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Review

In Vitro Fertilisation and Intracytoplasmic Sperm Injection predictive factors: A review of the effect of female age, ovarian reserve, male age, and male factor on IVF/ICSI treatment outcomes

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ABSTRACT

The development of assisted reproductive technology has allowed offspring in infertile couples, and specifically, allowed infertile men to conceive through Intracytoplasmic Sperm Injection (ICSI). Despite the proven efficacy of *In Vitro* Fertilisation (IVF) and ICSI, many factors can influence its success. In this review we present an analysis on the effect of Female age, Ovarian Reserve, Male age and Male factor on the outcomes of IVF/ICSI, to determine if and which can be applied to the practical context. A literature search on PubMed, EMBASE and MEDLINE for relevant articles was elaborated until July 2021, leading to the selection of 234 articles based on their titles. After reading through the abstracts, those that evaluated IVF/ICSI predicting factors were selected. Finally, only those approaching female age, ovarian reserve, male age and male factor were considered in this review. Higher female age and baseline ovarian markers alterations such as lower anti-Müllerian hormone and antral follicular count, and higher basal follicle-stimulating hormone, were associated with poorer outcomes. The predictive value of Male age and Male factor presented varied results across literature. The multifactorial nature of male fertility makes evaluation difficult. Although the first assessment of male infertility is based on sperm concentration, motility and morphology, semen parameters have shown low prognostic value, whilst sperm DNA alterations gain importance. Nevertheless, results remain controversial. While some factors have proven to predict IVF/ICSI success, other need to be further studied to be applied to practical context to allow the best prognosis possible.

Keywords: *in vitro* fertilisation, intracytoplasmic sperm injection, ART treatment outcomes, male infertility, female infertility, ovarian reserve

INTRODUCTION

Since the beginning of this century, a decline in the number of children and a temporal delay in childbearing have been verified in developed countries. Among other causes, the growth in contraception, female emancipation, increase in economic wealth, personal education, life expectancy, and social normalization of divorce and mixed families, have contributed to delayed parenthood (De Brucker *et al*., 2014; Meijerink *et al*., 2016; O'Brien *et al*., 2017; Carrasquillo *et al*., 2019). Both fecundity and fertility decrease with increasing age (O'Brien *et al*., 2017), causally turning couples to assisted reproductive technology (ART) to conceive.

The development of ART has not only allowed infertile couples the possibility of offspring, but also, allowed infertile men who were previously excluded from treatments, to conceive through Intracytoplasmic Sperm Injection (ICSI) (Palermo *et al*., 1992). Since the initial use of *In Vitro* Fertilisation (IVF) in couples with bilateral tubal occlusion (Steptoe & Edwards, 1978), improvement of the ICSI technique (Tesarik & Sousa, 1995) and the introduction of ICSI for couples with male subfertility (Hamberger *et al*., 1998), the indications for IVF/ICSI have evolved, being widely used in various settings. Consequently, studies on the predicting factors of ART outcomes broadly address these techniques of assisted reproduction and will be our object of revision.

In this review, we chose to address factors that are believed to most influence ART outcomes, before initiating a stimulation protocol. Although largely studied and investigated, the effect of female age, ovarian reserve, male age and male factor infertility still present varied results and contradicting conclusions. Thus, our goal was to establish common ground of literature depicting these subjects, allowing patients and practitioners, before initiating IVF/ICSI, to detain knowledge of possible difficulties in their programs. The possibility of predicting and adapting beforehand, changing the approach in a particular couple, is the key to the best prognosis in ART.

Among all the predicting factors of ART outcomes that are studied, female age is the most frequently addressed, being currently proven that increasing female age results in decreased fertility (De Brucker *et al*., 2014). Nevertheless, the reason for loss of fertility with female aging is not known to full extent. Possible mechanisms include the decreasing of ovarian reserve, poorer oocyte quality, lower embryo implantation rates, altered hormonal environment resulting in ovulatory dysfunction, and uterine alterations (Tan *et al*., 2014).

The primary value of ovarian reserve markers is to provide a more accurate estimate of potential treatment success for patients, allowing optimization and individualization of therapy prior to the commencement of treatment. It is not only important to predict those who can expect low outcomes, warning poor responders and modifying stimulation approaches, but also to identify high responding patients at risk for ovarian hyperstimulation syndrome (Nelson *et al*., 2007; Lee *et al*., 2009; Brodin *et al*., 2013; Brugo Olmedo *et al*., 2013; Li *et al*., 2016; Liao *et al*., 2016; Reijnders *et al*., 2016). On one hand, studies have shown that markers of ovarian reserve are useful in the individualization of stimulation, but on the other, further association with clinical pregnancy rate (CPR) and live birth rate (LBR) have varied and been disputed (Lee *et al*., 2009; Brodin *et al*., 2013; Amsiejiene *et al*., 2017; Azizi *et al*., 2019).

Whilst several studies have examined the effect of male age on ART outcomes, the results are inconclusive and contradictory (Tsai *et al*., 2013; Beguería *et al*., 2014; Meijerink *et al*., 2016; Wu *et al*., 2016; Ma *et al*., 2018; Park *et al*., 2018), contrasting with the knowledge detained on the effects of maternal age (Abdel Raheem *et*

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al., 2013; Zhu *et al*., 2016; Mariappen *et al*., 2018; Park *et al*., 2018). Although advanced male age seems to influence male reproductive function to a lesser extent than the respective female age effects (Park *et al*., 2018), the interpretation of the impact of paternal age is challenging due to bias introduced by female age (Bartolacci *et al*., 2018).

Male factor infertility has been identified as a predictive factor of the cumulative chance of achieving a live birth (LB) (McLernon *et al*., 2016; Metello *et al*., 2019). The difficulty in the evaluation of outcomes and data when using male factor may appear due to the multifactorial nature of male reproduction (Park *et al*., 2018). In the human testis, ageing results in reproductive hormonal and cellular changes that can influence semen quality in volume, motility, concentration and morphology (Nijs *et al*., 2011; Tsai *et al*., 2013; Wu *et al*., 2016; Mariappen *et al*., 2018; McPherson *et al*., 2018; Carrasquillo *et al*., 2019). Hormonally, this could be attributed to the decrease of steroid levels with advancing age, indicating altered hypothalamic-pituitary-testicular axis regulations (Park *et al*., 2018; Carrasquillo *et al*., 2019). Additionally, there is an increase in gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and decrease in testosterone levels (Park *et al*., 2018).

Increased male age has been linked to changes in epigenetic factors, leading to alterations at a molecular level, and to negative effects on post-fertilisation development (Mariappen *et al*., 2018). Investigating the effect of male age on fertility is growing important by each day, due to the increasing choice to be a father at an older age (Nijs *et al*., 2011; Mariappen *et al*., 2018). According to the Sixth Edition of the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Sperm (WHO, 2021), the management of a subfertile couple can be guided by clinical assessment and semen analyses. Despite the existence of reference ranges for sperm parameters, these do not allow the clear distinction of subfertile and infertile men and thus should not be the single predictor in ART prognosis. WHO has concluded that several parameters should be used, as true fertility potential as a couple, is what defines them as fertile or infertile (WHO, 2021).

MATERIALS AND METHODS

A literature search on PubMed, EMBASE and MEDLINE for relevant articles was elaborated until July 2021. Combinations of the following terms were used as keywords: "ICSI", "IVF", "IVF/ICSI" and "predicting factor", leading to the selection of 234 articles based on their titles. After reading through the abstracts, 182 articles were selected. Articles were considered relevant if they provided a clear investigation on the effect of intrinsic and extrinsic variables on ICSI outcomes, and then read entirely. Finally, those evaluating the effect of female age, male age, male factor, and ovarian reserve on IVF/ ICSI results were considered in this review, totalizing 96 papers.

Articles were included if they consisted in original articles written in English, Portuguese or French and excluded if review articles, meta-analyses, incomplete or inaccessible articles, or if written in another language. Due to their theoretical importance, 14 additional papers were read and added to this review (Steptoe & Edwards, 1978; Tesarik & Sousa, 1995; Hamberger *et al*., 1998; Sergerie *et al.*, 2005; Ferraretti *et al*., 2011; The Practice Committee of the American Society for Reproductive Medicine & the Practice Committee of the Society for Assisted Reproductive Technology, 2013; Neri *et al*., 2014; Bucar *et al.*, 2015; Majzoub *et al.,* 2017; Esteves, 2018; Calhaz-Jorge *et al*., 2020; Clarke *et al*., 2021; CFM, 2021; WHO, 2021). These include specialized guidelines and recommendations, and articles of historical or theoretical importance.

RESULTS

Female age

Due to the surge in the use of ART, a parallel increase in the investigation of the effects of female age is seen. A common approach that was found in original articles studying the repercussion of female age on IVF/ICSI outcomes was the stratification of the cohort by age. The methods and design model of each study vary and whilst some study female age alone, others associate female age to other possible predicting factors.

Within those whose object of evaluation was female age only, a negative relationship between IVF/ICSI outcomes and rising female age was predominant (Malizia *et al*., 2009; Cetinkaya *et al*., 2013; De Brucker *et al*., 2014; Tan *et al*., 2014; Yilmaz *et al*., 2017; Mahesan *et al*., 2018). When evaluating the success of any ART technique, the LBR is the ultimate and most desired outcome. Cumulative LBR has been compared by dividing women in groups according to age (Malizia *et al*., 2009; De Brucker *et al*., 2014). Crude cumulative LBR (number of women achieving live-birth divided by the number of women who started treatment with ICSI) after one and six cycles, was found to decrease from 20-29, 30-37 to 38-39 years (De Brucker *et al*., 2014). The decline in cumulative LBR was also found when dividing women in under 35 years, 35-37, 38-39, and ≥40 years (Malizia *et al*., 2009). In this case, the cumulative LBR among women 39 years of age or younger who were treated up to six cycles seemed to be similar to or higher than the cumulative LBR in the general population, suggesting that IVF was successful in the treatment of infertility but also implying that women aged 40 or over may find increased difficulty in reversing age-dependent decrease in fertility (Malizia *et al*., 2009).

Advanced maternal age has been further addressed, in which the overall CPR and LBR declined from women aged 43 to women aged 44, with no clinical pregnancy (CP) achieved in women 45 years of age or older. Cancellation rates and miscarriage risk were statistically lower in the 43-year-old group compared to older ages. Such suggests that women 45 years and beyond do not benefit from IVF/ICSI using their own oocytes (Cetinkaya *et al*., 2013). Along with the decrease in CPR and LBR, various other outcomes were shown to be associated with the elevation of female age such as higher miscarriage rate (Tan *et al*., 2014), lower proportion of cycles reaching embryo transfer, decreased number of oocytes retrieved (Tan *et al*., 2014; Yilmaz *et al*., 2017; Mahesan *et al*., 2018), increased risk of embryo aneuploidy (Bilibio *et al*., 2021), downward trend in fertilisation rate (FR) (Tan *et al*., 2014), and multiple pregnancy incidence (Tan *et al*., 2014; Mahesan *et al*., 2018). In addition, older women had significantly longer stimulation and lower number of normally fertilized (2 pronuclei, 2PN and 2 polar bodies, 2PB) zygotes (2PN zygotes) (Yilmaz *et al*., 2017; Mahesan *et al*., 2018).

We also reviewed papers that studied female age along with other variables. Most demonstrated the trend expected (Shen *et al*., 2003; Kovacs *et al*., 2003; Pinto *et al*., 2009; Nelson & Lawlor, 2011; Huang *et al*., 2012; Maman *et al*., 2012; Ramezanzadeh *et al*., 2012; Berger *et al*., 2014; Hamdine *et al*., 2015; Coelho Neto *et al*., 2015; Nouri *et al*., 2015; Meijerink *et al*., 2016; Amsiejiene *et al*., 2017; Bocca *et al*., 2017; Hassan *et al*., 2017; McPherson *et al*., 2018; Peuranpää *et al*., 2020), but others found no or a varied relationship between female age and IVF/ICSI (Busnelli *et al*., 2014; Chen *et al*., 2015; Lefebvre *et al*., 2015; Niinimäki *et al*., 2015; Borges *et al*., 2017; Reljič *et al*., 2017).

In the course of analysing the studies that are consistent with the deleterious effect of female age, it was verified once again that increasing female age decreased the mean number of retrieved oocytes (Pinto *et al*., 2009; Maman *et al*., 2012; Ramezanzadeh *et al*., 2012; O'Brien *et al*., 2017; Sahin *et al*., 2021), embryo quality (Ramezanzadeh *et al*., 2012; Bocca *et al*., 2017), embryo cleavage rate (Ramezanzadeh *et al*., 2012), FR (O'Brien *et al*., 2017), biochemical pregnancy rate (BPR) (O'Brien *et al*., 2017), CPR (Kovacs *et al*., 2003; Shen *et al*., 2003; Pinto *et al*., 2009; Huang *et al*., 2012; Maman *et al*., 2012; Berger *et al*., 2014; Coelho Neto *et al*., 2015; Nouri *et al*., 2015; Meijerink *et al*., 2016; Amsiejiene *et al*., 2017; Bocca *et al*., 2017; Hassan *et al*., 2017; O'Brien *et al*., 2017; McPherson *et al*., 2018; Peuranpää *et al*., 2020) and LBR (Nelson & Lawlor, 2011; Hamdine *et al*., 2015; Nouri *et al*., 2015; McPherson *et al*., 2018; Peuranpää *et al*., 2020; Wen *et al*., 2021), and was associated to poor response cycles (Maman *et al*., 2012), higher cancellation rate (Borges *et al.*, 2017), higher miscarriage rate (Peuranpää *et al*., 2020; Sahin *et al*., 2021) and higher risk of macrosomia (Nelson & Lawlor, 2011). On the other hand, other studies showed no or mixed outcomes with female age such as no variation in cancellation rate (Lefebvre *et al*., 2015), FR (Ramezanzadeh *et al*., 2012), LBR (Busnelli *et al*., 2014; Niinimäki *et al*., 2015), or congenital birth defects (Chen *et al*., 2015). However, these variations could be due to the studies methods and different age cohorts. For example, Busnelli *et al*. (2014) considered only a particular population of poor responders according to the Bologna criteria, and Niinimäki *et al*. (2015) considered women from 20-35 years, whilst Lefebvre *et al*. (2015) divided female age in <38 and 38-40 years. The diversity of age cohorts exists across literature.

Different results have surged regarding gestational age at delivery, preterm deliveries and neonatal birth weight. Whilst some authors associated preterm deliveries (<37 weeks) and low birth weight (<2500g) with increasing female age (Yilmaz *et al*., 2017), others reported no differences in gestational age, preterm deliveries and neonatal weight between different female ages (Mahesan *et al*., 2018).

It is interesting to also investigate the relationship between younger female age and IVF/ICSI outcomes. Humm *et al.* (2015) found that women under 25 years had the lowest cumulative FR and LBR. This initiated a discussion concerning inherent issues with younger women's oocytes, such as increased aneuploidy prevalence, or the possibility that couples with women <25 years were more likely to be diagnosed with male factor infertility. Additionally, it is possible that very young women are subject of a less aggressive stimulation approach due to the higher risk of over response or multiple gestation. Thus, the optimal age suggested by the authors for couples with women using their own oocytes was 25 to <30 years (Humm *et al*., 2015).

Out of the 43 countries from Europe evaluated, 34 have legal age limits for treatment. Maximum female age is a legal limit in 18 countries, ranging from 45 years in Denmark and Belgium (embryo replacement and insemination are allowed up to 47 years) to 51 in Bulgaria. There are no legal age limits in Finland, Germany and Norway, while current legislation in France sets a female upper limit at "normal reproductive age", Spain at the "age of the menopause", and the Netherlands at age 49 (Calhaz-Jorge *et al*., 2020). Portugal (Calhaz-Jorge *et al*., 2020) and Brazil (CFM, 2021) both have an upper legal limit of < 50 years.

Ovarian reserve

The decline of fertility with advanced female age is acknowledged. However, the etiology is still uncertain, with the decline in oocyte quantity and quality being a potential cause for poorer pregnancy outcomes (La Marca *et al*., 2017). The quantity and quality of residual ovarian follicles and oocytes is referred to as the ovarian reserve (Yin *et al*., 2019) and is a potential predictor of IVF/ICSI outcomes. Thus, the existence of ovarian reserve markers gains primary value. Currently, a variety of biochemical and sonographic tests allow this evaluation (Nelson *et al*., 2007; Lee *et al*., 2009). Serum levels of FSH and anti-Müllerian hormone (AMH), and the antral follicle count (AFC), are considered baseline factors predicting the ovarian reserve (Nelson *et al*., 2007; Lee *et al*., 2009; Fridén *et al*., 2011; Brugo Olmedo *et al*., 2013; Li *et al*., 2016; Yin *et al*., 2019). The third criterion of the Bologna ESHRE consensus group defines diminished ovarian reserve (DOR) as women presenting either an AFC ≤ 5-7 or an AMH assay ≤ 0.5-1 ng/mL (Ferraretti *et al*., 2011). Ovarian volume and blood flow, ovarian stimulatory test, gonadotropin agonist stimulation test (Lee *et al*., 2011), menstrual cycle lengths, levels of basal gonadotropins (Brodin *et al*., 2013), estradiol and inhibin B concentrations can also assess the ovarian reserve (Lee *et al*., 2011; Li *et al*., 2016).

It is important to distinguish DOR from poor ovarian responders (POR). POR are defined by the ESHRE Bologna consensus when at least two of the three characteristics are present: advanced maternal age (≥ 40 years) or any other risk factors for POR (all the known genetic or acquired conditions possibly linked to a reduced amount of resting follicle), a previous poor ovarian response (≤ 3 oocytes with a conventional stimulation protocol) or an abnormal ovarian reserve test (AFC <5-7 or AMH < 0.5-1.1 ng/mL) (Ferraretti *et al*., 2011).

Despite the proven relationship between female age and decline in reproductive capacity, namely ovarian reserve (Zhou *et al*., 2020), the rate of fertility decline can vary considerably among women of the same age, signalling that ovarian ageing may not be merely and parallelly associated to chronological ageing (Lee *et al*., 2011). Early ovarian ageing is thought to be caused by deficient initial follicle number, follicle dysfunction, accelerated follicle atresia (Lin *et al*., 2014). Oocyte quality is a more complex part of the ovarian reserve than oocyte quantity. Nevertheless, the association between female age and declining fertility is possibly due to abnormalities of the oocyte, with the possibility that a reduced ovarian reserve can directly lead to reduced oocyte quality (La Marca *et al*., 2017).

During the process of studying the predictive value of ovarian markers in IVF/ICSI outcomes, most studies resort to dividing each marker into groups based on the marker levels measured. Due to its most recent discovery and already referred advantages, AMH has become largely investigated, and, therefore, will be the first and main object of our review of ovarian reserve markers.

In the recent years AMH has been shown to represent a reliable marker of ovarian reserve and ovarian stimulation response (Fridén *et al*., 2011; Brugo Olmedo *et al*., 2013). Although knowledge of the physiologic role of AMH remains limited, various studies have demonstrated its importance as regulator of ovarian activity (Azizi *et al*., 2019). AMH, a member of the transforming growth factor-β family, has the primary role of regression of the Müllerian duct in the male fetus during early testis differentiation, persisting after completion of the male reproductive system, and commencing in females in early fetal life, produced by ovarian granulosa cells (Nelson *et al*., 2007; Brugo Olmedo *et al*., 2013) of pre-antral and small antral follicles (Nelson *et al*., 2007; Lee *et al*., 2009; Fridén *et al*., 2011; Brugo Olmedo *et al*., 2013; La Marca *et al*., 2017). Thus, AMH can be directly indicative of the pool of such follicles, the ovarian reserve (Fridén *et al*., 2011). The hormone has also been detected in the follicular fluid, where, via autocrine and paracrine actions it could impact on the quality of oocytes.

Additionally, as granulosa cells exert important roles in folliculogenesis, AMH could also influence the quality of oocytes (Azizi *et al*., 2019).

Besides predicting the ovarian reserve, AMH may be a potential predictor of the ovarian response to FSH, as AMH expression declines as antral follicles increase in size (FSH-dependent final stages of follicular growth) and is absent from atretic follicles, suggesting that the basal levels of AMH may represent the total developing follicular cohort (Nelson *et al*., 2007). Moreover, unlike basal follicle-stimulating hormone (bFSH), AMH has an insignificant variation during the menstrual cycle and therefore no restriction of measurement to a particular stage of the cycle (Nelson *et al*., 2007; Fridén *et al*., 2011; Brugo Olmedo *et al*., 2013).

When divided into groups (based on lower and higher serum levels of AMH), studies found that high levels of AMH were associated with a higher ovarian response (≥ 15 retrieved oocytes) (Li *et al*., 2016) or excessive ovarian response (≥ 21 oocytes) (Nelson *et al*., 2007). On the contrary, AMH levels were significantly lower in non and poor (≤ 2 oocytes) responders (Nelson *et al*., 2007).

High AMH levels were also associated with lower cycle cancellation rates (Fridén *et al*., 2011; Lee *et al*., 2011; Brodin *et al*., 2013; Brugo Olmedo *et al*., 2013; Lin *et al*., 2014), good response rate (≥ 5 retrieved oocytes) (Brugo Olmedo *et al*., 2013), higher mean number of retrieved oocytes (Lee *et al.*, 2009; Fridén *et al*., 2011; Brugo Olmedo *et al*., 2013; Li *et al*., 2016; Daney de Marcillac *et al*., 2017; Azizi *et al*., 2019) and metaphase II oocytes (Azizi *et al*., 2019), and higher CPR (Azizi *et al*., 2019; Fridén *et al*., 2011; Lee *et al*., 2011; Brodin *et al*., 2013; Brugo Olmedo *et al*., 2013).

AMH levels were also shown to be positively associated with AFC and mean menstrual cycle length (Brodin *et al*., 2013), oocyte yield (Nelson *et al*., 2007; Brodin *et al*., 2013), embryo score (Brodin *et al*., 2013), number of high quality embryos transferred (Reijnders *et al*., 2016), implantation rate (IR) (Fridén *et al*., 2011; Reijnders *et al*., 2016), and LBR (Nelson *et al*., 2007; Fridén *et al*., 2011; Brodin *et al*., 2013; Reijnders *et al*., 2016; O'Brien *et al*., 2017). A positive age-independent relationship between AMH levels and the rate of euploid blastocysts was also found, enforcing that an increased ovarian reserve is associated with an increased rate of blastocyst euploidy (La Marca *et al*., 2017). Still confirming the importance of AMH evaluation, several studies showed an inverse relationship between AMH and total gonadotropin, recombinant stimulating hormone/human menopausal gonadotropin dose given at stimulation (Fridén *et al*., 2011; Brodin *et al*., 2013), and between AMH and bFSH (Nelson *et al*., 2007; Brodin *et al*., 2013).

Mean and median AMH values show a progressive decline with advancing age (Nelson *et al*., 2007; Lee *et al*., 2011; O'Brien *et al*., 2017). However, whilst in females with lower age (≤ 35 years) AMH levels were not correlated to BPR, CPR or LBR, in females with advanced age (≥40) higher AMH levels were associated to higher BPR and CPR (O'Brien *et al*., 2017). Parallelly, it was described that AMH was a predictor of LB in women ≥35 years of age and in couples without male factor (Lee *et al*., 2009). Two studies investigating AMH as a predicting factor described no significant differences found between groups of low or high serum AMH levels regarding the CP rate (Lin *et al*., 2014; Amsiejiene *et al.*, 2017) and LBR per embryo transfer (Lin *et al*., 2014). However, they considered women ≤ 35 years. Another study found no statistically different AMH values when comparing pregnant and non-pregnant women ≤ 38 years old (Cohen *et al*., 2017). Additionally, individualized controlled ovarian stimulation protocols tailored to patient AMH values were associated to higher mean number of retrieved oocytes, and higher CPR, implantation rate (IR) and LBR in women of advanced age (> 40 years). Nevertheless, in these women, no differences seemed to exist between the duration of stimulation, embryo transfer rate, number of transferred embryos, and abortion rate (Liao *et al*., 2016). In this light, the prognostic value of AMH as an ovarian reserve marker appears to be greater for women of advanced age than for women of younger age, being theorized that age protects low responders from the deleterious effects of a poor ovarian response. Thus, the uncertainty behind the significance of a low AMH level in infertile women is being slowly lifted as literature heavily studies this subject. Despite this, data is still preliminary, and no women should be denied ART solely based on AMH levels. The main relationships of female age and AMH with these ART outcomes are resumed in Table 1.

Determination of bFSH levels on cycle day 3 is used in many ART units, being first described by Muasher *et al*. (1988) and since been described in many studies as associated to, when elevated, a poorer response to ovarian stimulation and lower quality oocytes, influencing the CPR and miscarriage rate (Martin *et al*., 1996; Sharif *et al*., 1998; Sahin *et al*., 2021). On the other hand, although circulating bFSH levels have classically been used to predict the fertility potential, clinical practice has revealed a limited usefulness and lack of precision (Fridén *et al*., 2011; Brugo Olmedo *et al*., 2013). The levels of bFSH were negatively correlated to the number of oocytes retrieved (Lee *et al*., 2009; Pinto *et al*., 2009; Abdalla & Thum, 2004; Li *et al*., 2016; Daney de Marcillac *et al*., 2017). Low serum levels of bFSH were associated to higher mean number of high quality embryos (Pinto *et al*., 2009), and higher FR (Jawed *et al*., 2016), pregnancy rates (Abdalla & Thum, 2004; Pinto *et al*., 2009; Sahin *et al*., 2021) and LBR (Abdalla & Thum, 2004; Sahin *et al*., 2021).

Similarly to AMH, bFSH levels do not seem to affect pregnancy rates and LBR in younger women, but for those patients aged >38, the pregnancy rate and LBR were significantly reduced as bFSH levels increased (Abdalla & Thum, 2004). The reduction in the CPR and LBR seen in women with high levels of bFSH was suggested be likely due to a reduced ovarian reserve (Abdalla & Thum, 2004; Zhou *et al*., 2020) rather than a reduced oocyte quality (Abdalla & Thum, 2004). Concordantly, high serum levels of bFSH were associated to more cycle cancellations (Abdalla & Thum, 2004; Brugo Olmedo *et al*., 2013), need of higher stimulation doses, and a lower number of 2PN zygotes, embryos available for transfer and embryos transferred (Abdalla & Thum, 2004). In other works, the levels of bFSH were not correlated with the FR and miscarriage rate (Abdalla & Thum, 2004). This can be explained by the fact that high bFSH does not originate ageing oocytes, but fewer are produced, and therefore support previous conclusions that an elevated bFSH level does not indicate deterioration of oocyte and embryo quality (Abdalla & Thum, 2004).

Thus, AMH appears as a superior predictor of ovarian response (Nelson *et al*., 2007), number of retrieved oocytes (Lee *et al*., 2009) and LB (Nelson *et al.,* 2007) than bFSH and age (Nelson *et al*., 2007), and seems to provide an additional item of discriminatory information (Daney de Marcillac *et al*., 2017).

The AFC is one of the first ovarian markers used, being positively correlated with the number of retrieved oocytes (Li *et al*., 2016) and known to decrease with age (Zhou *et al*., 2020). The number of antral follicles, despite not characterizing oocyte quality, represents a good estimator of the primordial follicle pool and, consequently, the quantitative aspect of ovarian reserve (Brugo Olmedo *et al.*, 2013). More recently, AFC of less than 5 was found to be associated to an increased risk of embryo aneuploidy (Bilibio *et al*., 2021). Yet, AFC is not associated to pregnancy (Cohen *et al*., 2017) or LB (Busnelli *et al*., 2014), indicating that

Table 1. Main relationships of female age and AMH levels with ART outcomes.

Increasing female age	Higher AMH levels	Lower AMH levels
Lower ovarian reserve	Higher antral follicle count (AFC)	Non responders
Higher poor response cycles	Lower bFSH levels	Poor responders
Longer stimulation time	Lower gonadotropin dose	
Higher cancellation rates	Higher ovarian response	
Lower number of retrieved oocytes (COC)	Excessive ovarian response	
Lower fertilisation rate (FR)	Higher good response rate	
Lower embryo cleavage rate	Lower cancellation rates	
Lower embryo quality	Higher number of COC	
Lower biochemical pregnancy rate (BPR)	Higher number of MII oocytes	
Lower clinical pregnancy rate (CPR)	Higher CPR	
Higher miscarriage rate	Higher embryo quality	
Lower cycles reaching embryo transfer	Higher implantation rate (IR)	
Higher embryo aneuploidy	Higher embryo euploidy	
Lower live birth rate (LBR)	Higher LBR	
≥45 years old: no pregnancy	Without correlation if ≤35 years:	
	BPR, CPR, LBR	
Predictor models:	With correlation if ≥40 years:	
>36 years: lower LBR	COC, BPR, IR, CPR, LBR	
>30 years: lower LBR		
>28 years: lower LBR	AMH values decrease with age	
	Prognostic value of AMH is higher in advanced age	

despite being an acknowledged ovarian reserve marker, the transposition to IVF/ICSI outcomes is a larger step, still to be enlightened. The main relationships of bFSH levels and AFC values with these ART outcomes are resumed in Table 2.

The development of prediction models

In addition, research is directed towards the development of models that can predict IVF/ICSI outcomes. These include female age (Nelson & Lawlor, 2011; Khader *et al*., 2013; Hamdine *et al*., 2015; Dhillon *et al*., 2016; McLernon *et al*., 2016; Vaegter *et al*., 2017; Leijdekkers *et al*., 2018; Metello *et al*., 2019; Tarín *et al*., 2020; Wen *et al*., 2021) and/or ovarian markers (Khader *et al*., 2013; Hamdine *et al*., 2015; Dhillon *et al*., 2016; Leijdekkers *et al*., 2018; Tarín *et al*., 2020) as one of their predictors.

Nelson & Lawlor (2011) showed a multivariable association of live birth, including a decrease in odds with increasing maternal age. Khader *et al*. (2013) confirmed, after external validation, a designed model where AMH and female age were independent predictors of LB. Hamdine *et al*. (2015) developed a model predicting cumulative LBR within one year that included age at first treatment and AMH (amongst type and duration of infertility, and number of previous ART treatments), with a non-linear declining relation. Dhillon *et al*. (2016) reported that increasing age (particularly above 36 years) was significantly associated with reduced chances of IVF/ICSI success and female age was included as a predictor in the final model for LB. McLernon *et al*. (2016) developed two clinical prediction models to estimate individualised cumulative chance of first LB over a maximum of six complete IVF cycles. One model used information available before starting treatment, and the other was based on additional information collected during the first attempt. Female age was described as one of the key pre-treatment predictors, as LB declined after age 30 and decreased linearly with increasing duration of infertility (McLernon *et al*., 2016). Vaegter *et al*. (2017) revealed a prediction model for LBR with a total of seven predictors, including female age (along embryo score, treatment history, number of oocytes/total dose of FSH, infertility cause and endometrial thickness), reporting a decrease in LBR after 28 years of age, another decrease at 35 years of age, equal rates between 36 and 37 years, and a subsequent decrease in higher ages. Leijdekkers *et al*. (2018) added AFC and AMH to previous McLernon *et al.* (2016) models, recalibrating and improving the pre-treatment model for prediction on LBR. Tarín *et al*. (2020) presented a prognostic model that included female age and AFC. Metello *et al*. (2019) reviewed pre-existing models and performed a univariate and multivariate analysis concerning different variables. Female age was a predictor of LB when exponentialized in univariate analysis, and predictor along with AMH and AFC, in multivariate analysis when categorized. These variables were transformed as these curves better describe the expected behaviour of those variables on reproductive outcomes after IVF/ICSI (Metello *et al*., 2019). Finally, Wen *et al*. (2021) developed a prediction model estimating live births that included female age, amongst male infertility factor.

Male age

When analysing previous studies, it turns out that the influence of male age on clinical outcomes is contradictory, varying in the results obtained and the ages considered.

Most studies found no association between male age and FR (Nijs *et al*., 2011; Abdel Raheem *et al*., 2013), BP (Beguería *et al*., 2014; Meijerink *et al*., 2016), CP (Abdel

Table 2. Main relationships of bFSH levels and AFC values with ART outcomes.

Higher bFSH levels	**Lower bFSH levels**	**Higher AFC**
Lower ovarian reserve	Higher FR	Higher number of COC
Higher poor response cycles	Higher embryo quality	
Higher gonadotropin dose	Higher LBR	AFC <5 correlates with:
Higher cancellation rates		Increased risk of embryo aneuploidy
Lower number of COC		No correlation with:
Lower MII quality		CPR, LBR
Lower FR		
Lower embryo quality		
Lower CPR		Decreases with age
Higher miscarriage rate		
Lower cycles reaching embryo transfer		
Lower embryos for transfer		
With correlation if >38 years old:		
Lower CPR, LBR		
No correlation if ≤38 years old:		
CPR, LBR		
AMH is a superior predictor than age or bFSH:		
Ovarian response, COC, LBR		
Increases with age		

Raheem *et al*., 2013; Beguería *et al*., 2014; Wu *et al*., 2016; Hassan *et al*., 2017; Ma *et al*., 2018; Mariappen *et al*., 2018; Park *et al*., 2018), IR (Park *et al*., 2018), miscarriage (Abdel Raheem *et al*., 2013; Beguería *et al*., 2014; Meijerink *et al*., 2016; Wu *et al*., 2016; Ma *et al*., 2018), ongoing pregnancy (Beguería *et al*., 2014; Meijerink *et al*., 2016; Wu *et al*., 2016; Bartolacci *et al*., 2018), LBR (Abdel Raheem *et al*., 2013; Beguería *et al*., 2014; Ma *et al*., 2018; Mariappen *et al*., 2018; Park *et al*., 2018), low neonatal birth weight (<2500g) in singletons (Ma *et al*., 2018), and preterm birth and birth defects (Wu *et al*., 2016).

However, some studies found that paternal age was an independent factor negatively affecting the FR (Bartolacci *et al*., 2018), CPR (Yu *et al*., 2019), IR (Yu *et al*., 2019), embryonic aneuploidy risk (Bilibio *et al*., 2021), LBR (McPherson *et al*., 2018; Yu *et al*., 2019; Wen *et al*., 2021) and term-birth rates (live-born infants between 37 and 41 weeks gestation) (McPherson *et al*., 2018). McPherson *et al*. (2018) described the evidence of negative associations between paternal age and both viable pregnancies and live births, with a 10% decrease in the probability of pregnancy in women who were aged 35 years with a male partner over 40 years, vs. a male partner aged under 30 years. When investigating the effect of paternal age in cryptozoospermic men, one study found that the IR, CPR and LBR were all higher in a male <35 years group than in a male ≥35 years group, regardless of sperm origin (Yu *et al*., 2019). Consequently, due to oxidation and damage to spermatozoa during transit through the male genital tract, the authors recommended the use of testicular sperm versus ejaculated sperm in men ≥35 years (Yu et al., 2019). The main relationships of male age with these ART outcomes are resumed in Table 3.

Table 3. Main relationships of male age with ART outcomes.

Higher male age	**Higher male age**
No correlations with:	**Correlations with:**
(majority of studies)	Lower FR
COC	Lower embryo quality
MII	Higher embryo aneuploidy
FR	Lower CPR
ECR	Lower IR
Embryo quality	
Blastocyst quality	Lower gestation age
Embryo aneuploidy	
BPR	≥35 years: Testicular sperm recommended
CPR	
IR	
Miscarriage rate	
Ongoing pregnancy rate	
LBR	
Gestation age	
Birth weight	
Birth defects	

Male age and oocyte, blastocyst and embryo parameters

Regarding oocyte, cleavage embryo parameters and blastocyst, most studies found no association with male age. Mariappen *et al*. (2018) described that the male counterpart had a lesser role in embryo quality, compared to the female counterpart, and Beguería et al showed that the morphologic embryo score, an indicator of quality of the cohort of embryos generated, was not associated to paternal age (Beguería *et al*., 2014). Meijerink *et al*. (2016) found no statistically significant differences between paternal age groups and the probability of availability of at least one high-quality embryo. Bartolacci *et al*. reported no association between male age and top-quality blastocyst formation rate (following specific criteria that categorized inner cells mass and multicellular trophectoderm). Additionally, no association to blastulation rate was described (Bartolacci *et al*., 2018). Other authors also found that male neither influenced embryo cleavage (Abdel Raheem *et al*., 2013), embryo aneuploidy (Carrasquillo *et al*., 2019) nor the number of embryos transferred (Meijerink *et al*., 2016). Wu *et al*. (2016) also reported no association with the number of fertilized oocytes and number of viable embryos. However, these authors observed that paternal age negatively influenced the number of high-quality embryos (Grade 1 embryos defined as 4-6 cells on day 2, 8-10 cells on day 3, equal size, fragmentation <20%, no multinucleated blastomeres), contradicting with the results of other studies previously described (Wu *et al*., 2016).

Male age and sperm parameters

The influence of male age on sperm parameters is more established and better understood. Male aging can directly damage sperm DNA and increase DNA methylation through excessive reactive oxygen species production, and then compromise spermatogenesis (Wu *et al*., 2016). Begueria *et al*. (2014) revealed a significant relationship between paternal age and all sperm parameters. They described that for every 5 years of age, sperm volume decreased by 0.22 ml, concentration increased by 3.1 million sperm/ml and the percentage of motile spermatozoa decreased by 1.2% (Beguería *et al*., 2014). Mariappen *et al*. (2018) reported no significant association between male age groups and sperm concentration, morphology and DNA fragmentation index (DFI) but identified a significant decrease in sperm motility. Specifically, males between 40-49 years had a 52% reduced chance to have normal sperm motility (≥32% progressively motile sperm), whilst males ≥50 years of age had a 79% reduced chance for normal motility (Mariappen *et al*., 2018). A close correlation between the patients age and the percentage of vacuoles in the motile sperm organelle morphology examination (MSOME), sperm aneuploidies and DNA fragmentation has been reported (Braga *et al*., 2011). Nevertheless, results remain contradictory. Some authors described no relationship between paternal age and any sperm parameter, such as concentration, motility or morphology, and no increase in DFI or immature chromatin (Nijs *et al*., 2011).

The contrast in results can be explained by several reasons. Firstly, only a few normal sperm are required for successful ICSI. Secondly, ICSI can surpass possible sperm alterations that are due to advanced male age (Yu *et al*., 2019). Finally, there is a bias introduced by female age and many studies do not exclude this factor, resulting in consequent biased results (McPherson *et al*., 2018). The main relationships of male age with sperm parameters are resumed in Table 4.

Concerning legal or biological restrictions given to participation of men in ART programs, the European Union detains variable legal age limits. Male maximum age is legally set in Portugal (60 years) and recommended in Finland (60 years) and Sweden (56 years), whereas in Brazil there is no upper limit defined (CFM, 2021). According to Swiss regulations, 'the potential father should be able to be alive until the child is 18 years-old'. In France no definition of numerical age limits exists, and it´s the responsibility of the centres to define in practice the legal concept of 'normal reproductive age'. Legal limits in third-party donations are set for sperm donors in most European countries - most commonly a lower age of 18 years and upper age of 40 years (Calhaz-Jorge *et al*., 2020) and 50 years in Brazil (CFM, 2021). The American Society for Reproductive Medicine (Practice Committee of the American Society for Reproductive Medicine & Practice Committee of the Society for Assisted Reproductive Technology, 2013), defined that semen donation should be restricted to men aged less than 40 years, and a working group composed of representatives from: the Association of Biomedical Andrologists, the Association of Clinical Embryologists, the British Andrology Society and the British Fertility Society set the maximum age at 46 (Clarke *et al*., 2021).

Table 4. Main relationships of male age with sperm parameters.

Higher male age	Higher male age
Correlations with:	**No correlations with:**
Excessive ROS production	DNA fragmentation
Increased DNA fragmentation	Immature chromatin
Increased DNA methylation	Sperm concentration
Increased sperm aneuploidies	Sperm motility
Sperm vacuoles	Sperm morphology
Decreased sperm concentration	
Decreased sperm motility	Differences may be due to:
Decreased sperm morphology	Sperm selection in ICSI
	Female age bias

Male infertility

Male infertility is assessed based on semen quality analysis according to WHO standards, which includes sperm concentration, motility and morphology (Dar *et al*., 2013; Oleszczuk *et al*., 2016; Sun *et al*., 2018). However, these tools have shown low predictive value in a diagnostic and prognostic manner (Sakkas *et al*., 1998; Dar *et al*., 2013; Oleszczuk *et al*., 2016; Sun *et al*., 2018). It is believed that such findings may be due to sperm DNA alterations that are not detected by these assessments, as sperm DNA damage has been associated to poorer ART outcomes (Bungum *et al*., 2012; Dar *et al*., 2013). Potential causes include advanced age, infection, chemotherapy, radiotherapy, cigarette smoking, drug use and increased levels of reactive oxygen species (Braga *et al*., 2011; Nijs *et al*., 2011; Sun *et al*., 2018). Sperm DNA fragmentation (sDNAfrag), has been suggested as one of the causes of male subfertility (Giwercman *et al*., 2010). It has been shown that the sperm DFI can be used to predict male infertility with a better diagnostic and prognostic value than the WHO parameters (Sun *et al*., 2018). Nonetheless, whereas some cases of male infertility were suggested to be caused by DNA defects that routine analysis failed to detect and these defects correlated negatively with conventional sperm parameters such as sperm motility (Sun *et al*., 2018), the true prognostic value of DNA defects in predicting ART outcomes remains uncertain (Jin *et al*., 2015).

The predictive value of sperm DNA fragmentation

When revising literature it was described that the sperm DFI has a negative effect on various outcomes (Braga *et al*., 2011; Zorn *et al*., 2012; Jin *et al*., 2015; Oleszczuk *et al*., 2016), while others described there is no relationship between these variables (Bungum *et al*., 2012; Dar *et al*., 2013; Jin *et al*., 2015; Oleszczuk *et al*., 2016; Sun *et al*., 2018; Antonouli *et al*., 2019).

In men with conserved spermatogenesis, data revealed lower sDNAfrag levels in testicular spermatozoa, driving its use in cases with high sDNAfrag levels, and recurrent implantation failure and pregnancy loss (Esteves, 2018).

Authors found a strong negative association between DNA damage and FR (Braga *et al*., 2011; Moubasher *et al*., 2021), CPR (Braga *et al*., 2011; Moubasher *et al.*, 2021) and IR (Braga *et al*., 2011). When dividing men based on the percentage of sDNAfrag (≤10%, 11-20% and ≥21%), a significant difference was found regarding the rates of high-quality day 3 embryos, blastocysts, implantation and clinical pregnancy, being lower in males with sDNAfrag ≥ 21%, relatively to the other groups (Van Montfoort *et al*., 2004). DNA denaturation was also associated with significantly lower natural pregnancy rates (Zorn *et al*., 2012). The FR and blastocyst rate have been reported as significantly reduced in obstructive azoospermia (OAZ) and nonobstructive azoospermia (NOAZ) (Mazzilli *et al*., 2017). This association could be due to elevated sDNAfrag of the spermatozoa injected (Ni *et al*., 2014; Alvarez Sedó *et al*., 2017).

Oppositely, other literature reports gave evidence that high levels of sDNAfrag were not associated with the FR (Lin *et al*., 2008; Dar *et al*., 2013; Sun *et al*., 2018; Antonouli *et al*., 2019), embryo quality rate (Lin *et al*., 2008; Sun *et al*., 2018), total number of blastocysts (Antonouli *et al*., 2019) and CPR (Lin *et al*., 2008; Dar *et al*., 2013; Oleszczuk *et al*., 2016; Sun *et al*., 2018; Antonouli *et al*., 2019). In addition, DFI was not associated to birth characteristics such as birth weight and gestational age (Bungum *et al*., 2012).

One study noted, within a higher sDNAfrag group, a significantly higher IR and CPR with ICSI compared to IVF (Bungum *et al*., 2007). The application of methods for selection of morphologically normal spermatozoa may result in the use of spermatozoa with lower sDNAfrag in ICSI (Dar *et al*., 2013; Bucar *et al*., 2015; Sun *et al*., 2018; Antonouli *et al*., 2019). In the same context, a higher proportion of healthy and fertile women was found in ICSI treatments in which better quality oocytes were retrieved, and therefore with better DNA repair capacity (Bungum *et al*., 2012; Oleszczuk *et al*., 2016; Sun *et al*., 2018). It is believed that at the zygote stage there exists a mechanism of sperm DNA damage repair via oocyte DNA repair enzymes and antiapoptotic proteins, which appear to be dependent on the oocyte´s cytoplasmic and genomic quality (Jin *et al*., 2015; Oleszczuk *et al*., 2016; Antonouli *et al*., 2019). This was corroborated in an investigation where sDNAfrag was a prognostic predictor of reduced CPR, LBR and IR in couples with reduced ovarian reserve but not in couples with normal ovarian reserve (Jin *et al*., 2015). This was also shown when the FR appeared to not be affected by the presence of a sperm higher DFI in ICSI groups compared to IVF groups (Oleszczuk *et al*., 2016). In addition, LBR was described as significantly lower in IVF when DFI was >20% but not in ICSI (Oleszczuk *et al*., 2016).

The lack of agreement regarding a cut-off value of DFI is due to, for example, the deficiency of standardized protocols, variation between facilities or laboratories and the variety of DNA testing methods (Jin *et al*., 2015; Moubasher *et al*., 2021), putting at stake its reproducibility. The methods developed and used to analyse sDNAfrag include: terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick and end-labeling (TUNEL) assay; sperm cromatin structure assay (SCSA); sperm cromatin dispersion (SCD) assay, the single cell gel electrophoresis (COMET) assay and DNA breakage detection-fluorescence in situ hybridization (DBD-FISH) technique (Oleszczuk *et al*., 2016; Sun *et al*., 2018; Antonouli *et al*., 2019). Additionally, different sDNAfrag percentages are used as thresholds. Numerous cut-offs for DFI have been reported in the literature, but no absolute upper limit that could define an unsuccessful pregnancy exists (Dar *et al*., 2013). Notwithstanding, for the TUNEL assay, recent efforts have consensually determined a cut-off of ≥ 20% (Sergerie *et al*., 2005; Zidi-Jrah *et al*., 2016; Majzoub *et al*., 2017). Nevertheless, the interpretation and conclusions from the review of those studies must be made with caution. The main relationships of sperm DNA fragmentation with these ART outcomes are resumed in Table 5.

The predictive value of conventional sperm parameters

When analysing conventional semen parameters, results also vary between studies. When comparing NOAZ and OAZ, the rates of CP and LB were lowest in the NOAZ group (Esteves & Agarwal, 2013). Whereas men with OAZ are expected to present a conserved sperm production, NOAZ men have an altered spermatogenesis, which may explain the above results. In addition, other authors observed that low spermatozoa concentration (<1M/ml) had a significant negative impact on the FR and blastocyst rate, but no effect on the rates of top-quality blastocysts and ongoing pregnancy (Bartolacci *et al*., 2018). Concordantly, a negative correlation was also observed between spermatozoa motility <5% and FR, but not with the blastocyst rates or with ongoing pregnancy rate (Bartolacci *et al*., 2018). It has been shown that although abnormal sperm parameters compromise fertilisation and blastulation rates, they do not impact the rate of euploid blastocysts obtained, or their implantation potential, explaining these findings (Mazzilli *et al*., 2017; Bartolacci *et al*., 2018). Also, this could be due to the limited availability of spermatozoa suitable for the treatment, leading to the selection of a suboptimal sperm for ICSI (Bartolacci *et al*., 2018).

Normal sperm motility was positively correlated with the FR, IR and CPR (Braga *et al*., 2011). Regarding morphology, a significant decrease in the rates of CP and LB for every 1% decrease in the rate of normal spermatozoa morphology was found (Vural *et al*., 2005), another reported significantly reduced FR in oligoastenoteratoazoospermia (OAT) (Mazzilli *et al*., 2017) and more recently a low percentage of spermatozoa with normal morphology was associated to an increased risk of embryonic aneuploidy (Bilibio *et al*., 2021) and lower FR and CPR (Moubasher *et al*., 2021). The IR has also been found to be affected by leucocytospermia (Vural *et al*., 2005), and abnormal spermatozoa morphology and the presence of large or multiple sperm vacuoles were shown to negatively influence the FR, IR and CPR (Braga *et al*., 2011).

On the contrary, several other studies did not identify any kind of relationship between spermatozoa concentration, motility or morphology and ART outcomes, such as CP and LB (Mazzilli *et al*., 2017; Mariappen *et al*., 2018). Additionally, the cause of azoospermia (OAZ or NOAZ), when performing Testicular sperm extraction (TESE)/ICSI, was, contrarily to the data above discussed, reported to not negatively affect the rates of fertilisation and embryo cleavage (Abdel Raheem *et al*., 2013), the rates of miscarriage (Abdel Raheem *et al*., 2013; Esteves & Agarwal, 2013), CPR and LBR (Abdel Raheem *et al*., 2013; Park *et al*., 2018), the rates of high-quality embryos and the mean number of high-quality embryos available for transfer (Park *et al*., 2018), and the rates of ectopic pregnancy, multiple pregnancy, gestational age, birth weight, preterm birth, low birth weight and very low birth weight (Esteves & Agarwal, 2013). Furthermore, no differences among men with moderate male factor, severe OAT, OAZ and NOAZ were found regarding the gestational age, birth weight and congenital malformations (Mazzilli *et al*., 2017).

Table 5. Main relationships of sperm DNA fragmentation (SDF) with ART outcomes.	
Higher SDF	**Higher SDF**
Correlations with:	**No correlations with:**
Lower FR	FR
Lower embryo quality rate	Embryo quality rate
Lower blastocyst rate	Total blastocysts
Lower IR	CPR
Lower CPR	Gestational age
Lower LBR	Birth weight
Due to lower SDF in testicular sperm, TESA is advised in:	
Recurrent implantation failures	
Recurrent pregnancy loss	
Regarding azoospermia, SDF is increased in:	
Obstructive azoospermia	
Secretory azoospermia	
Regarding IVF/ICSI:	
IVF: lower IR, CPR	
ICSI: higher IR, CPR	
Differences may be due to:	
sperm selection in ICSI	
fertile women associated with higher DNA repair capacity	
Reduced ovarian reserve associated with:	
Lower IR, CPR, LBR	
Normal ovarian reserve associated with:	
Higher IR, CPR, LBR	

When evaluating men with complete teratozoospermia undergoing ICSI, no significant differences were found in the rates of BP, CP, spontaneous miscarriage and live birth, reaffirming the importance of sperm selection in ICSI for male factor infertility (Pereira *et al*., 2015). The ability of ICSI to achieve normal outcome parameters can be explained, not only by the processes already described, but by the certainty of introducing the oocyte activating factor (Neri *et al*., 2014). Recently, within a cohort of couples experiencing IVF/ICSI, those with male factor infertility had slightly increased chances of success compared to those without (Wen *et al*., 2021). Moreover, as no significant differences in the rates of pregnancy and miscarriage were observed when comparing couples with male or tubal factor, but FR and IR were higher, results further suggest that ICSI can surpass male factor infertility limitations (Borges et al., 2017). The main relationships of sperm parameters with these ART outcomes are resumed in Table 6.

CONCLUSION

The increasing phenomenon of delayed parenthood, associated to the inherent effects of aging on reproductive capacity, has led to an increase in the search of ART to conceive. Thus, the investigation on the possibility of factors, existing before initiating a stimulation protocol, that could influence IVF and ICSI outcomes, has grown in literature, reporting various but also contradictory results. In an effort to analyse these findings and establish a consensus in literature, we reviewed the effect of female age, male age, ovarian reserve and male factor on IVF/ICSI success.

Increasing female age is associated to lower LBR and CPR. The levels of bFSH and AMH and the AFC are considered baseline factors predicting the ovarian reserve. Higher AMH levels are associated to higher CPR and LBR. However, the prognostic value of AMH as an ovarian reserve marker is greater for women of advanced age than for women of younger age, as age could protect low responders from the deleterious effects of a poor ovarian response. Lower bFSH levels are associated to higher FR, CPR and LBR. Similarly to AMH, bFSH levels do not seem to affect pregnancy and LB rates in younger women. This can be explained by the fact that high bFSH does not originate ageing oocytes (with poorer quality) but is instead related to fewer oocytes produced. The AFC is positively correlated with the number of retrieved oocytes, decreases with age, and characterizes the ovarian reserve quantitatively.

Most studies report no association between male age and CP, IR and LBR, but others contradict these results. The contrast in results can be explained by several hypotheses: only a few normal spermatozoa are required for successful ICSI; ICSI can surpass possible sperm alterations due to advanced male age; the male age cohorts used in different studies vary; and due to the bias introduced by female age. Male age reveals a significant relationship with all sperm parameters and can directly damage sperm DNA and compromise spermatogenesis, but no association to oocyte, blastocyst and embryo parameters were found.

Male infertility is assessed based on semen analysis according to WHO standards. However, these tools have shown low predictive value in a diagnostic and prognostic manner, possibly due to sperm DNA alterations. Sperm

Table 6. Main relationships of sperm parameters with ART outcomes.

Presence of correlations	ART Outcomes
Obstructive azoospermia (OAZ) vs secretory azoospermia (SAZ)	Higher CPR, LBR
	No association with:
	FR, ECR, high quality embryos, number of embryos for ET, CPR, multiple pregnancy, ectopic pregnancy, miscarriage rate, LBR, gestational age,
birth weight, congenital malformations	
Low spermatozoa concentration (< 1M/ml)	Lower FR, Blastocyst rate
	No association with:
	high quality blastocysts, Embryo aneuploidy, IR, ongoing pregnancy
Low spermatozoa motility (<5%)	Lower FR
	No association with:
	blastocyst rate, embryo aneuploidy, IR, ongoing pregnancy
Normal spermatozoa motility	Higher FR, IR, CPR
Spermatozoa morphology	Negative correlation with:
	FR, embryo euploidy, IR, CPR, LBR
Complete teratozoospermia	No association with:
	BPR, CPR, miscarriage rate, LBR
Oligoasthenoteratozoospermia (OAT)	Lower FR
Severe OAT	No association with:
	Gestational age, birth weight, congenital malformations
Spermatozoa parameters	No association with: CPR, LBR
Male factor vs tubar factor	Higher FR, IR
	No association with:
	CPR, miscarriage rate
Moderate male factor	No association with:
	Gestational age, birth weight, congenital malformations
Discrepancies rule the importance of sperm selection in ICSI in overcoming male factors	

DNA fragmentation could be used in the future to assist WHO parameters in predict male infertility with a better diagnostic and prognostic value.

The knowledge of possible factors that could influence the success of a couple's IVF/ICSI program can allow the optimization and individualization of therapy, prior to the commencement of treatment, establishing the best prognosis possible and increasing the chances of reaching a live birth.

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CONFLICT OF INTEREST

None.

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