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Assisted Reproductive Techniques – Promises and Problems

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ABSTRACT

Assisted reproductive techniques (ART) are procedures in which the oocyte is handled before replacement, either as an oocyte or embryo. The current success rates and pregnancy outcomes of in-vitro fertilisation (IVF), gamete intra-fallopian transfer (GIFT), zygote intra-fallopian transfer (ZIFT), intra-cytoplasmic sperm injection (ICSI), donor oocyte and frozen embryo programmes are reviewed. Some problems associated with assisted reproductive procedures are also discussed.

Keywords: assisted reproductive techniques, pregnancy rates, complications

INTRODUCTION

Since the birth of Louise Brown in 1978[1], assisted reproductive techniques have been used to treat subfertile women all over the world. These assisted reproductive techniques (ART) refer to procedures in which the oocyte is handled before replacement, either as an oocyte or embryo[2]. In this paper, we will review the current success rates and pregnancy outcomes of in-vitro fertilisation (IVF), gamete intra-fallopian transfer (GIFT), zygote intra-fallopian transfer (ZIFT), intra-cytoplasmic sperm injection (ICSI), donor oocyte and frozen embryo programmes. We will also discuss some of the problems associated with assisted reproductive procedures.

Although IVF was initially developed for patients suffering from tubal infertility, it is currently employed for a wide range of indications including male factor problems, endometriosis and unexplained infertility. To date, IVF data from several national registries have been published[3-5]. The results of IVF programmes carried out in the United States, Canada, Asia-Oceania and France are shown in Table I. In general, patients undergoing IVF can expect pregnancy rates of 17% to 23% per cycle and corresponding delivery rates of 13% to 18% per cycle. The incidence of spontaneous abortion is similar to the 15% to 20% seen in the general population[6]. Furthermore, the occurrence of birth defects is not increased over the background incidence of 2% to 5%[7]. However, the incidence of ectopic pregnancy is 2 to 3 times higher than the 2% quoted for the general population[8], and the incidence of multiple pregnancies is also greater than the 11 per 1000 deliveries reported in the literature[9].

Several authors[10,11] have also published data on the cumulative pregnancy rates (CPR) after IVF treatments. Check et al[10] reported pregnancy rates of up to 28% in the first cycle, 51% after three cycles and 66% after six cycles. Dor et al[11] also published data that demonstrated a constant rise in the cumulative pregnancy rates during the six initial IVF cycles (56% CPR). Life-table analysis of the IVF programmes in Australia[12] also revealed cumulative pregnancy rates of 18.6% in the first month of attempt to 63% at six months and 84% at 1 year. Data from the French IVF programmes[13] also showed similar increases in CPR over a 10-month period. Although life-table analysis and CPR are useful for assessing IVF success, several problems have been highlighted in the literature. An important assumption in the use of life-table analysis is that women who stop IVF treatment before the occurrence of pregnancy have the same probability of getting pregnant as those who continue. However, women who cease treatment for emotional reasons and those who stop further treatment because of poor IVF results (eg. poor embryo quality), cannot be expected to have the same probability of achieving a pregnancy. This and other confounding factors have been discussed by several authors[11,14].

Much has been published in the literature concerning factors that may affect IVF pregnancy rates. Numerous variables have been investigated including the type of infertility[15,16], use of GnRH-agonists[17], presence of a hydrosalphinx[18,19], duration of infertility[5,16], previous pregnancy[16] and the use of luteal phase support[20]. Although the above data has been at times confusing, it is now clearly recognised that patient age has a crucial effect on IVF success rates. Templeton et al[16] analysed 36,961 IVF treatment cycles carried out in the United Kingdom and reported a sharp decline in pregnancy rates in older women. Compared with livebirth rates of 17% per cycle started at age 30, the corresponding success rates at ages 40 and 45 were only 7% and 2% respectively. Data from the French national IVF registry (1986 – 1990)[5] also showed lower IVF pregnancy rates with increasing patient age. Dor et al[11] analysed 2,252 IVF treatment cycles at the Chaim Sheba Medical Centre and reported significantly lower cumulative pregnancy rates in women aged $\geq$ 40 years. Dawood[21], in an analysis of the 1991 – 1993 IVF data from the United States and Canada, reported that the delivery rate per retrieval was halved in women $\geq$ 40 years old compared with those $<$ 40 years old

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Table I – Reported pregnancy rates and outcome for IVF procedures

	US and Canada (1994)	Asia/Oceania (1993)	France (1990)
Cycles	26,961	24,571	18,639
Cancellation (%)	13.8	11.9	7.8
Pregnancy per cycle (%)	22.6	16.6	17.2
Pregnancy per oocyte retrieval (%)	26.2	18.9	18.7
Deliveries per cycle (%)	18.2	13.3	-
Deliveries per oocyte retrieval (%)	21.0	15.1	-
Spontaneous abortions (%)	19	14	17.7
Ectopic pregnancy (%)	4	5.8	-
Multiple pregnancy rate (%)	36	18.8	28
Birth defect per neonate delivered (%)	2.7	1.3	3.5

Data for United States and Canada obtained from IVF Registry of Society of Assisted Reproductive Technology[3]

Data for Asia/Oceania taken from Chang YS, et al[4]

Data for France obtained from French in vitro National (FIVNAT) Registry[5]

Table II – Reported pregnancy rates and outcome for GIFT procedures

	Meirow and Shenker[27]	US and Canada (1994)	Asia/ Oceania (1993)
Cycles	43,966	4,214	5,240
Pregnancy per cycle (%)	24	32	25
Pregnancy per retrieval (%)	29	36	28
Deliveries per cycle (%)	-	25	19.5
Deliveries per retrieval (%)	23	28.5	22
Spontaneous abortions (%)	22	22.5	18
Ectopic pregnancy (%)	5.5	3.3	2.9
Multiple pregnancy rate (%)	24.5	37	19.4
Birth defect per neonate delivered (%)	2.8	1.8	1.5

Source of data for US and Canada[3]
Source of data for Asia/Oceania[4]

Table III – Reported outcomes for ZIFT procedures

	US and Canada (1994)	Asia/Oceania (1993)
Cycles	962	1,005
Cancellation	13.6	6.6
Pregnancy per cycle (%)	28.8	17
Pregnancy per retrieval (%)	34.7	18.2
Deliveries per cycle (%)	24.2	13.8
Deliveries per retrieval (%)	29.1	14.8
Spontaneous abortions (%)	16.2	14
Ectopic pregnancy (%)	3.2	4.7
Multiple pregnancy rate (%)	35	15.8
Brith defect per neonate delivered (%)	2.4	2.1

Source of data for US and Canada[3]
Cource of data for Asis/Oceania[4]

with no male factor. These poorer IVF results in older women have been attributed to higher cancellations rates[21], poorer oocyte quality[22,23], possible decreased endometrial receptivity[16,24] and a higher incidence of miscarriages[25].

Gamete intra-fallopian transfer (GIFT) has been recognised as a mode of treatment for subfertility since 1986[26]. Meirow and Schenker[27] reviewed 43,966 GIFT cycles carried out worldwide from 1986 to 1993 and reported that GIFT accounted for 13.5% of all ART procedures. Current indications for GIFT include mild to moderate endometriosis, male factor disease and unexplained infertility. The pregnancy rates for GIFT cycles carried out in the United States, Canada and Asia-Oceania are shown in Table II. The pregnancy rate per GIFT cycle has been reported to range from 24% to 32% with delivery rates per cycle of 20% to 25%. The incidence of spontaneous abortions and birth defects are not greater than those expected in the general population. However, like IVF, the incidence of ectopic gestation and multiple pregnancy have increased. Kovacs[12] performed life-table analysis of 6,308 GIFT procedures done in 1989 in Australia. The author reported cumulative pregnancy rates of 29% in the first cycle, 60% after 3 cycles and 80.5% after 6 cycles. Once again, advanced patient age was reported to adversely affect GIFT success rates. Data from the United States and Canada[28] have also shown that cycle cancellation rates were higher and delivery rates per cycle were lower (11% – 12%) in women aged $\geq$ 40 years as compared to delivery rates (30% – 32%) in patients $<$ 40 years old.

Zygote intra-fallopian transfer (ZIFT) procedures have been recommended for patients with oligoasthenoteratozoospermia and those who have failed GIFT cycles[29]. In 1993, 1,005 ZIFT cycles were performed in centres in Asia-Oceania[4]. The most common indications published in that report were male factor and unexplained infertility. The reported outcomes of ZIFT procedures in the United States, Canada and Asia-Oceania are shown in Table III. In general, pregnancy rates of 17% – 29% and delivery rates of 14% – 24% per cycle of ZIFT may be expected. As with IVF and GIFT, the incidence of miscarriage and birth defects in ZIFT pregnancies are not higher than those expected for the general population. However, as with IVF and GIFT, the ectopic gestation and multiple pregnancy rates associated with ZIFT procedures are increased.

Although IVF has helped in the treatment of male factor infertility, there were still couples with sperm parameters which were just too poor for even IVF to be successful. Thus a major advancement in the management of male infertility has been the introduction of gamete micro manipulation, especially intracytoplasmic sperm injection (ICSI). Ever since the first report of successful pregnancies after ICSI by Palermo et al[30] in 1992, many centres around the world have employed this technique and have published their data[31-34]. A summary of the fertilisation and pregnancy rates achieved with ICSI from 4 centres is shown in Table IV. The fertilisation

Table IV – Fertilisation and pregnancy rates for intracytoplasmic sperm injection (ICSI)

	Tournaye et al[31]	McLachlan et al[32]	Hamberger et al[33]	Bourne et al[34]
No. of patients	919	156	456	263
No. of cycles	1,275	171	538	296
Fertilisation per injected metaphase II oocyte (%)	66.4	47.7	57.7	69
Clinical pregnancy rate per cycle (%)	28.4	17	26.5	10.8
Clinical pregnancy rate per transfer (%)	31.3	17.6	29	12

Table V – Reported success rates and pregnancy outcomes for donor oocyte programmes

	US and Canada (1994)	Asia/Oceania (1992 – 1993)
Cycles	3,119	201
Pregnancy per cycle (%)	36.5	33
Pregnancy per retrieval (%)	57.4	33
Deliveries per cycle (%)	30	25
Deliveries per retrieval (%)	47	26
Spontaneous abortions (%)	17.4	20
Ectopic pregnancy (%)	1.4	1.3
Multiple pregnancy rate (%)	39.7	26
Birth defect per neonate delivered (%)	2.1	2.9

Source of data for US and Canada[3]
Source of data for Asia/Oceania from Chang Y S et al[4]

rates using ICSI range from 48% – 69% and clinical pregnancy rates of up to 31% per cycle are possible. Tournaye et al[31] have also reported no significant differences in the clinical pregnancy rates per transfer after ICSI with ejaculated, epididymal or even testicular sperm. However, other authors have reported lower fertilisation rates, poorer embryo development and fewer embryos available for cryopreservation when epididymal and testicular sperm are used for ICSI. Wisanto et al[35] reported the outcome of 424 pregnancies after ICSI. Although there was a 23.3% incidence of early pregnancy loss before 16 weeks, they reported no increase in chromosomal abnormalities or major congenital malformations as compared to the general population. The obstetric outcome of the pregnancies in terms of prematurity, low birth weight and perinatal mortality were similar to pregnancies obtained after IVF and other ART procedures.

Trounson et al[36] were the first to report successful embryo donation in humans in 1983. Donor oocytes are used for patients with premature ovarian failure or gonadal agenesis; those with certain genetic disorders eg. Huntington's chorea and for IVF failures as a result of abnormal oocytes[37]. The reported success rates and pregnancy outcome for donor oocyte programmes carried out in the United States, Canada and Asia-Oceania are shown in Table V. Pregnancy rates per cycle range from 33% – 37% with corresponding delivery rates of 25% – 30% per cycle. Compared to the general population, there is no increased risk of spontaneous abortions, ectopic pregnancy and birth defects associated with oocyte donation programmes. However, the increased risk of multiple pregnancy is due to the higher number of embryos transferred, as is inherent in any ART procedure.

Trounson and Mohr[38] were the first to report a successful pregnancy after transfer of a frozen-thawed embryo in 1983. Currently, it is possible to successfully freeze early stage one to four-cell embryos with 1,2 propanediol[39] and blastocysts using gylcerol[40] as the cryoprotectant. Embryo cryopreservation has the potential to reduce the risk of mutiple pregnancies by freezing excess embryos instead of replacing them. The excess embryos may then be used to increase the number of embryo transfers and thus increase pregnancy rates per retrieval. The ongoing pregnancy rates after IVF have been reported to be doubled by the addition of cryopreservation[41]. Instead of discarding excess embryos, cryopreservation can also reduce patient time and expense involved in additional stimulation cycles[42]. In addition, the ability to cryopreserve embryos and then transfer them in a subsequent non-stimulated cycle has been shown to reduce the risk of ovarian hyperstimulation syndrome[43]. Furthermore, some authors[44] have also reported that the endometrium, in an unstimulated or natural cycle, may be more favourable for implantation than in hyperstimulated cycles.

The reported outcome of cryopreserved embryo transfers performed in the United States, Canada and Asia-Oceania are shown in Table VI. In general, patients undergoing transfer of cryopreserved embryos can expect pregnancy rates of 13% – 18% per transfer and delivery rates of 10% – 14% per transfer. The incidence of early pregnancy loss and birth defects were not higher than those seen in the general population. However, the ectopic gestation and multiple pregnancy rates were increased. Although 60% – 80% of embryos survive cryopreservation[46,47], some workers have suggested that cryopreservation may affect embryo quality[48] and reduce the reproductive potential of surviving embryos[49]. However, other studies have shown no detrimental effects on surviving cryopreserved embryos[42,47]. There are also uncertainties over the choice of embryo transfers in either the natural or hormone replacement cycles. The advantages and problems of both options have been discussed by Yee et al[50].

Although ART has brought hope to many women suffering from infertility, these procedures are not without their problems. With the need for daily injections, repeated hormonal monitoring and follicular scanning, oocyte recovery, embryo replacement, and luteal support, it is no wonder that

Table VI – Outcome of cryopreserved embryo transfers

	US and Canada (1993, 1994)	Asia/Oceania (1993)
Cycles	13,865	5,562
Transfer of frozen-thawed embryos (%)	13,095 (94.4%)	5,349 (96%)
Pregnancy per transfer (%)	17.6	12.8
Deliveries per transfer (%)	14.3	9.9
Spontaneous abortions (%)	20.3	19
Ectopic pregnancy (%)	2.4	3.5
Multiple pregnancy (%)	27	9.6
Birth defect per neonate delivered (%)	2.2	1.9

Source for US and Canada[3,45]
Source for Asia/Oceania from Chang Y S et al[4]

patients who undergo such programmes are emotionally taxed and stressed to the limit. Also involved are complex religious, ethical and legal issues that accompany these techniques[51].

Another drawback of ART is its financial cost. The cost of a successful IVF pregnancy has been calculated to range from US$22,000 – $212,000[52-54]. Workers have tried various ways to reduce the cost of IVF, including:

a) the use of clomiphene citrate in place of human gonadotrophins to reduce the cost of ovarian hyperstimulation[55];
b) reducing the number of blood tests and ultrasound scans performed during monitoring[56];
c) performing oocyte retrieval and embryo replacements in an office setting; and
d) reducing the manpower needs by limiting oocyte recovery to only 3 days a week[57].

Other authors have sought to use cheaper alternatives. For patients with unexplained infertility, Simon et al[58] reported that superovulation with menotropin treatment was as successful and less costly than IVF or GIFT. In a literature review, Dawood compared the efficacy of superovulation and intrauterine insemination (IUI) with GIFT, in the treatment of unexplained infertility[21]. Although some studies[59,60] have reported higher fecundity rates with superovulation and IUI compared to GIFT; there are other multicenter studies that have shown no significant differences[61,62]. When comparing superovulation and IUI with IVF in the treatment of unexplained infertility, Crosignani et al[61] found similar fecundity rates for both treatments. Peterson et al[54] also reported that one to four cycles of human menopausal gonadotrophin (hMG) and IUI were just as effective as one cycle of IVF in obtaining a pregnancy in patients with unexplained infertility. It was estimated that the total expenditure per hMG and IUI pregnancy was US$7,907 as compared to US$22,811 per IVF pregnancy. Thus in unexplained infertility, although the superiority of IVF and GIFT over superovulation with IUI has not been firmly established, the current cost-calculations do suggest that 4 cycles of hMG and IUI be tried before resorting to IVF or GIFT. However more prospective trials are needed to settle the cost-effectiveness issue of ART versus superovulation and IUI in the management of unexplained infertility.

Another recognised limitation of IVF are the poor success rates. In general, most centres report delivery rates of less than 30% per cycle of treatment. This may be related to the fact that in the normal fertile female, embryos are usually at the blastocyst stage when they reach the uterus just before implantation. However in IVF, the embryos are replaced early at the 2 to 4-cell stage instead of 5-day old blastocysts. This discordance could account for the poor IVF success rates. The reason why IVF programmes transfer day 2, 4-cell stage embryos instead of blastocysts is because current in-vitro culture systems are suboptimal and only 18% – 28% of embryos cultured in such systems reach the blastocyst stage[63,64]. In an attempt to simulate the in-vivo environment of early conception, Bongso et al[65,66] used human tubal ampullary monolayers as a coculture system to generate blastocysts and reported up to 69% blastocyst development rates. These results were confirmed by Yeung et al[67] and various other workers using other coculture systems including bovine oviductal cells, vero cells and Buffalo rat liver cells which they reported, increased blastulation rates[68]. Several investigators[69-71] using various coculture systems have also reported higher pregnancy rates (35% – 55%) per cycle compared to those achieved with conventional culture media (17% – 32%). The ultimate goal of coculture systems would be to replace a single high quality cocultured blastocyst; thereby increasing pregnancy rates and reducing the risk of multiple pregnancies.

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic condition that is caused by the gonadotrophin injections used for ovarian hyperstimulation in ART cycles. This complication has been reported in 1% – 10% of IVF cycles[72,73] and is characterised by ovarian enlargement, ascites, abdominal distention, hydrothorax and tissue oedema[74]. Severe OHSS has been reported in 0.5% – 2% of IVF cycles[72,75] and can lead to hypovolaemia, oliguria, electrolyte imbalance, respiratory distress and thromboembolism[75]. Although many preventative strategies have been put forward, none has been totally successful in the prevention of OHSS[74]. Thus all reproductive specialists involved in ART must be constantly aware of this complication and familiarise themselves with the treatment options available[74].

There are also problems associated with pregnancies conceived through ART programmes. The incidence of ectopic pregnancy is increased with IVF, GIFT and ZIFT procedures. Worldwide data[3-4,27] suggest that the ectopic pregnancy rates associated with IVF (4% – 5.8%), GIFT (2.9% – 5.5%) and ZIFT (3.2% – 4.7%) are two to three times higher than in the general population[8]. Multiple pregnancies are increased in IVF and GIFT programmes because of the multiple embryos and oocytes replaced. Data from national registries have reported multiple

pregnancy rates of 19% – 37% per cycle of IVF or GIFT[3-5,27]. This is important as most of the obstetrical and perinatal complications associated with ART pregnancies are caused by multiple gestations. Von During et al[76] compared the obstetric and neonatal outcomes for twins and triplets conceived through IVF with singleton pregnancies in Norway from 1988 to 1991. They found that the IVF multiple pregnancies had increased rates of preterm delivery, low birth weight, intra-uterine growth retardation with higher neonatal and infant mortality rates compared to singleton pregnancies. Other workers[77] have also documented the increased incidence of developmental disability, cerebral palsy, mental retardation, learning disabilities and behavioural problems in children resulting from multiple pregnancies. In an attempt to reduce the frequency of multiple gestations in ART programmes, several countries have resorted to legislation to limit the number of embryos transferred. In the United Kingdom, Germany and Singapore, only 3 embryos per transfer is allowed. In the Nordic countries, only 2 embryos are transferred except in special circumstances. These measures are not surprising considering the costs associated with multiple gestations. For triplet births, it has been estimated to cost between US$21,000 – $36,000 per baby[78,79]. Perinatal health-care costs have also been calculated to be 11 times higher for triplets and 4 times higher for twins when compared to those for a singleton birth[78].

Singleton pregnancies resulting from ART have also been shown to be at risk. Tanbo et al[80] compared the obstetric outcome of 355 singleton ART pregnancies with 643 spontaneous singleton pregnancies and reported increased frequencies of pregnancy induced hypertension, placenta praevia, caesarean section, preterm delivery, low birthweight and small for gestational age infants in the ART group. The French in-vitro National study[81] analysed data on 3,889 singleton births conceived through IVF carried out from 1986 – 1990. Although they failed to detect an increased incidence of pre-eclampsia and placenta praevia in the IVF pregnancies as compared to the general population, they did document increased rates of caesarean delivery, preterm labour and low birth weight ($\leqslant$ 2500g and $\leqslant$ 1500g) in the IVF group. The stillbirth and perinatal mortality rates (6 and 6.5 per 1,000 respectively) were also not different from those of the general population (6.1 and 6.4 per 1,000).

More recently, there have been fears that ovulation inducing agents used in ART programmes may increase the risk of ovarian cancer. In 1971, Fathalla[82] first mooted the idea of "incessant ovulation" and ovarian cancer. According to the theory, each episode of ovulation would lead to minor trauma to the ovarian surface epithelium and this repetitive surface injury over time could lead to the development of ovarian cancer. On this basis, it has been hypothesised that the hyperstimulatory effects of fertility drugs used in ART programmes, might have potential neoplastic effects on the ovary. Heightened concern about the risk of ovarian cancer and the use of fertility drugs was caused by a series of publications in 1992[83-85]. The authors analysed 12 case-control studies of ovarian cancer and reported that infertile women using fertility drugs had nearly three times the risk (relative risk RR = 2.8, 95% confidence interval CI = 1.3 – 6.1) for invasive epithelial ovarian cancers as women without a history of infertility. Conversely, infertile women not using infertility drugs were found to have no increased risk (RR = 0.9, 95% CI = 0.66 – 1.3). However, these results have been challenged[86]. Bristow and Karian 1996[87] have critically reviewed the literature on ovulation induction and risk of ovarian cancer. Based on their analysis of four case-control, three retrospective cohort studies as well as a large meta-analysis of three additional case-control studies, they concluded that subfertile women, especially those with refractory infertility, are at particular risk of developing ovarian cancer. However, the current data does not support a causal relationship between the use of fertility drugs and ovarian cancer. Thus more prospective studies with carefully selected control groups are still needed to address this uncertainty.

REFERENCES

1. Steptoe PC, Edwards RG. Birth after reimplantation of a human embryo [letter]. Lancet 1978; 2:366.
2. Ng SC, Bongso A, Chan CLK et al. The future of assisted reproductive technologies. Ann Acad Med Singapore 1990; 19:841-4.
3. Society for assisted reproductive technology. Assisted reproductive technology in the United States and Canada: 1994 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil Steril 1996; 66: No. 5, 697-705.
4. Chang YS, Kim SH, Moon SY, Lee JY. Current status of assisted reproductive technology in Asia and Oceania. J Obstet Gynaecol Res 1996; 22:305-30.
5. French national IVF registry: analysis of 1986 to 1990 data. Fertil Steril 1993; 59(3):587-95.
6. WHO (1970) Spontaneous and induced abortions. WHO technical report series no. 41, Geneva.
7. Pretorius DH. An overview of congenital malformations. In: Nyberg DA, Mahony BS, Pretorius DH eds. Diagnostic ultrasound of fetal anomalies. Chicago: Year Book Medical Publishers. 1990; 21-38.
8. Rosevear S. Bleeding in early pregnancy. In: James DK, Steer PJ, Weirer CP, Goniks B eds. High risk pregnancy. Management options. London: W B Saunders 1994; 75-100.
9. Crowther CA. Multiple pregnancy including delivery. In: James DK, Steer PJ, Weirer CP, Goniks B eds. High risk pregnancy. Management options. London: W B Saunders 1994; 137-49.
10. Check J H et al. Comparison of the cumulative probability of pregnancy after in-vitro fertilization embryo transfer by infertility factor and age. Fertil Steril 1994; 61:257-61.
11. Dor J, Seidman DS, Ben-Shlomo I, Levran D, Ben-Rafael Z, Mashiach S. Cumulative pregnancy rate following in-vitro fertilization: the significance of age and infertility aetiology. Human Reproduction 1996; 11(2):425-8.
12. Kovacs GT. The likelihood of pregnancy with IVF and GIFT in Australia and New Zealand. Med J Aust 1993; 158:805-7.
13. Cohen J. The efficiency and efficacy of IVF and GIFT. Hum Reprod 1991; 6:613-8.

14. Stolwijk AM, Hamilton CJCM, Hollanders JMG, Bastiaans LA, Zielhuis GA. A more realistic approach to the cumulative pregnancy rate after in-vitro fertilization. Human Reproduction 1996; 11(3):660-3.
15. Alsalili Mansour, Yuzpe AA, Tummon IS, Parker J, Martin JSB, et al. Confounding variables affecting in vitro fertilization success: A decade of experience. Journal of Assisted Reproduction and Genetics, 1996; 12 (2):88-92.
16. Templeton A, Morris JK, Parslow W. Factors that affect outcome of in-vitro fertilisation treatment. Lancet 1996; 348:1402-6.
17. Huges EG, Fedorkow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA. The routine use of gonadotropin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: a meta-analysis of randomized controlled trials. Fertil Steril 1992; 58:888-96.
18. Shelton KE, Butler L, Toner JP, Oehninger S, Muasher SJ. Salpingectomy improves the pregnancy rate in in-vitro fertilization patients with hydrosalpinx. Human Reproduction 1996; 11(3):523-5.
19. Sharara FI, Scott RT Jr, Marut EL, Queenan JT Jr. In-vitro fertilization outcome in women with hydrosalpinx. Human Reproduction 1996; 11(3):526-30.
20. Soliman S, Daya S, Collins J, Hughes EG. The role of luteal phase support in infertility treatment: a meta-analysis of randomized trials. Fertil Steril 1994; 61:1068-76.
21. Dawood MY. In vitro fertilization, gamete intrafallopian transfer, and superovulation with intrauterine insemination: Efficacy and potential health hazards on babies delivered. Am J Obstet Gynecol 1996; 174 (4):1208-17.
22. Sauer MV, Paulson RJ, Ary BA, Lobo RA. Three hundred cycles of oocyte donation at the University of Southern California: assessing the effect of age and infertility diagnosis on pregnancy and implantation rates. J Assist Reprod Genet 1994; 11:92-6.
23. Navot D, Bergh PA, Williams AM, Garrisis JG, Guzman I, Sandler B, Grufeld L. Poor oocycte quality rather than implantation failure as a cause of age-related decline in female fertility. Lancet 1991; 337:1375-7.
24. Yaron Y, Botchan A, Amit A, et al. Endometrial receptivity: the age related decline in pregnancy rates and the effect of ovarian function. Fertil Steril 1993; 60:314-8.
25. Wildschut HIJ. Sociodemographic Factors: Age/Parity, Social class and Ethnicity. In: James DK, Steer PJ, Weirer CP, Goniks B eds. High risk pregnancy. Management options. London: W B Saunders 1994:35-49.
26. Asch RH, Balmaceda JP, Ellsworth LR et al. Preliminary experiences with gamete intrafallopian transfer (GIFT). Fertil Steril 1986; 45:366-71.
27. Meirow D, Schenker JG. Appraisal of gamete intrafallopian transfer. Euro J Obstet & Gynecol and Reproductive Biology 1995; 58:59-65.
28. Society for assisted reproductive technology. Assisted reproductive technology in the United States and Canada: 1993 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil Steril 1995; 64:13-21.
29. Ratnam SS and Ng SC. Assisted reproductive techniques, and their place in subfertility management. In: Ratnam, Ng SC, Sen DK, Arulkumaran eds. Contributions to obstetrics and gynaecology volume 1. Singapore Churchill Livingstone 1991; 3-24.
30. Palermo G, Joris H, Devroey P, an Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992; 340:17-8.
31. Tournye H, Liu J, Nagy Z, Joris H, Wisanto A, Bonduelle M, et al. Intracytoplasmic sperm injection (ICSI): the Brussels Experience. Reprod Fertil Dev 1995; 7:269-79.
32. McLachlan RI, Fuscaldo G, Rho H, Poulos C, Dalrymple J, JacksonP et al. Clinical results from intracytoplasmic sperm injection at Monash IVF. Reprod Fertil Dev 1995; 7:247-53.
33. Hamberger L, Sjogren A, Lundin K, Soderlund B, Nilsson L, Bergh C, et al. Microfertilization techniques-The Swedish experience. Reprod Fertil Dev 1995; 7:263-8.
34. Bourne H, Richings N, Harari O, Watkins W, Speirs LS, Lan W, et al. The use of intracytoplasmic sperm injection for the treatment of severe and extreme male infertility. Reprod Fertil Dev 1995; 7:237-45.
35. Wisanto A, Magnus M, Bonduelle M, Liu J, Camus M, Tournaye H, et al. Obstetric outcome of 424 pregnancies after intracytoplasmic sperm injection. Human Reproduction 1996; 10(10):2713-8.
36. Trounson A, Leeton JF, Besanko M, et al. Pregnancy establishment in an infertile patient after transfer of a donated embryo fertilised in vitro. Br Med J 1983; 286:835-8.
37. Chan CLK, Devendra S. Embryos and oocyte donation. In: Ratnam, Ng SC, Sen DK, Arulkumaran eds. Contributions to obstetrics and gynaecology volume 1. Singapore Churchill Livingstone 1991; 89-103.
38. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature 1983; 305:707-9.
39. Mandelbaum J, Junca AM, Plachot M, Alnot MO, Alvarez S, Debache C, et al. Human embryo cryopreservation, extrinsic and intrinsic parameters of success. Hum Reprod 1987; 2:709-15.
40. Cohen J, Simons RF, Edwards RG, Fehilly CB, Fishel SB. Pregnancies following the frozen storage of expanding human blastocysts. J In Vitro Fert Embryo Transf 1985; 2:59-64.
41. Wang XJ, Ledger W, Payne D, Jeffrey R, Matthews CD. The contribution of embryo cryopreservation to in-vitro fertilization/gamete intra-fallopian transfer: 8 years experience. Hum Reprod 1994; 9:103-9.
42. Fugger E, Bustillo M, Katz L, Dorfmann A, Bender S and Schulman J. Embryonic development and pregnancy from fresh and cryopreserved sibling pronucleate human zygotes. Fertil Steril 1988; 50:273-8.
43. Navot D, Bergh PA, Laufer N. Ovarian hyperstimulation syndrome in novel reproductive technologies. Fertil Steril 1992; 58:249-61.
44. Paulson RJ, Sauer MV, Lobo RA. Embryo implantation after human in vitro fertilization: importance of endomtrial receptivity. Fertil Steril 1990; 53:870-5.
45. Assisted reproductive technology in the United States and Canada:1993 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil Steril 1995; 64:13-21.
46. Fugger E, Bustillo M, Dorfmann A, and Schulman J. Human preimplantation embryo cryopreservation: selected aspects. Hum Reprod 1991; 6:131-5.
47. Testart J, Lassalle B, Belaisch-Allart J, Forman R, Hazout A, Volante M, et al. Human embryo viability related to freezing and thawing procedures. Am J Obstet Gynecol 1987; 157:168-71.
48. Selick CE, Hofmann GE, Albano C, Horowitz GM, Copperman AB, Garrisi GJ, Navot D. Embryo quality and pregnancy potential of fresh compared with frozen embryos - is freezing detrimental to high quality embryos? Hum Reprod 1995; 10:392-5.
49. Levran D, Dor J, Rudak E, et al. Pregnancy potential of human oocytes - the effect of cryopreservation. N Eng J Med 1990; 323:1153-6.
50. Yee B, MD, Lin YP, MD, Chacon RR, BS, Soubra S, BS, Rosen GF, MD, Cassidenti DL, MD. A simplified method of timing frozen embryo transfers. Am J Obstet Gynaecol 1995; 172:1844-50.
51. Marshall BMS. Ethical issues in ART. Annals Academy of Medicine 1992; 21(4):582-8.
52. Trad F, Hornstein MD, Barbieri RL. In vitro fertilization: A cost-effective alternative for infertile couples? Journal of Assisted Reproduction and Genetics 1995; 12(7):418-21.
53. Neumann PJ, Johannesson M. Willingness to pay for in vitro fertilization: a pilot test using contingent valuation. Med Care 1994; 32:686-99.

54. Peterson CM, Hatasaka HH, Jones KP, Poulson AM, Carrell DT, Urry RL. Ovulation induction with gonadotrophins and intrauterine insemination compared with in vitro fertilization and no therapy: a prospective nonrandomized, cohort study and meta analysis. Fertil Steril 1994; 62(3):535-44.
55. Harrison RF, Kondaveeti U, Barry-Kinsella C, Gordon A, Drury L, Cottell E, et al. Should gonadotropin-releasing hormone down regulation therapy be routine in in vitro fertilization? Fertil Steril 1994; 62(3):568-74.
56. Wikland M, Borg J, Hamberger I, Svalander P. Simplification of IVF: minimal monitoring and the use of subcutaneous highly purified FSH administration for ovulation induction. Hum Reprod 1994; 9:1430-6.
57. Dimistry ES, Bates SA, Oskarsson T, Margara R, Winston RM. Programming in vitro fertilization for 3 or 5 day week. Fertil Steril 1991; 55:934-8.
58. Simon A, Avidan B, Mordel N, Lewin A, Samueluf A, Zajicek G, et al. The value of menotropin treatment for unexplained infertility prior to an in-vitro fertilization attempt. Hum Reprod 1991; 6:222-6.
59. Wessels PH, Cronje HS, Oosthuizen AP, Trumpelmann MD, Grobler S, Hamlett DK. Cost-effectiveness of gamete intra-fallopian transfer in comparison with induction of ovulation with gonadotropins in the treatment of female infertility: a clinical trial. Fertil Steril 1992; 57:163-7.
60. Abyholm T, Tanbo T, Dale PO, Magnus O. In vivo fertilization procedures in infertile women with patent fallopian tubes: a comparison of gamete intrafallopian transfer, combined intrauterine and intraperitoneal insemination, and controlled ovarian hyperstimulation alone. J Assist Reprod Genet 1992; 9:19-23.
61. Crosignani PG, Walters DE, Soliani A. The ESHRE multicentre trial on the treatment of unexplained infertility: a preliminary report. Hum Reprod 1991; 6:953-8.
62. Mills MS, Eddowes HA, Cahill DJ, et al. A prospective controlled study of in-vitro fertilization, gamete intra-fallopian transfer and intrauterine insemination combined with superovulation. Hum Reprod 1992; 7:490-4.
63. Bolton V, Hawes SM, Taylor CT et al. Development of spare human preimplantation embryos in vitro: an analysis of the correlations among gross morphology, cleavage rates and development to the blastocyst. Journal of IVF and ET 1989; 6:30-5.
64. Fehilly CB, Cohen J, Simons R F, et al. Cryopreservation of cleaving embryos and expanded blastocysts in the human: a comparative study. Fertil Steril 1985; 44:638-44.
65. Bongso A, Ng SC, Sathananthan H, Ng PL, Rauff M, Ratnam SS. Improved quality of human embryos when cocultured with human ampullary cells. Hum Reprod 1989; 4:706-13.
66. Bongso A, Fong CY, Ng SC, Ratnam SS. Human ampullary cocultures for blastocyst transfer in assisted reproduction. Ann Acad Med Singapore 1992; 21:571-75.
67. Yeung WSB, Ho PC, Lau EYL, Chan STH. Improved development of human embryos in vitro by a human oviductal cell coculture system. Hum Reprod 1992; 7:1144-9.
68. Wiemer KE, Hoffman DI, Maxson WS, et al. Embryonic morphology and rate of implantation of human embryos following coculture on bovine oviductal epithelial cells. Hum Reprod 1993; 8:91-101.
69. Bongso A, Ng SC, Fong CY, et al. Improved pregnancy rate after transfer of embryos grown in human fallopian tubal cell coculture. Fertil Steril 1992; 58:569-74.
70. Wiemer KE, Cohen J, Amborski GF, et al. In vitro development and implantation of human embryo following culture on the fetal bovine uterine fibroblast cells. Hum Reprod 1989; 4:595-600.
71. Morgan KF, Wiemer KE, Godke RA. Retrospective analysis of cocultured human embryos using videocinematography and image analysis. 49th Annual Meeting of the American Fertility Society, Toronto, Program Supplement 1993.
72. Forman RG, Frydman R, Egan D. Severe ovarian hyperstimulation syndrome using agonists of gonadotropin releasing hormone for in vitro fertilisation: a European series and a proposal for prevention. Fertil Steril 1990; 53:502-9.
73. MacDougall MJ, Tan SL, Jacobs HS. In vitro fertilisation and the ovarian hyperstimulation syndrome. Hum Reprod 1992; 7:579-600.
74. Brindsen PR, Wada L, Tan SL, Balen A, Jacobs HS. Diagnosis, prevention and management of ovarian hyperstimulation syndrome. Br J Obstet Gynaecol 1995; 102:767-72.
75. Navot D, Bergh PA, Laufer N. Ovarian hyperstimulation syndrome in novel reproductive technologies: prevention and treatment. Fertil Steril 1992; 58:249-61.
76. Von VD, Maltau JM, Forsdahl F, Abyholm T, Kolvik R, Ertzeid G, et al. Pregnancies, births and babies after in vitro fertilization in Norway, 1988-91. J Norwegian Med Assoc 1995; 115:2054-60.
77. Allen MC. Factors affecting development outcome. In multiple pregnancy, epidemiology, gestation and perinatal outcome, 1st edn. Keith LG, Papiernik E, Keith DM, Luke B. New York: Parthenon Publishing Group; 1995:599-612.
78. Callahan TL, Hall JE, Ettner SL, Christiansen CL, Greene MF, Crowley WF. The economic impact of multiple-gestation pregnancies and the contribution of assisted-reproduction techniques to their incidence. N Engl J Med 1994; 331:244-9.
79. Chelmow D, Penzias AS, Kaufman G, Cetrulo C. Costs of triplet pregnancy. Am J Obstet Gynecol 1995; 172:677-82.
80. Tanbo T, Dale PO, Lunde O, Mor N, Abyholm T. Obstetric outcome in singleton pregnancies after assisted reproduction. Obstet Gynecol 1995; 86:188-92.
81. FIVNAT (French In-vitro National): Pregnancies and births resulting from in vitro fertilization: French national registry, analysis of data 1986 to 1990. Fertil Steril 1995; 64:746-56.
82. Fathalla MF. Incessant ovulation - a factor in ovarian neoplasia? Lancet 1971; 2:163-5.
83. Whittemore AS, Harris R, Itnyre J. The Collaborative Ovarian Cancer Group. Characteristics relating to ovarian cancer risk: Collaborative analysis of 12 US case-control studies II. Invasive epithelial ovarian cancers in white women. Am J Epidemiol 1992; 136:1184-203.
84. Whittemore AS, Harris R, Itnyre J, Halpern J. The Collaborative Ovarian Cancer Group. Characteristics relating to ovarian cancer risk: Collaborative analysis of 12 US case-control studies. I. Methods. Am J Epidemiol 1992; 136:1175-83.
85. Harris R, Whittemore AS, Itnyre J. The Collaborative Ovarian Cancer Group. Characteristics relating to ovarian cancer risk: Collaborative analysis of 12 US case-control studies. III. Epithelial tumour of low malignant potential in white women. Am J Epidemiol 1992; 136:1204
86. Cohen J, Forman R, Harlap S, Johannisson E, Lunefeld B, deMouzon J, et al. IFFS expert group report on the Whittemore study related to the risk of ovarian cancer associated with use of fertility agents. Hum Reprod 1993; 8:996-9.
87. Bristow RE, Karlan BY. Ovilation induction, infertility, and ovarian risk. Fertil Steril 1996; 66(4)499-507.