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The activity (calcium oscillator?) responsible for human oocyte activation after injection with round spermatids is associated with spermatid nuclei

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Objective: To compare the oocyte-activating ability of whole human round spermatids and their isolated nuclei.

Design: Prospective study using sibling oocytes from patients undergoing spermatid conception and intracytoplasmic sperm injection treatment cycles.

Setting: Private assisted reproduction laboratories and a university department.

Patient(s): Couples with male infertility.

Intervention(s): Sibling oocytes were injected either with whole round spermatids or with their isolated nuclei, followed by artificial triggering of oocyte activation with calcium ionophore. Other sibling oocytes were injected either with isolated spermatid cytoplasm or with whole mature spermatozoa.

Main Outcome Measure(s): Numbers of activated oocytes and cleaving embryos.

Result(s): After oocyte activation was boosted with calcium ionophore, whole spermatids and isolated spermatid nuclei were equally effective in supporting oocyte activation and the formation of pronuclei, whereas no control oocyte was activated under the same conditions after injection of isolated spermatid cytoplasmic compartments. Cleavage rates were lower after the injection of isolated spermatid nuclei than after the injection of whole spermatids.

Conclusion(s): The factor responsible for human oocyte activation after round spermatid injection is associated with spermatid nuclei. The requirement for the artificial trigger (calcium ionophore) suggests that this factor is identical to the male gamete activity previously characterized as calcium oscillator. (Fertil Steril® 2000;74:1245–7. ©2000 by American Society for Reproductive Medicine.)

Key Words: Spermatid injection, spermatid nucleus, oocyte activation, calcium oscillator

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Several lines of evidence suggest that human oocyte activation during fertilization is mediated by a two-component mechanism (1). The first-acting component, activated during the period when the sperm and oocyte plasma membranes have established a tight contact or immediately after the beginning of gamete fusion, has been termed the "trigger" and is required for the first sperm-induced increase in the free ooplasmic calcium concentration. Subsequently, a trigger-independent, sperm-derived factor termed the "oscillator" alters the functional parameters of the oocyte's internal calcium stores so as to make them capable of supporting the ongoing, largely autonomous series of calcium oscillations (1).

When the spermatozoon is injected directly into the ooplasm, the trigger function is taken over by an artificial calcium influx (pseudotrigger) produced by the micromanipulation itself, and the success or failure of oocyte activation is thus mainly dependent on the intactness of the oscillator function (1). The fact that human oocytes can also be fertilized after being injected with round spermatids instead of spermatozoa (2) indicates the presence of the oscillator function in these cells. However, the subcellular localization of this activity (nuclear versus cytoplasmic) is not known.

In our spermatid conception program, there have been several cases in which very limited numbers of round spermatids were available,

and some of these spermatids disintegrated during manipulation in a way that the cytoplasm was lost and only the nucleus could be recovered. In the absence of intact spermatids available for the injection of all oocytes retrieved from the patient's wife, those oocytes for which no intact spermatids were available were injected with isolated spermatid nuclei. These situations inadvertently created a self-controlled experimental model in which fertilization and cleavage results could be compared for sibling oocytes injected with either whole round spermatids or their isolated nuclei. In this report, we complete these data by adding a voluntarily constituted control group consisting of oocytes injected with isolated cytoplasmic compartments of round spermatids retrieved from the testicular tissue of men with obstructive azoospermia.

MATERIALS AND METHODS

This study deals with five spermatid conception treatment cycles, performed with five infertile couples, in which part of the spermatids were inadvertently enucleated during micromanipulation and there were not sufficient intact spermatids to inject all the mature oocytes recovered from the patient's wife. Informed consent was obtained from the couples concerned for injecting the remaining oocytes with isolated spermatid nuclei. Spermatogenesis was completely arrested at the round spermatid stage in all cases. No reproductive pathology was detected in the patients' wives.

Additional oocytes were donated by three women undergoing an intracytoplasmic sperm injection treatment cycle, also devoid of any detectable reproductive pathology, who produced >15 mature oocytes each. These oocytes served as controls to be injected with isolated spermatid cytoplasm, prepared from round spermatids recovered from testicular biopsy samples from three consenting men with obstructive azoospermia. Institutional Review Board approval was obtained for these experiments.

Testicular tissue samples from the five men with nonobstructive azoospermia were obtained by open testicular biopsy and subjected to in vitro culture, as described (3). The concentrations of FSH and T in the culture medium were 50 IU/L and 1 μmol/L, respectively, and the duration of the culture was 24 hours. No signs of spermatid elongation were noted at the end of the culture in any of these samples. The spouses' oocytes, recovered by transvaginal ultrasound-guided follicle aspiration after previous GnRH agonist treatment and ovarian stimulation with gonadotropins, were injected with round spermatids 4–8 hours after recovery, using previously described techniques and instruments (2).

Isolated nuclei, apparently devoid of cytoplasmic remnants, which were released inadvertently from some spermatids during this manipulation, were injected into oocytes with the use of the same techniques and instruments. In the control series of injections, round spermatids from men with obstructive azoospermia were repeatedly aspirated in and out of the microinjection needle until the nucleus was released. Unlike the inadvertent spermatid disintegration, the entire cytoplasmic compartment of these intentionally enucleated spermatids usually could be recovered. The cytoplasm corresponding to one spermatid was injected into each donor oocyte by applying the same technique as for the injection of whole spermatids or their isolated nuclei.

In one case, the spermatids were labeled with Mito-Tracker (Molecular Probes, Eugene, OR) before nucleus isolation, as described previously (4), to visualize spermatid mitochondria. Fifteen nuclei isolated from these labeled spermatids were subsequently analyzed by fluorescence microscopy, and no associated mitochondrial tag could be detected in any of them.

Between 15 and 30 minutes after injection, oocytes of all three groups were exposed for 10 minutes to 10 μmol/L ionophore A23187 (Sigma, St. Louis, MO) in IVF-50 medium (Scandinavian IVF Science, Gothenborg, Sweden). They were then washed free of the ionophore and incubated further in the same medium. Oocyte activation was assessed at 8, 12, and 16 hours after injection. An oocyte was considered activated if it displayed one pronucleus (in the case of oocytes injected with isolated spermatid cytoplasmic components) or two pronuclei (in the case of oocytes injected with whole spermatids or with spermatid nuclei) at one or more of these three observation times. Cleavage of activated oocytes was assessed on the following day.

TABLE 1

Comparison of the oocyte-activating ability of whole round spermatids, isolated spermatid nuclei, isolated spermatid cytoplasm, and whole mature spermatozoa.

Entity injected	Oocytes			Cleaved embryos
	Injected	Surviving	Activated	
Whole spermatid	26[a]	22	15	14
Spermatid nucleus	22[a]	20	12	4
Spermatid cytoplasm	10[b]	9	0	0
Spermatozoon[c]	39[d]	37	32	30

[a,b] Pairs of oocytes with the same superscript were sibling oocytes.
[c] Unlike the three previous groups, oocytes injected with whole mature spermatozoa were not treated with ionophore A23187.

Tesarik. Human oocyte activation and spermid nuclei. Fertil Steril 2000.

RESULTS

The injection of sibling oocytes with either whole round spermatids or their isolated nuclei gave similar fertilization rates (68% and 60% of the oocytes that survived the manipulation, respectively) (Table 1). However, none of the 10 donor oocytes injected with isolated spermatid cytoplasm developed signs of activation, contrasting with high rates of

activation achieved by injecting intact spermatozoa into the rest of the oocyte cohorts from the three patients who donated oocytes for this experiment (Table 1). Interestingly, most (93%) of the oocytes activated by whole round spermatids cleaved, whereas this was the case in only 33% of the oocytes fertilized by isolated spermatid nuclei (Table 1).

DISCUSSION

Activation of human oocytes injected with nuclei isolated from human round spermatids has been reported previously (5). However, the present report is the first controlled study to compare the fertilizing potential of whole human round spermatids and their isolated nuclei in sibling oocytes subjected to the same treatment conditions and manipulations. The boosting of oocyte activation after spermatid injection by a short incubation of oocytes with calcium ionophore, applied to all oocytes involved in this study, augments fertilization rates and has recently been introduced as part of our standard protocol.

The data from our control group show clearly that the ionophore treatment by itself is insufficient to activate the human oocyte. Nevertheless, this treatment reinforces the artificial pseudotrigger in this particular mode of human fertilization, in which the efficiency of the spermatid-associated oscillator is often lower than in mature spermatozoa (1). The calcium oscillation–promoting activity of the spermatid nucleus–associated oocyte-activating factor has not been addressed in this study and remains to be tested. However, it was observed previously that, under the same conditions as those in this study, only those spermatid-injected human oocytes that showed calcium oscillations subsequently developed pronuclei (J. Tesarik, unpublished observations).

The reason for the lower cleavage rate of the oocytes fertilized with isolated spermatid nuclei (18% of oocytes surviving the injection) as compared with those fertilized with whole spermatids (64% of oocytes surviving injection) is not clear and may be related to the absence of the spermatid-derived centrosomal material in the former case. Centrosomal material is expected to remain associated with nuclei after enucleation, but total or partial loss of this material during this procedure may occur in some cases. On the other hand, our data with MitoTracker labeling suggest that spermatid mitochondria are not associated with isolated spermatid nuclei after the enucleation procedure. It remains to be elucidated whether there is any specific role for spermatid mitochondria in the processes of spermatid-induced oocyte activation and early development after fertilization.

These observations contrast with those of Yamanaka et al. (5), who reported cleavage in 30 of 49 oocytes (61%) successfully injected with round spermatid nuclei without applying any additional treatment to boost oocyte activation. In our experience (unpublished data), significantly fewer oocytes are fertilized with whole round spermatids when activation is not boosted with calcium ionophore, and we have never obtained fertilization with an isolated round spermatid nucleus under these conditions.

In conclusion, these data show that relatively high fertilization rates can be achieved with both whole human round spermatids and their isolated nuclei, provided that oocyte activation is boosted with calcium ionophore. Further study is needed to confirm that this nucleus-associated factor acts as a calcium oscillator, to elucidate its molecular identity, to find out how it is targeted to the nucleus during spermiogenesis, and to determine with what structural nuclear components it is associated.

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