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TOPIC PAPER

The male biological clock

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Abstract Do men have biological clocks that affect their hormone levels, fertility, and the genetic quality of their sperm? Women can no longer be viewed as solely responsible for age-related fertility and genetic problems. The effects of andropause and advanced paternal age on fertility and offspring are still under investigation. Further research is needed to fully characterize the associated risks and to treat the underlying abnormalities. A better understanding of the cellular and biochemical mechanisms of "gonadal" aging is important in order to determine safe, effective ways to delay this process and "rewind" the male biological clock. The benefits may include decreasing the potential for adverse genetic consequences in offspring, improvement in the sexual and reproductive health of aging males, and increase a *woman's* chance of having healthy children by correcting defects in the *male* reproductive system.

Andropause

The term "biological clock" is commonly associated with women and the decline in estrogen and oocyte production that occurs with menopause. The biological clock encompasses the decline in sex hormones, decline in fertility, and increased risk of pregnancy loss and congenital anomalies that are associated with advanced maternal age. Interestingly, the term "biological clock" is not commonly associated with advanced paternal age; yet, testosterone and fertility do decline in the male. In addition, advanced paternal age has recently been associated with pregnancy loss and genetic abnormalities. The objective of this review is to address these features of male aging and to expand the concept of a biological clock to include both sexes.

As with women, the levels of sex hormones in men decline with age [1]. The roughly 1% per year decline in testosterone levels after age 30 has been termed "andropause"[2]. Longitudinal studies have demonstrated that abnormally low testosterone levels are present in elderly men [1, 3, 4]. Additionally, there have been numerous cross-sectional investigations also documenting lower concentrations of total testosterone and/or free testosterone in older men [5, 6]. Most recently, the Massachusetts Male Aging Study, a large population-based random-sample cohort, reported that in a cohort of 1,709 healthy men aged 40–70 with a mean age of 55.2 years, the mean total testosterone value was 520 ng/mL. In follow-up data approximately a decade later, 1,156 healthy men with a mean age of 62.7 years had mean total testosterone value of 450 ng/mL [3]. Feldman et al. quantified the decreasing testosterone levels as a cross-sectional decline of 0.8% per year of age and a longitudinal decline of 1.6% per year within the follow-up data. Additionally, Feldman et al. reported that the rate of decline was the same in apparently healthy men as well as men reporting a chronic illness, obesity, alcoholism, prescription medication, or prostate problems.

The decline in testosterone with age puts a greater number of elderly men at risk for hypogonadism.

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Rhoden and Morgentaler estimated that between 2 and 4 million men in the United States alone suffer from hypogonadism (defined as serum total testosterone levels lower than 325 ng per deciliter) [7].

Because of the increasing prevalence of abnormally low levels of testosterone in elderly men, it is worthwhile to assess the relationship between hypogonadism and aging. The Baltimore Longitudinal Study on Aging, a large-scale study measuring total and free testosterone levels in 890 healthy men without any reported infertility or varicoceles, determined that hypogonadal testosterone levels were present in approximately 20% of men over 60, 30% over 70, and 50% over 80 years of age [1]. The study also found that 78% of men identified as hypogonadal by a single testosterone determination had low total testosterone levels on all subsequent samples. Multivariate analysis confirmed age as an independent predictor of a longitudinal decline in both total and free testosterone. This age-related decrease in testosterone and potential hypogonadism may result in decreased libido and erectile dysfunction, loss of muscle mass and strength, weight gain, and declining cognitive function. In addition, hypogonadism is also associated with type II diabetes, musculoskeletal frailty, cardiovascular disease, and the metabolic syndrome.

Advanced paternal age and fertility

Accompanying this decline in sex hormone levels, a reduction in fertility has also been associated with increasing age. In women, a decline in oocyte production occurs in the late thirties to early forties. This decreased oocyte production occurs in association with the decline in estrogen production. Additionally, the risk for genetic abnormalities in their offspring also increases with advanced maternal age. Men, on the contrary, can continue to father children well beyond 40 years of age, and to date there is no significant cessation of spermatogenesis. As paternal age continues to increase, many investigators have explored the effect of age on seminal parameters with the central question: is advanced paternal age associated with diminished semen quality, risk of infertility, or congenital anomalies?

In the murine model, studies have demonstrated that aging is correlated with histologic changes in testicular architecture and a decline in semen quality. Tanemura et al. [8] demonstrated that "older" mice (at the age of 18 months) have several age-related changes, including an increased number of vacuoles in germ cells and a thinner seminiferous epithelium. By the age of 30 months, extremely thin seminiferous epithelia with very few spermatocytes or spermatids were found. In another study, Wang et al. [9] found that total sperm production was significantly reduced in 22- and 30-month-old rats. Further studies also documented an increased incidence of preimplantation losses, mutation frequencies, and anueploidy in the offspring of older male mice [10–12].

With the abundant evidence on the association between aging and male infertility in mice, the appropriate question is if such a correlation exists in men. A comprehensive review by Kidd et al. [13] evaluated the effects of male aging on semen quality in men; the authors reviewed all studies published between January 1, 1980 and December 31, 1999 to examine the outcome parameters of semen volume, sperm concentration, sperm motility, sperm morphology, pregnancy rate, and time to pregnancy/subfecundity in the aging male.

The majority of the literature reviewed demonstrated a decrease in semen volume with increasing age. Of sixteen published studies, eleven reported decreases in semen quality with increasing age. Two of these studies adjusted for the confounder of duration of abstinence and found that a statistically significant correlation still exists between decreasing semen volume and increasing age; they reported decreases of 0.15–0.5% for each increase in year of age [14, 15]. When comparing men under the age of 30 with men aged 50 or older, most of the studies document decreases of 20–30% in semen volume between the two groups [13]. This correlation between advanced paternal age and decreased semen volume suggests that age contributes to a decline in semen quality and resultant in infertility.

Unlike semen volume, the relationship between increasing age and sperm concentration remains unclear. Of the 21 studies examining the association between age and sperm concentration, none were able to document a clear relationship between these parameters [13]. Five studies did note the correlation of decreasing sperm concentration with increasing age. However, only one of these studies controlled for abstinence and year of birth as potential confounders; this study found a decrease in sperm concentration of 3.3% per year of age and also a 66% decrease in concentration from age 30 to 50 years [16]. Eight studies documented that with increasing age, sperm concentration actually increased linearly from 0.03 to 3.3% per year of age. Yet, only two of these studies adjusted for duration of abstinence [14, 15]. Due to these conflicting reports and the presence of significant confounders, no clear association between sperm concentration and increasing paternal age can be documented.

In contrast, the association of advanced paternal age and decreased sperm motility is clearly elucidated in the current literature. Kidd et al. [13] reviewed 19 studies examining the relationship between the percentage of motile sperm and age; the majority of these studies (17/19) used visual assessment of sperm motility by light microscopy. Thirteen of the nineteen studies found a decrease in sperm motility with increasing age; five of these studies adjusted for duration of abstinence and the observed changes were statistically significant [14, 16–19]. When these studies compared men age 50 or older to men under the age of 30, they reported a 3–37% decline in motility. Four studies reviewed by Kidd et al. found no association between age and sperm motility and two studies reported a positive correlation; none of these studies adjusted for duration of infertility. Thus, the extensive body of evidence suggests that an inverse correlation exists between age and sperm motility.

Similarly, advanced paternal age is also associated with the presence of abnormal sperm morphology. Kidd et al. [13] reviewed 14 studies examining the relationship between age and sperm morphology. There were nine studies documenting a decrease in the percent of normal sperm with increasing age, five of which were found to be statistically significant. Controlling for the potential confounders of duration of abstinence and year of birth, Auger et al. [16] reported that the percent of normal sperm decreased by 0.9% per year of age. While Andolz et al. [15] reported a decline of 0.2% per year of age. When studies utilized age as a categorical variable, there was an observable trend with increasing age group and decreasing percent normal sperm. Five studies found no association between percent normal sperm and age, but none of these studies were found to be statistically significant. Despite the variation among morphological criteria to assess sperm abnormalities, there was a substantial amount of evidence linking increasing age to decreasing percent normal sperm.

The associations between increasing age and decreasing semen volume, sperm motility, and sperm morphology suggest that semen quality diminishes with advanced paternal age. Whether age is analyzed as a continuous or categorical variable, the body of evidence suggests that a trend exists between increasing male age and decreasing semen quality. This correlation may be attributed, at least in part, to degenerative changes to the prostate that occur with age, such as a decrease in protein and water content [20]. Additionally, age-related degenerative changes to the germinal epithelium can impact sperm morphology [21]. Acknowledging that increasing male age is associated with adverse semen parameters, it becomes imperative to determine how advanced paternal age affects fertility and the offspring conceived.

Of 11 studies evaluating the association between male age and subfecundity, nine studies found that the time to pregnancy increases with increased male age [13]. In addition, seven studies reporting a direct correlation between paternal age and time to pregnancy reached statistical significance. The increased risks of subfecundity with older age groups ranged from 11 to 250%. However, four studies did not adjust for female age in the analysis and female age is a well-established independent predictor affecting the ability to conceive. Of the studies that controlled for female partner age, the pregnancy rate in the cohort of older men over 50 years of age was 23–38% lower than that of men less than 30 years of age [18, 22]. After adjusting for duration of infertility and irregular ovulation, Mathieu et al. [22] demonstrated that after stratifying men into cohorts of age 35 or greater and less than age 30, there was a 60% decrease in the chance of initiating a pregnancy for the older men. Since this study was conducted for couples undergoing intrauterine artificial insemination, female age was found to be a poor prognostic factor in predicting a successful pregnancy. Although, two studies failed to document an association between male age and time to pregnancy, the weight of the evidence suggests a strong correlation between paternal age and time required for a couple to achieve pregnancy [13].

This correlation between advanced paternal age and the risk of delayed conception was confirmed in a study including 8,515 planned pregnancies. This study demonstrated that older men were significantly less likely to impregnate their partners in less than 6 or less than 12 months, compared to their younger counterparts. After adjusting for various confounding factors such as maternal age, Body mass index, tobacco exposure, education, duration of cohabitation, oral contraceptive use, and paternal alcohol consumption, paternal age remained significantly associated with time to conception. The odds ratio for conception within 6 months decreased by 2% per year of age and for conception within 12 months decreased by 3% per year of age. After comparing their study with the existent literature, the authors concluded that the probability a fertile couple will take greater than 12 months to conceive nearly doubles from approximately 8% when a man is less than 25 years of age to approximately 15% when he is greater than 35 years of age [23].

Currently, many studies evaluating the relationship between of advanced paternal age and infertility are available in the literature. These studies vary in their

adjustment of potential confounders, age-group stratifications, and patient population. For these reasons, no definitive conclusion regarding the linearity of the relationship between male age and semen parameters and/or fertility can be proven. Nevertheless, advanced paternal age is associated with a decline in semen quality and fertility status. As paternal age continues to increase, a greater understanding of the effect of advanced paternal age and the risk of infertility is essential.

Advanced paternal age and pregnancy outcomes

Population studies within the United States reveal a significant increase in paternal age with many couples postponing childbearing until their mid-thirties to mid-forties. According to the CDC birth statistics, the average maternal age in 2003 was 25.1 representing an increase from the average maternal age of 21.4 years in 1974. A trend towards advanced parental age is simultaneously occurring in American men. The birth rate amongst men 25–44 years has been steadily increasing since the 1970s while the birth rate of men less than 25 years has been decreasing [24]. An improved understanding of the effects of increased parental age on the developing fetus and newborn is imperative for counseling older couples preparing for childbearing. Advanced paternal age has been suggested to result in increased spontaneous abortions, autosomal dominant disorders, trisomy 21, and recently schizophrenia.

Women aged 35 years or greater are at higher risk than younger women for adverse reproductive events including infertility, abortion, congenital abnormalities, and perinatal mortality. Paternal age often correlates with maternal age and can confound the effects of advanced maternal age. The first connection between spontaneous abortion and paternal age was raised during an analysis of fetal death certificates in 1939 [25]. Recent data confirms the association between advanced paternal age and the risk of spontaneous abortion. A prospective study of 5,121 American women revealed that the risk of spontaneous abortion increased with advanced paternal age [26]. A prospective analysis of 23,821 women from the Danish National Birth Cohort further demonstrated that pregnancies fathered by men 50 years or older had almost twice the risk of spontaneous abortion when compared with pregnancies with younger fathers after adjustment for maternal age, reproductive history, and maternal lifestyle during pregnancy [27]. Although the correlation between advanced paternal age and spontaneous abortion is well demonstrated, there remains debate within the current literature regarding which trimester is at greatest risk with advanced paternal age. De La Rochebrochard and colleagues utilized the unique single variable to "couple age" to elucidate the interaction between maternal and paternal age and the risk of spontaneous abortion [28]. The retrospective data collected from this multicenter European study revealed that the effects of advanced paternal age and maternal age are cumulative; the risk of spontaneous abortion is highest if both partners are advanced in age. It is theorized that this increased risk of spontaneous abortions associated with advanced maternal or paternal age reflects chromosomal abnormalities in the developing fetus.

In this regard, advanced parental age is a recognized risk factor for many genetic abnormalities in the developing newborn. In men, advancing age decreases semen volume, percent normal sperm, and sperm motility [18]. While these factors adversely affect fertility, the genetic integrity of the sperm is also at risk. In contrast to oogenesis, spermatozoa are continuously produced and undergo lifelong replication, meiosis, and spermatogenesis [29]. This continued replication allows for spontaneous mutations within the paternal cell line. Apoptosis of sperm with damaged DNA is an essential aspect of spermatogenesis that ensures selection of normal sperm DNA [30]. As men age, the rate of genetic abnormalities that occur during spermatogenesis increases. Investigation of advanced paternal age in the murine model reveals age dependent effects on the meiotic and premeitotic phase of sperm development. These abnormalities in replication result in both aneuploidy and structural abnormalities in male germ cells [10]. Additionally, the frequency of these numerical and structural aberrations in sperm chromosomes increases with increasing paternal age in humans [31]. This age-related increase in sperm cells with highly damaged DNA results from both increased double strand DNA breaks and decreased apoptosis during spermatogenesis [32].

As a result, advanced paternal age is associated with many autosomal dominant disorders such as Apert syndrome, achondroplasia, osteogenesis imperfecta, progeria, Marfan syndrome, Waardenburg's syndrome, and thanatophoric dysplasia [33]. Apert syndrome results from an autosomal dominant mutation on chromosome 10, mutating the fibroblast growth factor receptor 2 (FGFR2). The incidence of sporadic Apert syndrome increases exponentially with paternal age resulting in part from an increased frequency of FGFR2 mutations in the sperm of older men [34]. Achondroplasia results from an autosomal dominant mutation on FGFR-3. Data from clinical achondroplasia

registries reveal that 50% of affected children were born to men 35 years or older. In addition, the rate of achondroplasia increased exponentially with increasing paternal age [35]. The Muenke-type craniosynostosis results from an autosomal dominant mutation in FGFR-3 resulting entirely from the paternal allele. The average paternal age of children with Muenke-type craniosynostosis has been reported as 34.7 years [36]. Therefore, men as young as 35 years are at higher risk for many autosomal dominant disorders, most notably costochondrodysplasias.

In addition to structural errors and resultant autosomal dominant disorders, aneuploidy errors in germ cell lines also occur at higher rates with advanced parental age. Trisomy 21 or Down syndrome is a common aneuploidy error that affects 1/800 to 1,000 newborns [37]. The association of advanced maternal age and trisomy 21 was first documented as early as 1933 [38]. Further amniocentesis data from the European collaborative study of Ferguson-Smith and Yates determined that the rate of trisomy 21 increases exponentially from a maternal age of 35 years [39]. Although the correlation between trisomy 21 and advanced maternal age is well documented, it is only recently that the affects of advanced paternal age have been elucidated. Investigation of the meiotic nondisjunctional error in trisomy 21 reveals the origin of the extra chromosome 21 to be paternal in 5–20% of cases [40, 41]. Despite this recognized paternal origin, to our knowledge no chromosomal studies evaluating parental origin of chromosomal defects in offspring and paternal age are available.

Early studies from the 1970s and 1980s failed to demonstrate a consistent effect related to advanced paternal age. Initial epidemiological studies utilizing city and state birth registries failed to show any correlation between trisomy 21 and advanced paternal age while confirming the effect of advanced maternal age [42, 43]. In contrast, an evaluation of the Medical Birth Registry of Norway from 1967 to1978 that included 685,000 total births and 693 cases of Down syndrome revealed an increased risk of Down syndrome with a paternal age of 50 years or greater [44]. As parental age is increasing in the United States, recent studies provide more men of advanced age to evaluate. The New York State Department of Health congenital malformations registry containing 3,419 trisomy 21 births was analyzed from 1983 to 1997 and demonstrated a 111 and 60% increase in women and men over 35 years, respectively. This contemporary evaluation revealed no affect of parental age on trisomy 21 until 35 years. A paternal age effect was apparent in association with a maternal age of 35 years or greater and was most pronounced when maternal age was 40 years or greater. The rate of Down syndrome with a combined parental age greater than 40 years was 60/10,000 births that represents a sixfold increase compared with parents less than 35 years [45]. Advanced paternal age in interaction with advanced maternal age significantly increases the risk of trisomy 21 and may possibly explain the exponential increase in trisomy 21 in women greater than 35 years.

In contrast to the effects of combined parental age in trisomy 21, schizophrenia is associated with spontaneous mutations arising in the paternal germ cells. Although schizophrenia is a complex disease of unclear etiology, there is substantial evidence for a genetic component [46]. Since most individuals with schizophrenia are born to unaffected parents and have reduced reproductive activity, it is unclear how the disease is maintained within the population [47]. Early studies from the 1960s and 1970s suggested an association between advanced paternal age and the development of schizophrenia [48, 49]. Recent studies have confirmed this association between advanced paternal age and schizophrenia in large population based studies [50]. A 12-year evaluation of the Jerusalem birth registry and the Israel psychiatric registry that included 658 individuals with schizophrenia revealed that the risk of schizophrenia increased monotonically with increasing paternal age. This increased risk culminated in a relative risk of 2.96 (95% confidence interval, 1.60–5.47) for offspring of men 50 years or greater [51]. Conversely, maternal age demonstrated no effect on the development of schizophrenia. A Sweden birth registry study revealed that the association between paternal age and schizophrenia was present families without previous history of the disorder, but not in those with a family history. Based upon the stronger association between paternal age and schizophrenia in people without a family history the authors suggested that accumulation of de novo mutations in paternal sperm contributed to the risk of schizophrenia [52].

Men of advanced paternal age, much like their female counterparts, are at greater risk for spontaneous abortion and genetic abnormalities. The continually replicating spermatogonia and the decreasing apoptotic rate are likely causes of the amplified meiotic and premeiotic errors in the male germ cell line. These chromosomal abnormalities increase in the aging male and potentially result in spontaneous abortion, autosomal dominant disorders, trisomy 21, and schizophrenia. In light of the trend towards delayed childbirth, the potential deleterious effects of advanced parental age become increasingly important. Therefore, appropriate prenatal counseling of older couples regarding the

effects of advanced parental age on the developing fetus and newborn is imperative.

Conclusion

This brief discussion of the aging male demonstrates an un-appreciated reality: men have biological clocks that affect their hormone levels, fertility, and the genetic quality of their sperm. Women should no longer be viewed as solely responsible for age-related fertility and genetic problems. The effects of andropause and advanced paternal age on fertility and offspring are still under investigation and further research is needed to fully characterize the associated risks and to treat the underlying abnormalities. A better understanding of the cellular and biochemical mechanisms of "gonadal" aging is highly important in order to determine safe, effective ways to delay this process and, in effect, "rewind" the male biological clock. Doing so will lessen the potential for adverse genetic consequences in offspring, improve the sexual and reproductive health of aging males, and increase a *woman's* chance of having healthy children by correcting defects in the *male* reproductive machinery [53].

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