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SYMPOSIUM: QUALITY MANAGEMENT IN ASSISTED REPRODUCTIVE TECHNOLOGY

Micromanipulation in assisted reproductive technology

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Dr Malter earned his PhD degree in Cell/Developmental Biology at Emory University. Over a 30-year career beginning in the large animal laboratory of Dr Ben Brackett and transitioning to human clinical work with Dr Jacques Cohen, Dr Malter has worked as a senior scientist at the New York Hospital and St Barnabas Hospital assisted reproduction programs, with the Reproductive Genetics Institute and the McGill Reproductive Center. He is currently the Director of Laboratories for the Fertility Center of the Carolinas in Greenville, SC and Fertility Solutions in Dedham, MA. His interests involve clinical and research aspects of human assisted reproduction and genetics.

Abstract Micromanipulation describes a set of tools and techniques for cellular microsurgery and manipulation. Micromanipulation techniques have played an important role in basic research and the development of clinical techniques in assisted reproductive technology. This work provides a review of the development and current practices involving micromanipulation in the human clinical assisted reproduction laboratory.

KEYWORDS: embryo biopsy, embryos, ICSI, micromanipulation, nuclear transfer, oocytes

Perhaps micromanipulation is not a worthwhile subject for isolated discussion? Micromanipulation simply describes a set of tools and techniques. However, these tools and techniques have played a key role in advancing knowledge in reproductive biology and expanding the repertoire of clinical methodology and options. They have essentially solved the dilemma of male factor infertility, they allow for the diagnosis and circumvention of inherited genetic conditions and they hold great promise for further advancement in the future. Therefore, I appreciate the opportunity to embark on this review of a subject I have had the great pleasure to have been intimately involved in for over 30 years. I hope to provide an analysis that puts the tools and techniques of micromanipulation in perspective with the science and clinical achievements that have resulted. We will begin with a historical perspective and then move into a categorical description of the various uses of micromanipulation in assisted reproductive technology (ART).

Historical perspective

No doubt from the first moments scientists looked through their microscopes into a new world, they wished to reach in and poke things about. Primitive simple manipulation devices – such as microscope-mounted needles – date back to the 18th

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century. Some of the earliest manipulative embryology experiments involved using such needles, already made from heat-pulled glass, to poke (and essentially destroy) individual cells of the early stages of simple aquatic organisms. Such destruction, in the case of these highly determinant embryos, resulted in satisfying downstream effects and manipulative embryology was born. Within 100 years, micromanipulation became a mature field with a substantial base of standard methodology and equipment, including the early versions of most current systems. Micromanipulation began to be used in a variety of reproductive developmental biology and animal husbandry settings. When the maturing science of ART was brought to bear in human clinical reproduction by Edwards and co-workers in the late 1970s, micromanipulation was not far behind. In the mid 1980s, several laboratories around the world began considering the application of micromanipulative techniques to human clinical material. The first efforts involved methodology to promote sperm–egg fusion and fertilization by circumventing the zona pellucida. After a few fits and starts, our group reported in 1989 on the first substantial series of pregnancies and healthy human births resulting from micromanipulated eggs in which the zona had been opened to supposedly facilitate fertilization in male factor cases (Cohen et al., 1988; Malter and Cohen, 1989a). Around the same time, other groups pursued simply injecting spermatozoa into the perivitelline space (Lacham et al., 1989; Ng et al., 1989). Within a few years, these techniques were quickly made obsolete by direct sperm injection, but human micromanipulation was established and other techniques addressing other clinical issues in human reproduction quickly followed. I will now review the historical development of micromanipulation techniques in greater detail as related to the stage of development.

Micromanipulation of eggs and spermatozoa

The interaction between egg and spermatozoa represents a primary mystery in developmental biology and is obviously of great clinical importance in both animal husbandry and human clinical reproduction. While we have still not solved this mystery, we have made great strides, and micromanipulation has played a key role in the associated detective work. Furthermore, using micromanipulation, obstacles to sperm–egg fusion and fertilization hampering reproductive success have been considerably ameliorated. The idea of simply sticking a sperm cell into an egg has had great appeal and perhaps represents the pinnacle of developmental biological "poking" (Markert, 1983). It seems quite amazing that this technique, which bypasses a great deal of natural sperm–zona–egg membrane interactions, can actually work as well as it does.

As sperm–egg fusion and fertilization began to be dissected by developmental biology and animal science researchers, early experimental attempts sought to first simply bypass the zona pellucida. Beth Talansky in Jon Gordon's laboratory performed the pioneering mammalian experiment in which an ingenious "hydraulic drilling" technique was used to burn a tiny hole in the zona of mouse eggs using a flow of acidified solution from a micropipette (Gordon and Talansky, 1986). This "zona drilling" resulted in substantially increased fertilization when the population of spermatozoa was compromised in various ways. The technique seemed a likely candidate for improving human clinical IVF. However, human eggs unfortunately were considerably more sensitive to the acidified media, and success was not obtained (Garrisi et al., 1990; Gordon et al., 1988). Our group, under the direction of Jacques Cohen, developed a simple mechanical alternative to dissect a gap in the human zona, which quickly resulted in facilitating healthy births in many mild male-factor couples (Cohen et al., 1988; Malter and Cohen, 1989a). This consistent clinical success demonstrated that micromanipulation could be integrated into the human ART laboratory setting and established a set of basic aspects, such as appropriately sized microtools for human eggs/embryos and strict temperature control to maintain the integrity of human eggs during the procedures. One lesson from these early manipulative developments was the failure of rodent eggs and embryos as a model for the human. As mentioned, the reaction of human eggs to acidified zona drilling was not commensurate with the prior rodent experiments. Another major discrepancy was the level of polyspermy observed when the zona barrier was compromised. Partial zona dissection of mouse eggs resulted in essentially no increase in polyspermy, whereas in the human it was considerable and basically rendered the technique unusable (Malter et al., 1989). This was of course a basic developmental biology finding in identifying a distinct difference between the two species in the basis of the polyspermy block. However, it was a frustrating cautionary tale in the attempt to model and develop human techniques in the mouse, and other developmental discrepancies between the species would continue to be identified.

The injection of a spermatozoon under the zona was another "bypass" technique that was successfully pursued, but early failures at direct cytoplasmic injection of spermatozoa in the human clinical setting put a damper on the pursuit of that idea (Lanzendorf et al., 1988; Sakkas et al., 1992). My co-worker Carol Keefer in Ben Brackett's large animal research laboratory had already produced the first mammals (Dutch belted rabbits) from direct cytoplasmic sperm injection in 1989 (Keefer, 1989). However, her success was hard fought and required a rather complex manipulative protocol and considerable skill, and had a restricted survival and success rate. Fortunately, Gianpiero Palermo and co-workers in Brussels observed random success when human eggs were fully pierced during clinical subzonal injection attempts. They pursued and refined the technique to create a working direct cytoplasmic injection technique somewhat ponderously termed intracytoplasmic sperm injection (ICSI) to delineate it from the subzonal procedure (Palermo et al., 1992). With ICSI, excellent fertilization and development was achieved with single sperm injection, providing a truly revolutionary functional clinical solution to even the most severe level of male factor infertility. It is worth noting that, again, human eggs and embryos fortunately have considerable developmental distinctions from other model systems. Direct sperm injection "works" much better in the human than in basically all other species in which the technique has been pursued, including other primates (Hewitson et al., 2000).

Another technique involving the egg is the manipulation of ooplasm. A variety of research and large animal experiments have used either membrane-bound cytoplast transfer or direct injection to modify the ooplasm. Some