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INVITED REVIEW

Sperm Biology

The paternal genome and the health of the assisted reproductive technology child

Sheena EM Lewis, Kishlay Kumar

As a number of children born by assisted reproductive technology (ART) are increasing each year across the developed world, the health of such offspring is a matter of public concern. Does the integrity of the paternal genome impact on offspring health? In societal terms, as birth rates fall, and the Western population become unsustainable, do the benefits outweigh the costs of creating and providing for this ART conceived subpopulation? There are little data to date to answer these questions. The long-term health of such children has largely been ignored, and success measured only by early (prebirth) outcomes such as embryo quality or pregnancy. However, there are powerful paradigms such as ageing and smoking that give vital clues as to the potential impact of unhealthy spermatozoa on disease risk, mental and physical health, fertility and mortality of these offspring.
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INTRODUCTION

Given that up to 5% of the children born in the Western world are currently conceived by assisted reproductive technology (ART) and the number of couples presenting with infertility is increasing 8%–9% year on year, the health of male and female gametes and the offspring born from them is paramount.[1] Although over 5 million babies have been born worldwide, success has always been measured in terms of early embryo quality and implantation; seldom are live birth rates cited and even less often is consideration given to the short or long-term health of the child. While these checkpoints are vital to fertility clinic survival and to individual couple satisfaction, they are inadequate for society at large. We need information on the birth-to-death health and wellbeing of children conceived by ART. In economic terms, we need to determine if the benefits outweigh the costs of creating and providing for this ART conceived subpopulation. The evidence begins, although it does not end with, the impact of measurable semen properties on ART outcomes. The impact of the paternal genome on the life of the offspring from cradle to grave is rather more important to both parents and Society.

THE EFFECTS OF SEMEN ANALYSIS PROPERTIES ON DIAGNOSIS OF MALE INFERTILITY AND PREDICTION OF ASSISTED REPRODUCTIVE TECHNOLOGY OUTCOMES

Fertility problems are almost equally divided between male and female partners with male infertility being indicated in more than 40% of couples presenting for treatment with ART. Traditional semen analysis is still used as a standard routine test to diagnose this condition although as a purely descriptive evaluation, it cannot discriminate between the sperm of fertile and infertile men.[2] This understanding is mirrored in the variable thresholds for normality (all "normal" values now lower) in the 5th edition of the WHO manual,[3] compared to the previous WHO editions. This reduction in the "normal" range may be a retrograde step with 15% of men previously classified as sub-fertile and treated accordingly, now being classified in the normal or fertile range and investigated no further.[4] The usefulness of a semen analysis to predict ART outcomes is also limited in *in vitro* fertilization (IVF)[5] and of no consequence in intracytoplasmic sperm injection (ICSI). In an attempt to find a better test, studies have been performed at the molecular level to assess sperm health over the past 20 years. From this research, two important issues have emerged. First, there is consensus that men in infertile couples have more sperm DNA damage than those in the general population or men who have recently fathered a child. Second, this damage points to a newly recognized, and potentially dangerous, although possibly preventable, cause of male infertility.

A plethora of studies has also concluded that DNA damage could be a more accurate diagnostic tool for male infertility. This criterion of sperm quality is also useful as a predictor of ART success at each checkpoint in the reproductive process including impaired fertilization, erratic preimplantation embryo development, miscarriage, and birth defects in the offspring.[6]

DNA damage in sperm is primarily from oxidative stress (OS).[6] In a semen sample from any man, most of the sperm look abnormal even under light microscopy.[3] The sperm that exhibits such OS are those morphologically abnormal cells that were should have been removed by apoptosis, but the process was only half finished. Other sperm experiencing OS are those with defective protamine packaging from abnormal spermiogenesis. Current WHO guidelines suggest that even in fertile men, 96% of their sperm are morphologically abnormal, many with excess residual cytoplasm, allowing them to generate excessive reactive oxygen species (ROS), which given their incomplete chromatin packaging, allows greater access to DNA and induction of damage.[6] Unfortunately, as sperm have few repair mechanisms,

Centre for Public Health, Queen's University Belfast, Grosvenor Road, BT12 6BJ,NI, UK.
Correspondence: Prof SEM Lewis (s.e.lewis@qub.ac.uk)
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DNA damage is commonly encountered in these cells even within fertile donor population.[6] However, it is the amount of damage that is most closely associated with poorer ART outcomes. For any test to be clinically useful, it must have a threshold value which provides adequate discriminatory power. Routine semen analysis falls short of these standards,[5,7,8] so improved assays are needed.

DNA damage has long been the recognized universal indicator of cell lethality in toxicology laboratories in the pharmaceutical industry. The same is true of sperm. Sperm DNA damage is a robust gauge of cellular poor health. With advances in ART, we can now achieve fertilization *in vitro* with sperm that would have been rejected *in vivo*. However, by using sperm with compromised DNA for assisted conception, we are risking the long-term health and wellbeing of children conceived by ART. As a matter of "best practice," we should be testing the DNA quality of male gametes before using them clinically.

THE STRENGTH OF SPERM DNA TESTING

DNA fragmentation testing has a high level of repeatability in comparison[9] to the high intra-individual variations of concentration, motility, and morphology as measured in a conventional semen analysis.[5] Previous studies have reported high levels of repeatability (CV ~ 10%) in men with low levels of DNA damage.[10,11] In a recent large study, the intra-individual CV for DNA damage (using the sperm chromatin structure assay) was higher (30%) but 85% of the men from infertile couples did not change subgroup from one test to the next, with respect to the clinical cut-off level of 30%, still supporting the concept that a single sperm DNA damage test has a high predictive value for assessing male infertility. Other studies comparing semen from fertile donors with men presenting for infertility investigations (using the Comet assay) report sensitivity of 63% and specificity of 98% for the diagnosis of male infertility. This resulted in a receiver operating characteristic (ROC) of 0.970. Similarly, in testing DNA damage in native semen to predict clinical pregnancy, the sensitivity was 95%, and specificity was 80% with a ROC of 0.905, again indicating a robust test. In the context of DNA damage testing, both sensitivity and specificity of the test are important as they correctly identify those without damage who would benefit from IVF and those with damage who can then be guided toward ICSI rather than IVF.

THE ORIGINS AND CHARACTER OF DNA DAMAGE

Sperm chromatin differs from somatic cells in both components and structure. During spermiogenesis, protamines[12] that are half the size of histones,[13] replace the majority of histones, and the chromatin is rearranged into unique supercoiled doughnut structures named toroids.[13] As the sperm transit through the epididymal tract, the protamines are cross-linked by disulfide bonds. This shrinks the chromatin volume to one-sixth of that in somatic cell nuclei. This added compaction gives protection to the sperm DNA against exogenous assault. This is seen in the high levels of irradiation needed to destroy sperm DNA, in comparison with somatic cells[14] and also by the high concentrations of hydrogen peroxide required to damage either sperm nuclear or mitochondrial genomes.[15] However, if this protamination is incomplete, as in some infertile men, the DNA strands that are scantily compacted are vulnerable to damage, observed as double and single strand breaks, abasic sites, inter-strand cross-links, and DNA-protein cross-links.[15]

Damaged DNA has been reported in sperm at all stages: from the testis, epididymis, and ejaculate. Sperm DNA first becomes susceptible to damage if chromatin packing is not completed during spermatogenesis.[16,17] Some strand breaks may be required to decrease the torsional stress that occurs for the tertiary structural reorganization during the elongation of spermatids. Normally, these nicks are transitory. If they are not corrected,[18,19] increased DNA damage will be present in ejaculated sperm. Epididymal transit is another hazard for sperm when they are vulnerable to damage if incomplete disulfide cross-linking has not been completed adequately.[20]

DNA repair occurs as sperm mature,[18] but it is terminated as transcription and translation cease following spermiogenesis.[21] This means that the damage that happens to DNA following germ cell differentiation into spermatocytes, spermatids, and spermatozoa cannot be corrected, although they may be undergoing apoptosis as reported by Fas expression or endonuclease activation.[21,22] This may explain the residual level of DNA damage observed even in semen from fertile donors.[23] Further genomic damage to healthy sperm can occur in the epididymis from adjacent ROS-producing sperm, epithelial epididymal cells or through commonly present toxic or inflammatory factors. This is seen in the lower levels of DNA damage reported in testicular sperm increasing in caudal epididymal and further in ejaculated sperm.[23–27] Suganuma's group[27] is also in agreement showing that a defective sperm experienced an increase in DNA damage during the passage through the epididymis. It is acknowledged that even the proximal epididymis has substantial proportions of senescent sperm[26] releasing ROS as they age and die[28] and damaging those adjacent to them. This may, at least in part, explain the higher levels of sperm DNA damage in the epididymis. As with other indications of sperm dysfunction, the importance of ROS, produced by either increased ROS generation or impaired antioxidant defense, as a primary instigator of sperm DNA damage is well established.[7] Sperm are particularly vulnerable to damage from ROS because of their high polyunsaturated fatty acid content and limited ability to repair damage. Sperm from infertile men are often associated with high levels of ROS caused by either increased production and/or impaired antioxidant defense.[29,30] Associations between OS and sperm DNA damage have been reported in numerous studies.[31]

Spontaneous germ-line mutations are not always deleterious. Their presence in animals has the benefit of creating genetic diversity and thus progressing evolution. However, in the process of creating mutations, molecules such as 8-oxoguanine (8-oxoG) are associated with ROS *in vivo* with very negative and immediate effects on the organism. In 2014, Ohno *et al.*[32] have designed an elegant study using a triple knockout mouse; TOY-KO in which three of the most important enzymes (Mth1/Ogg1/Mutyh) that mammals possess to prevent 8-oxoG induced mutations were knocked out. This caused spontaneous and inheritable G to T mutations at different stages in the germ cell lineage with the mutations distributed throughout the chromosomes. The TOY-KO mice were viable and fertile, but they had reduced survival, reduced fecundity, and a variety of life-shortening cancers.

We have known for some time, again from animal models that the oocyte can provide limited repair to damaged sperm DNA postfertilization.[33,34] It has also been observed in a small study[35] where the pregnancy rate of a mixture of IVF and ISCI cycles was not reduced by poor quality sperm DNA with donor oocytes suggesting that young fertile oocytes can repair some DNA damage in sperm from older men. However, it has often been reported that damaged DNA does not impact on early ART outcomes with ICSI, in contrast to poorer results using IVF. In this context, Meseguer *et al.*[36] investigated the effect of oxidative sperm DNA damage on ICSI outcomes using donor oocytes. Here, they reported that such damage led to an impairment of blastocyst formation. Clearly, these studies need to be extended in order to more fully resolve the oocyte's ability to repair different kinds of DNA damage in sperm.

What is not yet clear is whether the oocyte can repair double-stranded or just single stranded DNA breaks. Furthermore, the threshold beyond which repair is impossible has not been investigated in humans, although a figure of 8% has been postulated in mice.[37] If inadequately repaired by the oocyte, such sperm DNA damage can predispose to mutations in the developing offspring.[31]

THE BIOLOGICAL IMPORTANCE OF DNA DAMAGE FOR OFFSPRING

To assess the biological importance of good quality sperm DNA on offspring health, we need to focus on animal models as such studies would be unethical in humans.

In reproductive technology, the literature is irrefutable in recognizing DNA damage in the male germ line, as a negative factor in relation to embryo quality and on-going pregnancy. In the dominant lethal assay, the male is given a toxicant, and the females, which have not been exposed, are examined over time to assess embryo number and quality and progression and miscarriage.[38] Yet, of even more concern, if the conception results in a live birth, there may be an increased incidence of cancers and morbidity in the offspring.

A large range of toxicants, and, in particular, compounds that induce OS, cause DNA damage in sperm, and this is strongly associated with embryo loss and growth impairment. This literature has been carefully reviewed by Aitken *et al*.[15] A milestone study, with particular relevance to ART is that of Fernández-Gonzalez *et al*.[39] This group performed ICSI with mouse spermatozoa in which DNA damage had been induced by cryo-injury. The aim of their study was to investigate the long-term consequences on offspring of using a damaged paternal genome. Cryo-injury led to DNA strand breaks and loss of telomeres. Fertilization by ICSI with these DNA-damaged sperm led to preimplantation embryo development, albeit at a slower rate than control animals. The number of offspring per litter was also reduced. Methylation of some epigenetically regulated genes was altered. Animals exhibited increased anxiety, lack of a habituation pattern and a defective spatial memory ($P < 0.05$). At 16 months, 33% of females produced with DNA-fragmented sperm presented some solid tumors in lungs and dermis. Moreover, 20% of the mice died during the first 5 months of life, with 25% of the surviving animals showing premature aging symptoms. The authors suggested that the oocytes had been able to partially repair fragmented DNA, producing blastocysts able to implant and produce live offspring. However, the repair was incomplete, thus leading to long-term pathologies. The fact that these anomalies were only observed in later life is of concern, given that the oldest ICSI children are only in their 20's.

Many compounds capable of inducing such damage are widely present in our everyday lives. These include spray adhesives and glues containing 1-bromopropane, fried food with acrylamide, radiation, chemotherapeutic agents, and metals such as nickel and iron that can lead to OS.[38,40]

Other potential toxicants occur in unexpected situations. Two examples of this are the widely used drugs; Sildenafil citrate for erectile dysfunction and streptozotocin that is used in treating certain diabetic cancers. Further, work from our laboratory has shown that men with type 1 and type 2 diabetes have already had high levels of oxidative damage in their sperm although their semen analyses may appear normal.[41,42] As Sildenafil citrate is often used in fertility clinics for male patients having difficulties in producing a semen sample and also in the treatment of young diabetic men to restore potency, we used an animal model to assess the effects of sildenafil-treated males on fertilization and embryo development in unexposed females. Pups were born following treatment although both fertilization and embryo development were impaired.[43]

The toxic effects on embryo development may be due to phosphodiesterase (PDE) inhibitor effects on the embryo's DNA synthesis and repair. Pentoxifylline (a PDE type 4 inhibitor), also used in fertility clinics to improve poor sperm motility has also been shown to inhibit DNA repair *in vitro* during the S and G2 phases in a human ovarian cell line.[44,45] Furthermore, DNA mutations can be increased with milrinone; a PDE type 3 inhibitor,[46] suggesting more than one pathway by which PDE inhibitors act on DNA. In support of these animal studies, a recent human study has reported a negative correlation between sperm DNA oxidative damage after fertilization and blastocyst formation.[36] Again, such impairments in the development do not preclude the birth of offspring that may have on-going health issues.

In order to elucidate the mechanisms by which human offspring health is affected by paternal genomic damage, we can use indirect, but powerful paradigms: paternal ageing and smoking.

AGEING AND PATERNAL GENOME

It has always been clear to women that there is a natural endpoint, albeit over a period of years, to their childbearing years. In contrast, men have been viewed traditionally as retaining their ability to father a child until their old age. However, insufficient studies have explored the impact of the advanced paternal age on the health and wellbeing of such offspring. Far from viewing advanced paternal age as a possible disadvantage, media coverage of older male celebrities with their latest children has been met with approval and acceptance. Second, in today's changing society, many men begin new partnerships with younger women who wish to have a family for the first time. Reproductive technologies have made this possible even for vasectomized men as ART with testicular sperm is a successful option. Furthermore, there appears to be a worldwide trend for leaving the initiation of a family until later in life.[47,48] It is difficult to perform studies on paternal age and child health. Paternal information is often missing from birth certificates; in the USA, for example, father's age is missing from ~ 15% of all births.[47]

Over the past decade, an interest in aged fathers has been developed, concomitant with the increased interest in andrology, in general. As men age, they do not stop producing sperm, however, the gametes they do produce exhibit a progressive loss of quality, particularly in terms of DNA integrity.[49,50] Spermatogenesis consists of replication of spermatogonial stem cells throughout a man's life. This involves countless cell divisions, and more mitotic cell divisions occur, the more chance of chromosomal errors occurring too. This has been reported in numerous recent reviews indicating a link between reduced genomic quality and mutational load in the male germ line and developmental abnormalities in the embryo; exemplified by age.[49,51] Although the types of DNA damage observed in the sperm of ageing males have not yet been fully characterized, it is clear that as men age, the risk of morbidity in their offspring increases.

For many years, paternal age has been recognized as the major contributor to poor offspring health, as reflected by spontaneous dominant genetic diseases such as achondroplasia or Apert syndrome and in a variety of complex polygenic neurological disorders such as epilepsy, spontaneous schizophrenia, autism, and bipolar disease.[52–54] Further, advanced paternal age is associated with a trinucleotide repeat associated diseases such as Huntington's disease and myotonic dystrophy.[54–57] Paternal age has also been linked with birth defects (neural tube defects, congenital cataracts, reduction defects of the upper limb and Down syndrome); however, these effects are not particularly

strong and tend to be inconsistent.[58,59] DNA lesions in the sperm of ageing fathers are also associated with an increased risk of cancer in the offspring.[60] In the cohort of all children (1.8 million) born alive in Denmark from January 1, 1978 to December 31, 2004[61] there was also a statistically significant increased risk of mortality (1.65, 95% confidence interval [CI]:1.2–2.18) in offspring under 5 years of age if fathers were 45+ years compared to 30–34 years. The risk was attributed to congenital malformations and malignancies. As older fathers are increasing in the first world, this association will become of even greater importance.

In a large population-based study[62] of 2.6 million Swedish children born between 1973 to 2001, the risk of psychiatric and academic morbidity as a result of advancing paternal age was explored. Quasi-experimental designs were used to compare differentially exposed siblings and cousins. Childhood and adolescent morbidity were also investigated. Comparing paternal ages of 20–24 years with 45 years and older, the authors reported an association between an increased risk of the following psychiatric syndromes with advancing age: autism, psychosis and bipolar disorders. When the offspring of fathers (20–24 years) were compared with those of fathers of > 45 years, this was also true but also included additional associations with psychiatric and academic morbidity in the form of suicide attempts, substance abuse problems and low educational attainment. The magnitude of risk was larger than in previous estimates. In support of these findings are those of another large population-based Scandinavian study[63] of 2.8 million children born in Denmark between 1955 and 2011. Again, offspring of older fathers were at an increased risk of mental retardation and autism spectrum disorders.

Although there is strong epidemiological evidence that the diseases listed above are more common in offspring from older fathers, the mechanisms by which they occur are still unclear. *De novo* genetic mutations that can occur during spermatogenesis and/or epigenetics, and specifically DNA methylation alterations are the most likely candidates. In a unique study in 2012 by Kong's group and published in *Nature* the effects of paternal age on fertility *de novo* mutations and disease risk were examined.[64] The group conducted a study of genome-wide mutation rate by sequencing the genomes of 219 individuals consisting of 78 trios (father, mother, and child). They reported that the diversity in mutation rates of single-nucleotide polymorphisms is dominated by the age of the father, not the mother, at conception of the child. The effect is an increase of about two mutations per year. This extrapolates to a 20-year-old father transferring about 25 *de novo* mutations to his offspring compared to a 40-year-old man who could pass on 65 *de novo* mutations. The implications of this study are controversial. While there may be a connection between advanced paternal age and polygenic psychiatric disorders, mutations are an important part of evolutionary diversity and the number of mutations is very small compared to the total numbers of genes in the overall DNA code. In addition, the group reported that the rate of human mutations was lower than previously estimated.

In a study by Carrell's group,[65] the influence of paternal aging on DNA methylation in human sperm was investigated using a methylation array approach in 17 fertile donors by comparing the sperm methylome of two samples collected 9–19 years apart from each individual. The group found numerous regions of methylation alterations (promotor or gene body) that were hypomethylated with age. Further, some of the age-related changes in sperm DNA methylation are located at genes previously associated with schizophrenia and bipolar disorder. Overall, it would appear that multifactorial genetic and epigenetic disorders that cause impaired brain function and lead to autism, schizophrenia, and academic morbidity are associated with a paternal age effect.

SMOKING

Cigarette smoking has been shown to increase oxidative DNA damage in sperm[66] male offspring fertility[67] and also cancer in offspring.[68,69] However, it has been difficult to separate effects of paternal and maternal smoking on offspring health. To address this limitation of earlier studies, Ji *et al.*[45] conducted a population-based, case-control study of childhood cancer in Shanghai, People's Republic of China, where the prevalence of cigarette smoking is high among men but very low among women with only 1% of young adult females being smokers.[70,71] This study provided a one-off chance to evaluate the role of paternal smoking, in the preconceptual period and the absence of maternal smoking on the cause of childhood cancer. The study included 642 childhood cancer case patients (<15 years of age) and their individually matched control subjects. Information about the exposure of the child to parental smoking was obtained by interviewing both parents.

The effect of paternal cigarette smoking on childhood cancer was shown to begin in the preconception period with elevated risks of childhood acute leukemia, lymphoma, brain tumors, and total cancers. The significantly increased relative risks to cancers were mostly observed in young children diagnosed before 5 years old. They speculated that paternal smoking induces prezygotic genetic damage that, in turn, acts as a predisposing factor for cancers. More recently, a meta-analysis of 12 studies was undertaken to search for associations between paternal smoking and childhood leukemia risk.[72] Paternal smoking at home was significantly (1.8, 95% CI: 1.1–2.8) associated with all leukemias. Following on, the effects of paternal smoking on general female childhood health through to adulthood was investigated using data from 35 370 participants in the Nurses' Health Study II.[73] Women whose fathers smoked during their mother's pregnancy were also at an increased risk of being overweight or obese during in adulthood with the risk increasing as with the number of cigarettes smoked compared with nonsmoking fathers. Paternal smoking during pregnancy was not associated with childhood body size. This association persisted after adjustment for maternal smoking.

Adding these two paradigms of ageing and smoking from epidemiological studies to the direct evidence showing cause and effect in animal models, generates a compelling package of data implicating the paternal genome in the health of progeny from fertilization, pregnancy, and its maintenance through to the short- and long-term fitness of children's lives.

THE HEALTH OF CHILDREN BORN BY *IN VITRO* FERTILIZATION AND INTRACYTOPLASMIC SPERM INJECTION

The number of children born as a result of ART has now risen to 7 million worldwide. In the first world countries, they account for up to 5% of all babies born, and the number of couples presenting with infertility is increasing 8%–9% each year across Europe.[1]

Until recently, the most important endpoint to most clinics and couples was a pregnancy but with time, we are realizing that there may be more health problems with ART conceived children and so studies on their on-going mental and physical wellbeing is imperative. From 1978 to 2003, a few sparse publications had indicated that there were no increased risks. However, by 2005, the first systematic review was carried out by Hansen *et al.*[74] of the 25 major studies about the prevalence of birth defects in infants conceived following IVF and/or ICSI compared with spontaneously conceived infants. All 25 studies indicated an increased risk of 30%–40% of birth defects related to ART.

Since then, the most detailed studies and reviews have been published by Bonduelle's group in Belgium.[75–78] There are many reports

of normal physical development up to 18 years in comparison with spontaneously conceived controls.[79–83] The studies of children born following ICSI are mostly cross-sectional evaluations at different ages from 5 to 12 years.[84–90] Menarche and pubertal development of IVF and ISCI children are comparable to spontaneously conceived children[91] although breast development in ICSI girls is delayed. All of these data came from children in Northern Europe. A comprehensive study from South Australia[92] compared birth defects of 6163 children born from ART with 302 811 children born to couples with no record of infertility. They found that the overall risk of any birth defect following ART was 8.3%, compared to the significantly lower, 5.8%, in spontaneously conceived children. The most common birth defects connected to ART included spina bifida, cerebral palsy, cleft palate and musculoskeletal, cardiovascular and gastrointestinal conditions. The group then divided the risks for IVF and ICSI and found that ICSI resulted in the highest rate at 9.9% compared IVF at 7.2%. They also reported that this additional risk with IVF could be explained by patient characteristics, such as age or weight unlike the risk for ICSI that could not be thus explained. Interestingly, this difference was not observed with frozen-thawed embryos, suggesting that the cryopreservation may act as a physiological filter whereby only the best embryos survive the assault.

Any concerns we have had about ICSI children have largely focused on boys and their potentially impaired fertility. To some surprise, in a large study in 2012, Belva *et al.*[77] reported that ICSI girls were at risk of increased central, peripheral and total adiposity although boys appeared to be less affected with only an increase in peripheral skinfold being observed. The authors recommended that monitoring of body fat patterns in adolescents born after ART should be compulsory in order to assess their risk for developing obesity and taking avoidance measures to prevent adverse health effects in adulthood. In childhood and at puberty, however, elevated blood pressure, higher fasting glucose levels, generalized vascular dysfunction, and altered body fat distribution have been described in offspring from both IVF and ICSI.[77–79] In a review of all the literature pertaining to health outcomes of IVF and ICSI children by the Evian Annual Reproduction workshop group (2014), the report concluded that IVF children have lower birth rates and higher peripheral fat, blood pressure, and fasting glucose levels than controls. However, the group was unable to ascertain if the differences were due to ART procedures *per se* or also to the duration of infertility and the age of the mother as well.[93]

In 2011, Belva *et al.*[76] examined the neonatal outcomes of 724 children born after ICSI using nonejaculated sperm. They reported that stillborn rates, prematurity rates and low birth weight rates were comparable in surgically retrieved and ejaculated sperm groups. A nonsignificant increase in major anomalies was reported in offspring born from surgically retrieved sperm compared to ejaculated sperm, but the risk was higher than in the general population.

Further health and safety reassurance was recently reported in another large prospective register-based cohort study, undertaken in Denmark between 1995 to 2003 of 33 139 children born after ART compared to 555 828 children who were spontaneous conceived. No increase in the incidence of mental health disorders was observed.[94]

In our haste to provide an infertility treatment for men with azoospermia, we neglected to perform the basic research to investigate the functions of seminal plasma on sperm health. We have assumed that seminal plasma's only function was as a vehicle to transport sperm from male to female reproductive tract. Some exciting research has recently come from Robertson's group in Adelaide showing that seminal plasma has many more functions and the absence of sperm/seminal plasma contact may have deleterious effects on sperm, oocyte, uterine environment and in turn, offspring health. In particular, the programming of future adiposity and metabolic phenotype in male offspring is dependent on this interaction. Using an animal model, the group investigated the effect on offspring of excluding the plasma fraction of seminal fluid by surgical excision of the seminal vesicle gland. Fertilization was reduced, and placental development was impaired but most importantly, male offspring were severely affected in terms of growth trajectory and metabolic obesity, distorted metabolic hormones, reduced glucose tolerance, and hypertension. This carefully designed study showed compelling evidence that these effects were due to both sperm damage and seminal fluid deficiency on the female tract. The absence of seminal plasma was associated with a down-regulation of the embryotrophic factors Lif, Csf2, Il6, and Egf concomitant with an up-regulation of the apoptosis-inducing factor Trail in the oviduct.[95]

Given that many of these differences will persist throughout the lifetime of these offspring, there is a public health obligation to monitor them. Increased public awareness and early screening/prevention or lifestyle interventions may safeguard the health of ART-conceived individuals. In addition, the impact of ART procedures such as ovarian stimulation, sperm manipulation, and gamete/embryo culture conditions, on the epigenetic status of the embryo, with implications for the long-term health of the offspring, require elucidation.

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COMPETING INTERESTS

None.

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