

The Influence of Advanced Age on the Outcome of Assisted Reproduction

JENNIFER E. DEW,^{1,2} RONALD A. DON,¹ GRAEME J. HUGHES,¹ TREVOR C. JOHNSON,¹ and STEPHEN J. STEIGRAD¹

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Purpose: Our purpose was to determine the influence of age on the outcome of assisted reproduction, with particular interest in women aged 40 years or older.

Methods: A retrospective review of the 779 patients enrolled in the Royal Hospital for Women Fertility Group fertility program between 1987 and 1994 was performed. The results for women aged 40 years or older were compared with those for women between 36 and 39 years and those younger than 36 years. The main outcome measures were pregnancy rate, pregnancy outcome, fertilization rate, and ovarian response.

Results: Compared with those in younger women, pregnancy rate, pregnancy outcome, fertilization rate, and ovarian response to controlled ovarian stimulation were significantly worse in women aged 40 years or older.

Conclusions: The outcome of assisted reproduction in women of 40 years of age or older was extremely poor. Compared with those in younger women, pregnancy outcome and ovarian response to controlled ovarian stimulation were significantly worse in women of 40 years or more.

KEY WORDS: age; assisted reproduction; infertility; pregnancy outcome.

INTRODUCTION

Advancing age is associated with reduced fecundity both in vivo and in vitro (1,2). The results of donor insemination programs support a natural decline in fecundity with advancing age (1,2). Two studies of artificial insemination of donor semen in women with azoospermic husbands found a significant decrease in female fertility from 31 years (3,4), with an even greater decrease from 35 years (3). Using only women

with azoospermic husbands, they controlled for reduced fecundity due to a decrease in frequency of sexual intercourse, male subfertility, and a possible decrease in male fertility with increasing age. These findings are supported by studies of both in vitro fertilization with embryo transfer (IVF/ET) and gamete intrafallopian transfer (GIFT) (3–13). Piette *et al.* (5) showed a significant decrease in assisted reproduction outcome from 37 years, while Segal and Casper (7) and Kenny (6) found a decline after 35 years. In vivo there is a further rapid decline in fertility between 40 and 44 years, becoming negligible between 45 and 49 years of age, with only 2–5% of women having successful pregnancies (4).

The “take-home baby rate” in women older than 40 years in assisted reproduction programs has been shown to be extremely low due to a reduced conception rate and an increase in pregnancy wastage (3,4,6–9,11,13–15).

In Australia, assisted reproductive technology is still readily available to women of all ages. In 1993, 4.4% of women undertaking IVF and 6.5% of women undertaking GIFT were aged 40 years or older (16). It has been our opinion that our results in this age group are very poor and that this group’s access to assisted reproduction should be restricted. In view of the findings of other fertility programs, a retrospective review of patients enrolled in the Royal Hospital for Women Fertility Group fertility program was conducted to determine the influence of age in our group so as to appropriately manage and counsel the elderly infertile couple.

MATERIALS AND METHODS

A retrospective review of patients enrolled for IVF/ET and GIFT by the Royal Hospital for Women Fertility Group fertility program was conducted to determine

¹ Department of Reproductive Medicine, Royal Hospital for Women, Randwick, NSW, Australia.

² To whom correspondence should be addressed at Royal Hospital for Women, Barker Street, Randwick, 2031 NSW, Australia.

the influence of age on the outcome of assisted reproduction, with particular interest in those women 40 years of age or older (group A). These results were compared with those for women between 36 and 39 years (group B) and woman younger than 36 years (group C). The patients' ages ranged from 20 to 49 years. All medical records between 1987 and 1994 were reviewed.

Patient recruitment was uniform during the study period. All years had a similar mean age at recruitment, cause of infertility, and mode of treatment. Older women were not more likely to be assigned to a particular group. GIFT has been used almost exclusively throughout the study period for unexplained infertility.

The clinical pregnancy rate per cycle commenced, live birth rate per cycle commenced, cumulative pregnancy rate, and cumulative delivery rate were chosen as outcome measures. These are the facts that most couples beginning assisted reproduction programs can relate to and want to know before they commence treatment: their chance of conceiving and having a successful pregnancy each cycle and their overall likelihood of success after a certain number of cycles. The cumulative pregnancy and delivery rates were calculated using the technique described by Tan *et al.* (11). Many studies have looked at results in terms of rates per ET or ovum pickup. However, this excludes cycles canceled because of a poor response or those in which no eggs were retrieved at ovum pickup. Cumulative pregnancy and delivery rates give the most accurate assessment of outcome.

The cause of the infertility was unexplained in 33%, male factor in 26%, tubal in 24%, cervical in 2%; and in 15% the pathology was multiple.

Ovarian stimulation was achieved using either clomiphene citrate (CC; Clomid; Marion Merrell Dow Australia Pty. Ltd., Frenchs Forest, NSW), 100 mg daily on days 1 to 5, and human menopausal gonadotropin (hMG; Pergonal; Serono Australia Pty. Ltd., Frenchs Forest, NSW) or leuporelin acetate (Lucrin; Abbott Australasia Pty. Ltd., Kurnell, NSW) and hMG or Metrodin (Serono Australia Pty. Ltd.), or hMG alone. Serum estradiol (E₂) and serial ultrasound examinations were used to monitor ovarian stimulation. Human chorionic gonadotropin (hCG), 10,000 U, was administered by intramuscular injection after day 10 if the E₂ level was higher than 7300 pmol/L and if there were three or more follicles greater than 15 mm in diameter.

Ovum retrieval was performed vaginally in the case of IVF/ET and the embryos were transferred 48 hrs later. Laparoscopic egg retrieval and transfer were used

for GIFT procedures. In 15.6% of patients one egg was transferred; in 26.1%, two eggs; in 39.7%, three eggs; and in 18.6%, four eggs.

A clinical pregnancy was confirmed by ultrasound or a positive β-hCG after at least 6 weeks' amenorrhoea.

The woman's age was recorded as the age at commencement of each cycle of assisted reproduction.

Statistical analysis was performed using the odds ratios (ORs)—the ratio between two groups tested to see if there is a significant difference between the groups. The 5% level of significance is taken as the cutoff point. ORs were chosen because, in addition to determining whether an association exists between two groups, they allow study of the nature and strength of the study. Statistics were calculated manually.

RESULTS

Of the 779 patients undergoing assisted reproduction, 104 (13.4%) were aged 40 years or older at commencement in the program. A total of 308 stimulated cycles was conducted in these women. Cycles commenced comprised 185 IVF, 114 GIFT, 9 zygote intrafallopian transfer (ZIFT), and 22 frozen embryo transfer (FET) cycles.

In women 40 and older there were 16 pregnancies, representing 5.2% of cycles and 15.4% of patients. Of these, eight resulted in miscarriage and two were tubal pregnancies (Table I). There were five births, resulting in two preterm and three term infants. In women older than 42 years of age there was only one pregnancy, which ended in a miscarriage. There is one currently unconfined pregnancy of the 22 FET cycles of women aged 40 year or older—the only FET pregnancy in this age group.

There was a cumulative pregnancy rate of 24.9% for three or more cycles and a cumulative delivery rate of 6.4% in women aged 40 or older (Fig 1).

The results for group A were compared with those for groups B and C (Table II). Pregnancy outcome deteriorated significantly with increasing age. In group

Table I. Pregnancy Outcome in Women 40 Years and Over

Number of patients	Pregnancy rate (%)	Early pregnancy loss (%)	Number who delivered
104	15.4 (n = 16)	63 (n = 10)	3.1 (n = 5)

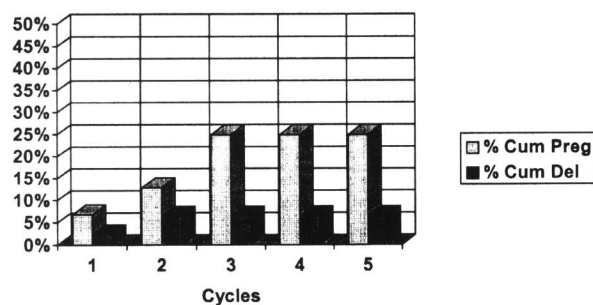


Fig. 1.

A there was a 5% pregnancy rate per cycle commenced, compared with 13% per cycle in group B (OR, 0.37; 95% CI, 0.20–0.67) and 14% in group C (OR, 0.33; 95% CI, 0.19–0.57). There were no multiple pregnancies in group A. This is contrasted with the 16% multiple pregnancy rate in group C (OR, 0.26; 95% CI, 0.01–4.71) and the 13% rate in group B (OR, 0.18; 95% CI, 0.01–3.53), which are not significant. Early pregnancy loss, which includes both spontaneous abortions and ectopic pregnancies, was significantly different between group A and groups B and C. Group A had a 62% early pregnancy loss, compared with 37% in group B (OR, 2.86; 95% CI, 1.85–9.58) and 27% in group C (OR, 4.4; 95% C, 1.45–13.33). The patient live birth rate per cycle also decreased with increasing age. In group A 3% had a live birth, compared to 9% in group B (OR, 0.53; 95% CI, 0.19–1.48), which was not significant. Twelve percent had a live birth in group C (OR, 0.38; 95% CI, 0.15–0.97), which was significantly different from group A.

The ovarian response to controlled ovarian stimulation was also reduced with increasing age, with a greater percentage of cycles being canceled and fewer eggs being collected per cycle (Table III).

Women in group A had an average of 3 cycles each, compared with 1.4 for both group B and group C. Older women also had significantly more cycles canceled.

Table II. Assisted Reproduction Results Compared with Age^a

	≥40 years (group A)	36–39 years (group B)	36 years (group C)
% pregnancy/cycle	5	13	14
Patient pregnancy rate (%)	15	18.5	19.5
% early pregnancy loss	62	37	27
% live births	3	9	12
% unconfirmed	1	3	6
% multiple pregnancies	0	13	16

^a Maximum four oocytes/embryos transferred.

Table III. Ovarian Stimulation Results Compared with Age

	≥40 years (group A)	36–39 years (group B)	36 years (group C)
% average number of cycles	3.0	1.4	1.4
% canceled cycles	29	20	16
Average number of eggs/ cycle	4.7	7.0	8.8
% fertilized	37	41	42
% at risk of OHSS (>13,000 pmol/L)	7	21	23

Twenty-nine percent of cycles were canceled in group A, compared with 20% in group B (OR, 1.64; 95% CI, 1.13–2.41) and 16% in group C (OR, 2.19; 95% CI, 1.57–3.03). The majority was canceled because of a poor response.

The average number of eggs collected per cycle decreased significantly with increasing age. Pearson's χ^2 test was used to determine whether there was a significant difference in the number of eggs collected between groups. An average of 4.7 eggs was collected per ovum pickup in group A, compared with 7.0 eggs in group B ($P = 0.05$) and 8.8 eggs in group C ($P = 0.05$).

There was a significant difference in fertilization rates between group A and groups B and C: 37% in group A, compared with 41% in group B (OR, 0.84; 95% CI, 0.72–0.99) and 42% in group C (OR, 0.83; 95% CI, 0.73–0.96) (Table III).

To assess further the ovarian response to controlled ovarian stimulation, the percentage of cycles in each age group that resulted in E_2 levels higher than 13,000 pmol/L, a level regarded as "at risk of ovarian hyperstimulation syndrome," was determined, as this level is regarded as an excessive response to ovarian stimulation. Seven percent of the cycles in group A exceeded this level, compared with 21% in group B (OR, 0.28; 95% CI, 0.15–0.51) and 23% in group C (OR, 0.24; 95% CI, 0.14–0.42). There was a significant difference between group A and groups B and C.

DISCUSSION

It is known that female fertility decreases with advancing age. As increasing numbers of women delay childbearing for various socioeconomic reasons, more older women will present for infertility treatment.

The results of this study show that the outcome of assisted reproduction in women aged 40 years or older is extremely poor. Pregnancy rate, pregnancy outcome,

fertilization rate, and ovarian response to stimulation diminished with increasing age. Despite a fertilization rate of 34%, our group had no live births in women older than 42 years. Al-Shawaf *et al.* (3) and Bopp *et al.* (9) found that women older than 43 years are unlikely to benefit from IVF or GIFT, with no live births being achieved. Abdalla *et al.* (14) had a 55% miscarriage rate in women aged 40 to 44 years and a 100% miscarriage rate in women between 45 and 49 years. Our miscarriage rate in women aged 40 or more years is 63%.

In this study the age groups selected (younger than 36 years, 36 to 39 years, and 40 years or older) were chosen because previous studies have compared results only in women of 40 years or more versus younger women (3,4,10,11,13,14), while others have shown a reduced fecundity from 35 years (6,7).

Many authors have addressed the effect of aging on the ovary and uterus and their contribution to the decline in pregnancy and birth rates with advancing age in assisted reproduction programs. Conflicting results have been found and numerous mechanisms implicated.

We found that the ovarian response decreased with increasing age. Despite requiring increasing doses of hMG, lower maximum E₂ levels were achieved, fewer eggs were collected per cycle, and a higher percentage of cycles was canceled. A diminished ovarian response to exogenous gonadotropin stimulation has been postulated as a contributing cause (4,5,9,13). These findings were supported by Bopp *et al.* (9) who found a decreased responsiveness of the ovary to gonadotropins and an increased cancellation rate associated with failure to achieve pregnancy. Wood *et al.* (4) found that the factors affecting fertility were a poor response to ovarian stimulation and a reduced number of eggs collected. However, Segal and Casper (7) demonstrated that in women aged 35 to 40 years the ovarian response is proportional to the amount of hMG used and appeared to be equivalent in women older or younger than 35 years.

Progressive follicular depletion and reduced granulosa cell function have also been suggested as factors contributing to a reduced fecundity with increasing age. Hughes *et al.* (17) suggested that early deterioration of granulosa cell function may be detected by a reduction in serum inhibin levels.

Deteriorating oocyte quality with increasing female age, leading to reduced fertilization rates, has also been postulated as a contributing factor (7,10,18). Causes of poor oocyte quality may include an increased proportion of chromosomally abnormal oocytes (12) and

a "thicker" zona pellucida, which may interfere with fertilization (19).

The results of oocyte donation (10,14,18) in women older than 40 years suggest that oocyte quality rather than uterine or endometrial factors is the predominant cause of reduced fertility in older women, as pregnancy and delivery rates are significantly higher and abortion rates lower when donated oocytes from younger women are used (10,14). The abortion rate has been shown to be related to the age of the donor rather than the age of the recipient, supporting the view that poor oocyte quality contributes to increased fetal loss in older women (14,18). Our study found a significant reduction in fertilization rates with increasing age.

However, even the results of oocyte donation studies are conflicting and suggest that declining uterine receptivity may contribute to the poor outcome in older women. When progesterone (P) supplementation is increased in women older than 40 years to prepare the endometrium for implantation in oocyte donation programs, the pregnancy rate improves (4,18). Supporting these findings, a study of endometrial biopsies has shown a significant increase in the incidence of delayed secretory maturation in women older than 35 years (18). Animal studies have shown that the concentration of endometrial estrogen and progesterone receptors decreases with age (7). Changes in endometrial vasculature and an increasing incidence of uterine pathology such as fibroids may also contribute to the reduced uterine responsiveness with advancing age.

The outcome of women aged 40 years and older in assisted reproduction programs may possibly be improved by transferring more than three oocytes to compensate for the poorer implantation rates. In older women the risk of multiple pregnancy appears to be minimal (13,19). Our study found no multiple pregnancies in women 40 years and older. Hormonal priming of the endometrium with P may also improve the implantation rate (4,18).

It is unknown what effect freezing embryos has in older women. This study did not investigate whether older embryos are less likely to survive freezing and thawing. It has been shown that there is a decline in male fertility with increasing age (20). However, in a recent study Gallardo *et al.* looked at the effect of age on sperm fecundability using oocyte donation and found that age up to 64 years did not affect the sperm's ability to fertilize oocytes (21).

We investigated factors that may contribute to the poor pregnancy outcome in assisted reproduction programs with advancing age. Our findings support the findings of other studies and show that the declining

birth rate with increasing age was related to the reduced conception rate, increased pregnancy wastage, declining ovarian response to stimulation, increased cancellation rate, reduced maximum E₂ level, and reduced fertilization rate. The live birth rate was extremely poor in women 40 years of age and older, with only five live births occurring of 308 cycles commenced. Before offering assisted reproduction to these women, the poor outcome and possibility of not succeeding should be explained to the couple and the possibility of using donor eggs discussed. However, there is a problem with a paucity of donors. In our unit there is currently a 5-year waiting list for a donor unless the couple has a known donor.

Although it is a difficult ethical decision, we suggest that women older than 42 should be advised against proceeding with infertility treatment, in view of the fact that no live births occurred in this age group, unless egg donation is available. However, it is difficult to enforce an age limit, as often couples are desperate to have a child, no matter how low their chances.

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