<table>
<thead>
<tr>
<th>Title</th>
<th>Laboratory aspects of assisted reproduction.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Yeung, WS; Ng, EH</td>
</tr>
<tr>
<td>Citation</td>
<td>Hong Kong Medical Journal = Xianggang Yi Xue Za Zhi / Hong Kong Academy Of Medicine, 2000, v. 6 n. 2, p. 163-168</td>
</tr>
<tr>
<td>Issued Date</td>
<td>2000</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10722/57405">http://hdl.handle.net/10722/57405</a></td>
</tr>
<tr>
<td>Rights</td>
<td>Hong Kong Medical Journal. Copyright © Hong Kong Medical Association.; This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.</td>
</tr>
</tbody>
</table>
Laboratory aspects of assisted reproduction

WSB Yeung, EHY Ng

A number of advances have been made concerning the laboratory aspects of assisted reproduction. Intracytoplasmic sperm injection has revolutionised the treatment of male infertility. With the development of better embryo culture media, blastocyst transfer is now possible and is likely to reduce high-order multiple pregnancy in assisted reproduction treatment. Pre-implantation genetic diagnosis has become an alternative to prenatal diagnosis. The recent use of molecular biology techniques to detect small genetic defects in men with severe male-factor infertility has provided information for the better counselling of these patients. Other techniques that are being developed are likely to have a tremendous impact on assisted reproduction treatment. These include in vitro maturation, follicle culture, and oocyte/ovarian tissue cryopreservation. The current status of the developments in the laboratory aspects of assisted reproduction is reviewed in this article.

HKMJ 2000;6:163-8

Key words: Blastocyst; Cell culture/methods; Fertilization in vitro/methods; Microinjections; Oocytes; Preimplantation diagnosis

Introduction

Assisted reproductive technology has revolutionised the treatment of infertility. In this paper, the major recent advances in the laboratory aspects of assisted reproduction will be reviewed in two parts. The first part will review the relatively recently developed techniques that have become widely used in assisted reproduction. The second part will focus on techniques that are currently being developed and are likely to have a significant impact on assisted reproductive technology.

Established techniques

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection (ICSI) has had a considerable impact on the treatment of male-factor infertility. The technique involves the injection of a single spermatozoon into an oocyte. Since its development in 1992,1 ICSI has helped subfertile couples at most assisted reproduction units. The fertilisation outcome after ICSI is little affected by most conventional criteria of sperm quality. Since the requirement for spermatozoa during ICSI is minimal, ICSI can be used in almost all men in whom spermatozoa can be found in the ejaculate, epididymis, or testis.

The first baby conceived using ICSI in Hong Kong was born in 1995 at the Queen Mary Hospital (QMH). In 1998, the fertilisation rate of the ICSI technique at the QMH was approximately 60%.2 The results of ICSI with spermatozoa obtained from the ejaculate, by microsurgical epididymal sperm aspiration, or by testicular sperm extraction are shown in Table 1.2

Studies of obstetric outcomes, the malformation rates, and karyotype abnormalities of children born after ICSI have been reported by a number of groups.3

Table 1. Outcomes of intracytoplasmic sperm injection at the Queen Mary Hospital (1998)2

<table>
<thead>
<tr>
<th></th>
<th>ICSI* alone</th>
<th>MESA† +</th>
<th>TESE‡ +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilisation rate</td>
<td>64.0%</td>
<td>62.4%</td>
<td>55.4%</td>
</tr>
<tr>
<td>Mean No. of embryos</td>
<td>2.5</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>per transfer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate per</td>
<td>21.5%</td>
<td>20.8%</td>
<td>18.2%</td>
</tr>
<tr>
<td>transfer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>30.4%</td>
<td>40.0%</td>
<td>-</td>
</tr>
<tr>
<td>rate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ICSI intracytoplasmic sperm injection
† MESA microsurgical epididymal sperm aspiration
‡ TESE testicular sperm extraction

Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong
WSB Yeung, PhD
EHY Ng, FHKAM (Obstetrics and Gynaecology)

Correspondence to: Dr WSB Yeung
In general, these children are not associated with a higher incidence of congenital malformations, compared with those derived from conventional in vitro fertilisation (IVF). However, a slight increase in the incidence of chromosomal abnormalities has been shown in a follow-up study of ICSI children.

Pre-implantation genetic diagnosis
The development of molecular biology techniques has made it possible to identify chromosomal and gene defects in just one or two cells. This advancement has led to the development of pre-implantation genetic diagnosis (PGD), which can detect many common chromosomal abnormalities and inherited single-gene defects. In this procedure, one or two blastomeres, usually from an eight-cell-stage embryo, are removed and the genetic make-up in the extracted blastomeres is determined. The fundamental assumption is that these blastomeres are identical to those in the rest of the embryo. Hence, only embryos from which extracted blastomeres show normal genomes are transferred. Similarly, the polar body of oocytes or zygotes can be used for PGD.

There are two methods for detecting genetic abnormalities in a single cell. Polymerase chain reaction (PCR) analysis is used to detect genetic abnormalities at gene level. Although this technique is very sensitive, it does not provide information about the chromosome copy number. In contrast, multicolour fluorescent in situ hybridisation (FISH) not only detects abnormalities in autosomes, but also the number of sex chromosomes. Currently, FISH is the preferred method of identifying the sex of an embryo and of detecting aneuploidy when women are of advanced maternal age. The detection of aneuploidy by PGD in human embryos has recently been shown to reduce embryo loss after implantation, although the implantation rates were not significantly improved.

Pre-implantation genetic diagnosis may not be absolutely accurate, because of the occurrence of chromosomal mosaicism in human embryos—that is, two or more blastomeres from the same embryo having chromosome complements that are different from the remaining blastomeres. Studies using FISH have shown that a significant number of normally developing embryos display chromosome mosaicism. These chromosomal abnormalities are related to the impairment of embryo development, advanced maternal age, embryo culture conditions, and hormonal stimulation. Another reason that PGD may not be absolutely accurate is the failure of PCR amplification of one of the gene alleles, usually termed as allelic dropout. To minimise this effect, PCR conditions need to be optimised for each specific gene allele prior to PGD.
It is now possible to screen for single-gene disorders and chromosomal abnormalities simultaneously in a single blastomere. Attempts have also been made to develop a simple method of karyotyping metaphase chromosomes of the human blastomere.

**Co-culture versus sequential culture**

It is generally accepted that the culture conditions for human embryos are suboptimal. Two methods have been used to solve this problem: co-culture of embryos with somatic cells and the use of different culture media for different stages of embryo development (sequential culture). Co-culture received considerable interest in the early 1990s, with various studies having shown that co-culture improves the morphological development and implantation rate of treated embryos. Co-culture is particularly helpful to infertile couples with recurrent implantation failure. The oviductal cell is the most logical somatic cell to be used for co-culture with early pre-implantation embryos, as the oviduct is the natural site in which these embryos grow during early development. We have previously shown that co-culture with human oviductal cells improves the maturation rate of blastocysts and decreases the fragmentation rate of developing human embryos, and that improvement is more marked when co-culture is started earlier. In a randomised controlled trial, co-culture with human oviductal cells also enhanced the implantation rate of human embryos in the assisted reproduction programme at the QMH. However, co-culture involves complicated laboratory techniques and screening, and hence it is not suitable for routine use.

Cleaving embryos normally develop in the oviduct, whereas the morulae and blastocysts develop in the uterine cavity. These two stages of embryo growth have different nutritive requirements, which match quite well with the constituents in the oviduct and the uterine cavity. Thus, it is not surprising that the use of sequential culture media specific for different stages of embryo development produces better blastocysts in vitro; the resultant pregnancy rate is at least as good as that seen with co-culture. Because of its simplicity, sequential culture is rapidly becoming the method of choice for producing human blastocysts.

**Blastocyst transfer**

Early-embryo culture media do not support the long-term growth of human embryos. Hence, assisted reproduction programmes usually involve the transfer of day-2 or day-3 cleaving embryos to the uterus—an unusual site for early embryo development. Co-culture and sequential culture allow the production of sufficient blastocysts to make blastocyst transfer practical. There are a number of advantages of blastocyst transfer. Firstly, blastocysts are transferred to a natural site of development so the development of embryos and the endometrium are better synchronised. This synchronisation may theoretically enhance later implantation. Secondly, in some women, the uterus may fail to give adequate support to cleaving embryos, thereby resulting in the failure of IVF. Blastocyst transfer may overcome this problem. Blastocyst culture may be useful in patients with multiple implantation failure, although these data await confirmation by randomised studies. Thirdly, blastocyst transfer allows better selection of embryos; only good-quality embryos are able to develop into blastocysts. As a result, fewer embryos can be transferred. High-order multiple pregnancy can be eliminated by the transfer of just two embryos. Fourthly, the risk of miscarriage may be reduced, because chromosomally abnormal embryos tend to have retarded development and are less likely to be selected for transfer.

A problem with blastocyst transfer is that only approximately half of the fertilised eggs develop into blastocysts. Some patients may therefore have no embryos available for transfer. By carefully selecting patients, the percentage of patients without blastocysts could be low. Although the failure of blastocyst formation in vitro may indicate poor quality of the oocyte, sperm, or embryo, it is also possible that the current sequential media are still not yet optimal. Some embryos that fail to reach the blastocyst stage in vitro might have given rise to offspring, had they been transferred earlier. Studies of methods that further improve currently used sequential culture system media are ongoing. At the QMH, a human oviductal cell–mouse embryo coculture system has been developed for this purpose. Our data show that human oviductal cells improve the development of mouse embryos in vitro by the production of at least three embryotrophic factors. In future, the supplementation of these factors to the culture medium may further enhance human embryo development.

With the increasing use of blastocyst culture and transfer as a routine treatment in human clinical in vitro fertilisation, there is an increasing need for a reliable procedure to cryopreserve blastocysts. The conventional slow-freezing protocol is suboptimal for blastocysts, as the implantation rate of frozen blastocysts is only approximately half of that reported for the transfer of fresh blastocysts. A new vitrification method is being developed, with promising results.

**Blastocyst transfer versus cleaving embryo transfer**

Although blastocyst transfer has provided good results, Edwards and Beard have questioned whether...
have spermia in the Chinese population in Hong Kong with the non-obstructive azoospermia or severe oligo-

data from the QMH have shown that 9.1% of men often found to be deleted in these men. Preliminary
demonstrated at the QMH. 8 Recent studies have shown that men with severe
investigated. 53 Transmissions of a DAZ deletion from the father to his ICSI-derived son has also been
demonstrated at the QMH. 8

Managing male genetic abnormalities
Recent studies have shown that men with severe male-factor infertility have a higher incidence of chromosomal or genetic abnormalities. Attention has been made to microdeletions in the Y chromosome of these men. The DAZ (deleted in azoospermia) gene is often found to be deleted in these men. Preliminary data from the QMH have shown that 9.1% of men with the non-obstructive azoospermia or severe oligo-
sperma in the Chinese population in Hong Kong have DAZ deletions. 2 Transmission of a DAZ deletion from the father to his ICSI-derived son has also been demonstrated at the QMH. 8

Developing techniques
In vitro oocyte maturation
While the use of ICSI overcomes the fertilisation prob-
lems associated with culturing immature oocytes, there is an increased interest in retrieving oocytes without
gonadotrophic stimulation or with limited gonado-
trophic stimulation and then maturing them in vitro for embryo transfer. The advantages of maturing oocytes in vitro are that only a minimal amount of expensive drugs are needed; there is no risk of ovarian hyperstimulation, deep vein thrombosis, or other possible long-term side effects of fertility drugs including ovarian cancer; and that a large pool of pre-antral and antral oocytes is theoretically available in the early follicular phase of the cycle. Human oocytes can be matured from small antral follicles, fertilised in vitro, and give rise to live births following embryo transfer. 38,49 Currently, the success rate with in vitro oocyte maturation is low and the culture system needs to be improved.

Oocyte cryopreservation
The ability to freeze and store human oocytes success-
fully allows the circumvention of moral, ethical, and legal problems associated with embryo freezing. Oocyte cryopreservation also preserves the reproductive potential of women who, for a variety of medical reasons, are likely to lose ovarian function prematurely. Banks of frozen donated oocytes would facilitate the donation process by waiving the requirement for donor-recipient synchrony.

Gook et al 50 have demonstrated that oocytes can survive the conventional embryo cryopreservation protocol without compromising spindle integrity, and with normal karyotype. 51 Pregnancies and live births of cryopreserved oocytes that have been fertilised with ICSI have been obtained. 52 However, the success rate of oocyte cryopreservation remains low and new methods for human oocyte cryopreservation are being investigated. 53

Cryopreservation of the ovarian cortex
An alternative to cryopreserving mature human oocytes is to cryopreserve ovarian tissue that contains imma-
ture oocytes within the ovarian cortex. This approach has the additional advantage over oocyte cryopreserva-
tion of preserving the fertility of cancer patients. Detecting the cancer early and immediately starting treatment with chemotherapy and/or radiotherapy improves the long-term survival for patients with many types of cancer. 54 Unfortunately, these treatments may render women either temporarily or permanently infertile and the urgency of such treatments precludes harvesting mature oocytes from many of these patients. Cryopreserving the ovarian cortex would be a suitable option in these cases. The transplantation of cryopre-
served ovarian tissue has been able to restore fertility in animals, including marmosets. 55-57 However, efficacy of this technique in humans has yet to be determined.
**Follicle culture**

In biopsy samples of ovarian cortex, the numbers of primordial follicles present are significantly higher than those in the later development stages. Cryopreserved ovarian tissue of cancer patients can be used for transplantation, but this would carry a risk of transmission of the malignancy. The other option is to culture the primordial and primary follicles that have come from the cryopreserved biopsy sample of cortical tissue. In studies of mice, live offspring have been born from oocytes that have matured from primordial follicles in vitro. Although it is possible to isolate primordial and primary human follicles, it has not been possible to establish long-term culture of these follicles. The development of human follicles in vitro is better in tissue slices than in the partially isolated form. In ovarian slices, human primordial and primary follicles can be cultured to reach secondary and occasionally early-antral follicles.

**Conclusion**

A number of advances have been made in the laboratory aspects of assisted reproduction in the past decade. These improvements have extended the range of patients to be treated by assisted reproduction. With the use of molecular biology, more is known about the genetic causes of infertility, so better counselling can be offered to the infertile patients. Although a better understanding of the development of the oocyte/follicle and embryo has allowed us to provide a better culture environment for the oocyte and embryo, further research is required to find the ideal culture media.

**References**

9. Page DC, Silber S, Brown LG. Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. Hum Reprod 1999;14:1722-6.


32. Sakkas D. The use of blastocyst culture to avoid inheritance of an abnormal paternal genome after ICSI. Hum Reprod 1999;14:4-5.


