Advances in Understanding the Male Gamete

The reproductive fitness of the human male gamete

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Introduction

Our understanding of the contribution of the male gamete to reproductive success has a long and intriguing history. It has been known since ancient times that the male provides a vital force that is essential for embryogenesis, however, the functions and relative contributions of the male and female contributions have been debated. For example, Aristotle wrote of the necessity of the male “fluids” (semen) in terms of “that which generates,” in contrast to the female fluids, which he described as “that out of which it generates.” In other words, components of both the male and female “fluids” were necessary and contributory to development of the offspring, but semen was the controlling force while female “fluids” provided the resources necessary for embryogenesis [1].

The first reports of the visualization of sperm within semen were made separately by Anton Leeuwenhoek, Nicolaas Hartszoeker, and Christian Huygens, beginning in 1677 [2]. The visualization of the small “animalcules” which we now know as spermatozoa was made at a time of philosophical debate over two competing theories of reproduction: “epigenesis,” whose proponents held that development (embryogenesis) resulted from a systematic progression of development from the components provided by the male and female fluids according to laws or principles, versus the theory of “preformation,” which held that either the sperm or ova contained a fully formed individual that was stimulated to grow under the influence of the mixture of the two fluids [3, 4]. The “preformist theory” of development was partially influenced by religious doctrine, and comprised two competing camps, the “ovists,” who believed that the preformed individual was contained within the ovum, and the “spermists,” who held that the sperm contained the preformed person. The “spermist theory” is often represented by a drawing made by Hartsoeker in his publication Dioptrique in 1694 in which a homunculus is seen within the sperm cell (Figure 1.1) [5]. Interestingly, Hartsoeker did not claim to have seen a person within a sperm cell, although others later would make such claims, rather he was suggesting what the possible appearance of such a “homunculus” may reflect [5, 6]. Nevertheless, the “preformist” era, and specifically the “spermist” view, was the pinnacle of emphasis of the role of the contribution of the sperm to embryogenesis.

Although the “preformist theory” of reproduction was subsequently disproved through classical descriptive and experimental studies, the relative contribution of the sperm versus the oocyte to embryogenesis has continued to be debated. Clearly, the oocyte contributes the environment and most support organelles, enzymes, energy sources, and other molecules for the first few cleavage cycles, which perhaps has in some ways minimized the view of the contribution of the sperm to the embryo as simply a static haploid set of chromosomes (and in the human a centrosome) that are controlled and regulated entirely by the oocyte. This view is reflected in the paucity of studies from the “early days” of human in vitro fertilization (IVF) in regards to the effects of sperm on embryo morphology and fitness as compared with the much greater focus on the effects of the oocyte or of embryo culture conditions on embryo quality [7]. Whether this bias is due simply to the progress of technological advances that promoted...
studies on culture conditions and oocyte development or underlying biases in scientific thought, or a combination of the two factors, can be debated. However, it is clear that the general trend of sperm biology research moved towards a focus on fertilization, first with a focus on biological events such as capacitation, zona binding and oocyte fertilization events, and later, based on technological advances, to assisted fertilization techniques, such as partial zona dissection (PZD), sub-zona injection (SUZI), and intracytoplasmic sperm injection (ICSI) [8].

Recent studies have demonstrated that the role of the male gamete in embryogenesis is significant in ways not previously understood [9]. Advances in our understanding of the biology of the sperm have highlighted genetic and epigenetic mechanisms that can preclude normal cleavage of the embryo, often observed as fragmented embryos that undergo cleavage arrest, or can result in serious health concerns for the offspring (Figure 1.2) [10–12]. These advances in understanding the biology of the gamete have also facilitated an increased appreciation of the potential influence of environmental influences on the gametes and resulting embryo, including such influences as aging, obesity, air quality, and drugs. This brief chapter will highlight some of these advances and concerns, which are discussed in detail in the remaining chapters.

Sperm biology

Recent studies have clearly demonstrated genetic mechanisms and defects contributing to subfertility. Clearly, diminished sperm DNA integrity, as defined by an increase of single- and double-strand DNA breaks, has been shown to be associated with embryo quality and IVF outcome [13, 14]. Since sperm lack the ability to self-repair DNA strand breaks, an accumulation of damage may overwhelm and affect the repair processes that occur during embryogenesis [15]. The emergence of sperm DNA damage as a potential cause of poor embryo development is important and relevant in its own right, but has also been beneficial in focusing more attention on sperm factors in general, as well as focusing the need for improved sperm preparation and selection techniques to be used prior to assisted reproduction technologies (ART) [16].

Structural alterations to sperm DNA have been shown to alter the reproductive potential of an individual. Such alterations may include defects ranging from whole chromosome losses or gains to sub-microscopic...
variations, and even individual base mutations. Large structural alterations, such as chromosome aneuploidies, often preclude normal embryogenesis and result in miscarriage [17]. While oocytes have long been known to be the major contributor to embryo aneuploidy, recent studies have highlighted the contribution of sperm to embryonic aneuploidy as well [18, 19]. While chromosome aneuploidy is seen in 2–3% of men evaluated for infertility, the percentage increases dramatically in men with oligozoospermia or azoospermia [20, 21]. Meiotic errors are increasingly frequent in aging women, however, clear evidence of such an effect has not been demonstrated in men [22]. Smaller, sub-microscopic variations to the genome, typically termed “structural variants,” have recently been reported to be related to male infertility and are becoming a focus of research by several laboratories [23–25].

While the effects of structural variations and point mutations on embryogenesis have not been demonstrated in humans, it is interesting to note that a recent study on the effects of mutation accumulation in Drosophila melanogaster demonstrated that an increased “mutation accumulation” results in a decrease in post-fertilization embryo potential [26]. This is intriguing in light of the observation that the incidence of rare polymorphisms is elevated in severely infertile men [27]. The observations of increased levels of rae polymorphisms and structural variations may indicate that some subfertile men carry a form of genetic instability, which may have profound implications for the embryo and offspring [28].

Gametes of oligozoospermic men have been reported to contain errors of imprinting, an observation that is emphasized by reports that there is a small, but very significant increase in the rate of imprinting disorders in offspring conceived by IVF [29–31]. A larger, programmatic epigenetic role has also been proposed for sperm [10, 29]. Recent studies have demonstrated that the sperm epigenome is uniquely marked at genes involved in embryogenesis, and that severe abnormalities are observed in the epigenetic marks at many development-related genes in the sperm of some men who consistently contribute to very poor embryo development when undergoing IVF [32–34]. These observations need to be studied in more depth, but they may suggest a major mechanism whereby sperm influence early embryogenesis. Importantly, these studies have also highlighted a major mechanism by which the environment and lifestyle factors may alter sperm epigenetics and reproductive potential [35].

Two other emerging areas of sperm biology are explored in this book, the role of the sperm centrosome and the possible function of non-coding RNAs carried by the sperm. In humans, the sperm provides the functional centrosome, of which the proximal centriole and the centrosomal proteins are functional from the first embryonic cleavage onwards [36]. While our understanding of centrosomal function is in its infancy, defects of centrosome function have been described, while other studies have focused on identifying models to evaluate and better understand the role of centrosomal proteins in normal embryogenesis [37–39]. Similarly, recent studies have begun to identify differences in the RNA transcripts present in sperm, both in coding and non-coding RNAs [40, 41]. While mRNAs may be more reflective of the status of spermatogenesis (a historical record of spermatogenesis), it is thought that small non-coding RNAs may be functional in embryogenesis [10, 42]. These two areas highlight the growing scope of sperm factors potentially affecting embryogenesis.

**What is the role of the male gamete in embryogenesis?**

The advances in our understanding of sperm biology, briefly highlighted above and discussed in detail in the following chapters, open the door to better answering questions relevant to the contribution of the male to
reproductive success. The following are some of the questions that are considered and are explored in the remaining chapters:

1. Do genetic factors, including structural variations and polymorphisms, affect embryogenesis, and if so, what is the mechanism?
2. Is the sperm epigenome programmed to influence or support early embryogenesis?
3. How might environmental factors, including diet and stress, affect the programmatic epigenome?
4. Is the epigenome responsible for transmission of an increased risk in late-onset diseases such as diabetes or heart disease?
5. How is the epigenome altered as a male ages?
6. Does aging affect genetic features of male reproductive fitness?
7. Is there an increased risk of late-onset diseases in children conceived by older men?
8. Is altered sperm centrosome function responsible for poor embryogenesis in some couples?
9. Do RNA transcripts carried by sperm have a function in embryogenesis?
10. Can DNA integrity of sperm be improved?
11. How do medications and supplements affect sperm integrity, especially in the aging male?
12. Does obesity affect sperm function?
13. What evidence is there of transmission of diseases through epigenetic mechanisms?
14. How does the variability of semen production affect interpretation of clinical data?
15. Is ICSI safe?
16. What medical and surgical therapies can improve sexual function in the older man?
17. How can safety of ART techniques be better monitored and assessed?
18. Can sperm selection techniques select sperm with increased fitness?

**Conclusions**

As noted above, our increased understanding of sperm biology has highlighted the potential of the male gamete to affect embryogenesis and reproductive success. Many important questions are being investigated, and some important answers are emerging. Naturally, the advances we are making are also stimulating new and profound questions. As discussed in the following chapters, the ramifications of our growing knowledge are profound.

**References**


The sperm genome: effect of aneuploidies, structural variations, single nucleotide changes, and DNA damage on embryogenesis and development

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Introduction

A principal role of the sperm is to serve as a vessel for the delivery of paternal genetic material to the oocyte. Following penetration of the cumulus complex and zona pellucida by the sperm, the sperm membrane binds to and fuses with the oolemma triggering oocyte activation, which results in the resumption of meiosis, extrusion of a second polar body, and formation of male and female pronuclei. Subsequently male and female pronuclei migrate together and fuse to form a single, diploid pronucleus that will undergo replication and undergo multiple rounds of mitosis to form an embryo that will, under ideal circumstances, result in a healthy offspring. Successful sexual reproduction depends on, among other factors, a normal sperm genome. Aberrations in the sperm genome including DNA damage, aneuploidies, gene mutations, and structural variations can result in failed fertilization, arrested or abnormal embryonic development, early or late miscarriage, or in rare cases the birth of genetically abnormal offspring. This chapter will discuss the known sperm genetic abnormalities that can impact embryogenesis, pregnancy, or offspring health.

Spermatogenesis

A brief review of the events required for successful spermatogenesis and fertilization is important in understanding how the complete sperm genome arises and how insults at different stages in development can lead to genetic anomalies that can be transmitted to the embryo.

Prenatal germ cell development

The initiation of germ cell development occurs in the early stages of embryogenesis with primordial germ cell (PGC) precursors arising in the yolk sac during gastrulation [1]. Primordial germ cells migrate from the epithelium of the yolk sac to the gonadal ridges in an amoeboid fashion during which time the cells continue to divide by mitosis [2]. Primordial germ cells are guided in their migration to the gonadal ridge by chemotactic molecules CXCR4, expressed on PGC surface and SDF1, secreted by gonadal ridge cells [3].

Upon reaching the gonadal ridge, PGCs colonize the region and begin sex-specific differentiation to form gonocytes [4]. While the timing of PGC development in the human is not well established, the cells are readily detectable in the developing embryo by 3 weeks gestation [5], and they have begun to colonize the gonadal ridge by the fifth week [4]. The gonads remain undifferentiated in terms of gender until week seven, at which time differentiation of the gonadal cortex and sexual differentiation begins [4].

Upon initiation of sexual differentiation seminiferous cords, precursors to seminiferous tubules begin to form and encompass PGCs and mesodermal cord cells in the medullary region of the gonads. The PGCs will eventually give rise to spermatozoa, while the mesodermal cord cells will give rise to Sertoli cells. Interstitial stromal tissue becomes vascularized, and precursors to Leydig cells develop [6]. Sexual differentiation continues, driven in part by the endocrine activities of Sertoli and Leydig cells. The number of fetal gonocytes doubles every 6 days between week 6 and week 9, increasing from about 3,000 to about 30,000 [7]. Between weeks 13–15 of development fetal gonocytes begin to differentiate into spermatogonia triggered by the downregulation of a
number of genes including KIT, OCT4, NANOG, and TFPAC2 [8]. As fetal development continues, testes continue to develop and begin their descent from the lumbar region near the kidneys, over the pubic bone, and through the abdominal canal to finally reach the scrotum by 35–40 weeks gestation.

Postnatal development

The differentiation of fetal gonocytes to prospermatogonia continues throughout fetal development and is finally completed in infancy [8]. Sertoli and Leydig cells increase in number in infants during the first 3 months after birth accompanied by a rise in testosterone and inhibin B levels [8]. The testes grow slowly prior to puberty, and germ cell development remains relatively quiescent.

Puberty is a process wherein secondary sexual characteristics are developed in a gradual and stepwise manner and culminates in reproductive competence [9]. Associated with puberty is a sudden increase in testicular size resulting from the formation of seminiferous tubules from the solid seminiferous cords, increase in size and activity of Sertoli cells, and the resumption of mitotic activity by the germ cells as spermatogenesis initiates. In addition, endocrine secretion activity by Leydig cells increases, which drives many of the morphologic changes that occur at puberty [9]. These events mark the onset of sexual maturity, the resumption of spermatogenesis, and the concomitant acquisition of fertility, which will continue throughout a man’s life.

At puberty, spermatogenesis is initiated and proceeds in three main phases. First, prospermatogonial stem cells enter mitosis to produce large numbers of spermatogonial stem cells in the mitotic proliferation phase. As these stem cells replicate morphologically distinct cells called A1 spermatogonia emerge marking the start of spermatogenesis.

Type A1 spermatogonia undergo several rounds of mitosis to form subsequent generations of type A spermatogonia, eventually giving rise to intermediate spermatogonia then type B spermatogonia, which undergo a final round of mitosis to form resting primary spermatocytes. The cells derived from a single A1 spermatogonium remain linked by thin cytoplasmic bridges, which persist until residual cytoplasm is shed just prior to the release of sperm into the lumen [10].

Following the proliferative stage, which occurs just inside the basement membrane within seminiferous tubules, primary spermatocytes undergo a round of DNA replication without cell division, they pass through Sertoli cell junctions toward the tubule lumen, and meiotic division begins. During the first meiotic prophase, crossing over and the exchange of genetic material between homologous chromosomes at recombination foci occurs. The event of homologous recombination is the basis for new combinations of alleles in each gamete, mixing genetic material from both paternal and maternal genomes. A minimum of one recombination site per chromosome is required for proper chromosomal segregation, and errors in meiotic recombination are a primary cause of aneuploidy in gametes [11]. As homologous chromosomes separate, cytokinesis results in two secondary spermatocytes to complete the first round of meiosis. Following the first meiotic division, sister chromatids separate followed by a second cytokinesis event resulting in haploid early round spermatids, which remain linked by cytoplasmic bridges.

The completion of meiosis is followed by dramatic nuclear and cytoplasmic remodeling events during the process of spermiogenesis. At the nuclear level, gene transcription ceases and DNA becomes more tightly compacted as the majority of nuclear histones are replaced first by transition proteins and finally by protamines. Also during this phase, each cell elongates, the tail and midpiece form, enzymes are packaged to form the acrosome, residual cytoplasm is shed and phagocytized by the Sertoli cell, cytoplasmic bridges dissolve, and mature spermatids are released into the lumen through the process of spermiation.

Following the completion of spermatogenesis and spermiation, spermatids move through the seminiferous tubules to the rete testis, through the vasa efferentia and into the epididymus where sperm are concentrated and undergo a process of maturation that renders spermatozoa motile and capable of fertilization.

Fertilization

Fertilization follows the long process of male germ cell development which began just a few weeks after conception with the migration of primordial germ cells to the gonadal ridge and culminates with spermiation and epididymal maturation of spermatids, the final step in the transmission of the male germine to the next generation involves the process of fertilization. Through copulation, semen, composed of seminal plasma and spermatozoa is deposited in the female reproductive tract. The seminal plasma serves a role
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in buffering the acidic vaginal pH as well as providing an energy source (fructose and sorbitol) for the sperm as well as antioxidants such as ascorbic acid and hypotaurine to guard against oxidative damage to sperm.

A small fraction of the spermatozoa deposited in the vagina will enter the cervix, and in the absence of progesterone, coincident with ovulation a few sperm will be allowed to penetrate the cervical mucus to eventually reach the uterus. In the female reproductive tract, sperm undergo a process of capacitation in which sperm surface glycoproteins are removed resulting in a change in the membrane properties of the sperm and the transition to a state of hyperactive motility and the ability to undergo the acrosome reaction [12]. Finally a few hours after coitus a few sperm (tens to hundreds) will reach the ampullary region of the oviduct where spermatozoa come in contact with a recently ovulated oocyte surrounded by a mass of cumulus cells [13].

As sperm penetrate the loosely packed cumulus cells they reach the zona pellucida, the proteoglycan structure surrounding the oocyte. Interaction of the spermatozoan with the zona pellucida protein ZP3 initiates the acrosome reaction, and by vesiculation of the acrosomal membranes enzymes are released enabling penetration of the zona pellucida by the sperm [14]. Following zona pellucida penetration, the sperm membrane fuses with the oolemma, and in addition to introducing a haploid genetic complement to the oocyte also triggers oocyte activation, characterized by a series of intracellular calcium spikes that initiate the cortical reaction which is critical for the prevention of polyspermy [15].

In addition, activation of the oocyte results in the resumption of meiosis in the oocyte, which precedes male and female pronuclear formation. Oocyte meiosis concludes with the extrusion of a second polar body, yielding a diploid zygote. As the sperm nucleus is introduced to the oocyte cytoplasm, the nuclear membrane breaks down, and chromatin decondensation occurs relatively rapidly as protamines are removed and replaced by maternally derived histones. At this point both maternal and paternal sets of chromosomes acquire a membrane and form pronuclei. Male and female pronuclei migrate toward the center of the zygote, and at the same time DNA replication of each haploid set of chromosomes occurs. As DNA replication is completed and the pronuclei come in close proximity, the membranes break down, and synapsis occurs marking the final event of fertilization and the initiation of embryonic cell division [16].

The role of the sperm genome in embryogenesis

The paternal genome contributes half of the genetic material to the offspring, and therefore, the genetic state of the spermatozoon can have profound impacts on the viability and health of the early embryo, the fetus, and finally the offspring. As the process of gametogenesis is very complex, and the entire sperm population arises from a single sperm and egg and subsequently from a small number of primordial germ cells, subtle defects in the originating gametes or early in the process of gametogenesis can have profound impacts on the spermatozoon population and ultimately on the next generation. Genetic defects in sperm such as aneuploidies and de novo mutations or structural variations will be directly transmitted to the early embryo, and these effects have been well documented. Another potential source for a disruptive genetic state in offspring is elevated DNA fragmentation in the sperm, a relatively common feature in infertile men.

Single nucleotide polymorphisms and point mutations in sperm

In terms of size, single nucleotide polymorphisms (SNPs) and point mutations represent the smallest type of genetic variation, however their impact can be significant. These single-base changes are the most abundant source of DNA sequence variation in the human genome. Recent whole genome sequencing studies have revealed approximately 3.3 million single nucleotide differences within a given individual [17]. Single nucleotide polymorphisms are often, albeit arbitrarily, defined as polymorphisms whose minor allele is present in > 1% of the population; by contrast, point mutations are rare or de novo changes in DNA sequence. Recently, using whole-genome sequencing of two parent–offspring trios, the rate of de novo point mutation was directly estimated to be on the order of \(1 \times 10^{-8}\) per base per generation. This translates to an average of 30 de novo mutations per gamete [18].

Both SNPs and mutations in coding regions can be silent, with no effect on amino acid sequence; or they can be missense, resulting in the change of an amino acid; or nonsense, introducing a premature stop codon in a coding region. Alternatively they can be located outside of coding regions of genes and can have no effect, or can alter gene regulation by affecting gene
regulatory elements such as promoters, enhancers, or microRNAs.

Since by definition each individual SNP occurs in a significant proportion of the population there are few such common variants that are individually causal of disease, but they can confer increased risk for disease propensity. There are numerous examples of SNPs that confer risk for diverse diseases, and the number of SNPs identified as being associated with various complex diseases has grown rapidly in the past decade with the significant discovery power of genomic tools including SNP microarrays and whole genome sequencing. The maternal and paternal contributions of SNPs to offspring will be approximately equal because half of the DNA is derived from each parent. While numerous studies have evaluated the involvement of SNPs in male factor infertility, none has evaluated the effect of SNPs on embryogenesis and early development. We recently reported a small but significant increase in the frequency of minor alleles in somatic DNA from azoospermic men compared with controls based on a pilot genome-wide SNP association study [19]. The implications of these findings are currently unclear and warrant further research.

Because spermatogenic progenitors undergo a significantly greater number of cell divisions than germ cell progenitors in the female germline, it was predicted in the mid-1900s that the male germline would be more mutagenic than the female germline [20], and in fact whole-genome sequence analysis of human and chimpanzee indicates a six-fold higher mutation rate in the male germline [21]. While this male-driven mutation process has been observed across several million years of evolution, mutation rates and the source of mutations within a single generation can vary greatly [18]. The implication of a general increase in mutation rates in male versus female gametes is that on average, the majority of de novo mutations in offspring will be derived from the sperm.

As with SNPs, the studies to identify sperm-derived mutations that affect embryogenesis or early development have not been performed, however there have been numerous genes identified by mouse knock-out studies that result in embryonic lethality, so clearly functional mutations in sperm in any number of genes or regulatory elements could be responsible for disorders in embryo development, miscarriage, or developmental problems. The huge number of genes required for normal development and the diversity of phenotypes associated with reproductive complications make the identification of causal de novo mutations a daunting task.

Advanced paternal age is a reported risk factor for over a dozen Mendelian diseases, as well as a small number of complex developmental disorders such as autism [22]. One straightforward interpretation of this observation is that mutations are distributed stochastically across the genome, and sperm from older fathers are more likely to harbor random de novo mutations in Mendelian disease loci. However, a fascinating mechanism for the paternal age effect has been recently uncovered in the study of Apert syndrome, achondroplasia, and Costello syndrome [23]. These diseases are caused by de novo gain-of-function mutations in the genes FGFR2, FGFR3, and HRAS, which cause clonal expansion of the spermatogonia in which they occur. This mechanism, which is reminiscent of oncogenesis, is mediated through the growth factor receptor-RAS signaling pathways and is likely to occur in all men. Over the lifetime of a human male, their frequency is reported to reach as high as 1/10,000 spermatogonia within the testis despite their inception in one or a few spermatogonia. It remains to be seen to what extent selfish germline mutations such as these contribute to the pathogenesis of common human disease, and whether their existence can be detected by deep sequencing of germ cells or individuals. Because growth factor receptor-RAS signaling is used extensively throughout the body to control cell proliferation, it seems inevitable that mutations that confer selective advantages to spermatogonia will also perturb embryogenesis.

Genomic structural variations in sperm

Structural variations include insertions, deletions, duplications, and inversions in the genome. The term "structural variant" (SV) typically refers to submicroscopic changes in DNA, while larger events are termed cytogenic or chromosomal abnormalities, which will be discussed in the following section. Early definitions described SVs as events > 1 kb in length [24], however this definition was primarily based on technical limitations in the ability to detect smaller events. The coming of age of routine whole-genome sequencing has resulted in the expansion of the definition of SVs to include much smaller events – down to 50 bp in size [25]. Structural variants can be
identifying using genomic microarray analysis or whole genome sequencing. Figure 2.1 illustrates deletions and duplications of the Y-chromosome identified by SNP microarray analysis.

While SVs can arise by a variety of mechanisms, a predominant source for de novo SVs occurs during meiotic recombination of meiotic prophase I. Low copy repeat regions serve as a substrate for the genesis of SVs through non-allelic homologous recombination (NAHR) [26]. Because both male and female gametes only undergo meiosis once during gametogenesis, the rate of SV formation via NAHR is likely to be equal between males and females for most genomic locations. While several groups have evaluated the role of SVs in spermatogenic impairment [27–30], the role of sperm-derived SVs on fertilization and embryo development is unclear at present.

The best-characterized SVs that affect male fertility are the deletions of the azoospermia factor (AZF) regions of the Y chromosome, present in a significant proportion of azoospermic and severe oligozoospermic men [31], and first identified as distal Yq deletions in a subset of azoospermic men through karyotypic analysis [32]. While sperm retrieval is often possible in men with specific AZF deletions (e.g. AZFc), and embryo development and pregnancy rates following ICSI in AZFc-deleted men are similar to rates in men without deletions, the deletion will be transmitted to all male offspring, who will likewise be infertile.

Many of the structural variations contributed by the sperm to the early embryo will have little or no effect on embryo development, however larger SVs, particularly those that impact genes or regulatory elements may have profound effects on embryo and fetal development, or may increase disease susceptibility in offspring [33]. Numerous groups are working to characterize the extent of SV throughout the genome and to understand the impact that specific SVs have on phenotype. A more thorough assessment of the incidence of SVs in the sperm of infertile men compared with fertile controls will be necessary to better predict potential long-term risks of assisted reproductive technology to offspring health.