FERTILITY AND STERILITY®
VOL. 73, NO. 5, MAY 2000

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

EDITOR'S CORNER

Round spermatid injection

Sherman J. Silber, M.D., Larry Johnson, Ph.D.,† Greta Verheyen, M.D.,‡ and Andre Van Steirteghem, Ph.D.‡*

Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, Missouri; Texas A & M University, College Station, Texas; and Centre for Reproductive Medicine, Dutch-speaking Free University, Brussels, Belgium

We have previously expressed skepticism about round spermatid injection (ROSI) in the current human IVF setting (1–3). We do not find round spermatids in TESE (testicular sperm extraction) specimens in which we do not also find elongated sperm with tails. We find many "round cells" in all TESE specimens, but in the absence of sperm, these are not round spermatids.

This finding has caused great concern with round spermatid enthusiasts. A major complaint is that "one should not restrict the label of round spermatid only to those cells in which an acrosomal vesicle is visible" (p. 2977) (4). However, our observation is based not only on phase-contrast evaluation of TESE microdroplets but also on detailed stained histology of many hundreds of testis specimens and on the previous literature on the histology and pathology of maturation arrest (5–10). The atlas of phase-contrast wet preparation cells published by Johnson et al. (5, 6) attests to the difficulty of distinguishing round spermatids from Leydig cells, Sertoli cell nuclei, and even spermatogonia without staining and whole tissue fixation. The only reason we concentrate on the albeit transient acrosomal vesicle with phase contrast is to provide an easy identification marker for wet microdroplet ICSI preparations. The absence of round spermatids in wet TESE preparations determined by phase-contrast visualization of the acrosomal vesicle in the intracytoplasmic sperm injection (ICSI) setting merely confirms what was already demonstrated in fixed, stained tissue specimens over many years (Figs. 1 and 2).

The acrosomal vesicle forms very early after meiosis, and in fact, the Golgi apparatus is already present in the pachytene spermatocyte (Fig. 3). In their atlas, Holstein and Roosen-Runge (11) did not even draw the evanescent Golgi prevesicle phase. One could argue that round spermatids may be arrested at this brief, prevesicle phase (i.e., before the acrosomal vesicle can be observed). But our stained histologic sections still reveal (without any need to see the acrosomal vesicle) that maturation arrest in humans is apparently a block at meiosis. Thus, we still see no evidence that round spermatid arrest is a common event in human maturation arrest.

We also question whether "human round spermatids can be identified in the native state by simply respecting the criteria of cell size (*approximately* that of red blood cells) . . . *and* by detecting the presence of a round nucleus surrounded by a rim of cytoplasm [italics added]" (p. 2977) in wet preparations with Hoffman optics (4). The notion that round spermatids can be readily distinguished from Sertoli cell nuclei, Leydig cells, spermatogonia, and other TESE components using standard Hoffman optics and without any need to identify an acrosomal vesicle—just by looking at size—is challenging. The Sertoli cell nucleus has a diameter of approximately 10 μm on average, and red blood cells and round spermatids have average diameters of approximately 8 μm. Spermatogonia average 9 μm, and pachytene spermatocytes are much larger, 12 μm on average. The intrinsic variability in diameter of many of these cells makes determining size an unreliable way to distinguish (for human clinical ICSI) which cells to inject into the human egg. Thus, the efforts of Verheyen et al. (1), who used phase contrast

Received December 7, 1999; revised and accepted January 26, 2000.

Reprint requests: Sherman J. Silber, M.D., Infertility Center of St. Louis, St. Luke's Hospital, 224 South Woods Mill Road, St. Louis, Missouri 63017 (FAX: 314-576-1442; E-mail: silber @infertile.com).

* Infertility Center of St. Louis, St. Luke's Hospital.

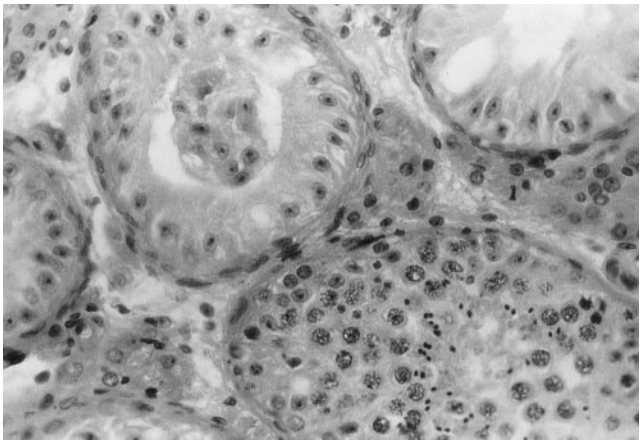
† Department of Veterinary Medicine, Texas A & M University.

‡ Centre for Reproductive Medicine, Dutch-Speaking Free University.

0015-0282/00/$20.00
PII S0015-0282(00)00488-X

FIGURE 1

A tubule with normal spermatogenesis in a testicular specimen that is otherwise Sertoli cell only. Each Sertoli cell nucleus at the base of the tubule is the same "cell" as the "round cell" in the wet testicular sperm extraction preparation (stain, hematoxylin and eosin; original magnification, ×400).



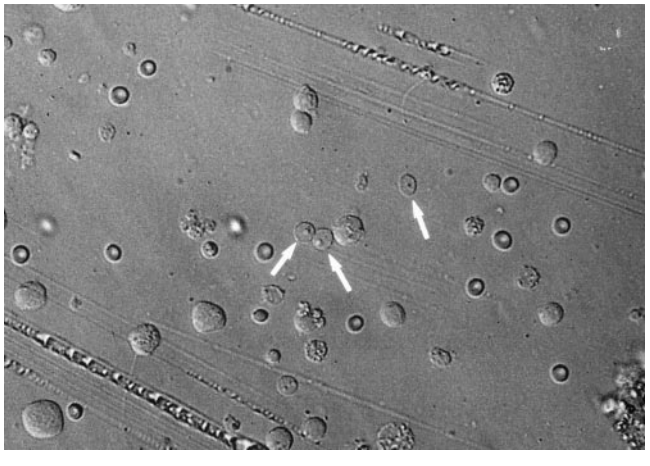
Silber. Round spermatid injection. Fertil Steril 2000.

with an inverted microscope, are a major step toward positive identification of round spermatids for ICSI.

A careful review of the E/M studies of fixed tissue specimens of normal spermatogenesis by Holstein et al. (11) shows how similar the size and appearance of the round spermatid can be to those of the Sertoli cell nucleus. With

FIGURE 2

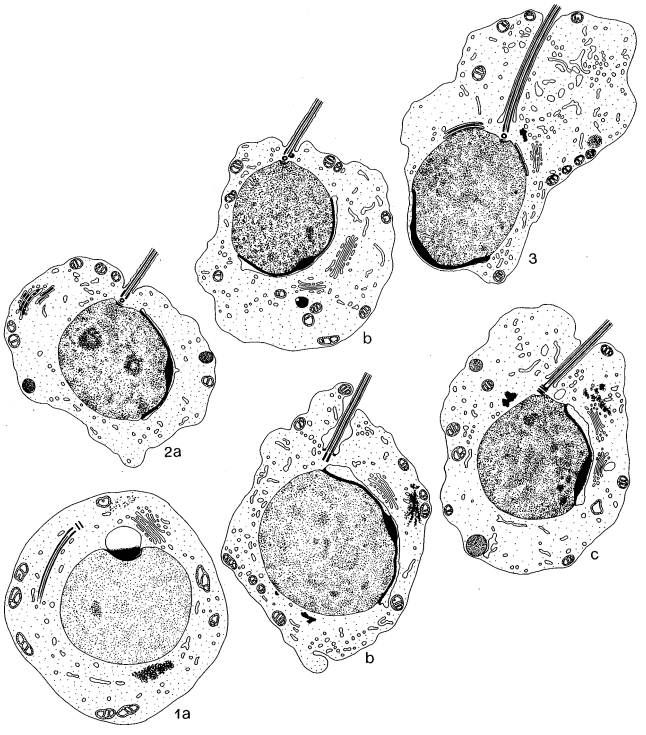
Sertoli cell only, viewed using Hoffman optics (wet prep.). *Arrows* indicate Sertoli cell nuclei (original magnification, ×400).



Silber. Round spermatid injection. Fertil Steril 2000.

FIGURE 3

Stages of spermiogenesis: stage 1 (*1a, b*, and *c*), stage 2 (*2a* and *b*), and stage 3 (elongated spermatid) (*3*) (11).



Silber. Round spermatid injection. Fertil Steril 2000.

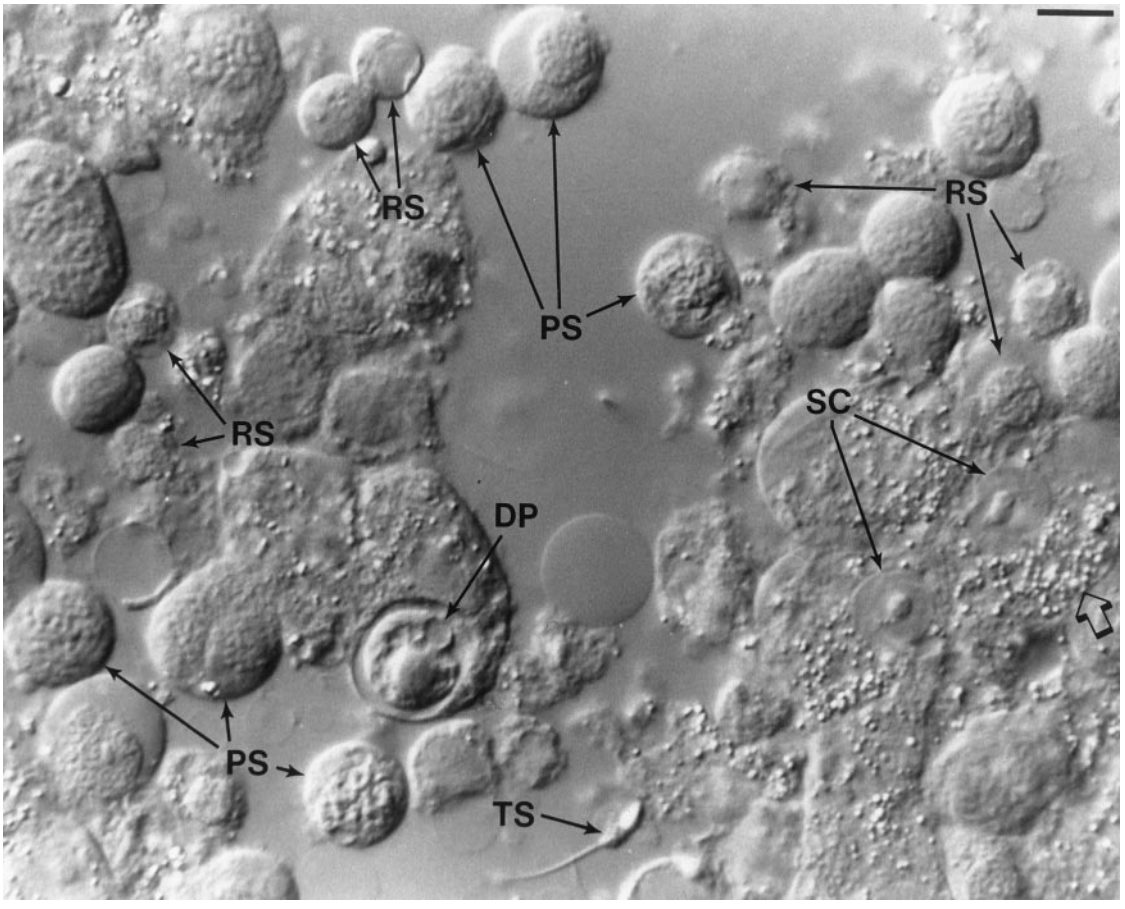
E/M, or with stained tissue sections, these distinctions are more easily seen. However, with wet preparations, the acrosomal vesicle seems to be the most reliable landmark. Nomarski differential contrast imaging of live human testicular cells does hold some promise for identifying even better various germ cells on wet preparation. It is easy to see how unreliable it is to try to distinguish a Sertoli cell nucleus from a round spermatid by size alone (Fig. 4).

A third issue is whether one can base one's view of ROSI on the well-known cyclic adenosine 3′:5′ monophosphate responsive element modulator (CREM)-mutant mouse model (12, 13). According to this model, the transcriptional activator CREM is required for germ cell–specific genes that participate in sperm structuring. This means that in homozygous CREM knockout mice, there is azoospermia caused by maturation arrest at some stage after meiosis. Most of these postmeiotic haploid germ cells in CREM-deficient mice undergo apoptosis before an acrosomal vesicle can form. Examination of a wet TESE-ICSI preparation with phase contrast might fail to reveal round spermatids if spermatogenic arrest in the human occurs just before the formation of the acrosomal vesicle, as in CREM-deficient mice.

Nonetheless, histologic sections (which are the gold standard even in the CREM literature) should still demonstrate

FIGURE 4

Dispersed, live, human testicular cells as observed by Nomarski optics. Sertoli cell nuclei (*SC*) are nonspherical, although some appear spherical. Sertoli cell cytoplasm is granular in appearance (*open arrow*). Germ cells with spherical nuclei include primary spermatocytes (*PS*) and round spermatids (*RS*). Testicular sperm (*TS*) are detached from other cells. Occasionally, degenerating germ cells (primary spermatocytes; *DP*) are observed in culture. Bar, 10 μm. (From Johnson L, Texas A & M University.)



Silber. Round spermatid injection. Fertil Steril 2000.

early round spermatid arrest in azoospermic men if they have this problem. Most histologists fail to find this early round spermatid arrest in humans, and others find that it is very rare (0.9%) (2, 7–10, 14). Furthermore, even in CREM-deficient mice, there actually were more advanced round spermatids (albeit in small numbers). Finally, what many ICSI programs determine to be viable early round spermatids are merely apoptotic cells, as also demonstrated in the CREM-mutant mouse model (1).

How do we reconcile these observational differences and why bother with this hairsplitting? Our reason for concern is that so many IVF centers are struggling to reproduce the clinical success reported by just a few investigators (4, 15–20). Some centers have experienced and reported consistently negative results with ROSI with desperate patients in whom no mature sperm or elongated spermatids could be found (21, 22). Many other centers perform ROSI regularly but understandably fail to report their negative results. We have visited these centers and seen the confusion regarding which round cells to inject. We are aware of the low success rate (1%) of ROSI even in the successful mouse model with normal spermatogenesis (23–25). Furthermore, round spermatid fertilization may be more favorable in mice, whose centrioles do not derive from the male, than in primates.

Schulze et al. (7) examined, using careful histologic methodology, biopsy specimens from 1,426 azoospermic men and found only 13 cases (0.9%) of spermatogenic arrest at the early ("round") spermatid stage. They concluded that "a complete maturation arrest at the stage of round spermatid is a rare phenomenon" (p. 625) (7). This agrees with early reports by Soderstrom and Suominen (26, 27). Some IVF centers have treated dozens of patients with ROSI every month and even seem to find what they think are round spermatids in almost all nonobstructive azoospermic cases in

which they do not find elongated spermatids. Yet pregnancies are not achieved.

We reexamined the histologic sections from all our azoospermic patients reported on in 1998 and 1999 (2) and still failed to identify a case of maturation arrest at the round spermatid stage. The classic histologic appearance we see in human maturation arrest reflects no normal progression beyond spermatocyte. There are also apoptotic cells with clumped chromatin. Figure 1 demonstrates the typical appearance of Sertoli cell–only tubules juxtaposed to tubules with normal spermatogenesis. The abundant round-appearing cells along the basement membrane of the tubule are simply Sertoli cell nuclei with their typical prominent nucleoli. These are the "round cells" typically seen in the majority of cases of nonobstructive azoospermia (2). We have found true round spermatids only when elongated forms are also present.

In conclusion, we applaud any effort to enable azoospermic men to father their own genetic children, and we are known for our efforts in that regard. However, we are concerned about the misuse of ROSI and overenthusiasm for an approach whose validity has yet to be clarified.

References

1. Verheyen G, Crabbe E, Joris H, Van Steirteghem A. Simple and reliable identification of the human round spermatid by inverted phase contrast microscopy. Hum Reprod 1998;13:1570–7.
2. Silber SJ, Johnson L. Are spermatid injections of any clinical value? ROSNI and ROSI revisited. Hum Reprod 1998;13:509–23.
3. Silber SJ, Verheyen G, Van Steirteghem AC. Spermatid conception [letter]. Hum Reprod 1998;13:2976–7.
4. Tesarik J. Spermatid conception [letter]. Hum Reprod 1998;13:2977–9.
5. Johnson L, Petty CS, Neaves W. A new approach to quantification of spermatogenesis and its application to germinal attrition during human spermiogenesis. Biol Reprod 1981;25:217–26.
6. Johnson L, Chaturvedi P, Williams JD. Missing generations of spermatocytes and spermatids in seminiferous epithelium contribute to low efficiency of spermatogenesis in humans. Biol Reprod 1992;47: 1091–8.
7. Schulze W, Hohenberg H, Knuth UA. Cryopreservation of testicular tissue: a highly effective method to provide sperm for successful TESE/ICSI procedures. Fertil Reprod Med 1998;621–6.
8. Soderstrom K-O, Suominen J. Histopathology and ultrastructure of meiotic arrest in human spermatogenesis. Arch Pathol Lab Med 1980; 104:476–82.
9. Silber SJ, Van Steirteghem A, Nagy Z, Liu J, Tournaye H, Devroey P. Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. Fertil Steril 1996;66:110–7.
10. Silber SJ, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC. Distribution of spermatogenesis in the testicles of azoospermic men: the presence or absence of spermatids in the testes of men with germinal failure. Hum Reprod 1997;12:2422–8.
11. Holstein AF, Roosen-Runge ED, editors. Atlas of human spermatogenesis. Berlin: Grosse Verlag, 1981.
12. Nantel F, Monaco L, Foulkes NS, Masquilier D, LeMeur M, Henriksen K, et al. Spermiogenesis deficiency and germ-cell apoptosis in CREM-mutant mice. Nature 1996;380:159–65.
13. Blendy JA, Kaestner KH, Weinbauer GF, Nieschlag E, Schutz G. Severe impairment of spermatogenesis in mice lacking the CREM gene. Nature 1996;380:162–5.
14. Brandell RA, Mielnik A, Liotta D, Ye Z, Veeck LL, Palermo GD, et al. AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test. Hum Reprod 1998;13:2812–5.
15. Antinori S, Versaci C, Dani G, Antinori M, Pozza D, Selman HA. Fertilization with human testicular spermatids: four successful pregnancies. Hum Reprod 1997;12:286–91.
16. Barak Y, Kogosowski A, Goldman S, Soffer Y, Gonen Y, Tesarik J. Pregnancy and birth after transfer of embryos that developed from single-nucleated zygotes obtained by injection of round spermatids into oocytes. Fertil Steril 1998;70:67–70.
17. Fishel S, Green S, Bishop M, Thorton S, Hunter S, Fleming S, et al. Pregnancy after intracytoplasmic injection of spermatid. Lancet 1995; 345:1641–2.
18. Tesarik J, Mendoza C, Testart J. Viable embryos from injection of round spermatids into oocytes [letter]. N Engl J Med 1995;333:525.
19. Tesarik J, Greco E, Mendoza C. ROSI, instructions for use: 1997 update. Hum Reprod 1998;13:519–23.
20. Amer M, Soliman E, el-Sadek M, Mendoza C, Tesarik J. Is complete spermiogenesis failure a good indication for spermatid conception [letter]? Lancet 1997;350:116.
21. Ghazzawi I, Taher M, Souso S. Pregnancies after round spermatid injection: a virtual reality! Hum Reprod 1998;13:Abstract Book 1 (O-180),46–7.
22. Ghunaim S, Shaban MA, Dakkak A, Tell B, Keilani S, Keilani Z. The outcome of round spermatid injection in assisted reproduction. Hum Reprod 1998;13:Abstract Book 1 (O-094),90–1.
23. Ogura A, Yanagimachi R. Round spermatid nuclei injected into hamster oocytes form pronuclei and participate in syngamy. Biol Reprod 1993;48:219–25.
24. Ogura A, Yanagimachi R, Usui N. Behaviour of hamster and mouse round spermatid nuclei incorporated into mature oocytes by electrofusion. Zygote 1993;1:1–8.
25. Ogura A, Matsuda J, Yanagimachi R. Birth of normal young after electrofusion of mouse oocytes with round spermatids. Proc Natl Acad Sci USA 1994;91:7460–2.
26. Pousette A, Leijonhufvud P, Arver S, Kvist U, Pelttari J, Hoog C. Presence of synaptonemal complex protein 1 transversal filament-like protein in human primary spermatocytes. Hum Reprod 1997;12: 2414–7.
27. Soderstrom K-O, Suominen J. Histopathology and ultrastructure of myotic arrest in human spermatogenesis. Arch Pathol Lab Med 1980; 104:476–82.