI ≥25 Kg/m^2 (Sarais, Pagliardini, et al., 2016), even after the transfer euploid embryos, showing that aneuploidy is not the major cause of miscarriage in obese women (Cozzolino et al., 2020).

Effects on male fertility

Paternal health may also play an important role in fertility outcomes. Obesity, drug use and nutritional deficiencies can negatively affect sperm count, motility and Deoxyribonucleic Acid (DNA) fragmentation (Lan et al., 2017). However, studies are still scarce, and the results are unclear, which raises concern regarding the reproductive consequences in men in whom the relationship between high BMI, adiposity and infertility is yet to be established (Håkonsen et al., 2011) (Sermondade et al., 2013).

The relationship between obesity and male infertility is thought to be multifactorial, with alterations to the normal function of the hypothalamic–pituitary–gonadal axis being one of them. Aromatization of steroids in peripheral tissues leads to hypogonadotropic hyperestrogenic hypogonadism, with a reduction in testosterone levels and an increase in estradiol, both leading to adverse effects on spermatogenesis (Colaci et al., 2013). Another hypothesis is a higher scrotal temperature caused by fat tissue accumulation (Sermondade et al., 2013). Some authors suggest that obesity may directly alter spermatogenesis and Sertoli cell function, which could lead to impaired sperm production (Sermondade et al., 2013). In contrast, when examining semen parameters, other study concluded that only semen volume, and no other semen parameters, was associated with both BMI and waist circumference, with men in the highest category of waist circumference having a 22% lower total sperm counts when compared with men in the lowest category (Eisenberg et al., 2014).

These conflicting results in the various studies in recent years may be due to the sample selection, as they generally only included men with diagnosed infertility, and the reliance on self-reported BMI. Moreover, previous meta-analyses assessed male adiposity only with BMI, which means that physically active muscular healthy males are grouped with overweight or obese men, which may lead to an underestimation in the association between overweight/obesity and semen quality (Ma et al., 2019). Finally, the semen parameters analyzed are only rough measures of male fertility and, as such, the relationship between true sperm function and fertility cannot be ascertained for sure by evaluating these parameters alone.

Pregnancy is a couple-dependent outcome and the combined effect of obesity in couples was accounted for only in a small number of studies (Arabipoor et al., 2019). One of them, in 2013, showed that women and men BMI are both independently responsible for negative effects on live-birth rates after IVF, although this relationship was less clear in cases of ICSI cycles (Petersen et al., 2013). In order to clarify the doubt left by Petersen et al., and since previous investigations did not differentiate their results according to the type of treatment, a more recent study concluded that a combined effect of couple's BMI did not influence the outcomes of ICSI (Arabipoor et al., 2019), raising one to question whether the ICSI procedure may overcome the potential influence of male obesity on ART.

Effects of weight loss on fertility outcomes

Previous data have demonstrated that a modest weight loss of between 5 to 10% in obese women may be effective in normalizing gynecological disturbances and in improving fertility by increasing ovulation and spontaneous pregnancy rates (Sneed et al., 2008) (Goldman et al., 2019) (Kluge et al., 2019). However, this minor weight reduction is unlikely to be beneficial in very obese women, who will probably require a superior weight loss. Health professionals have hesitated whether to recommend weight loss programs due to concerns regarding the safety of weight reduction immediately prior to conception and the lack of experience in this population, namely with only a limited number of studies documenting the effect of weight loss in obese women with infertility (Rothberg et al., 2016). Furthermore, while weight reduction seemed to be beneficial in PCOS individuals trying spontaneous conception, studies in these patients to date have not entirely assessed the impact of a decrease of BMI on IVF outcomes (Bailey et al., 2014). Therefore, the real effect of weight loss in fertility outcomes remains largely unknown.

Some authors suggest that the usual strategies of counselling, improved diet and exercise had a minimal effect on weight loss and that bariatric surgery could lead to better results. The problem of this approach is the concern regarding the potential malnutrition or malabsorption and its effects later on during pregnancy (Caughey, 2015), such as previous reports alluding to an association with small-for-gestational-age infants, shorter duration of pregnancy, cesarean delivery, maternal death and neonatal adverse outcomes (Luck et al., 2017). According to the American College of Obstetricians and Gynecologists (ACOG), women should be counselled to postpone conception until 12 to 24 months after surgery, which is the period when the most rapid weight loss occurs (Caughey, 2015).

A reduced number of studies have investigated the effect of weight loss in obese infertile women undergoing ART, suggesting an improvement in ART outcomes in obese women who trained regularly, and a negative association between poorer diet composition and infertility, both independent of weight change (Lan et al., 2017). Nutritional deficiencies from caloric restriction immediately prior to ART can affect fertilization. Nevertheless, a modest preconception weight loss can improve natural pregnancy in infertile women, which suggests that lifestyle changes potentially can improve fertility (Lan et al., 2017).

In a multicenter randomized trial including obese infertile women, a lifestyle modification program consisting of 6 months of diet with a reduction of energy intake by 600 kcal daily with the assistance of an online diet diary together with moderate to intense physical activity of at least 30 minutes two or three times/week was tested. Motivational counseling was also provided. This lifestyle program prior to infertility treatment did not result in higher rates of live-births after 24 months follow-up, when compared with women who received immediate infertility treatment. Moreover, the time to achieve pregnancy and the rates of neonatal outcomes were similar in both groups (Mutsaerts et al., 2016). A more recent randomized controlled trial also failed to find any increase in live-birth rates after weight loss (Einarsson et al., 2017). Meanwhile, Sacha et al. (2018) found that from 148 women enquired about the willingness to try to lose weight before ART, 70% with over-weight and 68% with obesity were presently trying to lose weight. Moreover, the majority of these women trusted that with intervention program they could lose more weight than alone, but also pointed to some disadvantages such as an increase in the time to pregnancy and overall treatment cost (Sacha et al., 2018).

Another study in 2017 developed a structured program which combined 12 weeks of low-calorie diet and physical exercise in obese women prior to a single IVF cycle and showed that this program led to weight reduction, a decrease of visceral adiposity and better cumulative live-birth rates (61.9%, versus 30.0% in the group who did not undergoes to lifestyle program) (Espinós et al., 2017). However, not all women had uniform outcomes, as around half of the women experienced a weight loss of <5%, which was a limiting factor in assessing the potential benefits of the intervention., Moreover, no obvious justification for the interindividual variability in the results of the intervention could be found. Greater or faster weight reduction would imply a more rigorous diet and exercise program or a longer intervention phase, which have been shown to rise the percentage of dropouts substantially, with women returning to their initial weight shortly after (Espinós et al., 2017). Moreover, excessively aggressive weight loss was also associated with a negative effect on ART outcomes and higher risk of unfavorable pregnancy results, such as miscarriage (Mutsaerts et al., 2016), suggesting that balance is critical. Finally, more recent data suggested that lifestyle interventions improved pregnancy rates although without actually leading to an improvement of live-birth rates, given that women had a higher risk of miscarriage (Espinós et al., 2020).

Women with class III and class IV obesity are frequently rejected for IVF treatment due to the potential worries around possible anesthetic and procedural complications during oocyte retrieval and embryo transfer, despite little being known regarding the incidence of these adverse outcomes during such procedures (Romanski et al., 2019). However, given the weak association between lifestyle interventions and improved ART outcomes, some have questioned whether there should be any BMI threshold to allow access to fertility treatments (Lan et al., 2017).

Regarding weight loss programs in males prior to conceiving, despite being still controversial, previous studies have shown that some endocrine abnormalities may be reversed. In a cohort study, weight loss was associated with an increase in total sperm count and semen volume, as well as an increase in testosterone levels in men participating in a 14-week program. However, it cannot be excluded that the actual changes in lifestyle may cause the observed progress in semen quality, rather than the reduction of weight per se (Håkonsen et al., 2011). Moreover, while weight loss was associated with an increase in total sperm count in a recent pilot study, other surveys reported a deterioration of semen parameters during the months after weight reduction by bariatric surgery (Sermondade et al., 2013).

A randomized trial including 97 infertile heterosexual couples was conducted involving a lifestyle intervention targeting their female partner with obesity. This study had the objective of enhance obese women's lifestyle but it later proved that the strategy proposed was still insufficient. Nonetheless, it was shown that the weight loss of male partners increased the probability of conception of the couple, so it could be important to enroll more actively male partners in interventions directed at women, in order to improve their fertility (Belan et al., 2019). Hence, it is equally important to determine the impact of lifestyle intervention on male partners of infertile couples to understand whether such may be correlated with better pregnancy outcomes.

Study aims

ACOG recommends that ‘‘optimal control of obesity begins before conception”. However, when infertility has already been diagnosed, patients are frequently reluctant to delay fertility treatments further in order to achieve weight loss because of an apprehension around the limited success of such intervention, concerns that a large amount of time is necessary to achieve adequate weight reduction, the false perception that ovulation induction is a quicker method to become pregnancy, and the belief that the risks of pregnancy associated with obesity are amenable (Rothberg et al., 2016). Moreover, most of the previous studies that analyzed the relationship between BMI and the results of ART did not contrast the relative importance of this factor against other relevant confounding factors, including the age of the woman performing IVF. That said, Sneed et al. suggests that pre-conceptional weight loss may only be considered in patients younger than 36, since in older women the leading effect of age on fertility and IVF outcomes become more important than the effects of weight. Specifically, these authors alluded to a potential effect modification of age on BMI, namely regarding the number of retrieved oocytes, mature oocytes, clinical pregnancy and live-birth rates.

Thus, while at first glance it would seem reasonable to delay treatment and recommend weight loss prior to ART, this recommendation may potentially hinder the success rates of patients at a more advanced age (Sneed et al., 2008). Moreover, only female partners were analyzed, leaving the potentially-compounding contribution of male age and BMI in heterosexual couples unexplored. Finally, CLBR was not considered the primary outcome.

Hence, with the present study, our main study question was **should couples who are overweight or obese attempt to lose weight prior to ART or should they start immediately?**

Material and Methods

Study design and sample

This retrospective analysis included patients undergoing their first ART cycle using autologous gametes between January 1st 2013 and February 1st 2018 in one of the 18 private ART clinics in the Iberian Peninsula belonging to the *Instituto Valenciano de Infertilidad* (IVI) group. Each subject was treated following the approval granted by the respective Ethics Committees for Clinical Research in both Portugal and Spain (study code 2007-LIS-051-SR). Only cycles in which a known live-birth outcome was reported after either a fresh or one of the subsequent frozen embryo transfers (FETs) using embryos generated from the same oocyte retrieval procedure and transferred within the study period were included.

Treatment cycles were excluded if entailed performing either natural or mild stimulation, used donated gametes (oocytes or sperm) or embryos. Cycles performed for fertility preservation were also excluded. Finally, those lost to follow-up or without a known live-birth outcome were also excluded from the analysis.

Treatment

ART are complex and consist of numerous decisive steps that involve the handling of gametes or embryos in order to achieve a pregnancy (National Center for Chronic Disease Prevention and Health Promotion - Division of Reproductive Health, 2019). In the present study, we only considered conventional IVF and ICSI. IVF entails the fertilization of extracted gametes in the laboratory and subsequent transfer of embryos into the uterus through the cervix. ICSI is a specific subtype of in vitro fertilization, consisting in the injection of a single sperm cell directly into an oocyte (National Center for Chronic Disease Prevention and Health Promotion - Division of Reproductive Health, 2019). ICSI is generally preferred to conventional IVF in the presence of severe male-factor infertility or in couples with previous failure or poor fertilization after conventional IVF (National Institute for Health and Care Excellence, 2013).

<u>Exogenous ovarian stimulation during ART</u>

Most ART cycles commence with the administration of exogenous ovarian stimulation, in which medication is given to stimulate multi-follicular growth while suppressing endogenous ovulation triggering by the hypothalamus-pituitary axis. The drugs administered include human menopausal gonadotropin (hMG) (Devroey et al., 2012), FSH (Pouwer et al., 2015), GnRH analogues (Siristatidis et al., 2015) and hCG (Checa et al., 2012).

In rare instances, natural cycle IVF, which consists in the monitoring of a spontaneous woman's cycle without medication, can also be performed (Nargund et al., 2001). However, in an attempt to improve the chances of conception (Siristatidis et al., 2015), exogenous stimulation is generally used to induce the development of multiple dominant follicles in order to mature a large number of high-quality oocytes and, subsequently, produce multiple embryos (Macklon et al., 2006). Conversely, mild ovarian stimulation or modified natural cycle IVF can also be performed with the administration of either clomiphene citrate or a low dose of exogenous gonadotropins. These approaches minimize the cost and duration of the treatment, while also reducing the risk of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS) (Allersma et al., 2013). However, women may require multiple attempts to achieve a pregnancy with this strategy because of the higher cancellation rates and the lower pregnancy rate per treatment (Verberg et al., 2008). Hence, conventional ovarian stimulation using conventional doses of exogenous drugs still represents more than 80% of the ART stimulation practices (Rongieres-Bertrand, 1999).

Conventional treatment protocols involve the use of gonadotropins usually from the beginning of menses onwards (Pouwer et al., 2015) maintaining a steady-state of circulating FSH that exceeds the threshold for multi-follicular development (Duijkers et al., 2002). In the late fifties, it was demonstrated that extracts derived from the hypophysis could be used to stimulate gonadal function and experiments with the extraction of both luteinizing hormone (LH) and FSH from urine of postmenopausal women led to the development of hMG (Macklon et al., 2006), which became widely used. However, the initial preparations contained multiple contaminating proteins, resulting in hypersensitivity reactions and painful administration (Macklon et al., 2006). Improved protein purification technologies allowed to produce purified urinary preparations. Later, recombinant preparations offered not only improved purity, but also consistency and large-scale availability (Macklon et al., 2006). However, in terms of efficacy, highly purified hMG still seems to be at least as effective as recombinant FSH (Devroey et al., 2012). Finally, the use of recombinant DNA technologies have also resulted in a new recombinant molecule, a long-acting FSH, named corifollitropin alfa (Fauser et al., 2009), making it possible to replace the first seven daily injections of exogenous FSH with a single injection. Corifollitropin alfa is as effective as recombinant FSH in terms of live-birth rate, which constitutes an advantage especially for older women and poor responders who frequently require high doses of gonadotropins (Cozzolino et al., 2019).

The administration of exogenous ovarian stimulation carries a high risk of premature LH surge, resulting in premature ovulation (Rongieres-Bertrand, 1999) (Allersma et al., 2013). Hence, GnRH analogues, which reversibly block pituitary function and prevent the occurrence of a premature LH surge, are an intricate part of ART. Originally, such down-regulation was mostly achieved with GnRH agonists, which are drugs that cause a LH and FSH hypersecretion that is followed by the depletion of pituitary storage and desensitization. The addition of such drugs to the stimulation protocol results in a significant improvement in treatment outcomes, including an increase in the number of oocytes retrieved (Testart et al., 1993), a decrease in the cancellation of cycles and higher pregnancy rates (E Fields et al., 2013). In the so-call long protocol, GnRH agonists are administered approximately two weeks before starting stimulation, usually during the mid-luteal phase (e.g. the 21st day) of the previous cycle, and continued until ovulation triggering (Allersma et al., 2013). However, GnRH antagonists can also be used. This latter approach is frequently considered as a more patient friendly option, since it causes an immediate, reversible and dose-related inhibition of gonadotrophin release, limiting its need for administration to the last days of stimulation, in which a premature LH surge is most likely to occur. Live-birth rates seem to be comparable between GnRH agonist and GnRH antagonist protocols, although GnRH antagonists substantially reduce the incidence of OHSS. There are two approaches in GnRH antagonist administration, the single-dose protocol, in which one injection of GnRH antagonist (Cetrotide® 3 mg) is administered in the late phase of ovarian stimulation, and the more commonly used multiple-dose regimen, that can be done in a fixed regimen (0.25 mg administered daily from stimulation day 6 onwards), or in a flexible regimen (based on the follicular size) (Huirne et al., 2007). It was demonstrated that there were higher pregnancy rates when using the fixed protocol, possibly justified by greater LH control (Kolibianakis et al., 2006).

Ovulation triggering and oocyte retrieval

After drug initiation, transvaginal ultrasound monitoring is needed to track the follicular development, and when the follicles achieve an appropriate size (in the case of this study, such was defined as the presence of ate least three follicles with at least 17 mm of mean diameter), final oocyte maturation and ovulation triggering is triggered (Farquhar & Marjoribanks, 2018) mostly using exogenous hCG (either purified from the urine of pregnant women or produced by recombinant DNA technology). Ovulation triggering is then followed by oocyte retrieval which consists of the surgical aspiration under transvaginal ultrasound guidance approximately 35-36 hours later. GnRH agonist can be used as another effective alternative to this final oocyte maturation and ovulation triggering, in order to decrease the risk of OHSS, with the same efficacy proven on IVF treatment (Santos-Ribeiro et al., 2016).

IVF and embryo transfer

The next step is the fertilization process itself, where aspirated oocytes are fertilized and cultivated in vitro. The sperm used for fertilization can be retrieved from the male either by masturbation of following surgical retrieval when the former is not feasible- (National Institute for Health and Care Excellence, 2013). After, fertilized embryos are left in culture media under tightly-regulated environmental conditions (Farquhar & Marjoribanks, 2018) to allow growth during a few days prior to being transferred into uterus, cryopreserved or discarded (whenever of insufficient morphological quality).

Embryos are commonly transferred in either the cleavage (3 days after fertilization) or blastocyst (5 to 7 days after fertilization) stages. The number of embryos transferred depends on multiple prognostic factors including maternal age, embryo quality and the predicted risk of multiple pregnancies. The American Society for Reproductive Medicine (ASRM) recommends that single-embryo transfer be considered in women < 35 years old with a favorable prognosis (≥ 1 high-quality embryos available for cryopreservation; euploid embryos; previous live-birth after IVF cycle), while the National Institute for Health and Clinical Excellence (NICE) recommends single-embryo transfer in first full IVF cycle in women < 37 years old.

Luteal phase support includes various options such as the administration of progesterone (preferred) or hCG (Farquhar & Marjoribanks, 2018), although hCG is now only seldomly used given since it seems to increase the risk of OHSS without improving pregnancy outcomes (National Institute for Health and Care Excellence, 2013).

Exposure/Independent variables assessed

Age of both the female and male partners and their preconceptional BMIs were the independent variables considered. These variables were available at the beginning of the treatment in all included cases. The main objective of our study was to assess the potential association between female/male age and female/male BMI with ART treatment outcomes. Hence, the age range of patients included in the study were intentionally wide, spanning from 18-50 years in women and 18-60 years in men, to maximize the exploration of the effect of these variables. Meanwhile, according to Centers for Disease Control and Prevention (CDC) and WHO, BMI is defined as an individual's weight (in kilograms) divided by the square of height (in meters). A BMI less than 18.5 kg/m^2 is within the underweight range, BMI from 18.5 to 25 kg/ m^2 is defined as normal, BMI from 25.0 to 30 kg/m^2 is within the overweight range and BMI of 30.0 kg/m^2 or higher is within the obese range. Normal weight couples, i.e. both male and female's BMI within the normal range (between 18.5 kg/ m^2 and 24.9 kg/ m^2), were considered the reference group, to survey the potential effect of overweight and obesity in ART outcomes. Similarly, to investigate the effect of age, those equal or below 30 years old were considered the reference group.

Covariates

There are several factors that may affect the results of ART cycles. A priori confounders could be the cause of infertility, the number of oocytes retrieved, the number and developmental stage of the embryos transferred and the transfer of a fresh or a thawed embryo. Furthermore, harmful habits of couples, such as smoking, should be accounted for when recorded.

Causes of infertility include female factors, male factors and unexplained factors. The main distinction should be made between ovulatory and anovulatory women. Ovulation disorders occurs when ovaries fail to produce a mature oocyte regularly, owing to hypothalamic dysfunction (hypothalamic amenorrhea; hypogonadotropic hypogonadism), polycystic ovary syndrome, ovarian failure or hyperprolactinemia

(National Institute for Health and Care Excellence, 2013). Furthermore, the presence of endometriosis or tubal occlusion may contribute to the failure of conception irrespective of ovarian function. Male factor infertility can affect the outcomes of pregnancy and may be responsible for the use of different types of treatment, such as ICSI. A diagnosis of male factor infertility is made if WHO criteria was not fulfilled during semen analysis (Cooper et al., 2010).

The number of transferred embryos may affect the outcomes of treatment and may increase the risk of pregnancy complications (Ishihara et al., 2014). Kissin et al. (2014) considered an association between a higher chance of a good perinatal outcome in women aged under 35 years with a favorable prognosis and a single embryo transfer (Kissin et al., 2014) (Practice Committee of the American Society for Reproductive Medicine, 2017).

Regarding status of embryo (fresh or frozen-thawed), it was found that there is a reduced risk of ectopic pregnancy, preterm birth, low birth-weight, small for gestational age and perinatal death in frozen-thawed embryo transfer, when compared with fresh embryo transfer (Qiu et al., 2019) (Li et al., 2019), all which can affect our results if unaccounted for.

Main Outcome Measures (Dependent)

The following endpoints were defined according to definitions of International Committee Monitoring Assisted Reproductive Technologies (ICMART) (Zegers-Hochschild et al., 2017), with gestational age being calculated according to the day of embryo transfer.

The primary outcome assessed was CLBR, defined as the delivery of at least one live-birth resulting from the initiated treatment cycle, including all fresh embryos transferred and/or eventual frozen embryos subsequently transferred, until the first delivery with a live-birth or all embryos are used, whichever occurred first. It is important to clarify that a live-birth referred to an individual newborn after 22 completed weeks.

The secondary pregnancy outcome evaluated included time to pregnancy (TTP) defined as the time taken to establish a clinical pregnancy measured in months. Regarding the offspring, the neonatal endpoints contemplated were birth weight, birth weight z-scores and prematurity.

Concerning birth weight, the following indicators were also evaluated: low birth weight was considered if a newborn weighed less than 2500 g, and very low birth weight if a newborn weighed less than 1500 g. Birth-weight z-score were calculated using the following formula: z-score = («observed value» - «sex-and-gestational-age-adjusted median value of the national reference population») / « sex-and-gestational-age-adjusted standard deviation value of reference population. Small for gestational age (SGA) was defined as a birth weight under the 10^{th} percentile for gestational age and large for gestational age (LGA) was defined as a birth weight above the 90^{th} percentile for gestational age (according to the sex-specific birth weight for a given gestational age reference). According to WHO, fetal macrosomia is defined as birth weight over 4000 g irrespective of gestational age. In order to calculate the z-scores, SGA and LGA, we used the INTERGROWTH-21st dataset as the reference population (J. Villar et al., 2014) (José Villar et al., 2016).

A preterm birth (PTB) was defined as a birth that occur after 22 weeks and before 37 completed weeks of gestational age, while very preterm birth was defined as a live-birth occurring before 34 weeks.

Statistical methodology

Database

The database was rigorously defined and only included the variables intended to be analyzed according to the study objectives. The centers contributing data which were included in the research were: IVI A Coruña, IVI Albacete, IVI Alicante, IVI Almería, IVI Barcelona, IVI Benalmádena, IVI Bilbao, IVI Burgos, IVI Cartagena, IVI Castellón, IVI Elche, IVI Faro, IVI Girona, IVI Ibiza, IVI Las Palmas, IVI Lleida, IVI Lisboa, IVI Madrid – Alcorcón, IVI Madrid – Aravaca, IVI Madrid – Centro, IVI Málaga, IVI Mallorca - Manacor, IVI Mallorca - Palma, IVI Murcia, IVI Pamplona, IVI Salamanca, IVI San Sebastián, IVI Santander, IVI Sevilla, IVI Tenerife, IVI Valencia, IVI Valencia - Alzira, IVI Valencia - Gandía, IVI Valencia - Torrent, IVI Valladolid, IVI Vigo, IVI Vitoria and IVI Zaragoza.

The necessary information was exported from SIVIS® to a table in Excel® format through a database query system. The exported data was anonymized in order to protect the patient's' clinical and personal information in accordance with European, Spanish and

Portuguese legislation regarding Biomedical Research. IVI Foundation was in charge of carrying out the process of extraction and anonymization of the data in the IVI centers. Finally, and previous to the statistical analysis, in order to review the quality of the extract information, an exploratory analysis of data was carried out. The anonymized database was only accessible to the statisticians involved.

Pre-analyses power analysis

Given the exploratory nature of the analysis and the unknown net effect of the four variables being studied, we were unable to determine with accuracy the ideal sample size for this study a priori. Moreover, since the four independent variables evaluated were continuous, this further complicated the estimation of an adequate sample. Nonetheless, for our main outcome of interest, CLBR, we estimated that at least 10000 cycles with a known liveborn outcome would be available and that the CLBR will be approximately 45%. Hence, this sample of large proportions would have >80% power to detect >2% differences in CLBR caused by one of the independent variables.

Statistical analysis

Categorical data was presented in absolute and relative frequencies, while continuous values were be summarized according to the normality of the distribution with either means and standard deviations or medians and interquartile ranges, as appropriate. Unadjusted between-group comparisons were performed using the chi-square test for categorical variables, and either the t-test or Kruskal-Wallis tests according to the normality of the distributions for continuous variables. Stata Software version 13.1 (StataCorp, College Station, Texas, USA) was used for statistical analysis, with a p-value <0.05 being considered as statistically significant and followed by Bonferroni-adjusted pairwise comparisons.

Confounder-adjustment was performed using multivariable regression analysis. The independent variables were first added to regression models as continuous variables. However, in order to avoid bias by assuming that the relationship between these continuous predictors and the ART outcomes is linear, and also in attempt to facilitate the application of the results into everyday clinical practice, we also ran models in which these variables were added as categorical variables or using fractional polynomials to assess potential non-linear relationships. These included the following analyses:

- Figure 1: we assessed the variation in the CLBR across female BMI, performing multivariable logistic regression and adjusting for female age, partner age, number of oocytes retrieved, ovulatory factor infertility, tubal factor infertility, uterine factor infertility, infertility diagnosis due endometriosis, other female infertility diagnoses, male factor infertility and female smoking habits;

- Figure 3: we evaluated the variation in the CLBR across female and male age, performing multivariable logistic regression adjusted for female BMI, number of oocytes retrieved, ovulatory factor infertility, tubal factor infertility, uterine factor infertility, infertility diagnosis due endometriosis, other female infertility diagnoses, male factor infertility and female smoking habits;

- Figure 4 and Table 4: we determined the variation in the predicted CLBR across female age and BMI, performing multivariable logistic regression and adjusting for partner age, number of oocytes retrieved, ovulatory factor infertility, tubal factor infertility, uterine factor infertility, infertility diagnosis due endometriosis, other female infertility diagnoses, male factor infertility and female smoking habits;

- Table 5: we assessed the relationship of CLBR with female and male BMI, performing multivariable logistic regression adjusted for female age, partner age, number of oocytes retrieved, ovulatory factor infertility, tubal factor infertility, uterine factor infertility, infertility diagnosis due endometriosis, other female infertility diagnoses, male factor infertility and female smoking habits;

- Table 2: we performed multivariable cox regression analysis to assess the mean time to pregnancy stratified by maternal BMI, adding the following confounding variables as the appropriate control variables to reduce bias: female age, partner age, number of oocytes retrieved, ovulatory factor infertility, tubal factor infertility, uterine factor infertility, infertility diagnosis due endometriosis, other female infertility diagnoses, male factor infertility and female smoking habits;

- Table 3: we assessed the variation in the neonatal outcomes across female BMI, performing multivariable linear or logistic regression, as appropriate, adjusted for female age, partner age, fresh embryo transfer, number of oocytes retrieved, ovulatory factor infertility, tubal factor infertility, uterine factor infertility, infertility diagnosis due endometriosis, other female infertility diagnoses, male factor infertility and female smoking habits (and new-born gender and gestational age for birth-weight <2500 g or >4000 g).

Results

The baseline characteristics are shown in **Table 1**. A total of 23.5% of women were overweight or obese. Owing to large sample size, multiple clinically small differences among the groups were still found to be statistically significant, including female and male age, and multiple female infertility diagnoses. The diagnosis of ovulatory factor infertility was more frequent in obese group (33.0% vs 24.3% in overweight group and 24.8% in reference group, p<0.01). Moreover, male factor infertility was also less frequently present in the underweight group (35.0% vs 40.2% in reference group, 41.1% in overweight group and 44.5% in obese group, p<0.01), while overweight and obese women were administered higher doses of gonadotropins.

The number of oocytes retrieved did not vary by BMI class, while the overweight group presented slightly fewer embryos when compared with the normal weight group. Among the fresh embryo transfers at blastocyst stage, women with obesity (43.9%) had fewer transfers than women with normal and low weight (50.7% and 54.6% in normal and low BMI, respectively, p<0.01).

Cumulative live-birth rate and time to pregnancy

The CLBR stratified by female BMI category is shown in **Figure 1.** Following multivariable logistic regression, when compared with the normal weight group, CLBR was lower in overweight (aOR 0.83, 95% CI 0.75-0.92) and obese (aOR 0.69, 95% CI 0.58-0.81) women. Conversely, no statistically significant difference with the underweight group was found.

A Kaplan-Meier curve presenting the time to an embryo transfer leading to a live-birth is shown in **Figure 2**, while the mean time is shown in **Table 2**. Time is measured in months after oocyte retrieval and the results correspond to a single treatment cycle, with more than one possible transfer. The average time to achieve a clinical pregnancy that eventually resulted in a live-birth for 50% of women corresponds to the beginning of the chart plateau in each BMI line. The mean time was higher than the reference group (3.1 months, 95% CI 2.9-3.2) for the overweight (3.5 months, 95% CI 3.1-3.9) and obese BMI groups (4.1 months, 95% CI 3.4-4.8).

Neonatal outcomes

Neonatal outcomes are shown in **Table 3.** As for birth-weight, compared with the average weight of newborns of normal BMI women (reference group), the weight in newborns of overweight women was on average 34.5 g higher (95% CI between 1.1 and 68.0 grams), while the weight of newborns of obese women was 96.1 g higher (95% CI between 39.9 and 152.4 grams). However, when assessing birth-weight z-scores (which is the most relevant measure for comparing individual weights accounting for gender and gestational age), the results revealed that, when compared to the reference group, birth-weight z-scores were 0.08 (95% CI between 0.00 and 0.17) greater in newborns of overweight mothers and 0.22 (95% CI between 0.08 and 0.36) greater in newborns of obese mothers. Furthermore, there was a higher frequency of LGA newborns in obese mother group (0.52, 95% CI between 0.08 and 0.95). Gestational age did not vary significantly among the groups.

Cumulative live-birth rate in relation with female/male age and female BMI

The influence of female and male age on CLBR is shown in **Figure 3**. There is an evident relationship between female age and CLBR, which increases from 25 to 30 years, being maximum at 30 years, and then decreasing, with this decrease being more pronounced between 35 and 45 years, of approximately 30%. Conversely, the role of the male age did not seem so evident. Male age decreased CLBR 1.09% per year (95% CI between 0.26% and 1.92%). The peak on the chart corresponds to the point at which the CLBR was highest (between 50 and 60%) and occurs when the woman is in her 30s, and in this age group the age of men did not seem to affect CLBR.

The relationship between female age and BMI in terms of predicted CLBR is shown in **Figure 4**. The peak on the chart corresponds to the point at which the CLBR was highest (between 50 and 60%) and occurred when the woman was between 30 and 31 years old, with a BMI among <18.5 and 24.9 kg/m^2. For the same age, CLBR decreased with an increase in BMI, greater than 24.9 Kg/m^2. As maternal age advanced, BMI had a less marked influence on CLBR.

In the **Table 4**, for female ages shadowed in green, a reduction from obesity/overweight subgroups to overweight/normal-weight subgroups within the following year was numerically still beneficial. Meanwhile, for the female ages shadowed in yellow, only a reduction from obesity to at least normal weight in the following year would be numerically beneficial. Finally, for women over 38 years-old, a year-long reduction in BMI group was no longer beneficial. As mentioned before, for heterosexual couples, a mean decrease in CLBRs per year of 1.09% (95% CI between 0.26% and 1.92%) should also be accounted for due to male ageing.

Cumulative live-birth rate in relation with female/male BMI

Only 1398 cycles had both female and male BMI registered. **Table 5** shows the results of the multivariable logistic regression model attempting to assess the relationship between female and male BMI and CLBR. Within this restricted sample, CLBR were lower in the category of female BMI $\geq$30 kg/m^2, when compared to the reference group (aOR 0.54, 95% CI 0.31-0.94). Regarding male BMI, this difference in relationship, when compared, was not statistically significant.

Discussion

The main finding of our study was verifying that reducing BMI within the following year from obesity or overweight to overweight or normal weight, respectively, is beneficial up to 35 years old. Conversely, between 36 and 38 years-old, only a larger reduction (from obesity to normal BMI) seemed beneficial. This paradigm changed in women above 38 years, where an advantage in postponing for a year the start of treatments in order to lose weight no longer remained.

Another objective of this study was to clarify the role of the male partner in a heterosexual couple regarding weight loss in relation to aging. Analyzing the literature about the effect of weight on male fertility, it is verified that the studies are centered on endocrine parameters, total sperm count, semen volume (Håkonsen et al., 2011) and semen quality (Lan et al., 2017). Evidence regarding CLBR as the primary outcome is scarce. In our study, male BMI had no statistically significant impact on CLBR. Moreover, concerning the age of male partner, it was observed that it influences the CLBR in a much less marked way than female age.

Clinical implications

Previous studies have shown that obese patients may benefit from a weight loss of at least 5% (Goldman et al., 2019). However, weight loss is a complex challenge for much of the overweight population, even more so when under a time limit to optimize conception. A large proportion of women were not willing to lose weight if the delay period for ART was longer than 3 months (Sacha et al., 2018). The challenge is even greater when significant loss (i.e., from obese to overweight or normal weight, as our results proposed) is required and if this loss takes more than one year or does not ultimately lead to the target weight, both factors may compound to decrease CLBR further. Moreover, as meaningful weight loss often takes at least a year to achieve (Kim et al., 2020), this poses an extra issue for women at older ages who seem not have such time to squander.

There is a diversity of therapeutic approaches for losing weight including lifestyle interventions, pharmacotherapy and bariatric surgery. However, the success of each alternative is heterogeneous in terms of weight reduction and maintenance, demonstrating a multifactorial and individualized response. Nevertheless, it has been pointed out that the main factor for the success of any intervention seems to be the motivation and compliance of the patients, which may often be directly associated with their physical, psychological and financial capacity (Severin et al., 2019).

The largest studies on weight loss in infertility focus essentially on lifestyle measures (Einarsson et al., 2017; Mutsaerts et al., 2016). These interventions are commonly the first option for health professionals, with a common duration spanning from 12 weeks to 6 months prior to a follow-up of up to one year (Kim et al., 2020). These strategies are based on caloric restriction for fat metabolization, involving physical exercise (moderate or intense aerobic activity), diets under the supervision of professionals from different areas, psychosocial therapy and education either with face-to-face consultations or using tele-health (Severin et al., 2019). According to previous systematic reviews and meta-analyses, lifestyle interventions improved ovulatory response and showed a higher rate of natural pregnancies (Kim et al., 2020), including in women who intended to start treatment after weight loss; however, no improvement in TTP and pregnancy rates following ART were observed (Best, Avenell, & Bhattacharya, 2017). Moreover, live-birth as the main outcome was seldomly reported in the studies and, when assessed, failed to show any difference (Mutsaerts et al., 2016) or, at best, showed only a trend towards improvement (Kim et al., 2020). Therefore, although lifestyle interventions may be attractive due to their ease of use and low cost, doubts remain regarding their efficacy (van Oers et al., 2017).

It is proven that it is possible to lose up to 10% in 4 to 6 months solely with lifestyle interventions (Severin et al., 2019). Nonetheless, in addition to this benefit being less likely in patients with extremely high BMIs, not everyone can achieve this percentage, as shown in the LIFEstyle Study, where only 38% of patients achieved weight loss between 5 and 10% of their initial weight (Mutsaerts et al., 2016), or in another observational study when only 25% of the patients reached the goal of $\geq$ 5% at the end of the dietetic intervention (Verberne et al., 2019). Even when patients manage to achieve this goal, there is a tendency after 6 months to reach a plateau followed frequently by a regaining of weight within the next 5 years (Severin et al., 2019). Verberne et al. (2019) also emphasizes that only 9% of patients completed treatment for a year or more, with the majority dropping out earlier. One potential solution to enhance the success of these programs is the motivational contribution of the partner to the behavioral change of the patient, that is, a couple-based intervention rather than individual intervention, since this can lead to a better support and compliance without any significant additional cost (Best, Avenell, Bhattacharya, et al., 2017).

All in all, the available evidence seems to show that no specific lifestyle intervention is the best when it comes to weight reduction (Best, Avenell, & Bhattacharya, 2017), but strategies that delayed fertility treatment (Espinós et al., 2020) and less intensive programs delivered by primary health care providers (Severin et al., 2019) had greater discontinuation rates. Thus, others have also proposed intensive programs that essentially entail periods of at least 12 weeks of intense energy restriction with liquid diets followed by a solid diet replacement in the next two weeks, to reach a significant weight loss above 15% from original weight and a BMI as close to normal as possible (Rothberg et al., 2016). This approach can be associated or not with moderate daily activity, although exercise may be more useful for maintaining low weight after a descent with an assertive caloric restriction (Legro, 2017). Studies have proven that intense weight loss is safe (Rothberg et al., 2016) and effective in increasing the rate of spontaneous pregnancy (Einarsson et al., 2017), contradicting the possible harms of excessive weight loss in a short period of time previously posited by Mutsaerts (Mutsaerts et al., 2016). This could mean that more intensive intervention programs could lead to better results. However, although these regimens lead to a great weight loss, almost three times more than in the LIFEstyle Study (Kluge et al., 2019), still only a small percentage reached a BMI below 25 kg/m^2 and the difference between groups regarding ART outcomes (including live-birth rates) was not statistically significant (Einarsson et al., 2017). Conversely, two studies observed that very rigid diets (vegan or ketogenic, for example) that can be difficult to accomplish and that they can also lead to higher discontinuation rates (Best, Avenell, & Bhattacharya, 2017) while strict caloric restriction (i.e., less than 800 kcal/day) may cause severe health outcomes and potential damage to oocytes (Legro, 2017), which can imply waiting a while before starting the ART treatment. Moreover, weight regain can also occur following intensive programs (Kluge et al., 2019). This data corroborates that even with an intensive well-planned lifestyle program, there seems to be weak evidence to an advantage in ART patients (Norman & Mol, 2018).

Another alternative to faster weight loss is pharmacotherapy, that may take place in the obese population having difficulties in losing or maintaining their weight loss in the long term (Legro, 2017). Several drugs have been already used for such purpose, including naltrexone/bupropion, liraglutide, orlistat, lorcaserin and phentermine/topiramate, leading to an additional weight loss (3-9% of the initial weight after one year) when combined with exercise or diet (Severin et al., 2019). However, in the subfertile female population, this approach has a major limitation due to concerns regarding risks of teratogenicity (Kang & Park, 2012) and lack of evidence of its long-term safety (Legro, 2017) with need for future trials. Therefore, in the meantime, it is considered safer to maintain lifestyle primary interventions as a first line, until better evidence regarding the administration of these drugs in the preconception period is available (Best, Avenell, & Bhattacharya, 2017).

Finally, bariatric surgery is also an effective weighting loss procedure indicated for extremely obese patients (BMI greater than 40, or 35 if associated with other comorbidities), following the failure of first-line interventions such as psychological and endocrine follow-up (Mahutte et al., 2018). Following ART, unoperated extremely obese patients demonstrated a much lower live-birth rate per transfer, when compared with a group of patients who underwent bariatric surgery and with another group of non-operated patients with the same BMI as the previous one after surgery (Grzegorczyk-Martin et al., 2020). Hence, women submitted to bariatric surgery seem to have the same chances to conceive as those of non-operated individuals with a comparable BMI (Grzegorczyk-Martin et al., 2020). However, it is important to note that live-birth rates were once more seldomly reported in most studies, and, moreover, the doubt regarding the impact in terms of safety of such a rapid weight loss to mother and fetus remains. Hence, these and other potential benefits of bariatric surgery – such as a decrease in the rates of gestational diabetes, hypertension and LGA/macrosomia – should be balanced with the inherent risk for the occurrence of SGA and preterm birth (van Oers et al., 2018). To this extent, small cohort studies have reported no differences in obstetric or neonatal outcomes between women conceiving within the first year after bariatric surgery and those who waited one or more year, a result which is of special importance for women with advanced age (Mahutte et al., 2018). Despite this, postponement of at least 6 months for the gastrointestinal tract to recover and to be able to ensure adequate nutritional intake for a pregnancy is still advised (Legro, 2017). Moreover, bariatric surgery is also an invasive and costly procedure, prone to surgical complications and consequent morbidity (Legro, 2017). In addition, the majority of analyzed patients undergoing fertility treatments belong to groups of BMI without surgical indication (Einarsson et al., 2017) and, even when surgery is recommended, only a small percentage of those who meet the criteria eventually opt for surgery (Legro, 2017).

Considering the advantages and disadvantages of all approaches, one can conclude that, on one hand, few interventions have achieved a normal BMI and/or control weight regain, while, on the other hand, even when successful, uncertainty regarding the outcomes in terms of CLBR or postnatal outcomes will continue to exist (Legro, 2017).

This set of evidence leads to the conclusion that pretreatment weight change cannot be seen as an absolute guarantee of benefit in terms of both pregnancy and perinatal outcomes, defying the theory advocated prior to these large prospective trials, making it increasingly difficult to justify delaying the onset of fertility treatment in an attempt to achieve significant weight loss (Norman & Mol, 2018).

Strengths and limitations

Regarding the limitations of our presented research, the main weakness is the retrospective nature of the study, which means that the groups may not be completely comparable and that there may be potential for unmeasured confounding. To minimize this factor, the outcomes studied were adjusted for known confounding factors using multivariable regression. The limited statistical power regarding the effect of male BMI is an additional limitation since it was not possible to obtain the BMI of the majority of the male members of the couple. Moreover, these results should not be extrapolated to treatment cycles using donor gametes, as these were excluded from the analysis. Therefore, future studies are needed to validate our results regarding the effect of male weight on CLBR. Another limitation is the reduced number of couples in the extreme BMI categories. Moreover, there were couples with unsuccessful embryos transfers who still had frozen embryos that were not thawed within the study period, and these couples could have lost weight followed by a successful transfer that was not captured.

In terms of strengths, the main asset of this study resided in the large sample size, including data from several clinics, contributing to the robustness and real-life clinical extrapolation of the results. Another strength is the use of CLBR as a primary outcome, which was seldomly used in previous studies and is very important from a patient's perspective.

Conclusion

This study provides further insight regarding the potential benefit of weight loss strategies in overweight or obese women younger than 35 years prior to ART, while those above this age may benefit from commencing treatment immediately.

We also concluded that male age and BMI seems to influence CLBR to a much lesser extent, although future studies are needed to validate these results.

Given the difficulty associated with weight loss programs and its conflict with the desire to treat infertility as soon as possible, these results may improve the counseling of couples regarding the ideal preparation and timing for ART.

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Conflict of interests

The authors have no conflicts of interest to declare.

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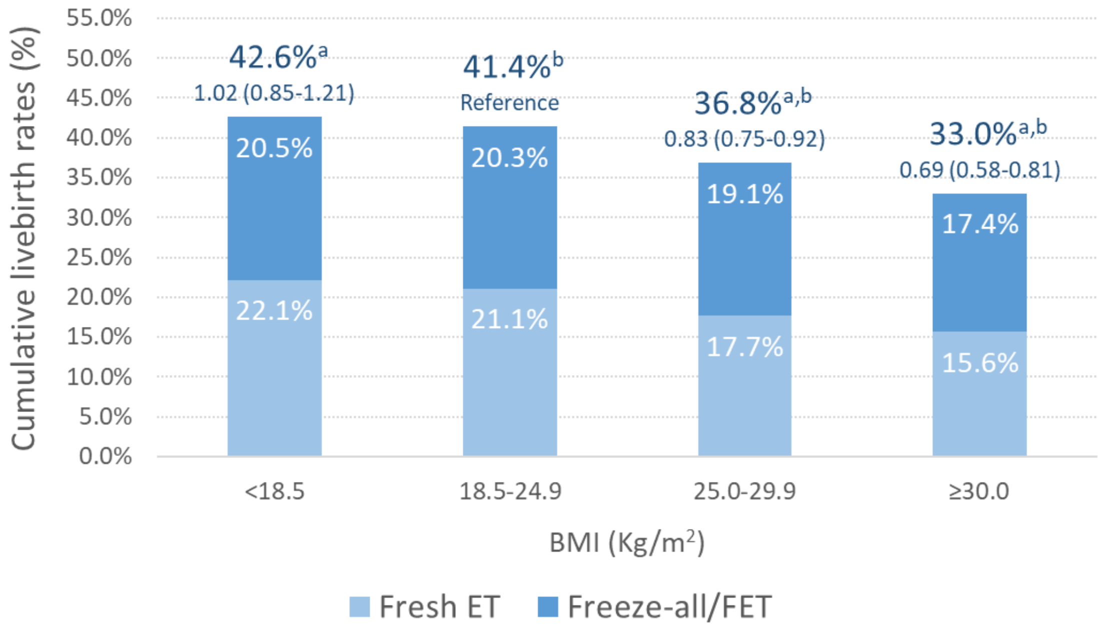
Appendix

Table 1 - Baseline characteristics

	Female BMI (kg/m^2)				
	<18.5 (n=671)	**18.5-24.9 (n=10243)**	**25.0-29.9 (n=2472)**	**≥30.0 (n=874)**	**p-value**
Female age (y)	**35,7 ± 3,9[a,b]**	**36,2 ± 4,0[a]**	**36,4 ± 4,2[b]**	36,1 ± 4,3	<0.01
<30	40 (6.0%)	538 (5.3%)	153 (6.2%)	66 (7.6%)	
30-34	211 (31.4%)	2808 (27.4%)	603 (24.4%)	222 (25.4%)	
35-37	184 (27.4%)	2831 (27.6%)	632 (25.6%)	211 (24.1%)	
38-39	123 (18.3%)	1801 (17.6%)	465 (18.8%)	168 (19.2%)	
40-41	78 (11.6%)	1357 (13.2%)	381 (15.4%)	141 (16.1%)	
≥42	35 (5.2%)	908 (8.9%)	238 (9.6%)	66 (7.6%)	
Female smoking habits					
No	494 (76.7%)	7459 (76.1%)	1747 (73.9%)	620 (74.2%)	
Current	119 (18.5%)	1889 (19.3%)	490 (20.7%)	168 (20.1%)	0.24
Passed	31 (4.8%)	450 (4.6%)	126 (5.3%)	48 (5.7%)	
Female infertility diagnoses					
Ovulatory factor	182 (27.3%)	**2514 (24.8%)[a]**	**596 (24.3%)[b]**	**287 (33.0%)[a,b]**	<0.01
Tubal factor	45 (6.8%)	**700 (6.9%)[a]**	**219 (8.9%)[a]**	67 (7.7%)	<0.01
Uterine factor	17 (2.6%)	**312 (3.1%)[a]**	**108 (4.4%)[a]**	31 (3.6%)	<0.01
Endometriosis	**63 (9.5%)[a]**	**830 (8.2%)[b]**	181 (7.4%)	**44 (5.1%)[a,b]**	<0.01
Other female diagnoses	15 (2.3%)	181 (1.8%)	48 (2.0%)	27 (3.1%)	0.05
Male age (y)	**38,1 ± 5,1[a]**	**38,3 ± 5,4[b]**	38,5 ± 5,6	**39,0 ± 6,1[a,b]**	<0.01
<30	20 (3.0%)	262 (2.6%)	70 (2.8%)	32 (3.7%)	
30-34	142 (21.2%)	2182 (21.3%)	482 (19.5%)	149 (17.0%)	
35-39	280 (41.7%)	4152 (40.5%)	948 (38.3%)	329 (37.6%)	
40-44	163 (24.3%)	2533 (24.7%)	696 (28.2%)	251 (28.7%)	
45-49	49 (7.3%)	761 (7.4%)	181 (7.3%)	70 (8.0%)	
50-54	11 (1.6%)	230 (2.2%)	61 (2.5%)	25 (2.9%)	
≥55	6 (0.9%)	123 (1.2%)	34 (1.4%)	18 (2.1%)	
Male factor infertility	**233 (35.0%)[a,b,c]**	**4074 (40.2%)[a]**	**1007 (41.1%)[b]**	**387 (44.5%)[c]**	<0.01
Total dose of gonadotropins	**2168,1 ± 879,8[a]**	**2252,9 ± 931,7[b]**	**2394,6 ± 905,2[a,b]**	**2604,9 ± 939,5[a,b]**	<0.01
Oocytes retrieved	10,7 ± 7,1	10,8 ± 7,2	10,7 ± 7,2	10,8 ± 7,7	0.91
Embryos transferred/cryopreserved	2,9 ± 2,9	**2,9 ± 3,0[a]**	**2,7 ± 2,9[a]**	2,6 ± 3,0	0.01
No viable embryos for transfer/cryopreservation	112 (16.7%)	**1937 (18.9%)[a]**	**525 (21.2%)[a]**	189 (21.6%)	<0.01
Fresh embryo transfer	346 (65.8%)	5136 (65.5%)	1174 (64.1%)	408 (63.5%)	0.51
Blastocyst stage embryo transfer	**189 (54.6%)[a]**	**2602 (50.7%)[b]**	568 (48.4%)	**179 (43.9%)[a,b]**	0.01
Single embryo transfer	150 (43.4%)	2028 (39.5%)	446 (38.0%)	151 (37.0%)	0.24

Numbers with the same superscript letter with p<0.05 following Bonferroni-adjusted pairwise comparison; BMI – Body mass index

Figure 1 - Cumulative Live-Birth Rate by female BMI



p<0.001, in which numbers with the same superscript letter present a pairwise comparison p<0.05); BMI – Body mass index

Figure 2 - Time to an embryo transfer leading to a live-birth

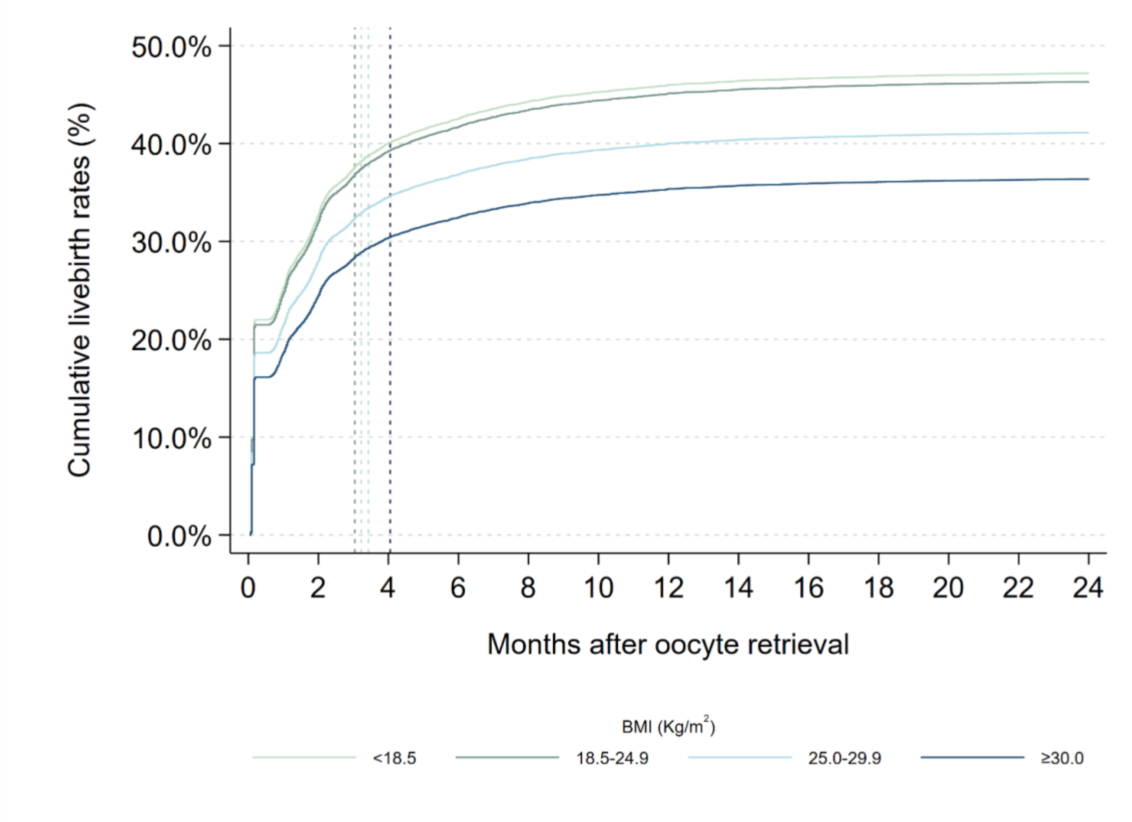


Table 2 - Mean time to pregnancy stratified by BMI category

Female BMI **(kg/m²)**	**<18.5**	**18.5 – 24.9**	**25.0-29.9**	**≥30.0**
Mean time to pregnancy (months)	3.3 (3.4-4.8)	**3.1 (2.9-3.2)[a,b]**	**3.5 (3.1-3.9)[a]**	**4.1 (3.4-4.8)[b]**

Numbers with the same superscript letter with p<0.05 following Bonferroni-adjusted pairwise comparison;
BMI – Body mass index

Table 3 - Neonatal outcomes stratified by female BMI

	Female BMI (kg/m²)			
	<18.5 (n=247)	**18.5 – 24.9 (n=3685)**	**25.0-29.9 (n=770)**	**≥30.0 (n=235)**
Birth-weight	2971.8 ± 554.5 -44.1 (-99.1;11.0)	3046.7 ± 620.2 Reference	3085.7 ± 616.5 **34.5 (1.1;68.0)**	3159.2 ± 660.1 **96.1 (39.9;152.4)**
Birth-weight <2500 g	37 (15.0%) -0.23 (-0.73;0.28)	599 (16.3%) Reference	111 (14.4%) -0.22 (-0.53;0.09)	31 (13.2%) -0.21 (-0.75;0.34)
Birth-weight >4000 g	4 (1.6%) -0.64 (-1.66;0.37)	139 (3.8%) Reference	30 (3.9%) 0.05 (-0.37;0.47)	12 (5.1%) 0.13 (-0.55;0.81)
Birth-weight z-score	-0.3 ± 0.9 **-0.14 (-0.28;-0.00)**	-0.2 ± 1.0 Reference	-0.1 ± 1.0 **0.08 (0.00;0.17)**	1.0 ± 1.3 **0.22 (0.08;0.36)**
Small for gestational age	35 (14.2%) -0.03 (-0.41;0.35)	534 (14.5%) Reference	97 (12.6%) -0.11 (-0.35;0.12)	33 (14.0%) 0.03 (-0.36;0.41)
Large for gestational age	12 (4.9%) -0.38 (-0.98;0.21)	271 (7.4%) Reference	67 (8.7%) 0.22 (-0.07;0.50)	29 (12.3%) **0.52 (0.08;0.95)**
Gestational age	270.4±14.7 -0.55 (-2.66;1.55)	271.2±15.9 Reference	271.3±16.2 0.13 (-1.14;1.41)	271.0±18.2 -0.14 (-2.29;2.01)
Delivery <37 weeks	34 (13.8%) -0.18 (-0.57;0.20)	588 (16.0%) Reference	108 (14.0%) -0.14 (-0.37;0.08)	28 (11.9%) -0.36 (-0.77;0.06)

BMI – Body mass index

Figure 3 - Influence of female and male age on CLBR

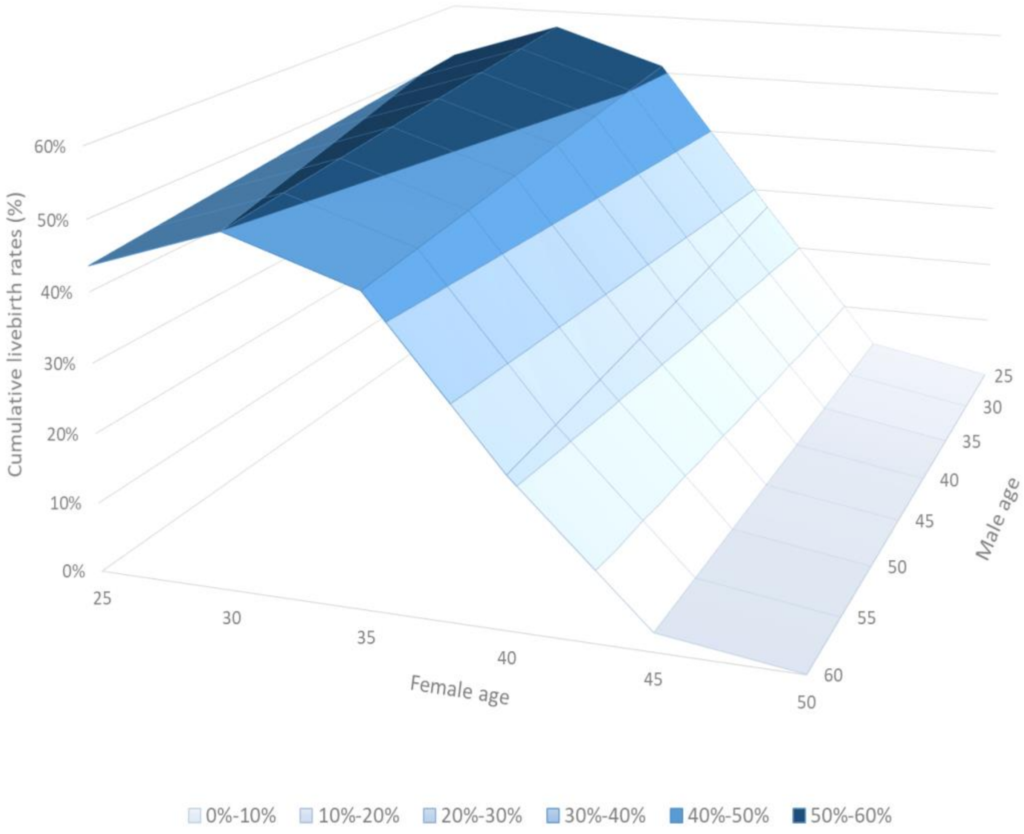
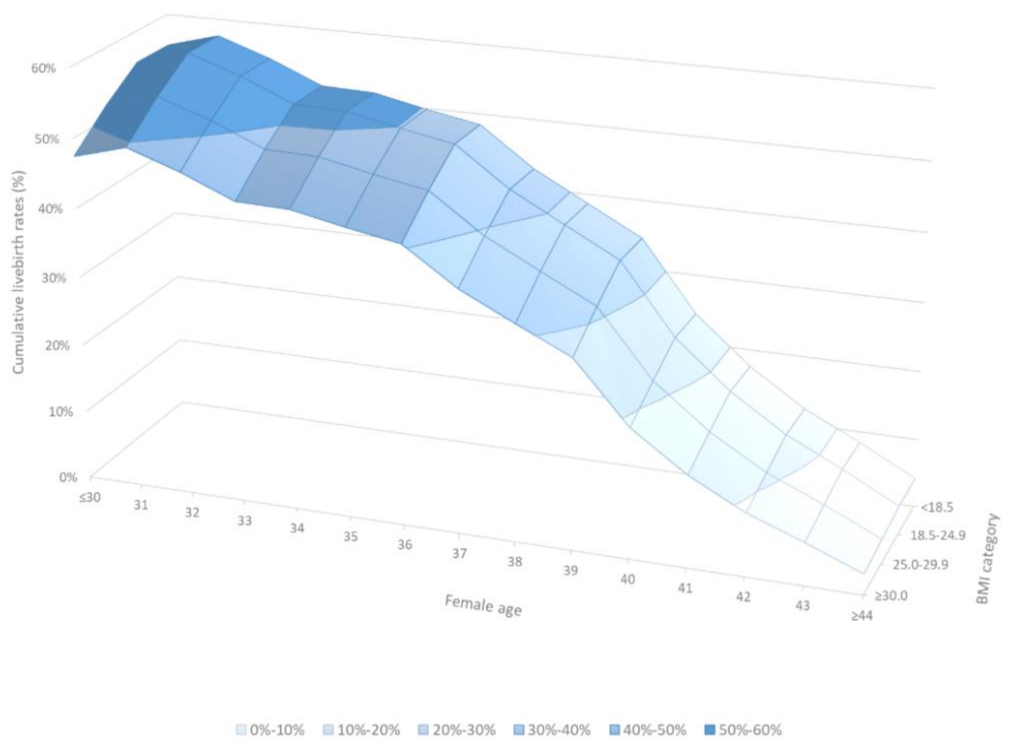


Table 4 - Relationship between female age and BMI in terms of predicted CLBR

		Female BMI (kg/m²)			
		<18.5	**18.5-24.9**	**25.0-29.9**	**≥30.0**
FEMALE AGE (y)	**≤30**	55%	56%	52%	47%
	31	58%	58%	54%	50%
	32	55%	55%	51%	47%
	33	52%	52%	48%	44%
	34	51%	52%	48%	43%
	35	50%	50%	46%	42%
	36	48%	48%	45%	41%
	37	43%	43%	39%	35%
	38	39%	39%	35%	31%
	39	34%	35%	31%	28%
	40	24%	24%	22%	19%
	41	18%	18%	16%	13%
	42	12%	12%	11%	9%
	43	8%	8%	7%	6%
	≥44	4%	4%	4%	3%

Figure 4 - Relationship between female age and BMI in terms of predicted CLBR



BMI – Body mass index; CLBR – Cumulative live-birth rate

Table 5 - CLBR stratified by female and male BMI

BMI category (kg/m^2)	**Female**	**Male**
<18.5	0.74 (0.40-1.39)	Reference
18.5-24.9	Reference	
25.0-29.9	0.78 (0.57-1.06)	0.85 (0.65-1.07)
>30.0	**0.54 (0.31-0.94)**	1.13 (0.75-1.71)

N = 1398 – both with BMI; BMI – Body mass index; CLBR – Cumulative live-birth rate

Since only 8 men had a BMI <18.5, they were characterized together with normal BMI (Reference group)