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Chapter

Paternal Effects on Embryonic, Fetal and Offspring Health: The Role of Epigenetics in the ICSI and ROSI Era

Jan Tesarik

Abstract

Paternal effects on the developmental potential of human embryos have been studied since the early 1990s, particularly with respect to newly emerging assisted reproduction technologies. Both genetic and epigenetic paternal effects can influence postfertilization development and cause implantation failure or miscarriage. However, it is only over the last few years that issues related to paternal effects associated with different assisted reproduction techniques on the health status of newborn and adult progeny have been focused. At the same time, new findings point out different, yet unexplored, areas of research into the potentially responsible factors, including the activity of the sperm-derived oocyte-activating factor and the oocyte signaling pathways mediating its action, the methylation status of both imprinted and non-imprinted genes, correct replacement of sperm nuclear protamines with oocyte-derived histones, the histone acetylation status, and the function of sperm-borne small RNAs. It is increasingly important to know how these developmentally important epigenetic regulators can be altered in the context of the current micromanipulation-assisted fertilization techniques, intracytoplasmic sperm injection (ICSI) and round spermatid injection (ROSI). Last but not least, transgenerational transmission of acquired, environmentally conditioned disorders from fathers to offspring is a newly emerging issue which warrants further research.

Keywords: paternal effects, embryonic health, fetal health, offspring health, epigenetics, ICSI, ROSI, assisted reproduction, epigenetic inheritance

1. Introduction

There are two types of paternal factors involved in problems of embryonic, fetal and offspring health: the genetic ones and the epigenetic ones. The terms "hard" and "soft" inheritance, first introduced in 1980 [1], are increasingly used to refer to the genetic and epigenetic inheritance, respectively [2, 3]. These terms reflect very nicely the nature of the two kinds of inheritance. The genetic inheritance, the hard one, is based on transmission of DNA sequences, and it is this type of inheritance which has been the main subject of human genetic studies for the past 60 years. On the other hand, a number of studies carried out over the past 20 years have revealed that phenotypes are affected by more complex layers of information besides DNA

sequences. These factors, interposed between the DNA sequence of a gene and its phenotypic expression, are termed epigenetic marks and can be modified by environmental exposures [4].

This chapter reviews the current knowledge about the factors affecting epigenetic marks in human gametes and embryos and about the ways how improper function of the male gametes during the process of fertilization can influence further embryonic and fetal development, with particular attention to possible adverse epigenetic effects related to the new micromanipulation-assisted fertilization technologies, intracytoplasmic sperm injection (ICSI) and round spermatid injection (ROSI).

2. Hard and soft hereditary elements

Hard hereditary elements are stable DNA sequences constituting specific genes that can only be modified by mutations or deletions. The system of soft hereditary elements is made up by a variety of molecules (epigenetic factors) that interact with each other and determine if, and to what extent, individual genes will be expressed at any given time of life. Early studies into epigenetic regulations in mammals largely focused on two constitutive events: genomic imprinting [5, 6] and X chromosome inactivation [7, 8]. However, later studies have pointed out that epigenetic regulation is a much more ample phenomenon than thought previously, involving a number of both imprinted and non-imprinted genes, and their role is particularly important in developmental processes, such as embryogenesis and organogenesis [9].

DNA methylation at the 5′ position of cytosin in CpG dinucleotides [2, 10] and histone acetylation are the two most widely known processes involved in epigenetic regulations. However, more recent studies have revealed other players in these complex processes, especially non-coding RNAs (siRNAs, microRNAs, and piRNAs) [11–18], some of which have been detected in the germline [14, 15, 17, 18], as well as factors regulating higher-level organization of chromatin [19, 20]. Some of these elements can be modified by environmental factors leading to alternate gene expression [18–20].

3. Gamete and embryo epigenetics

In spite of the ample knowledge about epigenetic mechanisms involved in gene expression control during the early embryogenesis fetal development and adult life [9], the environmental epigenetic inheritance through gametes was initially thought impossible because of the belief that all epigenetic marks, including DNA methylation, histone acetylation status and small RNAs, are completely erased and subsequently reset during germline reprogramming [21]. In mammals, these events take place both in the germline and in zygote immediately after fertilization [21, 22]. However, it is now known that this reset is not complete, and some parental imprinted loci can resist the global demethylation after fertilization, owing to the action of different mechanisms [23–26]. These findings explain previous observations on transgenerational transmission of environmentally conditioned disorders. For instance, the incidence of effects of parental ionizing irradiation on genomic instability in the offspring is too high to be explained by radiation-induced mutations which occur at a substantially lower rate [27]. Epigenetic inheritance through gametes can also explain transgenerational transmission of obesity [28, 29], diabetes [30, 31], and some types of cancer [32, 33].

All these data converge to suggest that epigenetic marks carried by the fertilizing spermatozoon are important for a variety of physiological and pathological processes that can affect offspring throughout life.

4. Potential sources of paternal epigenetic issues

It has been known since the early 2000 that human embryonic development is subject to paternal effects that can affect not only the early postfertilization events but also later phases of preimplantation and postimplantaion development [34–36]. Two types of paternal effects were distinguished: the early paternal effect and the late paternal effect [35]. The early paternal effect was reflected by an impairment of postfertilization development as early as the 1-cell zygote stage and subsequently was often associated with irregular cleavage divisions and blastomere fragmentation [34]. The late paternal effect was not detectable during the early cleavage stages, but became manifest after the 8-cell stage [35, 36]. Unlike the early paternal effect, the late paternal effect was often, though not always, associated with abnormally increased levels of DNA fragmentation in the father's sperm [35]. Both types of paternal effect reduced the chance of pregnancy [36].

In view of more recent data about sperm epigenetics, the meaning of these early observations can now be extended and reinterpreted. Since both the early and the late paternal effect were evaluated only in patients treated by intracytoplasmic sperm injection (ICSI), facilitating fertilization with spermatozoa carrying different morphological and functional abnormalities, which would not be capable of fertilizing oocytes by their proper means, the analysis of the resulting embryos can yield valuable information about the impact of sperm abnormalities and immaturity on fertilization and early development.

4.1 Fertilization with abnormal spermatozoa

ICSI has made it possible to achieve fertilization, embryonic and fetal development and childbirth by using spermatozoa with severe morphological and functional abnormalities. However, these abnormalities cannot be blamed for most of the negative paternal effects observed in ICSI-derived embryos. In fact, data have shown that fertilization with spermatozoa from certain individuals consistently leads to the formation of embryos with developmental abnormalities detectable as early as the 1-cell zygote stage [34]. Interestingly, these abnormalities almost always included abnormal pattern of the formation of nucleolar precursor bodies (NPBs) in the zygote pronuclei [34], as characterized previously [37]. The process of NPB assembly in human zygotes requires an early onset of RNA synthesis activity in the adjacent chromatin regions [38, 39]. The nature of the RNA molecules synthesized at this stage is unknown, but they are likely to be non-coding ones, since the first signs of embryonic gene expression can only be detected between the 4-cell and the 8-cell stage of the human preimplantation development [40–42]. These non-coding RNA species may be involved in the early epigenetic events that condition further embryonic development. Interestingly, the assembly of NPBs and the accompanying pronuclear RNA synthesis coincide with the assembly of microtubule organizing centers in human zygotes [43], and abnormalities of NPB assembly are associated with abnormal development of human preimplantation embryos [37, 44] and an increased risk of embryo aneuploidy [45].

Some conditions potentially responsible for sperm epigenetic abnormalities, such as advanced paternal age [46], tobacco smoking [47], and various lifestyle factors including dietary habits, physical activity or alcohol consumption [48], have been pointed out. However, a more comprehensive analysis of factors involved in these phenomena remains a big challenge for future research.

4.2 Fertilization with immature male germ cells

The final stage of sperm maturation is achieved during sperm passage through the epididymis. However, spermatozoa recovered directly from the testis [49, 50] and even round spermatids [51–55] are able to fertilize human oocytes and generate normal offspring when incorporated into oocytes via ICSI and ROSI, respectively. Moreover, live offspring was born in mice after fertilization with secondary spermatocytes [56], and in humans after fertilization with round spermatids developed in vitro from germ cells of men with spermatogenic arrest at the primary spermatocyte stage [57, 58].

Recent reports on the postnatal development of 108 babies born after fertilization of oocytes by round spermatid injection (ROSI), 90 of them in Japan and 18 in Spain [59], did not show any significant differences as compared with naturally conceived babies in either physical or cognitive development during the first 2 years after birth, and none of them developed any of the syndromes associated with genomic imprinting defects [59]. Thus, the use of immature male germ cells for fertilization, in spite of the still relatively low success rates, does not appear to be associated with an increased risk of epigenetic abnormalities in the offspring.

4.3 Disorders of paternally induced oocyte activation

As discussed in the previous sections, human embryonic development appears to be particularly sensitive to epigenetic events taking place during an early phase of the fertilization process, referred to as oocyte activation. Oocyte activation has been extensively studied since the mid-1990s, especially in relation with the new technologies. Both ICSI and ROSI avoid the initial contact between the surfaces of the fertilizing spermatozoon and the oocyte preceding their fusion during natural fertilization. Yet, this contact activates a series of signal transduction events that participate in physiological oocyte activation [60, 61].

These early signal transduction events are obviously by-passed when oocytes are fertilized by ICSI. This shortcut, however, does not prevent fertilization in most mammalian species. In fact, sperm-induced oocyte activation is driven by repeated rises of free cytosolic Ca^{2+} ions, referred to as calcium oscillations, mediated by periodic release and uptake of calcium by two types of calcium stores (basically endoplasmic reticulum), one opened by inositol trisphosphate (IP3) and the other by the very increase in free cytosolic calcium in its vicinity [62, 63]. In order to sustain the periodic calcium oscillations, the oocyte's calcium stores have to be sensitized by a soluble factor released from the fertilizing spermatozoon, initially called "oscillin" and later identified as a special form of phospholipase C (PLC) referred to as PLCζ [64]. Even in the absence of the initial contact between the sperm and oocyte surfaces, the release of PLCζ from the injected spermatozoon to the oocyte cytoplasm, together with an early extracellular calcium influx produced by the ICSI procedure itself, is sufficient to sustain calcium oscillations needed for proper oocyte activation [65]. However, the temporal pattern of the oscillations after ICSI (**Figure 1**) is slightly different from that following sperm-oocyte fusion (**Figure 2**). The release of PLCζ from the spermatozoon to the oocyte cytoplasm

marks the spatial pattern of the first calcium rise, which propagates in a wave-like manner from the sperm position site across the oocyte (**Figure 3**).

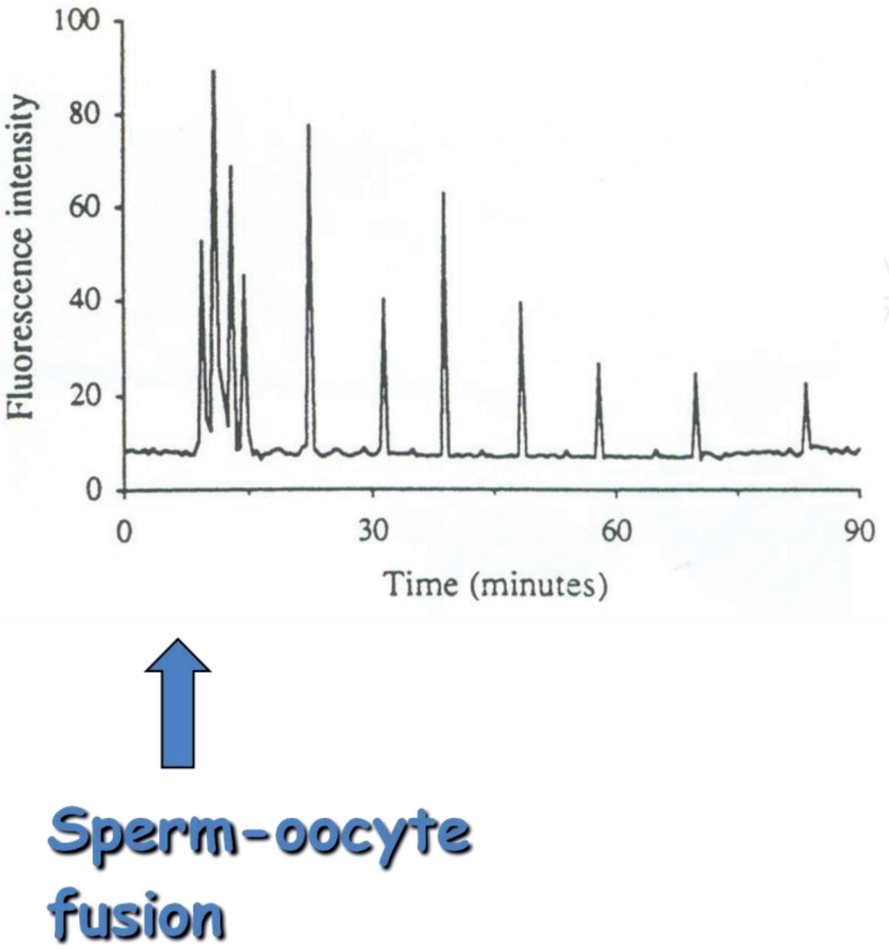

Figure 1.
Oscillations of free cytosolic Ca^{2+} concentration induced by sperm-oocyte fusion, recorded by confocal microscopy in a living human oocyte loaded with fluorescent calcium indicator Fluo-3 as described [65].

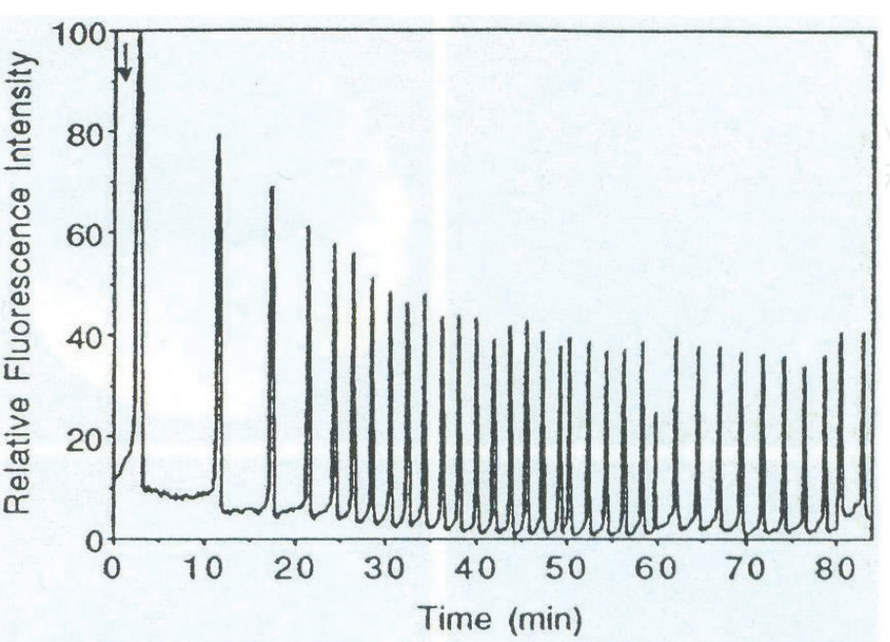

Figure 2.
Oscillations of free cytosolic Ca^{2+} concentration after ICSI (arrow), recorded by confocal microscopy in a living human oocyte loaded with fluorescent calcium indicator Fluo-3 as described [65].

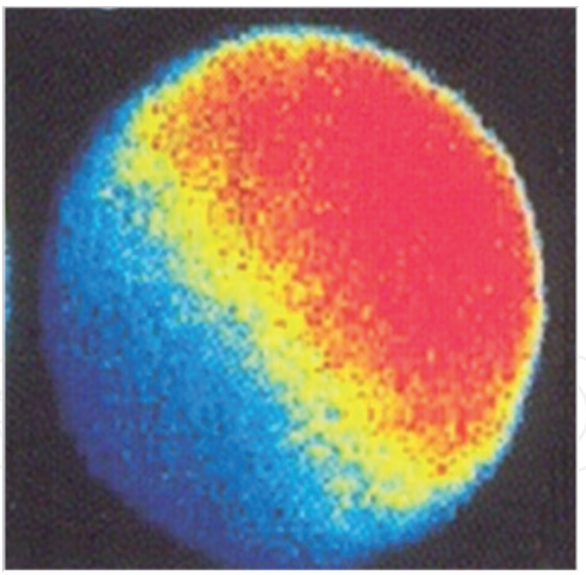

Figure 3.
Spatial propagation of the first sperm-induced increase in free cytosolic Ca^{2+} concentration, recorded by confocal microscopy in a living human oocyte loaded with fluorescent calcium indicator Fluo-3 as described [65].

In spite of the fact that the slight difference in the temporal pattern of the first sperm-induced calcium rise does not appear to have any impact on zygote and embryo development, the frequency and duration of the ongoing calcium rises have been shown to affect embryo development in different mammalian species [66–68] including the human [69]. Recent findings have pointed out the possibility that inherent abnormalities of the sperm-born PLCζ, but also in the oocyte response mechanisms, including steps downstream of the calcium releasing machinery, may influence mitotic divisions and gene expression during subsequent development and have to be considered an additional epigenetic risk factors to be taken into consideration in relation with the current-assisted fertilization technologies [70].

5. Current clinical experience

Several studies have suggested that both IVF and ICSI may increase the risk of certain types of birth defects in general [71–73] and heart defects in particular [74, 75]. However, there does not appear to exist a significant difference between the children conceived by conventional IVF and by ICSI [76, 77]. During the early years of its use, ICSI was preferentially employed in cases of poor sperm quality, while conventional IVF was used in cases with normal or nearly normal sperm, and the lack of significant differences between the outcomes of conventional IVF and ICSI suggests that sperm quality, as reflected by spermogram, spermocytogram and other standard sperm evaluation methods, has little, if any, impact on the health of offspring. Hence, the trend toward a higher risk of birth defects after IVF and ICSI as compared with natural conception is likely to be related to different, largely sperm-independent factors, such as the underlying cause of infertility, higher maternal age or a higher incidence of twin pregnancies [75, 78].

Even though there is no strong correlation between the conventional sperm parameters and ICSI outcome, sperm abnormalities, especially morphological ones, were shown to be associated with different genetic and epigenetic abnormalities [79], such as increased sperm DNA fragmentation [80] or abnormal patterns of DNA methylation [81]. Morphological abnormalities of the human sperm head

have been shown to be associated with different types of genetic abnormalities [79], increased sperm DNA fragmentation [80] and different potentially harmful epigenetic factors, such as abnormal patterns of DNA methylation [81] and the absence or defective function of the sperm-derived oocyte-activating factor [82]. These observations explain the findings of increased implantation and pregnancy rate and decreased miscarriage rate [83–85], as well as a significantly decreased risk of major birth defects [86, 87], with the use of high-magnification ICSI (IMSI) as compared with conventional ICSI, although some studies failed to confirm these differences [88, 89].

As to fertilization by ICSI with immature (testicular) spermatozoa and by round spermatid injection (ROSI), the initial fears that incomplete or defective DNA methylation and chromatin configuration of these immature germ cells might cause syndromes related to genomic imprinting abnormalities [90] were not confirmed. In fact, no increase in the frequency of health problems caused by genomic imprinting abnormalities, such as Beckwith-Wiedemann, Prader-Willi, and Angelman syndromes, has been detected in children born after ICSI with testicular spermatozoa [91] and ROSI [59]. A recent study [92] has suggested that the supposed increase of imprinting errors, present in the sperm of infertile patients, does not have an obvious influence on assisted reproduction outcome or the imprinting of offspring, probably because the imprinting errors in sperm are selectively discarded or corrected during development [20, 93].

In contrast to the reassuring clinical data concerning the potential epigenetic risk of using abnormal and immature male germ cells for fertilization, there is increasing concern about the possible transmission of epigenetic abnormalities and diseases acquired during the father's life via his spermatozoa. This risk is difficult to evaluate with the use of currently available diagnostic methods because it is not necessarily associated either with sperm morphology or with its DNA integrity. It was actually demonstrated in humans that nutritional status and physical activity levels were associated with dynamic epigenetic changes in spermatozoa, including DNA methylation patterns and small RNA expression [94–97]. This kind of acquired epigenetic changes in spermatozoa is suspected to mediate transgenerational epigenetic inheritance of neurological disorders [95] and susceptibility to diabetes [31] and obesity [96, 97], and this list does not appear to be definitive, since new evidence of environmentally driven sperm-borne epigenetic factors, which are capable of altering the phenotype of the next generation, is emerging on a large scale. Paternal aging [46] and smoking [47] were also shown to affect sperm DNA methylation patterns, with still ill-defined developmental and health consequences for the offspring.

6. Future prospects for paternal epigenetic diagnosis and treatment

Abnormal patterns of sperm DNA methylation, small RNA expression, and chromatin configuration can now be detected with a relative ease. However, many of these abnormalities lack clinical significance because they might be corrected during fertilization and postfertilization development. The mechanisms responsible for this repair are still largely hypothetical and poorly understood. However, they are very likely to exist because a recent publication, based on the analysis of 1280 IVF-related treatment cycles, did not show any influence of either male age or sperm parameters on clinical pregnancy and live birth outcomes [92], in spite of the existence of data suggesting that there are very clear consequences of aging in the sperm epigenome that can be directly detected in DNA methylation patterns [46].

Obviously, further studies are needed to assess whether any effects of male age and sperm parameters on the offspring health status can be detected later in life.

In order to be able to distinguish between "benign" paternal epigenetic alterations that can be repaired spontaneously, on the one hand, and clinically relevant alterations that can cause negative effects on the embryonic, fetal and offspring health, studies are needed to relate DNA methylation status of specific genes and the expression pattern of specific small RNAs with specific developmental abnormalities. This work can be done by analyzing nucleic acids extracted directly from sperm cells or by using the "liquid biopsy" approach, based on the use of soluble nucleic acids isolated from blood plasma or seminal fluid. This latter approach is particularly interesting in azoospermic men so as to avoid the need for testicular biopsy to obtain a sample.

The identification of the developmentally relevant sperm epigenetic abnormalities is a necessary pre-requisite to design possible therapeutic interventions. These may go from relatively simple to more complex ones. It has been shown in the mouse that several waves of microRNAs and tRNA fragments are shipped to sperm during post-testicular maturation in the epididymis [98]. If some pathogenic paternal epigenetic signals are conveyed to sperm essentially during epididymal passage, ICSI with spermatozoa retrieved surgically from the testis, the technique already used with success in men with elevated levels of sperm DNA fragmentation [99, 100], might be a relatively simple and immediately available solution. If this approach is not possible, other, more sophisticated technologies, such as injecting specific microRNA molecules, capable of repairing specific epigenetic defects, into the early zygote [98] or induction of DNA methylation of the genes of interest by a Dnmt3-type *de novo* DNA methyltransferase targeted to the corresponding sperm DNA sequence by a nuclease-inactivated CRISPR variant (dCas9) [101], may be explored.

7. Conclusions

Since the availability of cell micromanipulation technologies enabling fertilization of human oocytes by ICSI with immature (testicular) spermatozoa and by round spermatid injection (ROSI), the role of paternal factors on embryonic, fetal, and offspring health needed a profound revision. While genetic abnormalities contributed by injected spermatozoa or spermatids can be controlled by preimplantation genetic testing and usually lead to a miscarriage, sperm-borne epigenetic abnormalities are much more difficult to detect and may be at the origin of different health problems throughout the offspring life. The current knowledge of the origin, nature, and mechanism of action of these sperm-borne epigenetic factors is outlined in this chapter. Surprisingly, in spite of multiple types of sperm epigenetic abnormalities associated with defective spermatogenesis and male aging, the current clinical experience is reassuring. In fact, no significant increase in the prevalence of diseases attributable to abnormal genomic imprinting was detected in children conceived by testicular spermatozoa or spermatids, probably because of the existence of efficient repair mechanisms acting in postfertilization stages of development.

By contrast, there is increasing evidence suggesting that transgenerational inheritance of paternally acquired epigenetic abnormalities via spermatozoa is more frequent than previously thought and can occur even in cases with normal conventional sperm parameters and during natural conception. The known pathologies transmitted in this way include neurological disorders, obesity, and diabetes, and their list is in continuous expansion. Future diagnostic and therapeutic possibilities applicable in these cases are discussed.

Conflict of interest

The author declares no conflict of interest related to this chapter.

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