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# Round spermatid injection (ROSI) and elongated spermatid injection (ELSI)

Nares Sukcharoen MD.

*Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.*

## ABSTRACT

Micromanipulation of male gametes for treatment of male infertility is a rapidly moving field. Injection of the spermatids have been recently developed and achieved successful outcome in the past few years. Both round spermatids injection (ROSI) and elongated spermatids injection (ELSI) seem to play an important role in the treatment of non-bstructive azoospermia. However, many problems limiting success rates and hindering a wide application of this technique are still unresolved. This article therefore reviews the current knowledge of spermatids injection and its controversial issues.

**Key words :** ROSI, ELSI, spermatogenesis, spermiogenesis, round spermatids, elongated spermatids, assisted reproduction

Spermatids are haploid cells resulting from the meiotic phase of spermatogenesis. These originally round cells (round spermatids) undergo a progressive transformation into spermatozoa through a series of complex morphological and molecular changes collectively termed spermiogenesis, which is the final phase of spermatogenesis. Intermediate stages of this transformation are sometimes called elongating and elongated spermatids. When spermiogenesis is blocked by a testicular pathology, patients do not produce mature spermatozoa, but they can produce spermatids at various developmental stages. The frequency of spermiogenesis failure among all causes of infertility has been estimated to be 1-2 per cent.[1]

Following the first animal study showing the capacity of the round spermatid nuclei to fertilize the hamster oocyte,[2] the possibility of using spermatids in human-assisted reproduction has been suggested as a novel approach to the restoration of fertility in cases of spermiogenesis failure.[1] This was followed by several reports about the fertilization, embryo development, pregnancy and full term delivery following spermatids intracytoplasmic injection in the mouse[3,4] and rabbit.[5] The first clinical attempt at fertilizing human oocytes with round spermatids has been reported in 1995.[6] In the same year, the first two successful treatment cycles with

However, there is a considerable discussion going on between experts in the field of spermiogenesis on the use of different stages of germ cells in ICSI. The debate focuses on the following issues :

The presence of round spermatids without the concomitant presence of elongated spermatids somewhere in the ejaculate or testicular biopsy is questionable. Could the detection of at least some elongated spermatids depend on the time invested to screen the material ? By definition of meiotic arrest, no haploid spermatids can be found. This condition may not be present throughout the whole testis, analogous to the condition in Sertoli cell only syndrome where foci of spermiogenesis are sometimes found. This could be related to the underlying cause of spermiogenetic pathology.

The detection of round spermatids seems to be a difficult activity in the hands of many groups. In the first place analysis of oocytes after ROSI (round spermatid injection) shows that Sertoli cells are frequently selected for injection, suggesting an identification failure of the round spermatid. In the second place there appears to be an increase in pathogenic activation of the oocyte after ROSI.

The proper evaluation of the actual therapeutic potential of spermatid conception is one of the controversial issues. It is important to define the developmental stage of the spermatids used in each injection. The number of stages should be sufficient to reflect the major morphological changes in spermiogenesis and should be limited to those forms that can be distinguished clearly by microscopic examination in the living state without special cytologic techniques. Previously, de Kretser classified the developmental stages of the spermatids according to the morphological findings.[30] (Figure 1) The acrosomal vesicle

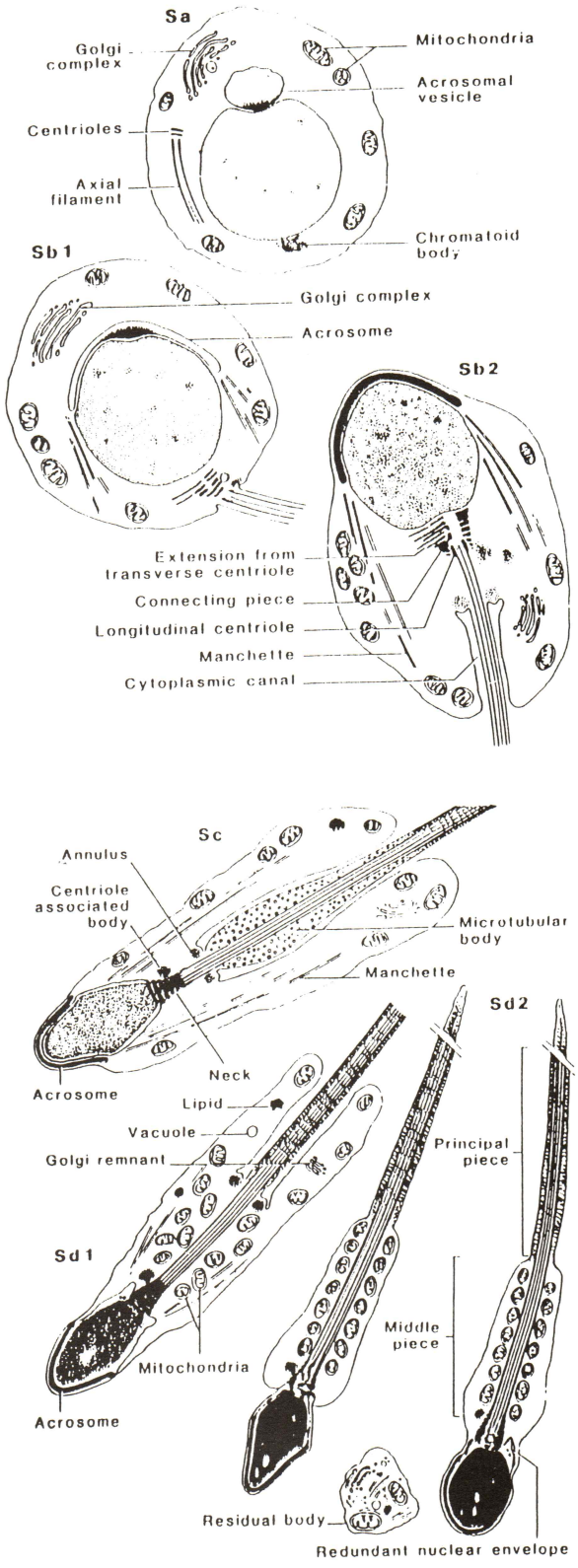



**Fig. 1.** The electron microscopic features of the stages of human spermatogenesis.[30]

can be seen in living round spermatids and its presence can be used to distinguish round spermatids at the Sa stage from those at the $Sb_1$ stage, when the acrosome takes a caplike form and is seen less clearly (without the application of special staining methods). The eccentric position of the nucleus, together with the beginning of spermatid elongation marks and $Sb_2$ stage, whereas the progressively decreasing nuclear size and the increasing degree of spermatid elongation can be used as criteria for the distinction between Sc, $Sd_1$ and $Sd_2$ elongated spermatids. The latter's morphology is identical to mature spermatozoa at this level of observation. There are many diagnositc methods for the detection of spermatid production by infertile men, such as, immunocytochemistry with selective or specific spermatogenic cell markers,[26,31] fluorescent in situ hybridization (FISH) to detect the haploid status of spermatids as opposed to other testicular cells[31,32] or a combination of both methods,[31] However, the spermatids can not be examined by these methods in the living state.

All these descriptions were based on observations of normal spermiogenesis. Abnormal development may not accommodate the classification based on the criteria for normal spermiogenesis. Until an appropriate classification for such pathological stages is available, it will be necessary to describe the cell morphology in detail. Even though the conditions under which spermatid injection is performed are not ideal for morphological analysis, morphological features of normal and abnormal germ cell development should be studied subsequently in more detail by appropriate techniques and should be related to the corresponding clinical data (e.g., fertilization rate, pregnancy rate, birth rate and molecular and clinical abnormalities detected in the progeny). The adoption of a clear terminology for individual stages of spermatid development is needed to avoid confusion about success rates reported from different centers.

Micromanipulation of male gametes for treatment of male infertility is a rapidly moving field. Injection of the spermatids have been recently developed in the past few years. Both round spermatids injection (ROSI) and elongated spermatids injection (ELSI) seem to play an important role in the treatment of non-obstructive azoospermia. We are still lacking in clinical and scientific data on using these immature germ cells injection. There are many problems about spermatids injection to be solved, such as poor fertilization and implanting rate, an appropriate case selection for such kind of treatment, etc. Therefore, basic science researches on spermatogenesis, spermiogenesis and cell biology of immature gametes are needed to improve the outcome of spermatids injection.

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