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ASSISTED REPRODUCTION

What is the role of assisted reproduction technology in the management of age-related infertility?

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Abstract
Although in the UK the upper age limit for National Health Service (NHS) provision of *in vitro* fertilisation (IVF) is 39 years of age there has been an increase in number of women having fertility treatment in their 40s. However, the success rates of IVF and intra-uterine insemination (IUI) in this group remain low. Human Fertilisation and Embryology Authority (HFEA) data from 2006 showed that the live-birth rate from IVF in the UK was 11% in the age group 40–42, 4.6% in the age group 43–44 and less than 4% in women over 44. We performed a literature search for studies using terms and combinations of terms in online databases and published meta-analyses reporting the outcome of interventions in older women. This review showed that assisted reproduction technologies (ARTs) continue to have low live-birth rates in women over 40. Trials showed that assisted hatching may increase the chance of pregnancy in women with poor history. Blastocyst transfer is associated with better outcome, whereas application of pre-implantation genetic screening (PGS) in older women has not increased the success rates. It appears that, with the exception of egg-donation, ART has no answer yet to age-related decline of female fertility.

Keywords: *Age, assisted reproduction technology, maternal age, older women, reproductive ageing*

Introduction

The average age at childbearing has continued to increase in the Western world. In the UK the mean age of giving birth was 29.3 years in 2007, compared with 28.6 in 2001 (Office for National Statistics, 2008). In the European Union, the mean woman's age at first child was 26.8 in 1994 and 28.2 in 2004 (Eurostat, 2006). Recent data from the UK have shown a 10-fold plus increase in women over 40 seeking fertility treatment from 1991 to 2006 (HFEA Facts and Figures, 2006).

This steady increase in the number of women having fertility treatment in their 40s has been documented despite the fact that these women do not qualify for National Health Service funded assisted reproduction treatment in the UK. The National Institute for Health and Clinical Excellence (NICE) Guideline Development Group based on complex cost-effectiveness modelling and on the Human Fertilisation and Embryology Authority (HFEA) statistics recommended that the upper age limit for National Health Service (NHS) provision of *in vitro* fertilisation (IVF) should be 39 years of age (National Collaborating Centre for Women's and Children's Health, 2004).

The age of over 39 for fertility treatment appears as a breaking point, as above this age the live birth effectiveness after IVF treatment falls to 50% of the plateau level of younger women (HFEA Facts and Figures, 2006).

The aim of this review is to identify whether advances in assisted reproduction technology (ART) can improve the poor natural fertility in this age group.

Material and methods

The review examined the most recent data on natural fertility of women 40 and over, based on demographic data in the Office for National Statistics (UK), Eurostat, World Health Organization (WHO) and National Vital Statistics System (USA) (Hamilton et al., 2007). We looked for the outcome

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of conventional fertility treatments such as intrauterine insemination (IUI) or IVF as reported by the HFEA and the American Society for Reproductive Medicine (ASRM). We carried out a literature search for primary studies in online databases (EMBASE and PUBMED) regarding interventions that have come across as improving the outcome of conventional fertility treatments in the age group of 40 years and over. For this literature search, we used the following key worlds: older women, assisted reproduction technology, reproductive ageing and maternal age.

We also searched for published guidelines from NICE, British Fertility Society (BFS), European Society of Human Reproduction and Embryology (ESHRE), ASRM and Royal College of Obstetricians and Gynaecologists (RCOG), based on recent systematic reviews.

Additional searches were carried out for evidence-based reviews in the Cochrane Library.

The level of evidence used is shown in Table I. This grading is used by the NICE (Table I).

What is normal fertility for a woman who is 40?

Anthropological studies in populations with limited birth control (natural fertility populations) showed that fecundity (biological capacity to conceive and carry a pregnancy at term) declines with maternal age (Wood, 1989).

Alongside the decline in egg quality, the major factor responsible for this decline in fecundity is the increase in early fetal loss with age. In a study of Bangladeshi women the probability of fetal loss increased from about 18% at age 18 to 45% by age 38. The total fecundability was constant until the age of 40 and then dropped rapidly to nearly zero by the age 46. Much of this fetal loss occurred because of chromosomal abnormalities of the ageing oocytes (O'Connor et al., 1998).

Table I. Hierarchy and strength of evidence according to the British National Institute of Clinical Excellence (NICE).

Hierarchy of evidence
1a. Systematic review and meta-analysis of randomised controlled trials (RCTs).
1b. At least one randomised controlled trial.
2a. At least one well-designed controlled study without randomisation.
2b. At least one other type of well-designed quasi-experimental study.
3. Well designed non-experimental descriptive studies, such as comparative studies, correlation studies or case studies.
4. Expert committee reports or opinions and/or clinical experience of respected authorities.

From demographic data, we can see the same decline in fertility with age in natural populations and among populations with effective use of contraception. This has been mainly attributed in the decline of fecundity rather with changes in coital frequency and marital duration (Wood, 1989). But can we calculate the conception rate in those couples in whom the woman's age is more than 40?

With a complex simulation model Leridon calculated the probability of conception under natural conditions within a year in relation to maternal age.

According to this model at 30 years, 75% of women will conceive within a year and have a pregnancy ending in a live birth, while at 35 and 40 years, 66% and 44% will conceive within a year, respectively (Leridon, 2004).

The outcome of IVF and IUI in women over 40

IVF/Intracytoplasmic sperm injection

The success of IVF declines with age and becomes particularly low in women over 40. In the UK the success rate (live birth/cycle started) of IVF (own eggs) during 2006 was 11.1% in the age group 40-42 (490 total live births), 4.6% in the age group 43-44 (41 total live births) and 4% (9 total live births) in women over 44 during 2006. On the other hand, the success rate was 18.6% in the age group 38–39, 26.4% in the age group 35–37 and 31% in women below 35. Although success rates have improved over the years the live birth rate still decreases with age (Figure 1).These results did not include natural cycles, pre-implantation genetic diagnosis (PGD) cycles and cycles where both fresh and frozen embryos were used in the same cycle (HFEA Facts and Figures, 2006).

The success rate of IVF in women over 40 using fresh non-donor eggs is similarly low in the USA. Data from 2005 showed that the success rate declines with each year of age. The success rate (live birth/cycle started) was 16.1% for age 40, 12.3% for age 41, 8.4% for age 42, 5.6% for age 43, 2.6% for age 44 and 0.8% for women aged over 44 (CDC. U.S., 2005).

IUI

Data analyses of intrauterine insemination cycles have demonstrated a decline in success rates with the advance of maternal age. Data for artificial insemination in 2193 nulliparous women with azoospermic husbands from 1973 to 1980 at the Centers d'Etude et de Conservation du Sperme Humain (CECOS) demonstrated a decrease in conception rate per cycle with the increase in the woman's age. The probability of success of artificial insemination for 12 cycles

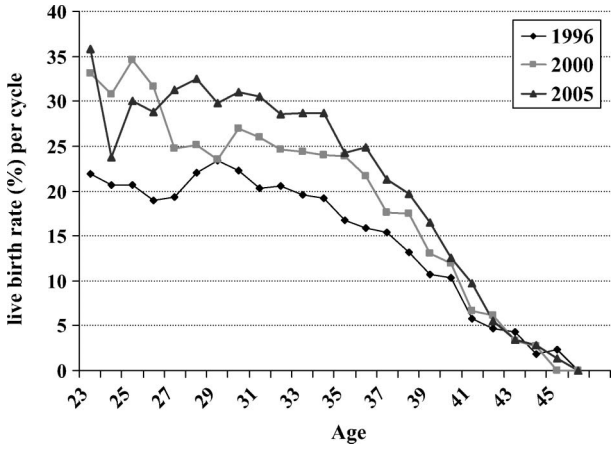

Figure 1. Success rates of IVF decline with age where a woman uses her own fresh eggs. Data taken from the HFEA register (1991–2006).

declined from 74% for those under 31 years, to 61% for the 31–35 age group ($p = 0.03$) and to 54% for those over 35 ($p = 0.001$) years (Evidence 3) (Schwartz & Mayaux, 1982).

There are only few published data of IUI for women over 40. A study of 281 women who underwent IUI treatment for cervical and or male factor infertility without ovarian stimulation showed very poor outcomes in women over 40. The cumulative probability of an ongoing pregnancy following three cycles of IUI was 28.2% for women below 40 and 0.0% in women above 40 (Evidence 2a) (Check et al., 2000).

In a retrospective study of 77 women over 40 (mean age 41.6), who underwent IUI combined with ovarian stimulation, the pregnancy rate was 5% per cycle and the live birth rate only 1.4% per cycle (Evidence 3) (Frederick et al., 1994).

Variability of fertility

ART has helped advance our understanding of the variability of ovarian ageing. Data from ART indicate that poor responders have poorer success rates and future fertility prognosis and go into menopause earlier (Nikolaou et al., 2002; De Boer et al., 2002; Lawson et al., 2003). The variability of response to stimulation, just like the variability of menopause, depends mainly on genetic factors (te Velde & Pearson, 2002; Nikolaou et al., 2008).

There is much less variability of fertility in women over 40 which is almost invariably low (Nikolaou, 2008).

However, there are some environmental and medical factors that contribute to a small degree to variability even in older age groups, such as smoking, history of endometriosis, previous ovarian surgery and chemotherapy, while a history of polycystic ovaries (PCO) may be protective (Evidence 3) (Nikolaou & Gilling-Smith, 2004).

Interventions that might improve fertility for women over 40

Mild stimulation IVF

There are no studies on the use of mild IVF specifically in women over 40 but only in younger groups. However, a prospective randomised study in patients younger than 38 demonstrated that the number of good morphology embryos was significantly higher after mild stimulation (51% versus 35% in the conventional group; $p = 0.04$) while the number of oocytes retrieved (and the embryos obtained) was higher in the conventional stimulation group. More impressive is the higher proportion of euploid embryos per patient (55% *versus* 37%; $p = 0.01$) and the lower proportion of mosaic embryos per patient (37% *versus* 65%; $p = 0.004$) in the mild stimulation group (Evidence 1b) (Baart et al., 2007).

Blastocyst tranfer

A recent meta analysis demonstrated that the probability of live birth following fresh IVF is higher after blastocyst-stage embryo transfer compared to cleavage-stage transfer (Evidence 1a) (Papanikolaou et al., 2008). In the USA, for the year 2005 in women over 40, success rates for day 5 embryo transfers were higher than for day 3 in women over 40. For the age group 41–42 the success rate for day 5 embryo transfer was 21% per transfer and for day 3 was 14.2%. In women over 43 the success rate for embryo transfer on day 5 was 11.2% and for embryo transfer on day 3 was 5.2%. However, the cycles which did not progress to the blastocyst stage because of embryo arrest between day 3 and day 5 were not accounted for in the success rates for day 5 transfers (Figure 2) (CDC. U.S., 2005).

Assisted zona hatching

Assisted hatching (thinning of the zona pellucida) was introduced by Cohen et al. (1990) as a method to improve the capacity of embryos to implant. It has been implemented in patients with poor prognosis (repeated implantation failures, advanced maternal age and in embryos with a thick zona pellucida).

The only study with selection criteria exclusively for age group over 40 was published by Schoolcraft et al. (1995). In this retrospective study of 38 cycles of IVF with assisted hatching, the delivery rate per oocyte retrieval was 48% compared to 11% in the controls ($p = 0.0003$). The implantation rate of

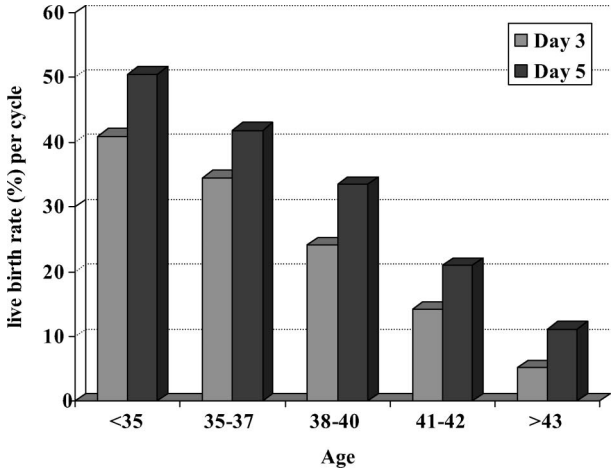

Figure 2. Live birth rate (%) per cycle of Day 3 and Day 5 embryo transfers (using fresh non-donor eggs or embryos), by woman's age in US during 2005.

hatched embryos was 22%, and in non-hatched embryos was 6%, ($p < 0.001$) (Evidence 2a) (Schoolcraft et al., 1995).

A randomised controlled trial (RCT) also examined a group of women over 40 and/or at least two previous failed IVF/intracytoplasmic sperm injection (ICSI) attempts. Acid Tyrode's solution was used to remove the zona pellucida. In that group the pregnancy rate was 23% *versus* 7.3% in the controls ($p < 0.05$) (Evidence 1b) (Mansour et al., 2000).

In 2006, a Cochrane meta analysis of 28 RCTs showed an increase in clinical pregnancy rate (OR 1.29, 95% CI 1.12–1.49) and significantly increased multiple pregnancy rates per woman after assisted zona hatching (AZH).Those RCTs included mainly patients with poor prognosis (over 35 years of age, embryos with thick zona pellucida and patients with previous IVF failures) (Evidence 1a) (Das et al., 2009).

Pre-implantation genetic screening

The evidence so far shows that the implementation of PGS does not increase IVF success rate in women over 40.

The first randomised trial was published by Staessen et al. (2004) and included 389 women above or equal to 37 years of age who received blastocyst transfer with PGS (chromosomes 13, 16, 18, 21, 22 X and Y). Embryos with at least five blastomeres were biopsied on day 3 and all women had ICSI. The embryo implantation rate was greater in the PGS group than the control group (17.1% *vs.* 11.5%) but this difference was not statistically significant. From 121 embryo transfers in the control group, 39 implantations had fetal heart beat. In the PGS group, 28 implantations had a heart beat out of 81 embryo transfers. The number of ongoing pregnancies beyond 12 weeks was 29 in the control group and 22 in the PGS group and the number of cycles with embryo transfer was decreased in the PGS group (54.7%), compared with the control group (85.8%) ($p < 0.001$) (Evidence 2a) (Staessen et al., 2004).

On the other hand, the results of a multicentre retrospective controlled study of 2279 cycles by Munné et al. (2006) showed that the spontaneous abortion rate was 22.2% after PGD compared to 40.6% in the controls ($p < 0.001$), in women over 40 ($n = 1024$) (Evidence 2a) (Munné et al., 2006).

The most recent RCT (double blinded) involved 408 women in the age range 35-41 (836 cycles of IVF). All the participants (controls, $n = 202$; PGS group, $n = 206$) had not had IVF before, received up to three cycles of treatment and had up to two embryos transferred on day 4. Only a single blastomere was biopsied when the embryo contained at least four blastomeres and a second biopsy was taken only if analysis of the first was unsuccessful. PGS involved chromosomes 1, 13, 16, 17, 18, 21, X and Y.

The viable pregnancy rate at 12 weeks per woman was 25% in the PGS and 37% in the control group, OR 0.69 [95% CI 0.51–0.93]. Live birth rate was 24% in the PGS group and 35% the control group. There was no difference in the miscarriage rate (18%) (Evidence 1b) (Mastenbroek et al., 2007).

One of the issues in this study is the age range 35–39 as it could be considered young for PGS. Another limitation is the lack of analysis for chromosomes 15 and 22, although they make up a significant contribution to aneuploidy rates. In addition, the undiagnosed embryos (20.1%) (failed biopsy or did not give results) which were transferred in PGS group may have contributed to the poor outcome since their implantation rate was low (6%).

Taking into consideration the two RCTs, the British Fertility Society guidelines suggested that clinicians should inform their patients that there is no evidence that PGS for advanced maternal age improves their likelihood of becoming pregnant and in some cases may result in a reduced chance of pregnancy and that further research needs to be carried out (Evidence 4) (Anderson & Pickering, 2008).

Egg donation

Oocyte donation has found wide application in cases of premature ovarian failure, advanced reproductive age, unexplained recurrent implantation failure and inherited conditions. The results from large databases showed good outcome in infertile older women.

Toner et al., in a retrospective cohort study, analysed data from 17,359 cycles of donated eggs that occurred in the USA between 1996 and 1998.

This analysis concluded that the success rate of egg donation is unaffected by the recipient's age up to the late 40s (clinical pregnancy rate 48%, delivery rate 40%). The reduction in success rates began at about age 48 years and became pronounced over the age of 50 (Evidence 2b) (Toner et al., 2002).

A retrospective data analysis compared the success rates of egg donation with women who had their own eggs transferred in the age group over 40. The age of the egg recipient had no effect on IVF outcomes. The probability of pregnancy decreased in women who underwent standard IVF with age but did not in those who had egg donation (the donors were younger than the recipients and the number of oocytes decreased with aging of the oocyte donor) (Evidence 3) (Stolwijk et al., 1997).

USA data showed that the percentage of transfers resulting in live births from cycles using embryos from donor eggs (donors were in their 20s or early 30s) varied only slightly for the age group 25–47. The average live birth rate per transfer was 52% (Figure 3) (CDC. U.S., 2005).

The application of oocyte donation in postmenopausal women has also been successful but the risk of antenatal complications and the psychological–social implications for the child have led to controversy. In 1997, The Ethics Committee of the American Society for Reproductive Medicine published guidelines which discouraged the use of egg donation in post-menopausal women (Ethics Committee of the American Society for Reproductive Medicine, 2004).

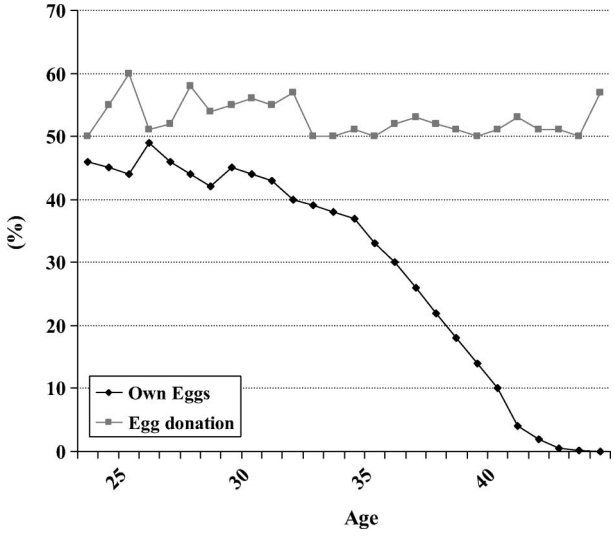

Figure 3. Live birth rate (%) per cycle using fresh embryos from own and donor eggs, by woman's age in US during 2005.

Dehydroepiandrosterone supplements

The positive effect of dehydroepiandrosterone (DHEA) in the ovarian reserve was first mentioned in a small series study (Casson et al., 2000). A larger case–control study of 190 women with poor ovarian function and age over 40 showed significantly higher cumulative pregnancy rates after DHEA administration. The cumulative clinical pregnancy rate was 28.1% in the treatment group *versus* 10.9% in the non-treatment group ($p < 0.01$). Almost half of the pregnancies in the study group occurred spontaneously before the planned IVF treatment. Within the patients reaching IVF the pregnancy rate in the treatment group was 20.6% *versus* 11.9%. Even the miscarriage rate was lower in the treatment group (20% *versus* 36%) (Evidence 3) (Barad et al., 2007).

Vesicle and ooplasm transfer

The nucleus from a recipient oocyte is injected to an enucleated donor egg and so far has been only successful in rabbits (Li et al., 2001). Ooplasm transfer involves injection of donor cytoplasm into the recipient s egg. So far, ooplasm transfer has only been applied in young women with recurrent implantation failures and has not yet been applied in women of advanced reproductive age (Barritt et al., 2001).

Discussion

ART has improved understanding of the age-related decline of fertility, as it is clear from gamete-donation data that the main culprit of infertility is deteriorating oocyte quality.

Conventional treatments such as IUI have very low success rates in older women with or without stimulation. Although IVF treatment has better success rates the outcome is poor and declines significantly with each year of age to nearly zero at the age of 46.

The main issue is that oocyte quality cannot be improved by conventional IVF; moreover, it has been suggested by Munné et al. (1997), and Katz-Jaffe et al. (2005), that ovarian stimulation protocols may affect embryo aneuploidy. It appears that reducing the duration and intensity of ovarian stimulation may interfere less with natural follicle selection and result in better oocyte quality (Evidence 3) (Munné et al., 1997; Katz-Jaffe et al., 2005).

Could mild stimulation facilitate the selection of good quality oocytes in women over 40, especially when the quality of the oocytes is expected to have declined? Such a possibility requires further research especially when RCTs have shown that PGS and

thus the degree of aneuploidy that this intervention can detect does not improve the pregnancy outcome in women with advanced reproductive age.

It also appears that there is no reliable tool to select the women with best prognosis from this age group. Studies have pointed out the failure of ovarian reserve tests to predict clinical pregnancy rates (Evidence 3) (Mol et al., 2006).

Even the stimulation characteristics of the first IVF cycle which is considered a good indicator of the ovarian reserve has proved unhelpful in predicting the possibility of clinical pregnancy rate in women over 40. A study with 97 patients aged 40–45 examined the characteristics of the first IVF cycle in relation to conception rates. This study showed that the number of oocytes recovered, the level of day 3 FSH, the duration of gonadotrophin stimulation, the serum E2 and progesterone levels on the day of hCG administration or the number of oocyte transferred did not correlate with conception rates in this group (Evidence 3) (Homburg et al., 2009).

But can the advances in assisted conception technology contribute in the improvement of the quality of the oocytes or of the embryos and increase the pregnancy rates in this age group?

Meta-analysis of RCTs has demonstrated that the application of AZH may improve the outcome in women with poor prognosis including those with advanced reproductive age. Data analysis also supports the implementation of day 5 embryo transfer as it has better success rates in all age groups including women over 40. However in this age group is not always possible to develop a day 5 blastocyst. Having a blastocyst to transfer reflects qualitatively better preserved ovarian reserve and therefore is a way to identify patients with better prognosis.

We did not find any published studies relating to adjuvant treatments that could significantly improve the fertility in women over 40 apart the use of DHEA. Case–control studies have shown higher cumulative pregnancy rates as well as lower miscarriage rates after DHEA supplementation in women over 40. However it's mechanism of action for the questionable improvement of the ovarian quality and reserve remains unknown. In addition, there have not been any studies in order to define the dose, length of treatment and side effects of the DHEA in women with poor ovarian reserve and old reproductive age. Further research and large RCTs are required to confirm the benefits and the mode of DHEA use in this age group and in women with poor ovarian reserve.

Oocyte donation remains the only method with high success rates (around 50% live birth rate per transfer) regardless of the recipient's age, although there is a small deterioration in the late 40s. But the lack of genetic contribution of the recipient can be an issue for the couple.

Women should be encouraged to seek early fertility assessment and treatment when it is clinically indicated. Wang et al. with a population-based study of 36,412 initiated first autologous fresh cycles, predicted the number of deliveries for women aged 35–44 years if they underwent first autologous treatment 1, 2 or 3 years earlier. According to this model, if 35 year old women would have a treatment 1 year earlier about 15% extra live births would be expected but in a 43 years old women having treatment 1 year earlier, 23.6% of extra live births would be expected (Wang et al., 2008).

Since the introduction of the NICE guidelines – that placed an age restriction on NHS funded ART – there has not been any robust evidence to show that ART can improve natural fertility in women over the age of 40. However, the application of IVF in these women could be beneficial in cases with a tubal and or male factor. In addition, there is no doubt that egg donation improves radically the outcome in this age group.

Considering the poor outcome of ARTs in women over 40 it is very important to include in the management of these couples education on normal ageing and fertility as well as advice on egg donation, adoption, on creative life without children and psychological support.

Conclusions

The ovarian senesce is the principal responsible for the decline in fertility in women above 40. If the only issue is age (not a male or tubal factor) IVF does not improve natural fertility. Mathematical models from epidemiologists are giving better success of live birth rate with natural conception than IVF.

Techniques such as mild stimulation IVF have not been applied in this age group mainly because of the expectant poor ovarian reserve although there is speculation that mild stimulation could select better quality oocytes. On the other hand, the application of pre-implantation genetic screening for the selection of good quality oocytes in elderly women remains controversial and further research is required. Analysis of data from trials showed an increase in clinical pregnancy rates after AZH in women with poor prognosis including those over 40 years of age.

Oocyte donation remains the most successful option for women with advanced reproductive age as the pregnancy rates do not alter significantly with the recipient's age. Of the various adjuvant treatments, DHEA supplementation seems interesting and requires further study. In terms of population strategy the key to the management of infertility in women over 40 is prevention with education of the women at a younger age regarding

normal ageing and fertility and the limitations of the ART.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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