Andrology-Open Access

Rabaça et al., Andrology (Los Angel) 2016, 5:1
http://dx.doi.org/10.4172/2167-0250.1000152

Review Open Access

# Aging of the Male Germline: Influence of Advanced Male Age on Reproductive Outcome

**Ana Rabaça , Carolina Ferreira and Rosália Sá***

*Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS), Unit for Multidisciplinary Research in Biomedicine (UMIB), University of Porto, Porto, Portugal*

***Corresponding author:** Rosália Sá Department of Microscopy, Laboratory of Cell Biology, ICBAS, University of Porto, UMIB, Rua Jorge Viterbo Ferreira 228, Building 1, Floor 2, Room 03, 4050-313 Porto, Portugal; Tel: +351-22 042 80005242; Fax: +351-22 042 80 90; E-mail: rmsa@icbas.up.pt

**Rec date:** January 6, 2016; **Acc date:** February 29, 2016; **Pub date:** March 8, 2016



## Abstract

The rise in life expectancy and the desire to establish a stable career prior to parenthood leads couples to a delayed childbearing. Advanced parental age at the time of pregnancy portrays several risks, with the majority of couples above 35 experiencing reproductive impairments. While the effect of women's age on fertility is well known, the influence of paternal age is less understood. Thus, efforts have been made to comprehend the influence of APA on pregnancy upkeep and disease risk for the offspring. Over the last decades numerous studies aimed to establish the relationship between APA and male fertility potential. It has been found that APA is related to testicular and hormonal alterations, along with a decreased sperm production, quality and DNA integrity. As expected, these alterations compromise the reproductive potential of older men and have been associated with several fecundity problems, including increased time to pregnancy and abortion rates, and decreased fertilization and pregnancy rates. Additionally, alterations in offspring health, like genetic disorders, mental illnesses, congenital anomalies and cancer, have also been linked to APA. As a result, APA influence on the reproductive outcome must be taken into consideration when counseling couples attending family planning consultations or pursuing fertility treatments. Nonetheless, to date, there are still no definite results regarding this problem and an age threshold has yet not been established and additional research to better elucidate the effects of APA on fertility and offspring's health is needed. Likewise, establishing an age threshold for APA would be of great value to males thinking of delaying parenthood, and to physicians for medical advice purposes.

**Keywords:** Advanced paternal age; Male reproductive potential; Male fecundity problems; Offspring health

## Introduction

The rise in life expectancy in developed countries, as well as the wish to establish a career prior to parenthood, leads numerous couples to a delayed childbearing. Advanced age at the time of pregnancy portrays clear risks, with a great majority of couples above 35 experiencing reproductive complications [1,2]. The effects of women's age on fertility are well established, being one of the most important factors to consider when addressing fertility issues. Indeed, women over 35 have a compromised fertility, with low oocyte numbers and overall reduced quality [3]. This biological clock of women's reproduction capacity restrains fertility after a certain age leading to a decreased capacity to conceive and prolonged time to achieve pregnancy [4,5]. Although the implications of advanced maternal age are well documented, the effects of paternal age on reproductive success and overall child health are yet to be fully disclosed.

Male fertility generally persists throughout life with male germ cells being continually produced [6,7]. As a result men tend to father children regardless of their age [8] raising concerns on the possible effects that may entail. Over the last decades, several studies have emerged, showing a relationship between advanced paternal age (APA) and male fertility. Aging is responsible for a general decay on the reproductive tissues and organs (Figure 1).

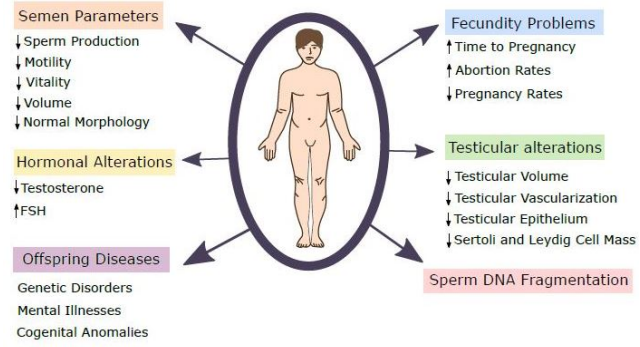


**Figure 1**: Effects of advanced paternal age on male fertility potential and offspring health. Male aging and its consequences on sperm parameters, hormonal balance, testis histology, fecundity potential, sperm DNA integrity and health of the offspring.

Older males experience a decrease in testicular volume [9,10] and histomorphological alterations [10] consistent with a decrease in Sertoli cell mass. However, this decrease in Sertoli cell mass was not trailed by a decrease in Sertoli cell functions. In fact, only a minor

decline in Sertoli cell functions was observed due to a compensatory increase in follicle-stimulating hormone (FSH) levels [11,12]. Additionally, a decrease in Leydig cell function led to a decrease in overall androgen levels, especially testosterone, what compromises reproductive functions [11,13,14]. Furthermore, it has also been reported that APA has significant metabolic effects on protein and carbohydrate metabolism, incidentally influencing insulin sensitivity [14].

Several studies have addressed the relationship between advanced age and the decline of semen production and quality. It has been shown that daily sperm production per testis gradually decays with age [15] followed by a decrease in semen volume, sperm vitality, and motility [16-19]. Variations in hormonal levels, especially FSH and testosterone, may partly explain this decrease in sperm quality [16] but further research is needed to clarify this matter.

There is an increasing body of evidence that APA may not only decrease sperm quality and quantity, but also affect the genomic integrity of spermatozoa [20]. This genomic instability, brought on by DNA replications errors of older germ cells, can severely impact the pregnancy and offspring, being associated with lower fertilization and pregnancy rates, pregnancy loss, birth defects and progeny diseases [21-24]. Furthermore, it has been demonstrated that time to achieve pregnancy is also highly affected by male aging [25]. These evidences raise serious concerns regarding the adverse impacts of APA on the offspring, as it has been confirmed that sperm DNA damage is closely associated with APA [26,27]. Although a decrease in male fertility occurs with age, there is still the need to define an age threshold for which the risk of an adverse outcome due to APA is greater than the gain.

It seems to be evident that APA is associated with several reproductive functions modifications, leading to a decreased sperm quality and DNA integrity, what may affect pregnancy outcome and offspring health. Thus, APA influence on the reproductive outcome must be taken into consideration when counseling couples attending family planning consultations or pursuing fertility treatments. Nonetheless, there is a lack of reports evidencing the exact relationship between APA-induced reproductive alterations and reproductive potential and the offspring diseases. Even though, efforts have been made to fully understand the exact influence of these alterations in fertilization ability, pregnancy maintenance and disease risk for the offspring. Hence, the purpose of this review is to provide an overview of the recent literature available on the influence of APA, during natural and assisted conceptions, on human fecundity, fertility, and offspring health.

## Age Influence on Testicular Functions

Although males do not experience an expiry of reproductive functions, such as menopausal women do, their reproductive organs suffer gradual alterations that have a real impact on fertility [28]. With each passing decade the testis undergo several histomorphological changes associated with a hormonal dysregulation and cellular mass loss [12,29].

Male aging is related to a decline in testicular and adrenal functions which results in decreased androgen concentrations [30,31]. Consequently, aging is closely related to male virility. In truth, advanced age males exhibit a higher incidence of erectile dysfunction, a decrease in muscle mass and strength, and increased tendency to develop atherosclerosis. Furthermore, these patients tend to experience a significant impairment in glucose metabolism, what has been related to a decline of testicular functions [10,14,32].

In healthy adult males, testis volume seems to remain fairly constant. However, there is a decrease of more than 30% in testis volume after their 8th life decade. Testosterone, FSH and inhibin B seem to be key players on these events. In effect, a decrease in inhibin B/FSH ratio seems to be responsible for a drop in testicular volume, accompanied with reduced Sertoli cell mass and function [12]. There is a connection between testis volume and spermatogenesis. Histological testicular studies have shown that, although variable, progressive alterations occur with general germ cell loss [33]. Advanced age has numerous effects on testes morphology being correlated to a stiffening of the basal membrane of the seminiferous tubules, a decrease of their epithelium, and a reduction of testes vascularization. In reality, aged testes display reduced numbers of type-A dark spermatogonia, and an increase in multinucleated spermatogonia and giant spermatids, as well as several multilayered spermatogonia, common features of cellular senescence [34]. These alterations lead to a reduced spermatogenesis efficiency, resulting in a lower sperm concentration [16]. Furthermore, Sertoli and Leydig cells are also reduced in numbers and present noticeable cytoplasmic alterations, which influence the testes architecture [8,35-37]. Although efforts have been made to define an age threshold for these changes, they seem to develop gradually throughout life and limits are yet to be defined [28].

Bearing all these testicular alterations in mind, it seems only natural that semen parameters can be affected by age related changes to the testes and that male fertility may suffer a noteworthy decline.

## Sperm Parameters Decline with Aging

Aging is related to a normal general decline in various bodily functions and organs, including the reproductive system, and semen parameters (Table 1).

| Parameter | Trend with Aging | Age Bound | Fertility Complications | Reference |
|---|---|---|---|---|
| Semen Volume | 0.5% per year | 50 years | ↓ sperm quality<br>Male factor infertility | [38-42] |
| Concentration | ↓ 3.3% per year | 40-50 years | Male fertility decline | [43-46] |
| Motility | ↓ 0.7% per year | - | Longer time to pregnancy<br>Male factor infertility | [17,40,46,47] |
| Morphology | ↓ 0.9% per year | 45 years | ↓fertilization capacity | [45,46,48,49] |
| -, not evaluated; ↓, decreased. | | | | |

**Table 1:** Effects of paternal age on semen parameters.

Thus, the effects of male aging on male fertility, especially on sperm parameters have been assessed by a great number of studies [8,28,50]. Even though, only a few accounted for possible bias from cofactors such as obesity, hypertension and smoking, it is noticeable that, as men grow older, there is an age-related decrease on the daily sperm production [9]. This deterioration is followed by a decrease in semen volume and overall spermatic quality, especially concerning sperm motility and morphology [40,44]. Although studies have demonstrated

that men over 45 years experience a gradual decline in sperm counts and quality, the general pattern is not constant, and as a result defining an age threshold is challenging [8,51]. Sperm parameters are negatively affected through a variety of mechanisms and a decline in sperm quality is normally related to several factors. As a result, it is difficult to predict what detrimental changes occur in sperm solely related to the aging process [8]. A possible mechanism for decreased sperm quality is the imbalance of reactive oxygen species (ROS) production at the mitochondrial level. A study conducted by Lissak and peers revealed that male aging is correlated to an increase in the oxidative potential of the seminal plasma [52]. These increase in oxidative stress can potentially cause a significant decline in sperm quality [28].

Additionally, there are several illnesses of complex etiology associated with age that can affect sperm function and, a decreased semen volume and less frequent ejaculations may be related to some of the morphological and motility changes observed in sperm from elderly patients [41,53]. Furthermore, aging is related to a natural accumulation of toxic products and reproductive tract infections that may end up compromising male fertility [42]. A study conducted by Rolf and peers revealed that males over the age of 40 display a 14% incidence of reproductive tract infections and a lower sperm count than men in their twenties. These infections seem to be closely related to a decreased semen volume, most likely due to seminal vesicles deficiency [42]. The seminal vesicles are responsible for the majority of fluid present in semen, therefore being crucial elements for male fertility. These secretions carry several core constituents needed for a proper spermatic function [34]. Research done by Rolf and peers revealed that although the levels of zinc and α-glucosidase, essential elements for sperm function and nutrition, remain fairly constant throughout life, fructose, a key preferred nutrient for sperm gradually decreases with aging [41]. Semen volume is a key aspect of male fertility. Although normally it is not a direct cause of male infertility and lower pregnancy rates [39], it affects spermatozoa quality and can play a major role in male factor infertility. Numerous studies have found a close relation between the aging process and semen volume decrease [40-42]. Though concrete numbers are still up to debate, studies have revealed that men tend to experience a semen volume decrease of 0.5% per year, summing up to a 20% decrease when comparing men of 50 to 30 years old [38,40].

Sperm concentration in a man's ejaculate is related to the likelihood of fathering children without needing the aid of Assisted Reproductive Technology (ART) treatments. Several studies have evaluated the variations in sperm concentration that occur with aging [44-46]. Authors have found a tendency for a continuous sperm concentration decline with aging, especially aggravated after the age of 45 years [45]. Actually, a decrease of 3.3% per year has been reported by Auger and peers while evaluating men with ages ranging 30 to 50 years old [43]. Nonetheless, a threshold for this parameter has not yet been established as others claim a more gradual decline in sperm concentrations throughout life, with no definite verging point [17]. A more recent study conducted by Stone and peers revealed that, males above 40 years old displayed a consistent significant decline in sperm concentration [46]. Interestingly, authors have found a negative correlation between sperm concentration and ROS levels in the semen. Indicating that ROS increase with aging may be a contributing factor for APA infertility issues.

Albeit reduced sperm concentration is a common anomaly found in infertile males with advanced-age, sperm quality is also affected by the natural aging process. Sperm motility seems to be one of the most affected sperm parameter by the aging process. Motility decrease in advanced-age patients is generally associated with alterations at the epididymis caused by several infections that may have occurred throughout life. As spermatozoa gain motility during mitochondria activation at their passage through the epididymis, it is highly affected by any age related alteration to this process [54]. Several studies have reported a significant decline in sperm motility associated with male aging [17,40,46]. In fact this decrease can reach 0.7% per year, and may be directly related to male factor infertility of advanced-age patients [40,47]. Studies have found that APA men are more likely to experience a compromised fertility with longer times to achieve pregnancy due to a decline in the total numbers of motile sperm present in the ejaculate [46].

Along with motility, sperm morphology is an essential parameter for assessing male fertility as it is believed to be correlated with the sperm ability to penetrate and fertilize the oocyte. Studies relating teratozoospermia index with advancing age have stated that after the age of 45 years, male's semen samples display a significant increase in sperm morphology alterations [45,46,48,49]. Furthermore, it has been revealed an annual decline of approximately 0.9% per year in spermatozoa with normal morphology [46]. Others have found an increase in the percentage of specific morphological anomalies, such as errors in the tail and head of spermatozoa, which tend to be present in several age groups, but are significantly more noticeable in advanced-age males [55]. However, the variable criteria found amongst studies exclude the possibility of clear comparisons between morphological anomalies found in spermatozoa and advancing age.

Evidences seem to indicate that aging and its related health alterations are associated with several modifications on male reproductive functions and sperm production. There are several fertility issues that can arise as a result of these alterations. However the true effects of aging on spermatogenesis remain unknown. Thus, it is important to understand the correlation between other several age-related fertility issues such as time to pregnancy (TTP), fertilization and miscarriage rates, and offspring's health.

## Male Age and Fecundity Problems

Clinical infertility is characterized by the incapacity to achieve pregnancy after 12 months of regular unprotected sexual intercourse [56]. The delay of the first pregnancy until a more advanced age, characteristic of industrialized societies, results in increased infertility issues and miscarriages [57]. While advanced male age has important effects on fertility, these variations are less documented when compared to women aging studies [28].

The influence of APA has been investigated regardless of the means to conception, including both natural and ART conceptions. We have summarized the influence of APA on TTP, fertilization/pregnancy, and abortion rates in Table 2.

### Time to pregnancy and pregnancy rates

TTP, the time taken to conceive, is usually used as a measure for fertility potential [56]. Frequently, increased TTP is associated with alterations in gametogenesis and fertilization [86], being an indicator of human infertility [87].

In relation to natural conceptions, few studies relate APA with alteration in TTP, fertilization, and pregnancy rates. Even though researchers have found an association between maternal age and an

increased TTP, the same was not verified regarding APA [67,68]. However, a more general approach by Ford and peers revealed that couples' infertility risk increased when males where above 35 years old [25]. Additionally, when men were above 40, pregnancy rates decreased approximately 10% in comparison to males below 24 [25]. A study conducted by Dunson and peers, which investigated the effects of paternal age in the probability of conception, while controlling for the maternal age bias, demonstrated that the probability of conception for men on their late thirties or older was decreased. In effect, women who had unprotected intercourse on their most fertile day of the menstrual cycle, displayed a decline in the probability of conception of 0.29 when man were aged 35 years old and 0.18 with 40 years old man [62]. Likewise other studies have found an increase in the time to achieve pregnancy when males presented an increased age [21,63].

| Type of conception | Paternal age (years) | Pregnancy / Fertilization | Abortion rates | Reference |
|---|---|---|---|---|
| Natural Conception | ≥ 35 | - | ↑ | [58-60] |
| | ≥ 40 | ↑ time to conception | ↑ | [25,61-64] |
| | | ↓ probability of conception | | |
| | > 45 | ↑ time to conception | - | [21] |
| | ≥ 50 | - | ↑ | [65,66] |
| | - | No effect | ↑ with each additional year | [67-69] |
| IUI | ≥ 35 | ↓ pregnancy rate | - | [70] |
| | ≥ 40 | ↓ pregnancy rate | ↑ | [71] |
| | - | No effect | No effect | [72] |
| IVF | ≤ 32 | ↓ pregnancy rate | - | [73] |
| | ≥ 39 | ↓pregnancy rate | - | [74] |
| | ≥ 40 | ↓ likelihood of conception | - | [75-77] |
| | | ↓ pregnancy rate | | |
| | > 50 | ↓ fertilization rate | - | [78] |
| | - | No effect | No effect | [79-81] |
| ICSI | ≥ 50 | ↓ fertilization rate | ↑ | [82,83] |
| | - | ↓ pregnancy rate in oligozoospermic men | - | [84,85] |
| | | No effect | | |

IUI: Intrauterine insemination; IVF: In vitro fertilization; ICSI: Intracytoplasmatic sperm injection; -: Not evaluated; ↑: Increased; ↓: Decreased.

**Table 2:** Effects of paternal age on time to pregnancy and pregnancy/fertilization and miscarriage rates.

Furthermore, when compared with men younger than 25 years, men of APA were 4.6 times more likely not to induce pregnancy until after 1 year of regular unprotected intercourse. After the examination of clinically infertile couples it was found that advanced-age males were 12.5 times more likely to exhibit fertility issues [21]. In fact, in men above 40 the probability of conceive decreases approximately 7% in relation to younger men [63]. The present results show that APA is responsible for inducing negative alterations in fertility/pregnancy rates and in TTP, independently of maternal age. ART treatments allow the purification of high quality gametes enabling infertile couples to conceive. Similarly to what occurs during natural conceptions, pregnancy and fertilization rates seem to be decreased by APA when ART treatments are implemented [77,88]. However, most studies did not control for the mother's age bias. In order to solve this issue, researchers have been using egg donation models to provide a relatively homogeneous pool of high quality oocytes, assuring that variations in sperm quality with male aging is the major variable [76]. The first evidence reporting an association between APA and pregnancy rates after ART treatments was discovered by Mathieu and peers [70]. While evaluating pregnancy rates of couples undergoing intrauterine insemination ( IUI ) they found that, after 5 cycles of IUI pregnancy rates displayed an 11.4% decrease in men older than 30 years, and a 26.8% drop after the age of 35, when comparing with men younger than 30 years old. These results were confirmed by other authors who evaluated the effect of APA during the same ART procedure [71]. Although some have found no significant differences in pregnancy rates in couples with APA [72], most studies appear to indicate a connection between APA and poor fecundity.

In a retrospective analysis of couples undergoing in vitro fertilization (IVF) treatments, with oocyte donation, it was identified a significant decline in sperm counts in men with APA [81]. Furthermore, Luna and peers have observed a decrease in fertilization rates after IVF cycles with men older than 50 years old that, however, had no impact on pregnancy rates [78]. Nonetheless, when men were above 60 years there was a negative association between APA and implantation rates. Similar results were obtained by de La Rochebrochard and peers, where failure to conceive was observed in individuals above 40 years old [75]. Others have described diminished pregnancy rates associated with increasing male age [77]. While males 35 years old and younger exhibited pregnancy rates of 53%, men between 36 and 40 years had pregnancy rates of 35%. It was also noted that man above 40 years had a significant pregnancy rate drop to about 25%. Additionally it was observed that with each additional year there was an 11% increase in the odds ratio of not reaching pregnancy, and a 12% increase of not achieving a successful live birth. A study by Girsh and peers comparing couples who achieve pregnancy through IVF treatments and couples who remain non-pregnant revealed that men in the pregnant group were significantly younger (43.2 ± 8.1) than those in the non-pregnant group (46.8 ± 7.8) [76]. The assessment of sperm morphology showed a significant higher prevalence of teratozoospermia in males of the non-pregnant group. Furthermore, men between 40 and 50 years old presented a decline in the quality of their semen parameters. Thus, a negative, but not significant, correlation between APA and pregnancy rates was reported, evidencing a possible correlation between decreased sperm parameters and failures in IVF cycles [76]. Likewise, others have observed a significant decrease in implantation and pregnancy rates in APA men [73,74]. Overall results have demonstrated that APA may negatively influence the reproductive outcome of IVF treatments.

Intracytoplasmic sperm injection (ICSI) cycles are a current practice for severe male infertility issues. Several studies have found no correlation between paternal age and pregnancy rates in ICSI treatments [83,85]. However, results are somewhat contradictory as men above 50 years are related to decreased fertilization rates in ICSI treatments but apparently pregnancy rate is not affected [82]. Nonetheless, a more recent study of couples undergoing ICSI cycles with fresh spermatozoa [84] has found an association between pregnancy rates and APA. Although no alteration have been found in normozoospermic patients, oligozoospermic men displayed a 5% decrease in implantation rates each proceeding year [84]. While results appear to be inconsistent, APA does not seem to be associated with decreased pregnancy rates during ICSI treatments, when involving normozoospermic males. These results may be explained by the technicalities of the procedure that involve the purification of high quality sperm, reducing the percentage of defected spermatozoa that may induce fecundity issues.

Previous studies that evaluated the correlation between TTP, as well as pregnancy/fertilization rates, and APA show conflicting results. These divergences may be explained by the practice of a different number of cycles between studies and other technical discrepancies. Even though the evidences provided to demonstrate the influence of paternal age on fertility outcomes of ART treatments are insufficient, the TTP of natural conceptions is negatively affected by APA. There is still no consensus concerning a limit age from which fecundity is really affected. Nonetheless, most studies considered 40 years old as the definition of APA. Interestingly, the majority of these studies presented an increase of almost 50% in TTP and a general decrease in fertilization and pregnancy rates of aging men. Hence, couples with APA males must be informed about the adverse effects of males' age in pregnancy outcomes.

### Spontaneous abortion rates

Similarly to TTP, spontaneous abortion is one of the most frequent adverse reproductive effects, influenced by advanced maternal age [89]. Interestingly, most of the fetal deaths occur during the first trimester of gestation and are defined as miscarriages. Deaths occurring after 20 weeks are generally described as "late fetal death", and the term "stillbirth" is commonly used for fetal deaths occurring after a 28 weeks gestation period [89]. Although male factors may also contribute to spontaneous abortion risk, few studies evaluating this situation are available. Nonetheless, reports have observed that paternal aging is associated with pregnancy loss after an established natural pregnancy.

Research evaluating spontaneous abortion rates found that paternal age has a significant effect on the risk of late fetal death, after an established natural pregnancy [69]. However, these authors considered that the negative effect of paternal age on pregnancy loss is linear, which may be incorrect. Other studies which were controlled for the maternal age effect verified that the risk of miscarriage increased with paternal age, particularly when fathers were aged >50 years old [65]. Similarly, Ford and peers analyzed risk factors for miscarriage in couples who achieved a recognized pregnancy, observing that the risk of miscarriage was higher in couples with males above 35 years old [58]. Similar results were found by several other authors that concluded that adverse pregnancy outcomes are related to APA, and reach a peak if both partners are of advanced age [59-61]. A case-control study by Kleinhaus and peers similarly verified a correlation between APA and miscarriage risk, independent of maternal age [64]. In this study, spontaneous abortion rates significantly increased in pregnancies conceived from fathers aged above 40 years old. These data demonstrates that during natural conceptions paternal age negatively impacts the embryo development, increasing miscarriage rates. As a result, it may be important to consider APA as a risk factor for pregnancy.

As well, some studies suggest that there is an association between APA and spontaneous abortion when couples resort to ART treatments [71,72]. Although there can be found some contradictory results, several studies have reported increase in miscarriage, sometimes to more than double, in couples with APA men that undertake ART treatments [71]. Moreover, some evidences suggest that APA may affect embryo development, due to a decrease in quality and genomic integrity of spermatozoa from older individuals. Controversially, a meta-analysis research by Dain and peers, and a research by Duran and peers, using donor oocytes, for IVF and IVF/ICSI procedures have stated that paternal aging is not associated with an increased risk of pregnancy loss after an established pregnancy [88,90]. However, although a study conducted by Frattarelli and peers has been involved in the previous meta-analysis, it is important to emphasize that these authors demonstrated that men above 50 years old displayed an increase in pregnancy loss and lower blastocyst formation and live birth rates [85]. In this situation, overall pregnancy loss rate significantly increased with paternal age, increasing from 24.4% in men below 50 years old to 41.5% in individuals above 50 years old. In a recent research using an oocyte donation model it was reported that APA has an adverse impact on ART outcomes [91]. APA men had increased abortion rates, when compared with younger men. Overall, research on this subject is contradictory and further investigations are needed to fully evaluate this issue.

As all available results report mixed outcomes, the relationship between APA and pregnancy loss remains unclear. Nevertheless, in some cases APA seems to have a negative effect on spontaneous abortion after established natural conceptions. On the other hand, the existing data in relation to ART treatments is less elucidative, and although some results seem to indicate a relation between pregnancy loss and APA, further studies are needed to evaluate this subject. Inclusive, results suggest increasing risk of spontaneous abortion with pregnancies resulting from APA males.

## Advanced Paternal Age and Spermatozoa Genetic Anomalies

The aging process is responsible for gradual reproductive functions alterations that cause a progressive decrease in sperm quality and may lead to subfertility and infertility issues. Studies have found a possible association between advanced paternal age and the presence of chromosomal abnormalities, DNA fragmentation and aneuploidy [92-94]. These genetic alterations may lead to adverse pregnancy outcomes, such as premature delivery and offspring anomalies [28,95,96]. In effect, higher levels of DNA breaks have been discovered in older men with a two-fold increase in fragmentation levels after the age of 60 years [95]. Furthermore, some studies have demonstrated that advanced age in the male is responsible for an imbalance in the sex ratio, altering the relative numbers of Y and X sperm [46,97]. The mechanisms behind this decrease in sperm quality are yet to be identified, still possibilities such as ROS production and epigenetic alterations are being evaluated [23,98]. A study by Jenkins and peers, that assessed alterations in the sperm methylome, has found an age associated increase of 5-methylcytosine in human spermatozoa. As cytosine residues is an important methylation marker associated with

gene regulation, its increase may contribute to a drop in sperm quality and raise the risk of offspring disorders associated with APA [23]. Others have focused their attention on sperm gene expression, finding altered transcript levels in several relevant spermatozoa genes [99,100] that can be correlated to male factor infertility. Furthermore, both clinical and animal studies have shown that sperm from advanced-age males is more sensitive to aggressive stimulus, such as oxidative stress related to paternal aging, than sperm from younger patients [101,102].

Sperm can be subjected to several intrinsic and extrinsic factors that lead to genetic alterations which severely affect sperm function and can be the main cause for male factor infertility. Nonetheless, sperm with altered DNA are unable to repair their genetic material due to an absence of DNA damage response mechanisms. In sperm, after transcription, DNA repair is stopped and cells are unable to repair any damage occurred after spermiogenesis [103]. However, oocytes and early embryos accumulate several DNA repair transcripts and are capable, to a certain extent, to repair sperm genetic damages [104]. Thus, the real consequences of defected sperm genetic material can be diminished if the repair mechanism of the oocyte and embryo is able to restore sperm genomic integrity [105]. In most cases, if the oocyte repair mechanism is insufficient and fails to correct sperm genetic errors, the embryo dies prematurely [106]. Still, this oocyte genomic control may fail and genetic errors may be introduced in the genome and result in offspring abnormalities [103]. A study by Ahmadi and collaborators has suggested that sperm genetic material has to present at least 92% integrity for the oocyte mechanism being able to repair it. Below this percentage, sperm DNA may not be fully repaired by the oocyte and lead to embryo loss and offspring anomalies [107]. Nevertheless, the oocyte and embryo capacity to respond to sperm genetic alterations is variable and there is no guarantee it will be effective repairing the chromosomal aberrations and DNA fragmentation found in sperm of APA males.

## Advanced Paternal Age and Offspring Health Complications

The fact that older parents are at increased risk of having children with genetic conditions has been well documented. In fact, several epidemiological studies have found a relationship between APA and numeral offspring illnesses, independently of maternal age, such as genetic disorders, mental illnesses, congenital anomalies and cancer (Table 3) [129-131]. APA is closely related to several sperm genetic aberrations that may evade oocyte repair mechanism and possibly be transmitted to the next generation. A genetic disorder associated with the effects of APA is achondroplasia (ACH). ACH is transmitted to the offspring in a dominant fashion being responsible for the most common presentation of dwarfism. Several studies have shown that fathers above 50 years old have a 10-fold increase in the probability of having a child affected by ACH when compared to those in their twenties [108]. This increase in the risk of ACH pass on may be explained in some extent by the elevated number of sperm DNA mutations found in the ACH site [132]. Crouzon and Pfeiffer syndromes, dominant autosomal diseases, are genetic heterogeneous disorders that affect the fibroblast growth-factor receptor 2 (FGFR2) [133]. Both illnesses are characterized by diverse clinical phenotypes that mainly involve severe cranial malformations. Mutations arising in the male germ line associated with APA have been linked with both these disorders [109,134]. Studies have found an increase in the number of sperm mutations in the FGFR2 gene site in males of advanced age, evidencing the risk for the genetic transmission of de novo mutations to the offspring [109,110].

| Offspring illness | | APA risk input | Age Bound (years) | Reference |
|---|---|---|---|---|
| Genetic Disorders | Achondroplasia | ↑ 10-fold | > 50 | [108] |
| | Crouzon/Pfeiffer syndrome | ↑4.1 odds-ratio | - | [109, 110] |
| | Klinefelter | ↑ 1.35 odds-ratio | - | [111] |
| Mental Illnesses | Schizophrenia | ↑ 3x | > 45 | [112-114] |
| | Bipolar Disorder | ↑ 37% | > 54 | [115, 116] |
| | Autism | ↑ 3x-5x | > 40 | [117-119] |
| Congenital Anomalies | Musculoskeletal Congenital Anomalies | ↑ 26% | > 50 | [120-122] |
| | Congenital Heart Defects | ↑ 69% | > 45 | [123] |
| Cancer | Leukemia | ↑ 1.31-1.49 relative risk | > 35 | [124-126] |
| | Central Nervous System | ↑ 15%<br>↑ 2.69 relative risk | > 40 | [125,127] |
| | Breast Cancer | ↑ 1.6 odds-ratio | ≥ 40 | [128] |

APA, advanced paternal age; -, not evaluated; ↑, increased.

**Table 3**: Advanced paternal age and offspring health complications.

Numeric chromosomic aberrations are severe genetic disorders that greatly impact life quality. There are growing evidences that some chromosomic disorders have been increasing in prevalence over the years and it has been hypothesized that these increase may be related to APA, a known risk factor for genetic anomalies [135].De Souza and peers performed an extensive analytic study to determine whether APA was related to some of the most common trisomy anomalies found in population or not [111]. After correcting their result for any other possible confounding factors, such as mothers' age, authors discovered a significant increase in Klinefelter syndrome associated with increasing paternal age, with odds ratio of 1.35.

While the association between APA and offspring genetic disorders is now well established, the influence of APA in offspring's mental health is still a recent notion. Nonetheless, several studies conducted recurring to animal models demonstrated a close relation between APA and an increasing risk for numerous mental conditions [136-138]. Schizophrenia is a mental disorder with symptoms normally appearing in late adolescence or early adulthood and being characterized by unclear or confused thinking, auditory hallucinations and psychosis onset [139]. A great number of studies that evaluated the age of the fathers of schizophrenic individuals have found a positive correlation between schizophrenia and APA [112]. Actually, a study by Malaspina and peers has shown that with each progressing decade of paternal age the risk of schizophrenia almost doubled and by the age of 45 the risk

values were tripled in relation to the ones found for younger males [113]. These results were in accordance to several others that likewise confirmed the detrimental effect of APA on schizophrenia risk for the offspring [114]. An additional mental illness linked to APA is the bipolar disorder. Bipolar disorder, also known as manic depression, is a mental condition characterized by a constant shift between periods of mania and periods of deep depression [140]. This mental health condition has also been associated with APA. A study conducted by Frans and peers has found that children from fathers above 54 years carry an increased risk of 37% to develop a bipolar condition early on their life [116]. Similarly, others have found an increase in the risk for offspring bipolar disorder if the father is of advanced age [115]. Austim, a mental disorder defined by restricted, repetitive forms of behavior, and substantial deficiencies in social functioning has also been related to APA. Studies have demonstrated that fathers above 40 years of age display a risk three to five times higher to experience an autism onset on their offspring than younger men [115,118,119]. Although the risk is higher when both the mother and father are of advanced age, APA is independently correlated to autism of the offspring [117,119].

There are numerous congenital malformations that have been associated with APA, namely abnormal limbs, neural tube defects, and heart malformations [122,141,142]. Several musculoskeletal congenital anomalies (CAs) have been positively linked to the father's advanced age and, although results display some inconsistencies, a number of studies have suggested paternal age as a key factor for an increased risk of CAs [120,122]. A study by Urhoj and colleagues revealed a 26% additional risk for CAs in fathers above 50 years old. Nonetheless, even though there seems to be an increased risk for CAs related to paternal age, authors suggest that other factors may also be contributing to this increase and perhaps APA is a minor contributor in these pathologies [121]. Congenital heart defects (CHDs), one of the most common congenital malformations, has also been connected to APA, most likely due to an accumulation of germ cell genetic alterations [123]. Although there are some discrepant results, in the majority of studies, authors observed increased risks of CHDs in men older than 45 years, when compared to the risk of younger fathers. A study by Su and peers has found that, when results were controlled for the mothers' age, men above 45 years old exhibited an increased risk of 69% for a specific subtype of CHD, patent ductus arteriosus [123].

Cancer is characterized by genetically abnormal cells, unable to form functional structures, with the ability to multiply indefinitely and invade the organism. Surprisingly, a correlation between the prevalence of several cancer types in the offspring and APA has been proposed over the last decades. Several studies have shown that APA is associated with an increased risk of leukemia in the offspring [124-126]. Additionally, central nervous system cancers are also associated with APA at conception [125,127]. Interestingly, a study conducted by Hemminkin and Kyyronen revealed that APA increased sporadic nervous system cancers by about 15% [127]. Furthermore, Choi and collaborators have found a strong correlation between APA and breast cancer prevalence in childhood, even when mother's age is controlled, with an odds ratio of 1.6 [128]. Remarkably, the majority of studies reported increased cancer incidences in the offspring of men who were above 40 years old at the time of conception. As APA is associated with a high prevalence of de novo mutations during cell division, the accumulation of chromosomal mutations during the maturation of germ cells may be the underling mechanism between male aging and increased cancer prevalence.

Although there are strong evidences suggesting a relationship between APA and several offspring health issues, much is still unknown. Further epidemiological studies must be conducted to clarify what are the consequences of APA on the offspring and what can be done to diminish the risks of genetic abnormalities transmission. Nonetheless, physicians should discuss the possible implications of APA with couples wanting to delay parenthood.

## Conclusion

Currently, the majority of couples choose to delay parenthood with dreams of achieving a stable career or higher education. Consequently, a high proportion of couples are experiencing subfertility and infertility issues, with a prolonged time to pregnancy, increased miscarriage rates, and increased health problems in the offspring. Furthermore, males of APA generally experience a decline in sexual activity that can negatively impact male fertility. While the use of ART treatments enables infertile couples to conceive, these treatments cannot fully compensate the alterations in male fertility potential induced by APA.

The influence of maternal age on reproductive alterations is well documented. However, the same is not observed for paternal age. Thus, this review provided an overview of recent literature concerning APA and reproductive outcomes. While several studies propose that APA has a significant negative impact on reproduction and can lead to fecundity problems and offspring diseases, there is a lack of strong evidences that can fully attest these suggestions. The reviewed results show that APA appears to be associated with fecundity problems, sperm genetic alterations and offspring health issues. Interestingly, these effects are independent of whether conception is natural or medically assisted. Even though efforts have been made to define the age from which APA affects fertility and fecundity are certain. This threshold has yet not been established. Nonetheless, most studies indicate that the majority of reproductive and health problems were observed in couples with males above 40 years old. In the future, additional research to better elucidate the detrimental effects of APA on fertility and offspring health is needed. Likewise, establishing an age threshold for APA would be of great value to males who are thinking of delaying parenthood, and to physicians for medical advice purposes.

## Acknowledgements

UMIB (Pest-OE/SAU/UI0215/2014) was funded by National Funds through FCT-Foundation for Science and Technology.

## Conflict Of Interest

Authors disclose no potential conflict of interest.

## References

1. te Velve ER (1991) Pregnancy in the 21st century: consistently later, consistently more artificial. University of Utrecht, The Netherlands.
2. van Zonneveld P, Scheffer GJ, Broekmans FJ, te Velde ER (2001) Hormones and reproductive aging. Maturitas 38: 83-91.
3. Corson SL (1998) Achieving and maintaining pregnancy after age 40. Int J Fertil Womens Med 43: 249-256.
4. Pal L, Santoro N (2003) Age-related decline in fertility. Endocrinol Metab Clin North Am 32: 669-688.
5. Cleary-Goldman J, Malone FD, Vidaver J, Ball RH, Nyberg DA, et al. (2005) Impact of maternal age on obstetric outcome. Obstet Gynecol 105: 983-990.

6. Amann RP (2008) The cycle of the seminiferous epithelium in humans: a need to revisit. J Androl 29: 469-487.
7. Sartorius GA, Nieschlag E (2010) Paternal age and reproduction. Hum Reprod Update 16: 65-79.
8. Zitzmann M (2013) Effects of age on male fertility. Best Pract Res Clin Endocrinol Metab 27: 617-628.
9. Handelsman DJ, Staraj S (1985) Testicular size: the effects of aging, malnutrition, and illness. J Androl 6: 144-151.
10. Sampson N, Untergasser G, Plas E, Berger P (2007) The ageing male reproductive tract. J Pathol 211: 206-218.
11. Baccarelli A, Morpurgo PS, Corsi A, Vaghi I, Fanelli M, et al. (2001) Activin A serum levels and aging of the pituitary-gonadal axis: a cross-sectional study in middle-aged and elderly healthy subjects. Exp Gerontol 36: 1403-1412.
12. Mahmoud AM, Goemaere S, El-Garem Y, Van Pottelbergh I, Comhaire FH, et al. (2003) Testicular volume in relation to hormonal indices of gonadal function in community-dwelling elderly men. J Clin Endocrinol Metab 88: 179-184.
13. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR; Baltimore Longitudinal Study of Aging (2001) Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab 86: 724-731.
14. Kaufman JM, Vermeulen A (2005) The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 26: 833-876.
15. Bray I, Gunnell D, Davey Smith G (2006) Advanced paternal age: how old is too old. J Epidemiol Community Health 60: 851-853.
16. Johnson L, Grumbles JS, Bagheri A, Petty CS (1990) Increased germ cell degeneration during postprophase of meiosis is related to increased serum follicle-stimulating hormone concentrations and reduced daily sperm production in aged men. Biol Reprod 42: 281-287.
17. Sloter E, Schmid TE, Marchetti F, Eskenazi B, Nath J, et al. (2006) Quantitative effects of male age on sperm motion. Hum Reprod 21: 2868-2875.
18. Levitas E, Lunenfeld E, Weisz N, Friger M, Potashnik G (2007) Relationship between age and semen parameters in men with normal sperm concentration: analysis of 6022 semen samples. Andrologia 39: 45-50.
19. Brahem S, Mehdi M, Elghezal H, Saad A (2011) The effects of male aging on semen quality, sperm DNA fragmentation and chromosomal abnormalities in an infertile population. J Assist Reprod Genet 28: 425-432.
20. Sloter E, Nath J, Eskenazi B, Wyrobek AJ (2004) Effects of male age on the frequencies of germinal and heritable chromosomal abnormalities in humans and rodents. Fertil Steril 81: 925-943.
21. Hassan MA, Killick SR (2003) Effect of male age on fertility: evidence for the decline in male fertility with increasing age. Fertil Steril 79 Suppl 3: 1520-1527.
22. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, et al. (2012) The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod 27: 2908-2917.
23. Jenkins TG, Aston KI, Pflueger C, Cairns BR, Carrell DT (2014) Age-associated sperm DNA methylation alterations: possible implications in offspring disease susceptibility. PLoS Genet 10: e1004458.
24. Simon L, Murphy K, Shamsi MB, Liu L, Emery B, et al. (2014) Paternal influence of sperm DNA integrity on early embryonic development. Hum Reprod 29: 2402-2412.
25. Ford WC, North K, Taylor H, Farrow A, Hull MG, et al. (2000) Increasing paternal age is associated with delayed conception in a large population of fertile couples: evidence for declining fecundity in older men. The ALSPAC Study Team (Avon Longitudinal Study of Pregnancy and Childhood). Hum Reprod 15: 1703-1708.
26. Varshini J, Srinag BS, Kalthur G, Krishnamurthy H, Kumar P, et al. (2012) Poor sperm quality and advancing age are associated with increased sperm DNA damage in infertile men. Andrologia 44 Suppl 1: 642-649.
27. Das M, Al-Hathal N, San-Gabriel M, Phillips S, Kadoch IJ, et al. (2013) High prevalence of isolated sperm DNA damage in infertile men with advanced paternal age. J Assist Reprod Genet 30: 843-848.
28. Wiener-Megnazi Z, Auslender R, Dirnfeld M (2012) Advanced paternal age and reproductive outcome. Asian J Androl 14: 69-76.
29. Plas E, Berger P, Hermann M, Pflüger H (2000) Effects of aging on male fertility. Exp Gerontol 35: 543-551.
30. Lamberts SW, van den Beld AW, van der Lely AJ (1997) The endocrinology of aging. Science 278: 419-424.
31. Oh JY, Barrett-Connor E, Wedick NM, Wingard DL; Rancho Bernardo Study (2002) Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. Diabetes Care 25: 55-60.
32. Mirone V, Ricci E, Gentile V, Basile Fasolo C, Parazzini F (2004) Determinants of erectile dysfunction risk in a large series of Italian men attending andrology clinics. Eur Urol 45: 87-91.
33. Fabbrini A, Francavilla S, Moscardelli S (1985) Structural and functional aspects of aging testis and sex accessory organs. J Endocrinol Invest 8 Suppl 2: 47-55.
34. Kühnert B, Nieschlag E (2004) Reproductive functions of the ageing male. Hum Reprod Update 10: 327-339.
35. Harbitz TB (1973) Morphometric studies of the Leydig cells in elderly men with special reference to the histology of the prostate. An analysis in an autopsy series. Acta Pathol Microbiol Scand A 81: 301-314.
36. Harbitz TB (1973) Morphometric studies of the Sertoli cells in elderly men with special reference to the histology of the prostate. An analysis in an autopsy series. Acta Pathol Microbiol Scand A 81: 703-714.
37. Paniagua R, Amat P, Nistal M, Martin A (1986) Ultrastructure of Leydig cells in human ageing testes. J Anat 146: 173-183.
38. Andolz P, Bielsa MA, Vila J (1999) Evolution of semen quality in North-eastern Spain: a study in 22,759 infertile men over a 36 year period. Hum Reprod 14: 731-735.
39. Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, et al. (1998) Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet 352: 1172-1177.
40. Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, et al. (2003) The association of age and semen quality in healthy men. Hum Reprod 18: 447-454.
41. Rolf C, Behre HM, Nieschlag E (1996) Reproductive parameters of older compared to younger men of infertile couples. Int J Androl 19: 135-142.
42. Rolf C, Kenkel S, Nieschlag E (2002) Age-related disease pattern in infertile men: increasing incidence of infections in older patients. Andrologia 34: 209-217.
43. Auger J, Kunstmann JM, Czyglik F, Jouannet P (1995) Decline in semen quality among fertile men in Paris during the past 20 years. N Engl J Med 332: 281-285.
44. Kidd SA, Eskenazi B, Wyrobek AJ (2001) Effects of male age on semen quality and fertility: a review of the literature. Fertil Steril 75: 237-248.
45. Pasqualotto FF, Sobreiro BP, Hallak J, Pasqualotto EB, Lucon AM (2005) Sperm concentration and normal sperm morphology decrease and follicle-stimulating hormone level increases with age. BJU Int 96: 1087-1091.
46. Stone BA, Alex A, Werlin LB, Marrs RP (2013) Age thresholds for changes in semen parameters in men. Fertil Steril 100: 952-958.
47. Fisch H, Goluboff ET, Olson JH, Feldshuh J, Broder SJ, et al. (1996) Semen analyses in 1,283 men from the United States over a 25-year period: no decline in quality. Fertil Steril 65: 1009-1014.
48. Li Y, Lin H, Li Y, Cao J (2011) Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. Fertil Steril 95: 116-123.
49. Whitcomb BW, Turzanski-Fortner R, Richter KS, Kipersztok S, Stillman RJ, et al. (2011) Contribution of male age to outcomes in assisted reproductive technologies. Fertil Steril 95: 147-151.

50. Zinaman MJ, Brown CC, Selevan SG, Clegg ED (2000) Semen quality and human fertility: a prospective study with healthy couples. J Androl 21: 145-153.
51. Hellstrom WJ, Overstreet JW, Sikka SC, Denne J, Ahuja S, et al. (2006) Semen and sperm reference ranges for men 45 years of age and older. J Androl 27: 421-428.
52. Lissak A, Wiener-Megnazi Z, Reznick AZ, Shnizer S, Ishai D, et al. (2004) Oxidative stress indices in seminal plasma, as measured by the thermochemiluminescence assay, correlate with sperm parameters. Fertil Steril 81: 792-797.
53. Hammoud AO, Wilde N, Gibson M, Parks A, Carrell DT, et al. (2008) Male obesity and alteration in sperm parameters. Fertil Steril 90: 2222-2225.
54. Jonge CJD, Barratt CLR (2006) The sperm cell: production, maturation, fertilization, regeneration Cambridge, UK ; New York: Cambridge University Press.
55. Schwartz D, Mayaux MJ, Spira A, Moscato ML, Jouannet P, et al. (1983) Semen characteristics as a function of age in 833 fertile men. Fertil Steril 39: 530-535.
56. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, et al. (2009) The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. Hum Reprod 24: 2683-2687.
57. Schmidt L, Sobotka T, Bentzen JG, Nyboe Andersen A; ESHRE Reproduction and Society Task Force (2012) Demographic and medical consequences of the postponement of parenthood. Hum Reprod Update 18: 29-43.
58. Ford JH, MacCormac L, Hiller J (1994) PALS (pregnancy and lifestyle study): association between occupational and environmental exposure to chemicals and reproductive outcome. Mutat Res 313: 153-164.
59. Slama R, Werwatz A, Boutou O, Ducot B, Spira A, et al. (2003) Does male age affect the risk of spontaneous abortion? An approach using semiparametric regression. Am J Epidemiol 157: 815-824.
60. Slama R, Bouyer J, Windham G, Fenster L, Werwatz A, et al. (2005) Influence of paternal age on the risk of spontaneous abortion. Am J Epidemiol 161: 816-823.
61. de la Rochebrochard E, Thonneau P (2002) Paternal age and maternal age are risk factors for miscarriage; results of a multicentre European study. Hum Reprod 17: 1649-1656.
62. Dunson DB, Colombo B, Baird DD (2002) Changes with age in the level and duration of fertility in the menstrual cycle. Hum Reprod 17: 1399-1403.
63. Dunson DB, Baird DD, Colombo B (2004) Increased infertility with age in men and women. Obstet Gynecol 103: 51-56.
64. Kleinhaus K, Perrin M, Friedlander Y, Paltiel O, Malaspina D, et al. (2006) Paternal age and spontaneous abortion. Obstet Gynecol 108: 369-377.
65. al-Ansary LA, Babay ZA (1994) Risk factors for spontaneous abortion: a preliminary study on Saudi women. J R Soc Health 114: 188-193.
66. Nybo Andersen AM, Hansen KD, Andersen PK, Davey Smith G (2004) Advanced paternal age and risk of fetal death: a cohort study. Am J Epidemiol 160: 1214-1222.
67. Joffe M, Li Z (1994) Male and female factors in fertility. Am J Epidemiol 140: 921-929.
68. Olsen J (1990) Subfecundity according to the age of the mother and the father. Dan Med Bull 37: 281-282.
69. Selvin S, Garfinkel J (1976) Paternal age, maternal age and birth order and the risk of a fetal loss. Hum Biol 48: 223-230.
70. Mathieu C, Ecochard R, Bied V, Lornage J, Czyba JC (1995) Cumulative conception rate following intrauterine artificial insemination with husband's spermatozoa: influence of husband's age. Hum Reprod 10: 1090-1097.
71. Belloc S, Cohen-Bacrie P, Benkhalifa M, Cohen-Bacrie M, De Mouzon J, et al. (2008) Effect of maternal and paternal age on pregnancy and miscarriage rates after intrauterine insemination. Reprod Biomed Online 17: 392-397.
72. Bellver J, Garrido N, Remohí J, Pellicer A, Meseguer M (2008) Influence of paternal age on assisted reproduction outcome. Reprod Biomed Online 17: 595-604.
73. Wu Y, Kang X, Zheng H, Liu H, Liu J (2015) Effect of Paternal Age on Reproductive Outcomes of In Vitro Fertilization. PLoS One 10: e0135734.
74. Campos I, Gómez E, Fernández-Valencia AL, Landeras J, González R, et al. (2008) Effects of men and recipients' age on the reproductive outcome of an oocyte donation program. J Assist Reprod Genet 25: 445-452.
75. de La Rochebrochard E, de Mouzon J, Thépot F, Thonneau P; French National IVF Registry (FIVNAT) Association (2006) Fathers over 40 and increased failure to conceive: the lessons of in vitro fertilization in France. Fertil Steril 85: 1420-1424.
76. Girsh E, Katz N, Genkin L, Girtler O, Bocker J, et al. (2008) Male age influences oocyte-donor program results. J Assist Reprod Genet 25: 137-143.
77. Klonoff-Cohen HS, Natarajan L (2004) The effect of advancing paternal age on pregnancy and live birth rates in couples undergoing in vitro fertilization or gamete intrafallopian transfer. Am J Obstet Gynecol 191: 507-514.
78. Luna M, Finkler E, Barritt J, Bar-Chama N, Sandler B, et al. (2009) Paternal age and assisted reproductive technology outcome in ovum recipients. Fertil Steril 92: 1772-1775.
79. Gallardo E, Simón C, Levy M, Guanes PP, Remohí J, et al. (1996) Effect of age on sperm fertility potential: oocyte donation as a model. Fertil Steril 66: 260-264.
80. Gu L, Zhang H, Yin L, Bu Z, Zhu G (2012) Effect of male age on the outcome of in vitro fertilization: oocyte donation as a model. J Assist Reprod Genet 29: 331-334.
81. Paulson RJ, Milligan RC, Sokol RZ (2001) The lack of influence of age on male fertility. Am J Obstet Gynecol 184: 818-822.
82. Aboulghar M, Mansour R, Al-Inany H, Abou-Setta AM, Aboulghar M, et al. (2007) Paternal age and outcome of intracytoplasmic sperm injection. 14: 588-592.
83. Frattarelli JL, Miller KA, Miller BT, Elkind-Hirsch K, Scott RT, Jr (2008) Male age negatively impacts embryo development and reproductive outcome in donor oocyte assisted reproductive technology cycles. Fertil Steril 90: 97-103.
84. Ferreira RC, Braga DP, Bonetti TC, Pasqualotto FF, Iaconelli A, Jr. et al. (2010) Negative influence of paternal age on clinical intracytoplasmic sperm injection cycle outcomes in oligozoospermic patients. Fertil Steril 93: 1870-1874.
85. Spandorfer SD, Avrech OM, Colombero LT, Palermo GD, Rosenwaks Z (1998) Effect of parental age on fertilization and pregnancy characteristics in couples treated by intracytoplasmic sperm injection. Hum Reprod 13: 334-338.
86. Baird DD, Wilcox AJ, Weinberg CR (1986) Use of time to pregnancy to study environmental exposures. Am J Epidemiol 124: 470-480.
87. Joffe M (1997) Time to pregnancy: a measure of reproductive function in either sex. Asclepios Project. Occup Environ Med 54: 289-295.
88. Dain L, Auslander R, Dirnfeld M (2011) The effect of paternal age on assisted reproduction outcome. Fertil Steril 95: 1-8.
89. Nybo AA, Wohlfahrt J, Christens P, Olsen J, Melbye M (2000) Is maternal age an independent risk factor for fetal loss. West J Med 173: 331.
90. Duran EH, Dowling-Lacey D, Bocca S, Stadtmauer L, Oehninger S (2010) Impact of male age on the outcome of assisted reproductive technology cycles using donor oocytes. Reprod Biomed Online 20: 848-856.
91. Robertshaw I, Khoury J, Abdallah ME, Warikoo P, Hofmann GE (2014) The effect of paternal age on outcome in assisted reproductive technology using the ovum donation model. Reprod Sci 21: 590-593.
92. Martin RH, Rademaker AW (1987) The effect of age on the frequency of sperm chromosomal abnormalities in normal men. Am J Hum Genet 41: 484-492.

93. Martin RH, Spriggs E, Ko E, Rademaker AW (1995) The relationship between paternal age, sex ratios, and aneuploidy frequencies in human sperm, as assessed by multicolor FISH. Am J Hum Genet 57: 1395-1399.
94. Bosch M, Rajmil O, Egozcue J, Templado C (2003) Linear increase of structural and numerical chromosome 9 abnormalities in human sperm regarding age. Eur J Hum Genet 11: 754-759.
95. Wyrobek AJ, Eskenazi B, Young S, Arnheim N, Tiemann-Boege I, et al. (2006) Advancing age has differential effects on DNA damage, chromatin integrity, gene mutations, and aneuploidies in sperm. Proc Natl Acad Sci U S A 103: 9601-9606.
96. Schmid TE, Eskenazi B, Baumgartner A, Marchetti F, Young S, et al. (2007) The effects of male age on sperm DNA damage in healthy non-smokers. Hum Reprod 22: 180-187.
97. NOVITSKI E (1953) The dependence of the secondary sex ratio in humans on the age of the father. Science 117: 531-533.
98. Ozkosem B, Feinstein SI, Fisher AB, O'Flaherty C (2015) Advancing age increases sperm chromatin damage and impairs fertility in peroxiredoxin 6 null mice. Redox Biol 5: 15-23.
99. Katz-Jaffe MG, Parks J, McCallie B, Schoolcraft WB (2013) Aging sperm negatively impacts in vivo and in vitro reproduction: a longitudinal murine study. Fertil Steril 100: 262-268.
100. Kovac JR, Pastuszak AW, Lamb DJ (2013) The use of genomics, proteomics, and metabolomics in identifying biomarkers of male infertility. Fertil Steril 99: 998-1007.
101. Zubkova EV, Wade M, Robaire B (2005) Changes in spermatozoal chromatin packaging and susceptibility to oxidative challenge during aging. Fertil Steril 84: 1191-1198.
102. Smith TB, De Iuliis GN, Lord T, Aitken RJ (2013) The senescence-accelerated mouse prone 8 as a model for oxidative stress and impaired DNA repair in the male germ line. Reproduction 146: 253-262.
103. González-Marín C, Gosálvez J, Roy R (2012) Types, causes, detection and repair of DNA fragmentation in animal and human sperm cells. Int J Mol Sci 13: 14026-14052.
104. Ashwood-Smith MJ, Edwards RG (1996) DNA repair by oocytes. Mol Hum Reprod 2: 46-51.
105. Genescà A, Caballín MR, Miró R, Benet J, Germà JR, et al. (1992) Repair of human sperm chromosome aberrations in the hamster egg. Hum Genet 89: 181-186.
106. Jurisicova A, Latham KE, Casper RF, Casper RF, Varmuza SL (1998) Expression and regulation of genes associated with cell death during murine preimplantation embryo development. Mol Reprod Dev 51: 243-253.
107. Ahmadi A, Ng SC (1999) Fertilizing ability of DNA-damaged spermatozoa. J Exp Zool 284: 696-704.
108. Risch N, Reich EW, Wishnick MM, McCarthy JG (1987) Spontaneous mutation and parental age in humans. Am J Hum Genet 41: 218-248.
109. Glaser RL, Jiang W, Boyadjiev SA, Tran AK, Zachary AA, et al. (2000) Paternal origin of FGFR2 mutations in sporadic cases of Crouzon syndrome and Pfeiffer syndrome. Am J Hum Genet 66: 768-777.
110. Goriely A, McVean GA, Röjmyr M, Ingemarsson B, Wilkie AO (2003) Evidence for selective advantage of pathogenic FGFR2 mutations in the male germ line. Science 301: 643-646.
111. De Souza E, Morris JK; EUROCAT Working Group (2010) Case-control analysis of paternal age and trisomic anomalies. Arch Dis Child 95: 893-897.
112. Hare EH, Moran PA (1979) Raised parental age in psychiatric patients: evidence for the constitutional hypothesis. Br J Psychiatry 134: 169-177.
113. Malaspina D, Harlap S, Fennig S, Heiman D, Nahon D, et al. (2001) Advancing paternal age and the risk of schizophrenia. Arch Gen Psychiatry 58: 361-367.
114. Miller B, Messias E, Miettunen J, Alaräisänen A, Järvelin MR, et al. (2011) Meta-analysis of paternal age and schizophrenia risk in male versus female offspring. Schizophr Bull 37: 1039-1047.
115. D'Onofrio BM, Rickert ME, Frans E, Kuja-Halkola R, Almqvist C, et al. (2014) Paternal age at childbearing and offspring psychiatric and academic morbidity. JAMA Psychiatry 71: 432-438.
116. Frans EM, Sandin S, Reichenberg A, Lichtenstein P, Långström N, et al. (2008) Advancing paternal age and bipolar disorder. Arch Gen Psychiatry 65: 1034-1040.
117. Lee BK, McGrath JJ (2015) Advancing parental age and autism: multifactorial pathways. Trends Mol Med 21: 118-125.
118. Reichenberg A, Gross R, Sandin S, Susser ES (2010) Advancing paternal and maternal age are both important for autism risk. Am J Public Health 100: 772-773.
119. Sandin S, Schendel D, Magnusson P, Hultman C, Surén P, et al. (2015) Autism risk associated with parental age and with increasing difference in age between the parents. Mol Psychiatry .
120. Barbosa-Buck CO, Orioli IM, da Graça Dutra M, Lopez-Camelo J, Castilla EE, et al. (2012) Clinical epidemiology of skeletal dysplasias in South America. Am J Med Genet A 158A: 1038-1045.
121. Urhoj SK, Mortensen LH, Nybo Andersen AM (2015) Advanced Paternal Age and Risk of Musculoskeletal Congenital Anomalies in Offspring. Birth Defects Res B Dev Reprod Toxicol 104: 273-280.
122. Yang Q, Wen SW, Leader A, Chen XK, Lipson J, et al. (2007) Paternal age and birth defects: how strong is the association. Hum Reprod 22: 696-701.
123. Su XJ, Yuan W, Huang GY, Olsen J, Li J (2015) Paternal age and offspring congenital heart defects: a national cohort study. PLoS One 10: e0121030.
124. Murray L, McCarron P, Bailie K, Middleton R, Davey Smith G, et al. (2002) Association of early life factors and acute lymphoblastic leukaemia in childhood: historical cohort study. Br J Cancer 86: 356-361.
125. Yip BH, Pawitan Y, Czene K (2006) Parental age and risk of childhood cancers: a population-based cohort study from Sweden. Int J Epidemiol 35: 1495-1503.
126. Sergentanis TN, Thomopoulos TP, Gialamas SP, Karalexi MA, et al. (2015) Risk for childhood leukemia associated with maternal and paternal age. Eur J Epidemiol 30: 1229-1261.
127. Hemminki K, Kyyrönen P (1999) Parental age and risk of sporadic and familial cancer in offspring: implications for germ cell mutagenesis. Epidemiology 10: 747-751.
128. Choi JY, Lee KM, Park SK, Noh DY, Ahn SH, et al. (2005) Association of paternal age at birth and the risk of breast cancer in offspring: a case control study. BMC Cancer 5: 143.
129. Frans EM, McGrath JJ, Sandin S, Lichtenstein P, Reichenberg A, et al. (2011) Advanced paternal and grandpaternal age and schizophrenia: a three-generation perspective. Schizophr Res 133: 120-124.
130. Hultman CM, Sandin S, Levine SZ, Lichtenstein P, Reichenberg A (2011) Advancing paternal age and risk of autism: new evidence from a population-based study and a meta-analysis of epidemiological studies. Mol Psychiatry 16: 1203-1212.
131. Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, et al. (2012) Rate of de novo mutations and the importance of father's age to disease risk. Nature 488: 471-475.
132. Shinde DN, Elmer DP, Calabrese P, Boulanger J, Arnheim N, et al. (2013) New evidence for positive selection helps explain the paternal age effect observed in achondroplasia. Hum Mol Genet 22: 4117-4126.
133. Passos-Bueno MR, Wilcox WR, Jabs EW, Sertié AL, Alonso LG, et al. (1999) Clinical spectrum of fibroblast growth factor receptor mutations. Hum Mutat 14: 115-125.
134. Wilkin DJ, Szabo JK, Cameron R, Henderson S, Bellus GA, et al. (1998) Mutations in fibroblast growth-factor receptor 3 in sporadic cases of achondroplasia occur exclusively on the paternally derived chromosome. Am J Hum Genet 63: 711-716.
135. Morris JK, Alberman E, Scott C, Jacobs P (2008) Is the prevalence of Klinefelter syndrome increasing? Eur J Hum Genet 16: 163-170.
136. Frans EM, Sandin S, Reichenberg A, Långström N, Lichtenstein P, et al. (2013) Autism risk across generations: a population-based study of advancing grandpaternal and paternal age. JAMA Psychiatry 70: 516-521.

137. Arslan RC, Penke L, Johnson W, Iacono WG, McGue M (2014) The effect of paternal age on offspring intelligence and personality when controlling for paternal trait level. PLoS One 9: e90097.

138. McGrath JJ, Petersen L, Agerbo E, Mors O, Mortensen PB, et al. (2014) A comprehensive assessment of parental age and psychiatric disorders. JAMA Psychiatry 71: 301-309.

139. van Os J, Kapur S (2009) Schizophrenia. Lancet 374: 635-645.

140. Van der Schot A, Kahn R, Ramsey N, Nolen W, Vink M (2010) Trait and state dependent functional impairments in bipolar disorder. Psychiatry Res 184: 135-142.

141. Kazaura M, Lie RT, Skjaerven R (2004) Paternal age and the risk of birth defects in Norway. Ann Epidemiol 14: 566-570.

142. Grewal J, Carmichael SL, Yang W, Shaw GM (2012) Paternal age and congenital malformations in offspring in California, 1989-2002. Matern Child Health J 16: 385-392.