Spermatozoon Effect on Embryo Development

Subjects: Others

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The intracytoplasmic sperm injection (ICSI) technique was invented to solve severe male infertility due to altered sperm parameters. It is applied worldwide for the treatment of couple infertility. ICSI is performed with any available spermatozoon from surgery or ejaculated samples, whatever are the sperm motility, morphology or quantity. The causes of male infertility are crucial in building a competent spermatozoa that will contribute to normal embryonic development and healthy offspring.

sperm DNA embryo development ICSI male infertility

1. Sperm Parameters

Intracytoplasmic sperm injection (ICSI) is the chosen fertilization method when at least one sperm parameter is altered, such as sperm count (oligozoospermia), sperm motility (asthenozoospermia), or sperm morphology (teratozoospermia).

Many ICSI studies failed to correlate sperm parameter alteration after ejaculation with embryo development [1][2][3], concluding that ICSI resolves male infertility due to the semen parameters alteration. Nevertheless, data correlating the morphology of the micro-injected spermatozoon in ICSI or IMSI outcomes evidenced the paternal effect on embryo development. In ICSI, the micro-injection of morphologically abnormal spermatozoa can result in decreased fertilization and implantation rates [4]. In IMSI, the increasing volume of vacuoles in the micro-injected sperm head was correlated with a decreasing blastocyst rate [5][6][7] and a delay in embryo development following the pronuclear fading time and continuing all along the cleavage stage [7][8]. The sperm head vacuoles would be the sign of incorrect sperm DNA packaging [9], protamine organization [10], and DNA fragmentation [11].

Globozoospermia is a rare morphological sperm abnormality characterized by a round-headed spermatozoon due to a lack of acrosome and a coiled tail [12]. For those less than 0.1% of infertile men with globozoospermia, ICSI is the only solution to becoming a father with their own gametes. Compared to non-globozoospermic sperm, the fertilization rate is diminished. Oocyte activation fails due to sperm-specific phospholipase PLCζ under-expression or inactivity [13], defective sperm chromatin condensation, and sperm DNA damage [14].

In absolute asthenozoospermia, the fertilization rate is significantly decreased with regard to sperm vitality and origin [15]. The combination of oligoasthenoteratozoospermia was found to correlate with a delay of 4-cell and 5-cell stages and cell division synchronization s1 and s2 [16].

2. Sperm DNA

In the later stages of spermatogenesis, the spermatozoon undergoes molecular remodeling. Histone proteins are substituted by protamines and haploid sperm DNA is broken on one or two strands in several parts to occupy as little space as possible inside the sperm head. The histone–protamine transition occurs at the epididymis level. In addition to the physiological sperm DNA fragmentation (SDF), several clinical and environmental factors are known to have negative impacts on sperm DNA integrity, increasing the percentage of SDF. Numerous studies emphasize the direct relationship between SDF and male infertility and increased miscarriage rate after IVF or ICSI [17]. Whereas low SDF can be repaired by the competent oocyte, high levels of SDF were correlated with embryo morphokinetic delay observable from the time of of pronuclei fading to the morula stage [18][19][20][21]. The consequences are lower blastulation and pregnancy rates [22]. The adverse effects are stronger when both sperm DNA stands are broken [23].

Maternal age is known as the main factor of embryonic aneuploidy [24] but spermatozoon can contribute to aneuploidy too, especially in males with reduced sperm concentration [25][26]. Increased paternal age over 50 years old is associated with damaged DNA, lower blastocyst rate, and a significantly higher number of trisomic embryos [27]. Obviously, the risk of aneuploid embryos is particularly high in males with abnormal karyotype due to gonosomal aneuploidy (47,XXY; 47,XYY). The percentage of euploid embryos to transfer after preimplantation genetic testing (PGT) in ICSI couples in which one member carries an abnormal karyotype (PGT-SR) varies according to the carrier partner (male or female) and the chromosomal abnormality [24]. When the structural chromosomal abnormality is carried by the male patient, the percentage of transferrable embryos is higher in Robertsonian translocation instead of reciprocal translocation. This result highlights that specific (structural) chromosomal abnormalities carried by the male partner are incompatible with embryo development and induce embryo arrest [24].

The Y chromosome is specific to males and can be microdeleted in Yq11 at the specific loci AZFa, AZFb, and AZFc, causing genetic infertility [28]. Minimal data correlating the effect of AZF microdeletion with embryo development and kinetics are available. Fertilization rate was found to be lower with sperm from AZF-microdeleted patients, but embryo development was similar [26].

In azoospermic patients with CBAVD due to cystic fibrosis mutation(s) and carrier couples that are candidates for PGT for a specific genetic disease, no study evidenced a correlation between the genetic trait and embryo development and kinetics.

3. Sperm RNA

Several molecular biology techniques, particularly RNA sequencing, have allowed the characterization of the whole RNA content of the sperm cell, which, as in other mammals, includes both coding and non-coding RNAs, such as mRNA, miRNA rRNA, piRNAs, lncRNA, siRNA, tRFs, and others [29][30][31][32]. Innovatively, some RNAs are encoded by genes packaged within the H2M4me3 histones, which are compatible with transcription. Hence, it has

been recently speculated that the spermatozoon might be able of de novo transcription, at least in specific DNA loci [33]. A number of transcripts encoding for growth factors, transcription factors, or protein kinases have been identified in human sperm to a different extent in terms of concentration in infertile patients compared to fertile controls [34]. Some sperm RNAs could be effectors of male infertility by their injection in the oocytes [35][36][37]. As such, these RNAs, including clusterin, calmegin, and the integrator complex subunit I mRNA, seem to be defective in unfertilized oocytes and would play a role in early embryogenesis [37][38]. From mice studies, non-coding RNAs acquired during the epididymal transit play a role in embryogenesis [39]. Regarding the embryo kinetics, the miRNA appears to delay the cleavage stage from 2-cell to 5-cell stages and decrease the percentage of high-quality embryos [40].

A pilot study analyzing levels of several sperm-carried mRNAs encoding for genes involved in fertilization events, oocyte activation, chromatin remodeling, and DNA repair in oligoozoospermic patients and normozoospermic controls reported significantly lower levels of 21 mRNAs (e.g., mRNA of AKAP4, PTK7 PLCζ, POU5F1) in oligoozoospermic patients. A total of 90% of the degenerated embryos did not reach the morula stage in those patients [41]. Furthermore, a study on normozoospermic males undergoing ICSI with young donor oocytes found 324 small RNAs (including 5′-tRF-Asp-GTC; 5′-tRF-Phe-GAA, let-7f-2-5p, miR-4755-3p, miR-92a-3p, etc.) differently expressed according to cases with high or low blastocyst rates [42].

Emerging evidence has addressed to sperm RNA a role in early embryo development and embryo kinetic [43].

4. Sperm Epigenetic

Epigenetics involves mitotically and/or meiotically inheritable changes in gene function without alterations in DNA sequences, enabling the transformation of the same genome into several different transcriptomes. Spermatozoa have a unique epigenetic signature with a specific methylation profile [44]. In the fraction of the sperm genome that does not undergo the histone–protamine transition, retained histones are subjected to chemical modifications, such as methylation, acetylation, phosphorylation, and ubiquitination [45][46], that regulate genome activation and silencing [47]. Data suggest that these epigenetic factors may affect transcriptional regulation during embryogenesis and contribute extra-genomically to early embryonic development. Moreover, alterations in this highly specialized chromatin architecture may be associated with male infertility (decreased sperm concentration, motility, and fertilization ability) and embryo developmental anomalies [48][49].

5. Source of Spermatozoa

The spermatozoa for micro-injecting in ICSI can be recovered from ejaculated specimen or chirurgical extractions (e.g., testicular sperm extraction TESE, testicular sperm aspiration TESA, microsurgical epididymal sperm aspiration MESA, percutaneous epididymal sperm aspiration PESA) in case of azoospermic men [50]. According to sperm origin, the fertilization rate is higher with ejaculated sperm, followed by epididymal, and then testicular samples [51]. Different studies underlined the effect of sperm recovery on embryo kinetics comparing data from

ejaculated semen with spermatozoon from testicular or epididymis. The micro-injection of spermatozoon recovered from the testicle (TESE, TESA) has an effect at the zygote stage with an earlier second polar body extrusion, a delay of pronuclei appearance, and a longer pronuclear stage [52][53][54]. At the cleavage stage, the embryos reach the 3-, 5-, 7-, 8-, and 9+ cell stages earlier in the case of testicular spermatozoon [53][54]. The morula and blastocyst stages are reached later [52][53][54]. A higher percentage of unequal cleavage from the 1-cell stage to the 3-cell stage are observed [52].

In the case of epididymal spermatozoon, embryo kinetics seem more similar to ejaculated semen, except for when they reach the 2-, 4-, and 6-cell stages. The blastocyst stage is delayed compared to testicular and ejaculated semen [53]. More blastocysts are obtained with ejaculated and normospermia compared to surgically extracted spermatozoon [53].

The effects of sperm recovery on embryo kinetics highlight, once again, the molecular aspects of spermatozoa maturation along the male genital tract. At the testicular level, the sperm DNA is enfolded with histones, is not yet compacted, and is less fragmented. The variation of the zygote kinetics for testicular spermatozoa compared to epididymis or ejaculated sperm is explained by the fact that the oocyte does not need to substitute protamines with histones or to repair fragmented DNA sperm as it would have done with a more mature sperm. The frequent unequal cleavage from the 1-cell stage to the 3-cell stage observed with testicular spermatozoa is explained by an incomplete maturation of centriole maturation in the testicular spermatozoon; this maturation being completed once the cell reaches epididymis [52]. In addition to the role of motility gain as the spermatozoon runs along the epididymis, the epididymis plays a role in molecular sperm maturation [55][56]. The incomplete molecular maturation of epididymis spermatozoa would explain the poor embryo development and lower ICSI outcomes compared to ejaculated sperm [43][57].

6. Sperm Cryopreservation

Even if semen cryopreservation decreases the levels of sperm mRNA [58][59], embryos from cryopreserved sperm have comparable development and kinetics compared to embryos from freshly ejaculated sperm [60][61]. The adverse effects of sperm cryopreservation are the loss of sperm motility and viability [62], but the sperm vitrification protocol is epigenetically safe and induces minor biological changes compared to conventional freezing [63].

The cryopreservation of testicular or epididymal sperm in patients with obstructive or non-obstructive azoospermia were found to have no impact on ICSI outcomes and embryo development compared to fresh testicular or epididymal samples [64][65].

7. Other Sperm Causes

According to scientific literature, other sperm causes can affect embryo development and kinetics. The high levels of reactive oxygen species in semen were associated with lower embryo quality and lower blastocyst rate [66]. The

abundance of sperm proteins, such as those of the chaperonin-containing T-complex, correlate with early embryo quality and could be considered a predictive biomarker of ICSI outcomes in couples with idiopathic infertility [67].

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