Contemporary Use of Intracytoplasmic Sperm Injection

Subjects: Reproductive Biology

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Intracytoplasmic sperm injection (ICSI) has been used for severe male factor infertility and non-male factors, such as unexplained infertility or advanced maternal age, without robust scientific evidence. However, applying ICSI blindly is not free of potential detrimental consequences since novel studies report possible health consequences to offspring. DNA methylation and epigenetic alterations in sperm cells of infertile men might help explain some of the adverse effects reported in ICSI studies on reproductive health in future generations.

assisted reproductive technology | human in vitro fertilization | embryo development | male infertility | intracytoplasmic sperm injection

1. Background

Over the past 40 years, assisted reproductive technology (ART) has evolved from an ambitious and experimental procedure to mainstream medicine. This has been obtained thanks to the constant advancements in ovarian stimulation and luteal phase support protocols, sperm preparation techniques, fertilization, and embryo culture methods, and importantly to the progress in cryopreservation of gametes and embryos, which improved pregnancy outcomes and live birth delivery. Worldwide, around 9 million children have been conceived by ART, and more than 3 million cycles are performed globally every year [1][2]. The IVF process is primarily dependent on three procedures: ovarian stimulation (OS), in vitro fertilization (IVF), or ICSI, and embryo culture. However, the process omits critical physiological reproductive steps and it includes a variable degree of invasiveness with unknown consequences. On this basis, the safety of these methods has been questioned. Historically, medically assisted reproduction (MAR) practices have been reported to be safe as most ART babies are healthy [3][4]. However, recent studies report that singletons born following IVF/ICSI treatments have an increased risk of adverse perinatal outcomes, which might be associated with epigenetic dysregulation, such as abnormal placentation or low birth weight [5][6].

Currently, there is an active debate that ART interventions, such as OS and extended embryo culture to the blastocyst stage, could promote adverse epigenetic effects, considering they occur when most epigenetic reprogramming takes place [7]. Due to the invasiveness of ICSI as a fertilization method, the technique is frequently debated as potentially causing epigenetic dysregulation. The procedure requires the injection of a single sperm cell directly into the oocyte cytoplasm using a narrow and sterile micropipette. This technique introduced in 1992 [8] represents one of the most remarkable changes in the field of MAR, allowing men with low sperm numbers and/or

abnormal sperm parameters to become biological fathers [9]. Nowadays, ICSI is an established procedure applied worldwide to treat couples with infertility. However, its application is still the object of debate, particularly concerning its potential adverse consequences on the health of the resulting offspring. In line with that, new data from epigenetics studies highlight possible associations between events occurring in early and adult-onset diseases and male infertility [10]. Male infertility is the primary indication for the treatment in around 30% of couples undergoing ART [11][12]. Given the importance of the sperm epigenome to early embryogenesis, the implications of using sperm from males with fertility problems for ICSI, have to be addressed.

The epigenetic regulation of gene activity represents a critical aspect of sperm function and related fertilizing ability [13]. Recent evidence has shown that disruption to the paternal epigenome can induce male infertility and transfer aberrant information to the embryo. One key element of controlling male gamete function involves post-translational modification of histones (PTMs), such as methylation (me), acetylation (ac), and phosphorylation (ph), which allows for activation or repression of underlying genes [14]. Histone PTMs are essential in governing cellular processes, such as transcription, DNA repair, DNA replication, and chromosome condensation [15].

2. Imprinting Alteration following ART

After fertilization, the zygote develops into a structure called the “blastocyst”, which includes about 200 cells, already differentiated into two types: the trophectoderm (TE) and the inner cell mass (ICM). The latter comprises a group of cells attached to the inside of the trophectoderm, which will eventually give rise to the fetus. TE cells are the blastocyst’s external layer, promoting the implantation process into the uterine lining and forming other extraembryonic tissues, including the placenta. Embryonic cells are guided toward their future lineages during early development through epigenetic reprogramming and subsequent re-establishment of cell-type-specific epigenetic signatures. This coincides with the period when gametes and embryos are cultivated inside the embryology laboratory. Therefore, during this critical time window, any artificial perturbations might lead to epigenetic modifications in the resultant offspring (**Figure 1** and **Figure 2**). Studies reported imprinted loci to be vulnerable to external environmental cues during in vitro embryo culture. For example, *KvDMR1* has been abnormally methylated in ART-related BWS in humans [16][17][18] and hypomethylated in ART-produced bovine progeny with large offspring syndrome (LOS) [19]. Additionally, several reports indicate that ART-related procedures, including OS, ICSI, and extended culture to the blastocyst stage, might promote epigenetic aberrations [16][20][21].

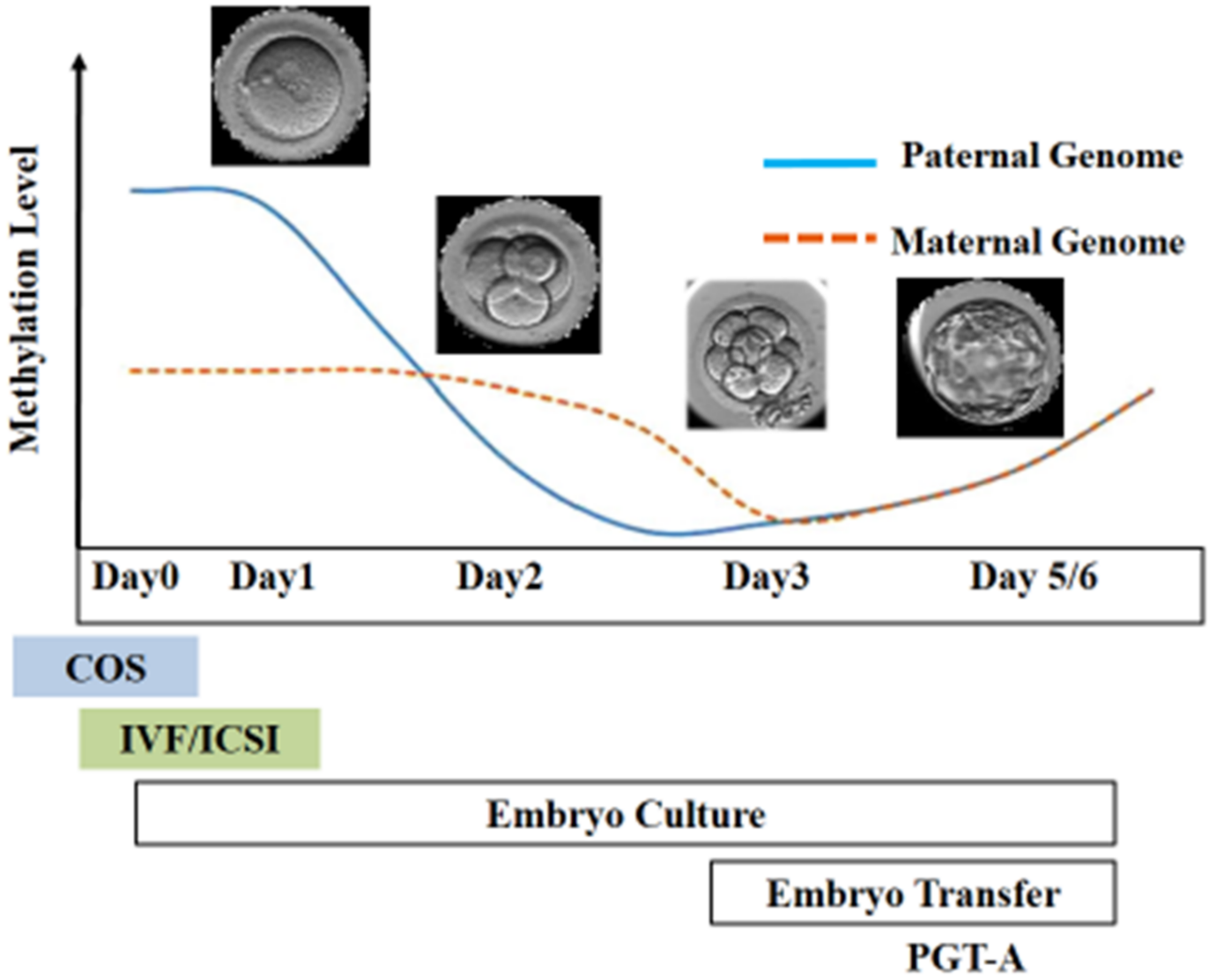

Figure 1. DNA methylation and epigenetic reprogramming during the early stage of embryo development. The paternal genome undergoes active demethylation post-fertilization, whereas the maternal genome is passively demethylated.

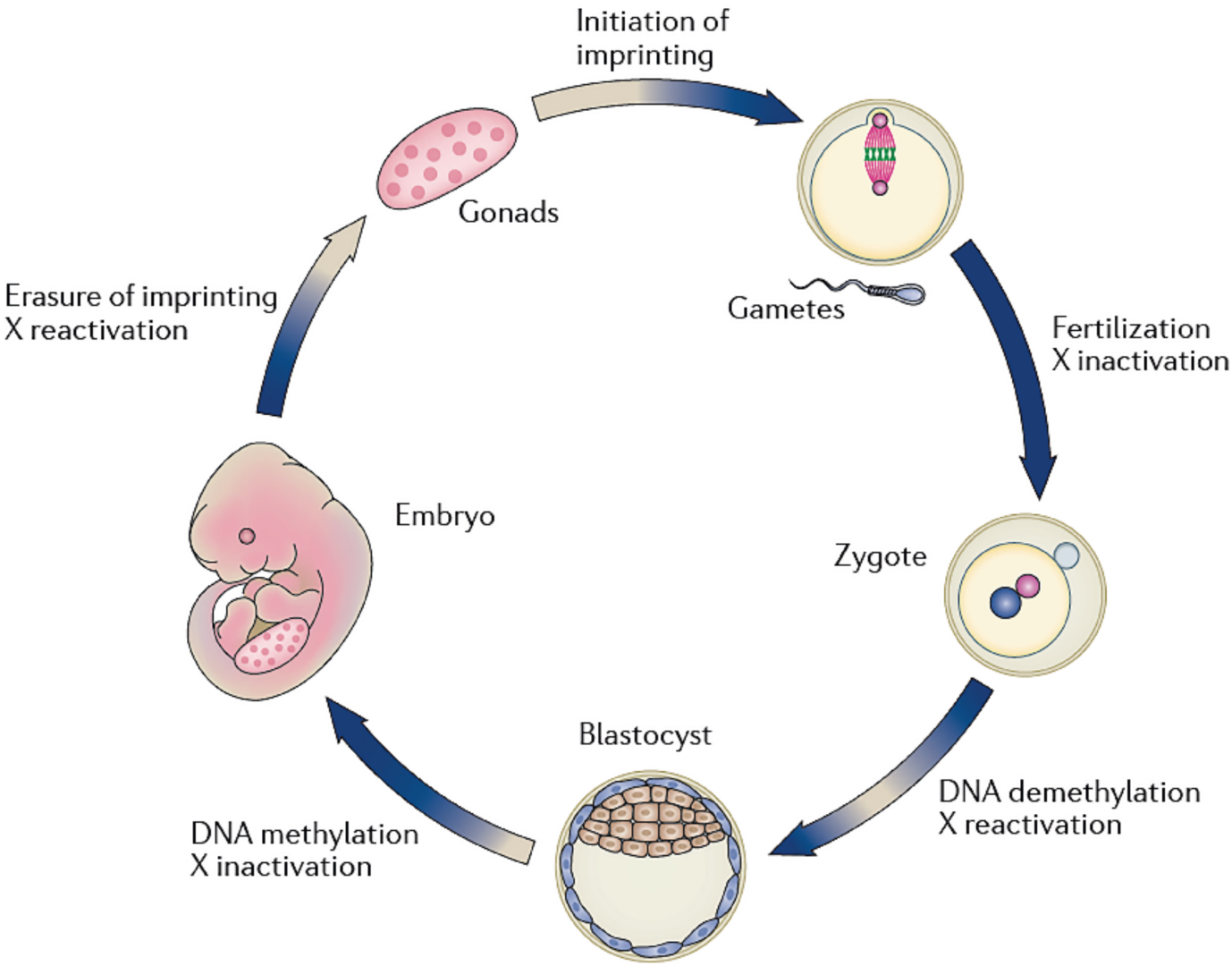

Figure 2. The epigenetic reprogramming cycle. The two major waves of epigenetic reprogramming occur during gametogenesis and after fertilization. During gametogenesis, most parental epigenetic marks are erased and re-established at the time of oogenesis and spermatogenesis. A second epigenetic reprogramming wave occurs soon after fertilization with a fast, active paternal demethylation and a slower, passive maternal demethylation. New methylation patterns are established at the blastocyst stage in the inner cell mass, while the trophectoderm stays relatively unmethylated. Adapted with permission from Ref [22].

3. Spermatogenesis, Epigenetics, and Infertility

Male fertility depends on the production of healthy sperm cells by the testis. This process is known as spermatogenesis and can be described by three main steps: first, the mitosis with the multiplication of the spermatogonia, then meiosis to reduce the number of chromosomes from diploid to haploid, and finally the spermiogenesis, which indicates the successful maturation of round spermatids into spermatozoa [23]. All of these processes are linked together and are responsible for normal sperm production; any alteration during spermatogenesis may cause a reduction in sperm quantity and quality. Recent evidence indicates that the dynamic

of epigenetic reprogramming and their regulatory systems are fundamental for normal spermatogenesis. Any disturbances of these epigenetic regulations might result in different infertility stages, which could be transferred to future generations [24][25]. Abnormal DNA methylation is linked with changes in histone formations, dysregulation of lncRNA, and abnormal protamination, which might induce male infertility. Along these lines, histone modifications have been investigated in mature sperm. Ben Maamar and coworkers examined the alterations in DNA methylation during the early stage of gametogenesis from primordial germ cells (PGCs) to sperm. Several DNA methylation regions at the different developmental stages were analyzed. The study recognized a compelling cascade of epigenetic changes during the early developmental stages, indicating alterations to regulate gene function and expression during gametogenesis [26]. Furthermore, even after spermatogenesis is completed with the formation of the sperm cells, extra maturation takes place in the epididymis [27][28]. The sperm cell, following the release into the seminiferous tubules and the rete testes, will cross the efferent ducts into the epididymis, where further maturation occurs. During this passage, the epididymal cells produce specific proteins acquired by the sperm to achieve motility after ejaculation. Therefore, the sperm's capability to achieve motility is mainly gained during epididymal transit [29][30]. Epigenetic regulation during epididymal maturation of the sperm cells remains to be clarified. Although the sperm nuclei are transcriptionally inactive due to the DNA compaction associated with protamines, it has been reported that environmental chemicals such as DDT or vinclozolin might induce epigenetic alterations, especially DNA methylation between caput and cauda epididymal sperm stage [31][32]. Indeed, during sperm epididymal maturation, histone modification and DNA methylation took place as additional epigenetic regulation, critically important for the sperm's function and formation [33].

4. ICSI for Male Factor Infertility

4.1. Oligoasthenoteratozoospermia

Although ICSI should be encouraged mainly in severe male infertility, it can be challenging to establish when a male factor is compulsory for the ICSI technique. Standard semen assessment is performed to confirm the severity of male infertility and advise ICSI, but it is well reported that sperm analysis has limitations; for example, it does not assess the function and physiology of the sperm, and genetic or epigenetic assessment [34]. Sperm number, morphology, and motility are typically evaluated to decide on the ICSI procedure rather than standard IVF insemination [35]. It is worth mentioning that high-quality studies investigating pregnancy outcomes and live birth rate (LBR) between ICSI and IVF in couples with oligoasthenoteratozoospermia are still missing. However, a study published in 2005 by Shuai and collaborators explored these concerns. The scholars observed no differences between the two insemination procedures (IVF and ICSI) in fertilization, implantation, and pregnancy rates in couples undergoing ART with men diagnosed with moderate oligoasthenoteratozoospermia [36]. Sperm morphology is another parameter broadly used to choose for ICSI. In 1986, Kruger and colleagues suggested using strict criteria for sperm abnormalities and advising ICSI when the proportion of normal sperm in the ejaculate was <4% [37]. Additional studies confirmed this evidence and proposed that at least 5% of sperm is needed to be morphologically normal to obtain an acceptable fertilization rate using standard IVF [38][39]. Therefore, ICSI rather than IVF has been routinely recommended in patients with reduced sperm morphology (<5%) [40].

4.2. Azoospermia

The term azoospermia indicates the absence of sperm cells in the ejaculate. It affects around 1% of the general male population and about 15% of infertile men [41]. There are two different types of azoospermia: obstructive and non-obstructive. In obstructive azoospermia, normal and complete spermatogenesis is typically found, and sperm can be surgically collected from the testis [42]. By contrast, non-obstructive azoospermia is associated with the testicular alterations that result in the failure of sperm production. Typical testicular histopathological features in males with non-obstructive azoospermia include germ cell aplasia, maturation arrest, or hypospermatogenesis. The procedures mostly applied to collect sperm from azoospermic patients are percutaneous acquisition and open surgery [43]. Following sperm retrieval, ICSI can be applied to achieve oocyte fertilization [44].

4.3. Antisperm Antibodies

The presence of seminal antisperm antibodies (ASAs) is typically associated with a gap or rupture of the blood–testis barrier in the reproductive tract, which can be linked with several conditions [45]. However, elevated levels of ASAs in semen samples are observed in about 5–12% of men undergoing ART, and might negatively affect fertility, reducing sperm motility, capacitation, acrosome reaction, and oocyte sperm bounding [46].

4.4. ICSI and Sperm DNA Fragmentation (SDF)

DNA fragmentation test is applied to assess the breakage of DNA strands inside the sperm head. This diagnostic test can predict fertility and normal embryo development and pregnancy outcomes than routine semen analysis parameters [47][48]. With the use of probes, sperm DNA breaks can be deeply scrutinized and quantified with the aid of fluorescence/optical microscopy or flow cytometry [48]. Sperm DNA fragmentation (SDF) is generally induced by oxidative stress resulting from environmental and lifestyle factors such as smoking, genital tract infections, obesity, and nutrition [49]. Moreover, SDF is frequently detected in men with infertility issues (e.g., varicocele), and it is more prevalent in those individuals than in fertile counterparts [50][51]. Scientific evidence indicates that a high level of SDF impairs the probabilities of success following ART [52][53].

4.5. Globozoospermia

This condition is described by the entire lack of the acrosomal vesicle in the sperm head, with alteration of the nuclear membrane, and midpiece defects, resulting in a round-shaped sperm head. It is an uncommon condition involving a small percentage of infertile men (about 0.1%) [54]. Despite having normal sperm count and motility, globozoospermic sperm cannot fertilize the oocyte: therefore, ICSI remains the favorable option available.

5. Use of ICSI for Couples with Partners Having Semen Analysis within Reference Ranges

One of the first Cochrane paper was published in 2004 by van Rumste and collaborators to investigate whether ICSI improves LBR compared to IVF in couples whose male partners had semen analysis within reference ranges.

The scholars showed a significantly higher fertilization rate in the IVF group but no difference in pregnancy, miscarriage, or LBR than ICSI insemination [55]. Subsequently, Bhattacharya and co-workers performed a multicenter randomized controlled study comparing clinical outcomes after ICSI or traditional IVF in couples with male partners having semen assessment within references ranges. The study randomly assigned 415 couples and was performed in four UK IVF units. Their results showed that the fertilization rate was higher with IVF than with ICSI (58% versus 47%; $p = 0.0001$). Standard IVF insemination provided an implantation rate of 30% compared to 22% for ICSI ($p = 0.03$). No significant difference was observed regarding the clinical pregnancy rate between IVF and ICSI (33% and 26%, respectively). Moreover, the overall laboratory time used was significantly shorter with IVF than with ICSI (22.9 min versus 38.1) [56]. Dang and co-workers reported similar results. They randomized 1064 patients undergoing ART to ICSI technique ($n = 532$) or standard IVF insemination ($n = 532$). After the first embryo transfer, LBR was 35% in the ICSI group versus 31% for couples assigned to conventional IVF ($p = 0.27$). They found higher TFF with IVF (6%) than with ICSI (5%). The study concluded that in couples undergoing ART with a male partner having so-called normal semen parameters, ICSI did not increase LBR compared with conventional IVF [57].

6. Contemporary Use (and Overuse) of ICSI

Since its first use almost 30 years ago, the application of ICSI as a fertilization method has raised steadily, even though the percentage of infertile couples with severe male factors has not increased [58]. Thus, it seems evident that currently, ICSI is applied broadly, even though there is no clear evidence of its benefit in couples without male factor infertility [55][56][58][59][60]. Boulet and collaborators analyzed data on ART between 1996 and 2012 and reported increased use of ICSI from 36.4% in 1996 to 76.2% in 2012, even though male-factor infertility remained unchanged at about 36% of cycles [61]. Another trial published by Dyer and colleagues analyzing the worldwide data on ART performed between 2008 and 2010 found that ICSI was used as a fertilization method in about 67% of about 4.5 million cycles completed [59]. However, there is considerable variation according to countries; in Asia, ICSI is applied in about 55% of the treatments, 65% of cases in Europe, 85% of patients in Latin America, and almost 100% of patients in the Middle East [59]. Moreover, in a large retrospective study performed in Australia between 2002 and 2013, analyzing about 585 thousand ART cycles, the scholars did not report any improvement when ICSI was used rather than standard IVF insemination for couples without obvious male infertility. They observed an LBR of about 10% lower with ICSI than IVF [62]. On this basis, one should ask why is ICSI preferred to standard IVF in routine practice for cases without a clear male factor? Possible factors to justify the broad ICSI application related to a general notion that ICSI reduces the risk of TFF. Naturally, fertilization failure is problematic to any couple undergoing ART; especially when counseling is not available and the physician is the person involved in delivering this bad news. In addition, in private settings, where the couple needs to pay for the treatment, the failed fertilization also represents a remarkable burden for the couple who will need to bear the costs of another cycle. The debate is ongoing [57] and the Practice Committee of the American Society for Reproductive Medicine (ASRM) has recently produced a committee opinion paper recommending against the extensive use of ICSI in couples undergoing MAR cycles without confirmed male factor infertility [63].

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