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Committee Opinion

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Round spermatid nucleus injection (ROSNI)

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BACKGROUND

ROSNI, or ROSI (round spermatid injection) is a method of assisted in vitro fertilization (IVF) in which precursors of mature sperm obtained by ejaculated specimens or testicular sperm extraction (TESE) are injected directly into oocytes as treatment for male infertility. ROSNI has been proposed as a treatment for men in whom mature sperm forms (elongating spermatids or spermatozoa) cannot be identified for intracytoplasmic sperm injection (ICSI), as in men with complete meiotic arrest. ROSNI has not been widely performed or as successful as ICSI, which revolutionized male infertility.

Because ROSNI involves the use of immature sperm, the procedure presents new technical challenges and raises new unresolved genetic concerns. The Practice Committee recommends that ROSNI be considered an experimental procedure that should be applied only in the setting of a clinical trial approved and overseen by a properly constituted Institutional Review Board.

SPERMATOGENESIS

Spermatogenesis can be divided into three major stages. The first stage entails mitotic division of the spermatogonia to yield diploid (2N) primary spermatocytes. In the second stage, primary spermatocytes undergo two successive meiotic divisions. The first gives rise to haploid (1N) secondary spermatocytes, and the second to round spermatids. In the final stage of spermatogenesis (termed *spermiogenesis*), round spermatids further mature, first becoming elongated spermatids (lacking a defined tail) and, finally, mature spermatozoa.

It has been proposed that differentiation of spermatids into mature spermatozoa serves only to provide the means to transport paternal genetic information to the oocyte. Therefore, in theory, the injected haploid genome of the round spermatid nucleus may be sufficient for fertilization and subsequent embryonic development in some species. In the mouse model, injection of round spermatids (1–4) and secondary spermatocytes (5) into oocytes has achieved fertilization and pregnancy with some consistency (6). However, in at least one strain of sterile male mice tested with ROSNI, the phenotypic abnormality causing sterility in the father was more severely expressed in the offspring, results that illustrate one potential risk of this technology (7).

UNRESOLVED ISSUES ASSOCIATED WITH ROSNI

Round Spermatid Identification

ROSNI has been used to achieve fertilization in human IVF, but with limited success (8–10). Accurate identification of round spermatid cells is one technical challenge of ROSNI. Using the standard optics present in most clinical IVF laboratories, it can be difficult to distinguish haploid round spermatid cells from diploid spermatogenic precursors and somatic cells. Phase contrast microscopy may simplify the task (11). Another technique recently described uses a fluorescent mitochondrial probe to specifically identify round spermatids in dispersed testicular cells (12). Originally developed for use in nonhuman primate species and cattle, the technique will require extensive testing to establish its safety before it can be applied in humans.

Oocyte Activation

Activation of the oocyte rarely occurs after spermatid injection alone. This observation suggests that another factor present in mature spermatozoa may serve to induce a calcium flux in the oocyte, resulting in its activation (13–15). Extraction and delivery of this factor or treatment with a calcium ionophore are two potential strategies for inducing oocyte activation, which is required for fertilization.

Embryonic Development

In primate species, the embryonic centrosome normally derives from spindle elements contributed by spermatozoa. Because round spermatids have not yet developed mature centriolar complexes with spindles, their injection into oocytes has the theoretical potential for unexpected and possibly adverse effects on subsequent embryonic development.

Genetic Abnormalities

Our current understanding of the molecular control mechanisms that regulate spermatogenesis, sperm transport, fertilization, and early embryonic development is severely limited. Consequently, nearly one quarter of all male infertility is categorized as idiopathic. Among men with nonobstructive azoospermia who are candidates for TESE, only approximately 17% have identifiable genetic abnormalities. Most affected men have chromosomal anomalies, predominantly Klinefelter syndrome (46,XXY); 7% to 10% harbor known Y chromosome microdeletions (16).

Among remaining azoospermic men, some are likely to have as yet unidentified autosomal genetic defects (17). Certainly, any genetic abnormality sufficiently severe to result in meiotic arrest (the only cause of infertility that could possibly benefit from application of ROSNI) might also have adverse effects on other normal cellular processes or other systemic manifestations. Offspring conceived via IVF with ROSNI may thus be at risk for infertility or even more severe genetic defects.

The health consequences for offspring conceived through ROSNI are largely unknown. An early report suggested that IVF with ICSI might be associated with an increased risk for sex chromosome abnormalities (18). Subsequent studies have suggested that ICSI has no intrinsic genetic risks (19–21) or that risk of birth defects is increased with IVF, but to no greater extent when ICSI is also performed using ejaculated sperm (22). However, data regarding the safety of ICSI using testicular sperm or spermatids obtained from men with nonobstructive azoospermia are far more limited.

Genomic imprinting (modification of gene expression by cytosine methylation) normally occurs during gametogenesis and may not be complete early in the round spermatid stage of development. Naturally occurring errors in imprinting may result in illness, such as type II Angelman syndrome (a genetic disorder characterized by mental retardation, lack of speech, and movement and behavior disorders). In mice, genomic imprinting is completed in the round spermatid

(23), but the exact stage of genomic imprinting in human spermatogenesis is unknown and must be defined before clinical application of ROSNI in human IVF.

Limited Success Rates

To date, the application of ROSNI in clinical IVF has had disappointing results. Although several reports have suggested the feasibility of ROSNI in human IVF (8–10, 24, 25), overall fertilization rates achieved with ROSNI are lower (45% to 50%) than with ICSI using mature sperm or elongating spermatids (69% to 74%) (10, 26–28). One report on the occurrence of significant congenital anomalies in ROSNI-conceived pregnancies raises further concerns (29). Although causality was not established, the report indicates that caution is warranted.

SUMMARY AND RECOMMENDATIONS

- ROSNI is a method of assisted fertilization in which precursors of mature spermatozoa are injected into oocytes.
- Accurate identification of round spermatids remains a technical challenge.
- Other important unresolved issues include impaired oocyte activation, potential adverse effects on the embryonic centrosome, and risk of infertility or more severe genetic defects in offspring.
- The health consequences of ROSNI for offspring are uncertain.
- Further research is required to better define the genetic causes of male infertility and to establish the safety of ROSNI and other novel assisted reproductive technologies.
- ROSNI should not be performed when more mature sperm forms (elongating spermatids or spermatozoa) can be identified and used for ICSI.
- Patients who may be candidates for ROSNI should receive careful and thorough pretreatment counseling to ensure they are clearly informed of the limitations and potential risks of the procedure.
- Application of ROSNI in clinical human IVF should be considered experimental and therefore requires approval and oversight by an appropriately constituted Institutional Review Board.

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