

Micromanipulation in Assisted Conception

This practical handbook provides an extremely comprehensive, highly illustrated and up-to-date guide to micromanipulation techniques in assisted conception in a clinical setting. It includes detailed, illustrated descriptions of all the common micromanipulation systems currently in use in in vitro fertilization (IVF) laboratories around the world and explains clearly how to optimize their successful use. The volume covers state-of-the-art techniques, including intracytoplasmic sperm injection (ICSI), and procedures such as assisted hatching and blastomere biopsy (for pre-implantation genetic diagnosis). Valuable information on troubleshooting the potential mechanical and technical difficulties that can arise is provided to help all practitioners of these techniques, including trainee embryologists and consultant obstetricians, and technicians and scientists involved in animal transgenesis and cloning. It will undoubtedly be of immense value to all doctors and scientists working with assisted reproductive technologies.

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Steven D. Fleming and Robert S. King

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Every effort has been made in preparing this book to provide accurate and up-to-date information which is in accord with accepted standards and practice at the time of publication. Nevertheless, the authors, editors and publisher can make no warranties that the information contained herein is totally free from error, not least because clinical standards are constantly changing through research and regulation. The authors, editors and publisher therefore disclaim all liability for direct or consequential damages resulting from the use of material contained within this book. Readers are strongly advised to pay careful attention to information provided by the manufacturer of any drugs or equipment that they plan to use.

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Foreword

This is no ordinary book. It is a detailed description, such as has never been compiled before, of the current status of micromanipulation procedures. It describes in detail the various pieces of equipment and supplies necessary to set up the sophisticated procedures used in the treatment of male infertility, in characterizing early-stage embryos, and in performing procedures of mouse transgenesis. It is gratifying to see that this work is grounded in the belief that the key to micromanipulation is understanding the instruments, selecting the appropriate equipment, and picking the ideal consumables.

Since the first success of in vitro fertilization (IVF) in 1978, the field has been transformed by a steady stream of discovery and technological progress that has led to the expansion of the indications, such as the treatment of severe male infertility by intracytoplasmic sperm injection (ICSI) and the identification of genetic disorders by pre-implantation genetic diagnosis (PGD). These discoveries and techniques are grouped under the term 'assisted reproduction techniques'. For the first time, this book describes in a clear and concise manner the hows, whys and therefores of such procedures. It has been written to be readable and usable by research fellows, embryologists and technicians who need some insight into the technical developments, and who wish to know the A to Z of micromanipulation as seen and performed by the two authors.

It is always exciting to browse through a new book, particularly a manual, but as we go along we often notice that the information is too polished, presented from an ideal standpoint, and dealing with theoretical situations. Such material makes a good book, but from a practical point of view often may not prove to be very useful. During the preparation of this manual, the authors could have been trapped by the irresistible drive to be comprehensive and make a large book that would have lost practical usefulness and contact with the reality of micromanipulation.

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Instead, Steven Fleming and Robert King have produced a work that stays on track. The authors deliver a quick, practical troubleshooting manual for the laboratory. This work will help scientists, embryologists and technicians feel secure in setting up their systems and dealing with the daily difficulties of micromanipulation. The manual integrates current successful procedures and some newer ones, such as nuclear transplantation and genetic engineering.

In short, the work is dynamic. There is an authoritative exposition of the different steps of micromanipulation, ranging from the routine of well-established procedures to the generation of new experimental animals. This manual represents a milestone in the literature of reproductive medicine and will benefit all who read it.

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Preface

A number of good books have been published on the scientific and technical aspects of micromanipulation. However, they are usually aimed at the experienced practitioner with limited benefit to those working in an environment that is often largely devoid of expert technical support. In contrast, the primary objective of this book is to provide an easy-to-follow, step-by-step guide to micromanipulation techniques, with an emphasis on troubleshooting the myriad difficulties inevitably encountered. Indeed, the original idea for this book arose from a chance meeting between the authors. We realized that we shared a common experience despite our diverse backgrounds. Having introduced students to the equipment and techniques involved in micromanipulation, much of our time was then spent acting on a long-distance consultancy basis once these students found themselves back in the 'real world' of a clinical assisted reproduction centre. In this respect, the authors have also recognized that many of these challenges are most likely common to those working within the related fields of human assisted reproduction, livestock production, endangered species preservation, and transgenic research. Therefore, an attempt has been made to direct the material at a broad readership wherever relevant.

It is important to appreciate that this is fundamentally a technical manual. Hence, the chapters are designed to be read not in any particular order but as required. Related information within the same chapter as well as within different chapters is cross-referenced wherever necessary. Abbreviations litter this field of work and, therefore, a list of abbreviations that relate specifically to the information in this book has been provided. Likewise, a glossary of terms has been provided to explain much of the terminology used within this book. Finally, full contact details for all the suppliers of equipment and consumables listed in this book have been provided; this list is by no means exhaustive, and neither is it intended to recommend one distributor over another.

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While every effort has been made to ensure the technical information contained in this book is as up to date as possible, it should be noted that manufacturers reserve the right to change product specifications, to discontinue old product lines, and to introduce new instruments without prior notice. New products will invariably be introduced in the future, and it is hoped that these instruments can be covered in future editions.

The authors welcome feedback and further discussion regarding content.

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Glossary

- Agglutination:** The sticking together of large numbers of motile spermatozoa due to the presence of anti-sperm antibodies.
- Aneuploidy:** A condition where there is a loss or gain of chromosomes resulting in an alteration to the normal complement within a cell.
- Assisted hatching:** The partial or complete removal of the zona pellucida by zona drilling or by enzymatic means, the rationale being that this will enhance the ability of the blastocyst to escape from the zona pellucida and implant into the uterus.
- Asthenozoospermia:** Lower than normal percentage of progressively motile spermatozoa in the ejaculate.
- Azoospermia:** The total absence of spermatozoa within the ejaculate.
- Blastomere:** A cell of a pre-implantation embryo, from the two-cell stage to the blastocyst.
- Blastomere biopsy:** Removal of one or more blastomeres from an embryo for pre-implantation genetic diagnosis.
- Calcium ionophore:** A drug, such as A23187, that opens calcium channels in the plasmalemma, allowing calcium to enter the cell.
- Cleavage:** Series of mitotic cell divisions by which a zygote is transformed into a blastocyst.
- Congenital bilateral absence of the vas deferens:** Abnormality, often associated with the cystic fibrosis mutation, in which the vas deferens fails to develop on both sides, resulting in obstructive azoospermia.
- Corona radiata:** The layer of granulosa cells immediately surrounding the oocyte and in contact with the zona pellucida. Also termed the zona radiata.
- Cryptozoospermia:** An ejaculate that appears azoospermic until concentrated down by centrifugation, after which a few spermatozoa can be identified.

- Cumulus oophorus:** The cloud-like layer of granulosa cells that surrounds the oocyte and connects it to the granulosa.
- Epididymis:** A long, narrow, coiled tube lying on the anterodorsal aspect of the testis. The epididymis connects the rete testis to the vas deferens and is the site where spermatozoa undergo further maturation.
- Fragile oocyte syndrome:** A condition where oocytes that appear morphologically normal usually degenerate following injection. These oocytes are typically very easy to inject, their oolemma offering little resistance.
- Globozoospermia:** A condition in which spermatozoa lack an acrosome due to impaired spermiogenesis.
- Headstage:** The moving section of a manipulator (as opposed to the controller), usually mounted on the illumination support limb of a microscope.
- Hypo-osmotic swelling test:** The immersion of spermatozoa within hypo-osmotic media to determine the integrity of their plasmalemma. Intact cells take up water from the surrounding media by osmosis and swell to accommodate their increase in volume.
- Hypospermatogenesis:** A lower-than-normal level of spermatogenesis.
- Immotile cilia syndrome:** A lack of motility or aberrant motility of the cilium or flagellum of the spermatozoon, due to abnormal development or function of the axoneme. One typical example of this syndrome is Kartagener's Syndrome, in which the dynein arms of the axoneme are either too short or absent.
- Manipulator:** Any device that allows a probe or micropipette to be positioned in one or more dimensions (*see also Micromanipulator*).
- Manipulator, coarse:** Distinct from a micromanipulator, this device allows only approximate positioning in one or more dimensions, i.e. it has a lower resolution than a micromanipulator.
- Micro-epididymal sperm aspiration:** The collection of spermatozoa from the epididymis by passing a hypodermic needle into the surgically exposed epididymis under local or general anaesthesia, and then applying gentle suction.
- Microinjection sperm transfer:** The transfer of one or more spermatozoa into the perivitelline space using an injection pipette, and subsequently termed subzonal insemination.
- Micromanipulator:** A device for positioning a probe or micropipette extremely precisely in three dimensions, often to resolutions of less than 1 μm . Usually used in conjunction with a *coarse manipulator*.
- Monopronucleate:** Having a single pronucleus.

- Multipronucleate:** Having more than two pronuclei.
- Non-obstructive azoospermia:** Azoospermia due to failed or impaired spermatogenesis.
- Normozoospermia:** Normal density of spermatozoa within the ejaculate with a normal percentage of progressive motility and normal morphology.
- Obstructive azoospermia:** Azoospermia due to a blockage in the reproductive tract.
- Oligozoospermia:** Lower-than-normal density of spermatozoa in the ejaculate.
- Oocyte:** A diploid (primary) or haploid (secondary) germ cell that gives rise to an ovum via meiosis.
- Oocyte activation:** The stimulation of the oocyte by the fertilizing spermatozoon that results in the completion of fertilization and initiation of cleavage.
- Oogenesis:** Production and growth of ova that occurs within the ovary.
- Oogonium:** A premeiotic diploid germ cell that gives rise to oocytes via mitosis.
- Oolemma:** Another term for the vitelline membrane, the membrane surrounding and secreted by an oocyte.
- Ooplasm:** The cytoplasm of an oocyte.
- Oscillin:** The original term used to describe the putative factor released from the spermatozoon at fertilization, responsible for causing the oscillations in intracellular calcium that bring about egg activation.
- Ovum:** Mature, haploid female gamete.
- Partial zona dissection:** Another term for *zona drilling* and originally devised to enhance the passage of spermatozoa into the perivitelline space during in vitro fertilization.
- Percutaneous epididymal sperm aspiration:** The collection of spermatozoa from the epididymis by passing a hypodermic needle through the skin and into the epididymis under local or general anaesthesia, and then applying gentle suction.
- Perivitelline space:** The space surrounding the oocyte, lying between the oolemma and zona pellucida.
- Phosphodiesterase inhibitors:** Drugs such as pentoxifylline and caffeine that inhibit the breakdown of cyclic adenosine monophosphate (cAMP) by phosphodiesterase. The consequent elevation in cAMP causes enhancement of sperm motility.
- Plasmalemma:** The cell membrane.

Polygyny: Polyploidy as a result of a failure of the fertilized oocyte to extrude the second polar body.

Polyploidy: A condition in which there is a gain of one or more sets of chromosomes, resulting in an increase in the normal complement within a cell.

Polyspermy: Entry of more than one spermatozoon into an oocyte during fertilization, resulting in polyploidy.

Pre-implantation genetic diagnosis: The use of molecular biological techniques, such as fluorescent in situ hybridization and the polymerase chain reaction, to determine the chromosomal and genetic constitution of an embryo prior to its transfer to the uterus.

Pronucleus: The nucleus of a gamete that appears within a zygote at fertilization, just prior to *syngamy*.

Rescue intracytoplasmic sperm injection (ICSI): ICSI performed upon an oocyte that has failed to fertilize following in vitro fertilization.

Retrograde ejaculation: Ejaculation into the bladder instead of through the urethra, due to failure of the sphincter muscle at the neck of the bladder.

Seminiferous tubules: The long coiled tubules within the testis where spermatogenesis occurs.

Sertoli cell: A large cell within the testis responsible for the sustenance of spermatogonia, spermatocytes and spermatids.

Sertoli cell-only syndrome: A condition in which spermatogonia are largely or totally absent within the seminiferous tubules.

Spermatid: Immature, haploid spermatozoon.

Spermatocyte: A diploid (primary) or haploid (secondary) germ cell that gives rise to spermatids via meiosis.

Spermatogenesis: Production and growth of spermatozoa that occurs within the testis.

Spermatogenic arrest: A condition in which spermatogenesis fails to continue beyond a certain stage of germ cell development for genetic or other reasons.

Spermatogonium: A premeiotic diploid germ cell that gives rise to spermatocytes via mitosis.

Spermatozoon: Mature, haploid male gamete.

Spermiogenesis: Maturation of spermatids into spermatozoa.

Split ejaculate: An ejaculate that has been collected into two separate receptacles, one containing the initial part and the other containing the remainder.

Strict criteria: Highly specific morphological criteria that spermatozoa must meet in order to be considered normal. Also termed Tygerberg strict criteria and Kruger strict criteria.

Subzonal insemination: Another, more commonly used term for *microinjection sperm transfer*.

Suction-mediated aspiration of the rete testis: The collection of spermatozoa from the rete testis by passing a hypodermic needle through the skin and into the rete testis under local or general anaesthesia, and then applying gentle suction.

Syngamy: The fusion of the male and female pronuclei.

Teratozoospermia: Lower-than-normal percentage of morphologically normal spermatozoa in the ejaculate.

Testicular sperm aspiration: The collection of spermatozoa from the testis by passing a hypodermic needle through the skin and into the testis under local or general anaesthesia, and then applying gentle suction.

Testicular sperm extraction: The collection of spermatozoa from the testis by excising part of the surgically exposed testis under local or general anaesthesia.

Tripronucleate: Having three pronuclei.

Vital dyes: Dyes that have a molecular size too large to cross an intact cell plasmalemma, used to determine cell viability.

x-axis: The horizontally oriented axis aligned from left to right, with respect to the microscope.

y-axis: The horizontally oriented axis aligned from front to back, with respect to the microscope.

z-axis: The vertically oriented (up-and-down) axis.

Zona drilling: The making of a passage through the *zona pellucida* by mechanical or chemical means, or with the use of a laser.

Zona pellucida: Acellular, striated glycoprotein membrane normally surrounding the oocyte.

Zona radiata: Another term for the corona radiata.

Zygote: The fertilized ovum, prior to cleavage.

Abbreviations

AH	assisted hatching
cAMP	cyclic adenosine monophosphate
CBAVD	congenital bilateral absence of the vas deferens
COC	cumulus–oocyte complex
DIC	differential interference contrast
DMEM	Dulbecco's modified Eagle's medium
EBSS	Earle's balanced salt solution
EDTA	ethylene diamine tetra-acetic acid
EKRB	enriched Krebs–Ringer bicarbonate
ELSI	elongated spermatid injection
EMEM	Eagle's modified minimal essential medium with Earle's Salts
ESC	embryonic stem cell
ET	embryo transfer
FACS	fluorescence-activated cell sorting
FBS	fetal bovine serum
FCS	fetal calf serum
FISH	fluorescent in situ hybridization
FSH	follicle-stimulating hormone
GIFT	gamete intrafallopian transfer
GV	germinal vesicle
GVBD	germinal vesicle breakdown
hCG	human chorionic gonadotrophin
HIC	high insemination concentration
HMEM	Hepes-buffered minimal essential medium
hMG	human menopausal gonadotrophin
HOST	hypo-osmotic swelling test
HSA	human serum albumin
HTF	human tubal fluid
ICM	inner cell mass

xviii Abbreviations

ICSI	intracytoplasmic sperm injection
IUI	intrauterine insemination
IVF	in vitro fertilization
IVM	in vitro maturation
LASU	laser setting-up device
LH	luteinizing hormone
LIF	leukaemia inhibitory factor
MEM	minimal essential medium
MESA	micro-epididymal sperm aspiration
MHC	major histocompatibility complex
MI	metaphase 1
MII	metaphase 2
MIST	microinjection sperm transfer
OHSS	ovarian hyperstimulation syndrome
PB	polar body
PB1	first polar body
PB2	second polar body
PBS	phosphate-buffered saline
PCOD	polycystic ovarian disease
PCR	polymerase chain reaction
PESA	percutaneous epididymal sperm aspiration
PGD	pre-implantation genetic diagnosis
PMSG	pregnant mare's serum gonadotrophin
PN	pronucleus
PVP	polyvinylpyrrolidone
PVS	perivitelline space
PZD	partial zona dissection
ROSI	round spermatid injection
ROSNi	round spermatid nucleus injection
SAS	screw-actuated syringe
SCNT	somatic cell nuclear transfer
SESI	secondary spermatocyte injection
SMART	suction-mediated aspiration of the rete testis
SSR	surgical sperm recovery
SUZI	subzonal insemination
TAE	Tris-acetate/ethylene diamine tetra-acetic acid
TCM	tissue culture medium
TESA	testicular sperm aspiration
TESE	testicular sperm extraction
TMC	total motile count

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Abbreviations **xix**

TNF	tumour necrosis factor
VSUG	velocity sedimentation under unit gravity
WHO	World Health Organization
ZD	zona drilling
ZIFT	zygote intrafallopian transfer
ZP	zona pellucida