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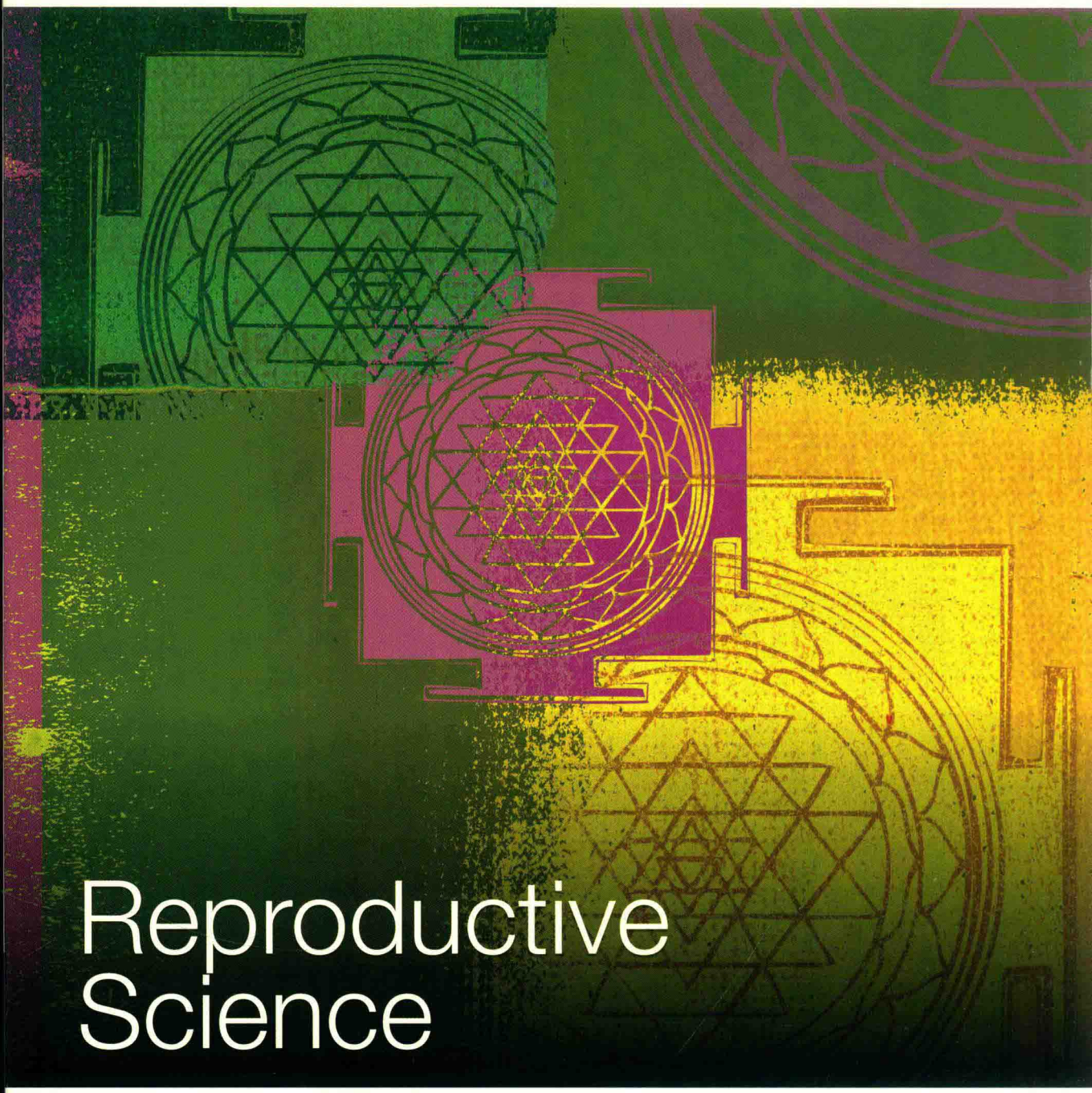

Reproductive Science

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Seth Guller, Carlo Bulletti, and Dominique de Ziegler

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Interventions in the prolongation of reproductive life in women

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Women may seek to prolong their reproductive span for a variety of reasons. For many this implies reproduction at a late age, possibly driven by lifestyle decisions, but for others affected by a natural or a cancer treatment–induced premature ovarian failure it may simply mean seeking to achieve the normal reproductive span. The range of interventions now available to address the issue of prolonging reproductive life has never been greater, although several of the approaches discussed remain in the realm of future application through being dependent on ongoing scientific developments.

Keywords: reproduction; ovarian failure; ovarian transplantation; preimplantation genetic screening; ovarian reserve; artificial oocytes; oocytes cryopreservation

Introduction

The considerable expansion of the range of techniques applicable to human reproduction has greatly extended the possibilities available for helping women who might seek prolongation of their reproductive capacity. These ongoing developments in reproductive technologies also continue to revolutionize the possibilities for those with reproductive difficulties. The pattern of technical innovation has tended to initially provide new approaches that are conceptually interesting but of limited success, which then gradually progress to being realistic options that can indeed play a part in clinical management. In some cases, the innovations remain the province of the originator and a small number of enthusiasts for an extended period until validation is achieved. That validation may require parallel technical advances to provide sufficiently good clinical outcomes that the technique is adopted by the field. An example is egg freezing, which was first reported in 1986; yet, it is only recently that freezing techniques based on vitrification have delivered sufficiently good outcomes for the field to accept that it is now a reasonable addition to mainstream options. An additional issue of importance is that many reproductive innovations are associated with elements of controversy, not helped by the promotion of these techniques to the public before they are validated to be effective. Some of this promotion appears to come from journalists reporting in the media the competitive announcements of researchers concerning the primacy of their innovations. Other undue promotion comes from clinicians who have adopted new techniques early, possibly ahead of clinical validation, and wish to promote their clinics to the public.

The average age of menopause is 50–51 years, but the extensive statistics on conception and pregnancy rates at different ages indicate that around a decade earlier, women are starting to experience reduced reproductive performance. Most couples will have completed their families by this age, but some are still seeking to reproduce beyond 40 years of age. Some are in this situation as a result of infertility or through establishing a relationship late in reproductive life, whereas others seek pregnancy late in reproductive life because of social circumstances, avoidable or otherwise. All of these groups would prefer to see the biological reproductive span extended. There are others who wish to extend their reproductive

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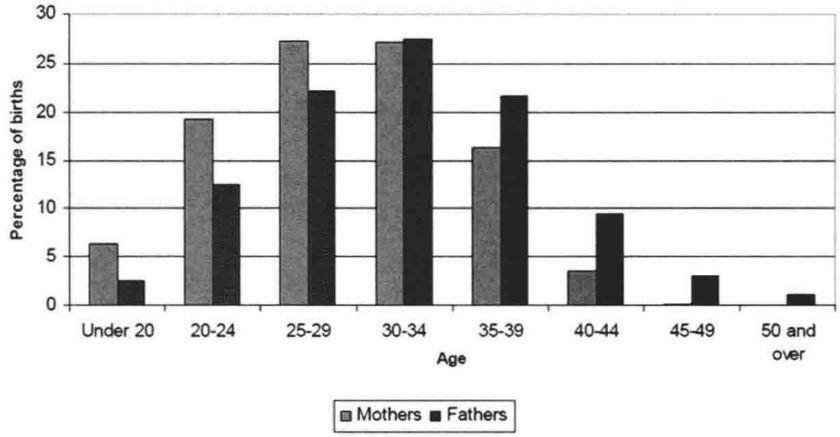

Figure 1. Percentage of live births by age group of mother and father, England and Wales.[35]

span because their reproductive span is actually reduced below what women can normally expect, either because of radiotherapy or chemotherapy in cancer treatment or because of a predicted or unexpected premature menopause. For these groups, the goal of extending their reproductive life is generally to provide the normal biological span that others take for granted. We now have a range of technologies that can help women toward these varied but related goals.

Those who have the practical option of completing their reproductive goal at younger ages are well advised to do so, but trends in contemporary society and subfertility are moving many women to the position that they may still be seeking to reproduce late in reproductive life.

The United Kingdom (UK) birth statistics on the age profile of parents shows that the age curve for fathers is displaced to the right compared to women with older age—a lesser constraint for men than women (Fig. 1). The histogram shows the expected decline in births to women over 40 years and very few births to women over 44 years of age, reflecting the biological constraint on female reproductive capacity. The social trend for women in the UK, being interested in later reproduction, is illustrated by the statistics that there were 9,336 live births to women over 40 years in 1989, whereas in 2009 there were 26,976; a 289% increase in 20 years. It is noteworthy that this trend to increasing maternal age shows stratification by family income as reflected by the father's occupation (Fig. 2).

The same trends are seen in the age pattern of women seeking *in vitro* fertilization (IVF) treatment in the UK. The Human Fertilization and Embryology Authority (HFEA) statistics show a shift to the right in the age spectrum of women having IVF in the UK comparing the most recent data (2007) with data from the start of HFEA (1992) (Fig. 3). Where the curves are not different is for women aged 45 and older. This has produced a consistent upward trend in the average age of women having IVF in the UK from 33.6 years in 1991 to 35.2 years in 2007, with the single exception of 1995, where the average age fell by 0.1 years (Table 1). Indeed, for women having donor insemination (DI) treatment, the trend in average age has been consistent in its upward trend from 31.9 years in 1991 to 35.1 years in 2007 without exception (Table 1).

The problem for older women is the impairment of reproductive performance in the older age categories. This is reflected in the HFEA outcome statistics for 2006 and 2007, where the success rates for women using their own eggs in IVF or DI treatments is noticeably reduced in women older than 39 years and especially where the woman is older than 42 years (Table 2).

It is well established that the principal cause of this age-related decline in women's reproductive potential is the decline in ovarian follicular potential rather than other factors. This is supported by the success age curve for egg donation treatment, in which an age-related decline is not observed (Fig. 4).

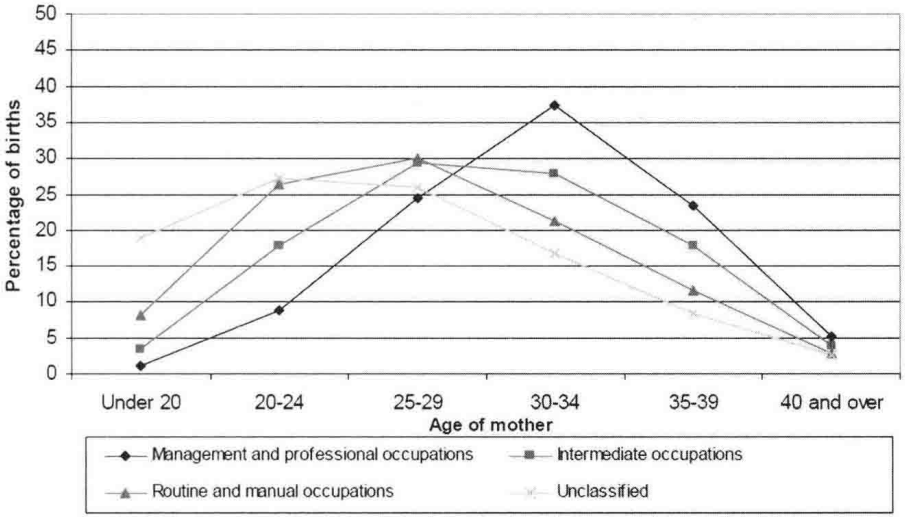

Figure 2. Percentage of live births in each age group (mothers) by father's socioeconomic group, England and Wales, 2008.[35]

The data on egg donation in older women should serve as a background for the discussion of how reproductive technologies might help women extend their reproductive span. This is an established and effective treatment but, by definition, does not enable a woman to reproduce using her own eggs. The techniques to be discussed seek to enable women to achieve the much-desired goal of reproduction that involves the woman's own genetic inheritance.

Egg donation in the UK is now dominated by women engaged with egg sharing programs, in which a woman has the costs of her own IVF defrayed or reduced by agreeing to provide some of her oocytes to a recipient, so long as there is a reasonable yield. This approach has been acceptable to some women against the background that access to state-funded IVF is limited. Women prepared to donate eggs in the UK additionally have to consider the regulations on gamete donor anonymity. The law now requires that identifying information can be provided on request to offspring of donor egg or sperm treatments once the offspring has reached 18 years of age. In the most recent HFEA analysis in 2007, more than 1,500 couples participated as recipients in donor egg cycles, and 450 births resulted.[2] However, the controversy associated with egg sharing and loss of donor anonymity ensures that the subject is not free from controversy and it remains unacceptable to many of the couples who would be eligible to use it. It is against this backcloth that we should look at technologies and strategies that might assist women who want to extend their reproductive life.

Technologies relevant to extending reproductive life

The range of technologies is varied and each has its own span of relevance for the different scenarios that women might face in seeking to extend their reproductive life. These might be summarized as follows:

- Embryo cryopreservation will be the principal technology used by those women who seek a pregnancy late in reproductive life, and who had IVF earlier in life, but who have not yet had replacement of all their frozen embryos.
- Assessment of follicular reserve and preimplantation genetic screening (PGS) are technologies that have seek to improve personal or clinical decision making aimed at increasing the effectiveness of late reproductive efforts.
- Ooctye cryopreservation is a technology that has been available for many years but has only relatively recently become clinically effective; and relevant to a number of late reproductive scenarios.
- Women facing cancer treatment that induces a risk of reduced or abolished ovarian function have options to consider if they aspire to future reproduction. Surgical ovarian transposition and suppression of ovarian

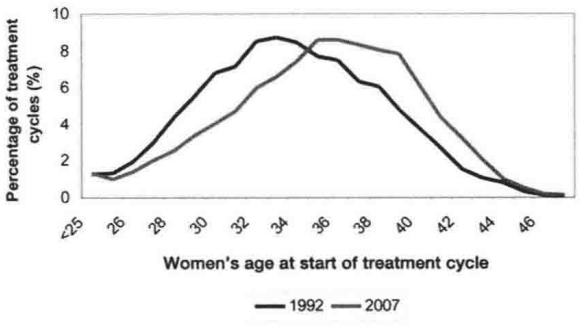

Figure 3. The age of women receiving IVF or intracytoplasmic sperm injection treatment in 1992 and 2007.[36]

activity during therapy are long established options where the aim is to minimize ovarian damage during chemotherapy or pelvic radiotherapy in women having oncology treatment. In addition, ovarian tissue cryopreservation, with later ovarian transplantation aimed at preserving viable ovarian tissue for reproductive use later in life, is a recently developed option for oncology patients.

- Hypothetical future options. These include modulation of the primordial follicle–primary follicle transition and the use of adult somatic cells in the generation of artificial gametes for reproductive use.

Embryo cryopreservation

Embryo cryopreservation will be the principal technology used by those women who seek a pregnancy late in reproductive life and who had IVF earlier in life but, for whatever reason, have not yet had replacement of all their frozen embryos. This technology is well established to provide good results.

The assessment of follicular reserve and PGS

These are technologies that seek to improve personal or clinical decision making aimed at increasing the effectiveness of late reproductive efforts, although they do not actually extend reproductive life. With both approaches there remains a significant question over whether they are yet valid options based on the current evidence.

Assessment of ovarian reserve

The nature of ovarian follicular biology determines that there is no practical intervention that can currently extend ovarian reproductive life *in vivo*. However, it is possible to seek to assess a woman's ovarian follicular reserve in the late reproductive phase to indicate the realistic potential for pregnancy. Predicting ovarian reserve does not directly augment reproductive potential but could improve decision making. If there can be effective assessment of ovarian reserve, then the age-specific chance of pregnancy late in reproductive life may be improved by discouraging women with poor ovarian reserve from attempting pregnancy, or, if they seek to try, then facilitate their clinic in addressing their likely lower ovarian response.

This has been an area of interest for many years with a progressive improvement in the markers available. The range of parameters studied has included basal levels of FSH, estradiol, inhibin B, and the LH/FSH ratio; FSH dynamics in stimulation tests; ultrasonic assessment of ovarian volume; antral follicle count (AFC) and ovarian blood flow; and, more recently, anti-Müllerian hormone (AMH). In some approaches, combinations of parameters from this list are employed, but these have generally not been more effective than the best single parameters. Inhibin B, AFC, and AMH are proving to be the most useful markers. Inhibin B is a product of preantral and early antral follicles, whereas AMH is a follicular product reflecting the whole of follicular development.

The evolving literature remains complex, with no single predominant option, and a practical approach is to turn to the review literature. We have notification that a formal systematic review of ovarian reserve tests for fertility prediction is underway with an International Collaboration Systematic Review Protocol already published.[3] Currently, we have the review by Maheshwari *et al.*[4] that indicates that the more successful tests are able to predict ovarian response to a gonadotrophin stimulation regimen in an IVF cycle (number of eggs retrieved), and that none of the available tests or combinations of tests has been shown to predict pregnancy or live birth with sufficient accuracy. The best effectiveness is for serum AMH or AFC in the prediction of oocyte yield, but not for the prediction of oocyte quality or pregnancy.

Preimplantation genetic screening

The concept behind the application of PGS to improving late reproductive potential is specific to IVF. PGS seeks to improve the IVF live birth yield once

Table 1. Average age (in years) of women treated by IVF or DI in the UK.[36]

Year	IVF	DI
1991	33.6	31.9
1992	33.8	31.9
1993	33.8	32.1
1994	33.8	32.2
1995	33.7	32.4
1996	33.8	32.5
1997	33.9	32.6
1998	33.9	33.0
1999	34.0	33.3
2000	34.2	33.6
2001	34.3	34.1
2002	34.5	34.2
2003	34.6	34.4
2004	34.8	34.6
2005	34.9	34.6
2006	35.1	35.0
2007	35.2	35.1

the treatment has progressed to the stage of embryos being selected for transfer. The idea that the embryological and genetic techniques developed for preimplantation genetic diagnosis (PGD) might be applied to embryos of older mothers to ensure that the transferred embryos do not have aneuploidy makes sense since as it is appreciated that in late reproductive life many pregnancies fail because of embryonic genetic abnormality, most notably aneuploidy. PGS potentially offers the ability to select for transfer only embryos free from detected anomaly.

This intervention potentially places the embryos at risk because it involves an invasive diagnostic process, so it has been especially important to thoroughly validate the efficacy of PGS in improving pregnancy outcomes. Thus far, this has not been achieved despite encouraging observational evidence with controls.[5] The randomized controlled trials that have addressed the effectiveness of PGS have not confirmed that it is a useful intervention. This was the conclusion of the current Cochrane Review, but that is limited to studies published up to 2005[6] and subsequent randomized trials have not changed the conclusion.[7–11] A *New England Journal of Medicine* editorial concluded that the evidence against the concept of PGS for maternal age was now strong.[12] This view was challenged on the basis of the methodological issues in that trial.[13] Braude and Flinter[1] have argued that the continued use of PGS simply for the indication of maternal potential could be unethical. However, there remains an international momentum behind PGS with the European data reported at intervals by the European Society of Human Reproduction and Embryology PGD Consortium.[14]

Ooctye cryopreservation

Oocyte cryopreservation is a technology that has been available for many years but has only relatively recently become clinically effective. It is relevant to a number of the late reproductive scenarios. The key social difference from the use of embryo cryopreservation is that the woman does not need to have an identified partner because no fertilization is being attempted.

Women who are concerned that they may in the future be seeking to conceive late in reproductive life and who wish to optimize their chance of successful late reproduction may turn to oocyte cryopreservation. This "fertility insurance" philosophy is relevant to several groups of women who might be interested to have their own "young eggs" available when they eventually seek to reproduce. These could be women who make a lifestyle decision to postpone reproduction, possibly because of career plans or for other personal reasons, or they could be women concerned that they do not have a partner and that this situation may not have changed until late in reproductive life. It is also relevant to some women at risk of premature ovarian failure as discussed later.

Women who are affected by, or are at particular risk of, premature ovarian failure face different considerations from those who wish to extend the normal reproductive span; however, some of the relevant tools may be the same for both groups. Where a woman is already known to be at increased risk of spontaneous premature ovarian failure, then she might wish to use assessment of ovarian follicular reserve to estimate her risk even though these tools are less validated in this situation. She might also decide to explore oocyte cryopreservation as "fertility insurance" if her ovarian function has not yet deteriorated. Where the premature ovarian failure was unanticipated, these options have little to offer and

Table 2. IVF and DI success rates by age group (years) in the UK.[36]

Age (years)	IVF 2007 (%)	(IVF 2006)	Age (years)	DI 2007 (%)	(DI 2006)
<35	32.3	(31%)	<35	14.3	(13.5%)
35–37	27.7	(26.4%)	35–39	12.1	(9.2%)
38–39	19.2	(18.6%)			
40–42	11.9	(11.1%)	40–42	4.6	(5.3%)
43–44	3.4	(4.6%)	43–44	1.4	(1.2%)
>44	3.1	(4.0%)	>44	0	(0%)

Note: Average success rate 2007 (2006) for IVF and DI treatment using own fresh eggs in the UK.

the only route to reproduction with likely success will be oocyte donation.

The first birth involving cryopreserved oocytes was in 1986,[1] but progress was slow. The low success rate of around 1–5% was a significant problem, but more recently, developments in cryotechnology have delivered more practicable pregnancy rates. The advance has been due to the development and validation of oocyte vitrification, which has produced superior results to slow freezing methods. In a prospective randomized study in a donor oocyte program, Cobo *et al.* reported that equivalent positive outcomes were achieved with fresh and vitrified cryopreserved oocytes.[16] The subject has been reviewed by Homburg *et al.*, who indicate that oocytes cryopreserved by vitrification are producing 90% oocyte survival rates, 75–90% fertilization rates, and 32–65% pregnancy rates per embryo transfer.[17] These statistics validate oocyte freezing as clinically relevant, and it is now likely that the application of "fertility insurance," as described earlier, will gain momentum while probably remaining controversial because of the "lifestyle" dimension. For example, a recent front cover of the "Body and Soul" section of *The Times* newspaper carried the caption, "The New Nest Egg. Is it ethical to stockpile embryos until you are rich enough to start a family?"[18]

Young women with cancer

In recent years, the landscape for young women with cancer has been changed by the increasing effectiveness of therapies. When faced with a reasonable chance of cure or very substantial periods of remission, it becomes relevant to consider parallel issues such as seeking to preserve the possibility of future fertility. For many, a key issue is to minimize the risk to ovarian function that can accompany cancer therapy if they are expecting to wish to have children in the future. Surgical ovarian transposition and suppression of ovarian activity during therapy are long-established options where the aim is to minimize ovarian damage during chemotherapy or pelvic radiotherapy in women having oncology treatment. In addition, ovarian tissue cryopreservation with later ovarian transplantation is a recently developed option for oncology patients aimed at preserving viable ovarian tissue for reproductive use later in life. Jeruss and Woodruff[19] have recently emphasized that in the planning of oncology treatment there needs to be a new paradigm of treatment and that this should involve an interdisciplinary approach that provides patients with accurate information on options for preserving fertility. These authors have additionally pointed out that even having the discussion about future fertility with young women facing cancer treatment provides a degree of valuable "generational hope" at this difficult time. Against the background that the American Society of Clinical Oncology and the American Society of Reproductive Medicine both recommend that fertility preservation issues are routinely discussed with all cancer patients of reproductive age, Forman *et al.*[20] conducted a survey of oncology practice at Duke University Medical Center. This indicated that a majority of the responding oncologists always or usually discuss the fertility impact of the cancer treatment, except when this is judged inappropriate. Interestingly, routine referral for specialist reproductive advice was uncommon with 45% never referring for this advice.[20]

Surgical transposition of the ovaries aimed at sparing them radiation exposure has been in use

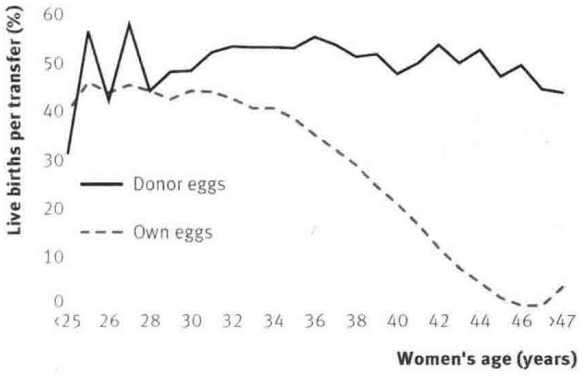

Figure 4. Live birth rates after embryo transfer using own or donor eggs, by age of recipient.[1]

since the 1950s. Initially, the transposition was performed at staging laparotomy before pelvic radiotherapy and now laparoscopic transposition is favored with good preservation of ovarian function achieved in many cases. For example, Morice *et al.*[21] reported 12 pregnancies achieved in a series of 37 women.

Ovarian suppression seeks to protect the ovaries by minimizing ovarian activity during cancer therapy. The most commonly used option is to use a GnRH agonist, because this removes the stimulus to ovarian follicular activity. The effectiveness of this intervention has recently been analyzed in a meta-analysis by Clowse *et al.*[22] This meta-analysis reports that there is a significantly higher chance of continued ovarian function after chemotherapy if a GnRH agonist is used compared to no treatment (Summary RR 1.68 [1.34–2.1]) and also a significantly higher chance of pregnancy (Summary RR 1.65 [1.03–2.6]).

The other reproductive technology of particular relevance to women with cancer is ovarian tissue cryopreservation and later autotransplantation of the ovarian tissue with the goal of achieving pregnancy. Early in 2004, Oktay *et al.*[23] reported that following such a heterotopic autotransplantation procedure, in which a 30-year-old woman who had been treated for breast cancer had her cryopreserved ovarian tissue transplanted under the skin of the abdominal wall, there had been spontaneous ovarian follicular activity from which oocytes had been retrieved and a four cell embryo resulted. Later in the same year, Donnez *et al.*[24] reported the first pregnancy following orthotopic autotransplantation of cryopreserved ovarian tissue. In this case, the ovarian tissue had been removed by laparoscopy in 1997 from a 25-year-old woman undergoing chemotherapy for Hodgkin's lymphoma. Subsequently, she was judged to have ovarian failure, and in 2003 the thawed cryopreserved ovarian tissue was reimplanted on the ovary by laparoscopy. Between five and nine months after transplantation, there were spontaneous menstrual cycles, and then a pregnancy was established with birth reported in 2004.[24] Oktay *et al.* continued with the heterotopic autotransplantation in the abdominal wall approach and reported several successful pregnancies by this method in a paper that also summarizes the progress of the field to date.[25] As these techniques progress, it is to be expected that tissue cryopreservation and thaw/replacement protocols will be optimized and that the ongoing debate between the pros and cons of orthotopic and heterotopic autotransplantation will be resolved, generating a standardized approach that can be of optimal benefit to young female cancer patients who desire to preserve their capacity for later reproduction.

Future possibilities

Where women have reached a state of ovarian failure and do not want to lose their own genetic link to their offspring, there may someday be options that are based on the use of biological technologies that are today not sufficiently developed and/or that are not currently judged to be acceptable for human reproductive use. These include modulation of the primordial follicle–primary follicle transition and the use of adult somatic cells in the generation of artificial gametes for reproductive use.

Modulation of the primordial follicle–primary follicle transition is currently not possible, but if it were clinically available, it would open up the possibility of reducing the numbers of follicles that go through the transition to the primary follicle stage from which ongoing development is inevitable.

The "saved up" primordial follicles could then be used later in reproductive life with a possible postponement of menopause. Women who decide, while young adults, that they could be confronted by the challenge of late reproduction might be interested in the manipulation of the process of ovarian aging through this approach. This is not presently possible and may indeed not be desirable, but research on ovarian biology is providing insights that explore the control of the key step in ovarian

follicular development, which is the primordial to primary follicle transition after which follicular development is inexorable under the influence of FSH. With the current state of development in this field, it is likely to be many years before any intervention to permit therapeutic modulation of primordial/primary follicle transition could be clinically available, if ever. The molecular control of ovarian follicular development in the context of ovarian aging has been reviewed by Hillier.[26] Indeed, if such an intervention does become possible, it will then introduce a new range of complexities. If fewer follicles were used up during the normal reproductive span, it might indeed make a significantly extended reproductive life span possible. On the other hand, it needs to be appreciated that we already know of potentially serious adverse consequences for women from extending the reproductive life span in terms of a higher risk of breast cancer in women with late menopause, and we know of the greater burden of genetic abnormality observed in pregnancies resulting from older oocytes.

Artificial oocytes

The other long-term development that might offer an alternative route to extended reproductive span is through the generation of artificial human oocytes derived by a variety of possible routed from adult somatic cells. This is a rapidly expanding field that cannot be addressed in detail here, but a summary is worthwhile.

The potential for female adult somatic cells to be used in reproduction has been an expanding field since the landmark report of the conception of Dolly the sheep, which involved a sheep somatic cell being used in somatic cell nuclear replacement to generate a "cloned" embryo.[27] Subsequently, there has been interest in the potential use of haploidized adult somatic cell nuclei in combination with donated recipient-enucleated oocytes to generate artificial oocytes. Although much of this research has been based in the mouse, Tesarik *et al.*[28] reported the generation of a small number of artificial human oocytes in which they confirmed haploidization of five chromosomes. Subsequently, Takeuchi *et al.*[29] used somatic cell nuclear replacement in enucleated human oocytes and induced haploidization. There were many difficulties but some of the oocytes underwent early preimplantation development but chaotic chromosome distribution was detected. The issues have previously been reviewed.[30]

Another route to artificial oocytes is through generating these in cultures of embryonic stem cells (ESCs) or induced pluripotent stem cells (iPS). Studies in mice have demonstrated that primordial germ cells can be derived from ESCs and that these can form oocyte-like structures and blastocyst-like structures.[31] Again in the mouse, Nicholas *et al.* have subsequently reported the full maturation of ESC-derived oocytes.[32] Human work is progressing on the generation of primordial germ cells with oocyte potential from ESCs and iPS.[33,34]

The human therapeutic use of artificial oocyte techniques will demand thorough scientific validation, as well as societal and legal acceptance, before it is ready for introduction into clinical practice.

Extending the reproductive span in women has many dimensions both for those with a normal expected reproductive span and for those with a shortened span as a result of premature menopause occurring naturally or in association with cancer treatment. Many of the options remain very much in the development stage and bring with them significant controversy.

Conflicts of interest

The author declares no conflicts of interest.

References

1. Braude, P. & F. Flinter. 2007. Use and misuse of preimplantation genetic testing. *BMJ* **335:** 752–754.
2. Fertility Facts and Figures 2007. HFEA (2007). London.
3. Johnson, N.P. *et al.* 2006. Ovarian reserve tests for predicting fertility outcomes for assisted reproductive technology: the International Systematic Collaboration of Ovarian Reserve Evaluation protocol for a systematic review of ovarian reserve test accuracy. *BJOG* **113:** 1472–1480.
4. Maheshwari, A., A. Gibreel & S. Bhattacharya. 2009. Screening for early ovarian ageing. In *Reproductive Ageing*. S. Bewley, W. Ledger & D. Nikolaou, Eds.: RCOG Press.
5. Gianaroli, L. *et al.* 1999. Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed. *Fertil. Steril.* **72:** 837–844.
6. Twisk, M. *et al.* 2006. Cochrane Review – Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection. The Cochrane Library.
7. Staessen, C. *et al.* 2004. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advances maternal age: a

prospective randomised controlled trial. *Hum. Reprod.* **19:** 2849–2858.
8. Stevens J. *et al.* 2004. Is aneuploidy screening for patients aged 35 or over beneficial? A prospective randomized trial. *Fertil. Steril.* **82**(Suppl. 2): 249.
9. Mastenbroek, S. *et al.* 2007. In vitro fertilization with preimplantation genetic screening. *NEJM* **357:** 9–17.
10. Hardarson, T. *et al.* 2008. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. *Hum. Reprod.* **23:** 2806–2812.
11. Schoolcraft, W.B. *et al.* 2008. Preimplantation aneuploidy testing for infertile patients of advanced maternal age: a randomized prospective trial. *Fertil. Steril.* **92:** 157–162.
12. Collins, J.A. 2007. Preimplantation genetic screening in older mothers. *NEJM* **357:** 61–63.
13. Cohen, J. & J. Grifo. 2007. Multicenter trial of preimplantation genetic screening reported in the New England Journal of Medicine: an in-depth look at the findings. *Reprod. Biomed. Online* **15:** 365–366.
14. Harper, J.C. *et al.* ESHRE PGD consortium data collection X: cycles from January to December 2007 with pregnancy follow-up to October 2008. *Hum. Reprod.* **11:** 2685–2707.
15. Chen, C. 1986. Pregnancy after human oocyte cryopreservation. *Lancet* **1:** 884–886.
16. Cobo, A. *et al.* 2008. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. *Fertil. Steril.* **89:** 1657–1664.
17. Homburg, R., F. Van Der Veen & S.J. Silber. Oocyte vitrification—Women's emancipation set in stone. *Fertil. Steril.* **91**(Suppl. 4): 1319–1320.
18. The Times – August 24, 2010. Body and Soul section front cover.
19. Jeruss, J.S. & T.K. Woodruff. 2009. Preservation of fertility in patients with cancer. *NEJM* **360:** 902–911.
20. Forman, E.J., C.K. Anders & M.A. Behera. 2009. Pilot survey of oncologists regarding treatment-related infertility and fertility preservation in female cancer patients. *J. Reprod. Med.* **54:** 203–207.
21. Morice, P. *et al.* 1998. Fertility results after ovarian transposition for pelvic malignancies treated by external irradiation or brachytherapy. *Hum. Reprod.* **13:** 660–663.
22. Clowse, M.E.B. *et al.* 2009. Ovarian preservation by GnRH agonists during chemotherapy: a meta-analysis. *J. Women Health* **18:** 311–319.
23. Oktay, K. *et al.* 2004. Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. *Lancet* **363:** 837–840.
24. Donnez, J. *et al.* 2004. Live birth after orthotopic transplantation of crypreserved ovarian tissue. *Lancet* **364:** 2093–2094.
25. Oktay, K., I. Türkçüoğlu & K.A. Rodriguez-Wallberg. 2010. Four spontaneous pregnancies and three live births following subcutaneous transplantation of frozen banked ovarian tissue: what is the explanation? *Fertil. Steril.* **95:** 804.e7–804.e10.
26. Hillier, S.G. 2009. The science of ovarian ageing: how might knowledge be translated into practice? In *Reproductive Ageing*. S. Bewley, W. Ledger & D. Nikolaou, Eds.: RCOG Press.
27. Wilmut, I. *et al.* 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature* **385:** 810–813.
28. Tesarik, J. *et al.* 2001. Fertilizable oocytes reconstructed from patient's somatic cell nuclei and donor ooplasts. *Reprod. BioMed.* **2:** 160–164.
29. Takeuchi, T. *et al.* 2005. Construction and fertilization of reconstituted human oocytes. *Reprod. BioMed.* **11:** 309–318.
30. Nagy, Z. *et al.* 2008. Symposium: genetic and epigenetic aspects of assisted reproduction. Development of artificial gametes. *Reprod. BioMed.* **16:** 539–544.
31. Hubner, K. *et al.* 2003. Derivation of oocytes from mouse embryonic stem cells. *Science* **300:** 1251–1256.
32. Nicholas, C.R. *et al.* 2009. Transplantation directs oocytes maturation from embryonic stem cells and provides a therapeutic strategy for female infertility. *Hum. Mol. Genet.* **18:** 4376–4389.
33. Equizabal, C. *et al.* 2009. Generation of primordial germ cells from pluripotent stem cells. *Differentiation* **78:** 116–123.
34. Park, T.S. *et al.* 2009. Derivation of primordial germ cells from human embryonic and induced pluripotent stem cells is significantly improved by coculture with human fetal gonadal cells. *Stem Cells* **27:** 783–795.
35. UK Office for National Statistics. 2008. Who is having babies? *Office for National Statistics Statistical Bulletin*, 1–11.
36. Human Fertilisation & Embryology Authority. 2007. Data on UK Fertility Treatment Patients-Long Term Trends. *HFEA–Fertility, Infertility, IVF, Embryo Research* http://www.hfea.gov.uk/2585.html#3019. Accessed February 25, 2011.

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Stem cells in endometrium and their role in the pathogenesis of endometriosis

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The human endometrium is a dynamic tissue that undergoes cycles of growth and regression with each menstrual cycle. Adult progenitor stem cells are likely responsible for this remarkable regenerative capacity; these same progenitor stem cells may also have an enhanced capacity to generate endometriosis if shed in a retrograde fashion. The progenitor stem cells reside in the uterus; however, less-committed mesenchymal stem cells may also travel from other tissues such as bone marrow to repopulate the progenitor population. Mesenchymal stem cells are also involved in the pathogenesis of endometriosis and may be the principle source of endometriosis outside of the peritoneal cavity when they differentiate into endometriosis in ectopic locations. Finally, besides progenitor stem cells, recent publications have identified multipotent stem cells in the endometrium. These multipotent stem cells are a readily available source of cells that are useful in tissue engineering and regenerative medicine. Endometrial stem cells have been used to generate chondrocytes, myocytes, neurons, and adiposites *in vitro* as well as to replace dopaminergic neurons in a murine model of Parkinson's disease.

Keywords: endometrium; endometriosis; stem cells

Stem cells in endometrium and endometriosis

Stem cells are undifferentiated cells that have the ability to self-renew as well as to produce more differentiated daughter cells.[1,2] Broadly, they can be divided into two categories: embryonic and adult. Embryonic stem cells are found in the inner cell mass of the blastocyst. Adult stem cells, derived from postembryonic cell lineages, have been described in a number of different organ systems and have been best characterized in the hematopoietic system.[1,3]

Embryonic and adult stem cells are classified by their ability to differentiate into cells of different cell lineages. Differentiation is defined as a change in cell phenotype because of expression of genes associated with cellular function rather than cell division.[4] Totipotent stem cells are fully undifferentiated and able to generate all embryonic germ layers (endoderm, mesoderm, and ectoderm) as well as the extra-embryonic tissues (trophoblast, placenta, and extra-embryonic membranes); the zygote is representative of this cell. The embryonic stem cells, in turn, are pluripotent stem cells that lie along a spectrum of differentiation and can produce cells of all three germ layers, but not the extra-embryonic tissues. As stem cells undergo differentiation and their cell lineages become more restricted, they are described as multipotent because they can produce multiple cell types within the same germ cell lineage, or unipotent, differentiating into a single cell lineage.[5]

Adult stem cells reside in an anatomic structure called the niche.[6] The stem cell niche is a microenvironment of surrounding support cells that signal to the stem cell population. The niche cells provide signals that maintain stem cells in an undifferentiated state, protecting them from differentiation, proliferation, and apoptotic cues. But they also sense

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the need for tissue replacement and communicate proliferative and differentiation signals to resident stem cells.[7]

Maintenance of the stem cell population requires cellular self-renewal, that is, the capacity to generate identical daughter cells, which can happen through asymmetric or symmetric division. In an asymmetric division, one stem cell produces an identical daughter cell and a more differentiated daughter, whereas in a symmetric division it produces two daughter stem cells or two transit amplifying (TA) progenitors. TA cells undergo repetitive cycles of cell divisions to increase in number while progressively acquiring markers of the differentiated cell type; consequently, they lose the ability for self-renewal.

Structure of the human endometrium

The human endometrium of the uterus comprises the endometrial mucosal lining, which is a highly regenerative tissue. It is composed primarily of two cell types—the epithelial cells (luminal and glandular) and the supporting mesenchymal cells (stromal cells)[8] as well as endothelial cells and leukocytes.[9] The endometrial–myometrial junction is irregular with no submucosal tissue to separate endometrial glandular tissue from the underlying smooth muscle of the myometrium.[10]

Functionally, the endometrium is composed of two layers—the outer functionalis layer and the inner basalis layer. The functionalis, comprising the upper two thirds, is composed of dense glandular tissue surrounded by a loose connective stroma. The inner basalis layer rests on the muscular subendometrial myometrium and contains primarily the base of the glands, dense stroma, and large vessels. This layer serves as a germinal compartment for generating the new functionalis each month.[8]

Evidence for progenitor stem cells in human endometrium

Adult stem cells are found throughout the whole body after embryonic development.[11] They have the potential for self-renewal, playing a critical role in replenishment and regeneration of damaged tissues, thereby contributing to the structural and functional maintenance of the organs and tissues. Similar events occur in the endometrium. During each menstrual cycle there is a vast growth of tissue and blood vessels.[12] Thus, following menstruation, the proliferative stage begins under the influence of increasing circulating estrogen levels. This, in turn, is followed by the secretory phase during which progesterone levels rise as the endometrium prepares for the possibility of fertilization and an implanting embryo. If this does not occur, then the functionalis and a small portion of the basalis endometrium are shed.[11] The shed blood and tissue contain a heterogeneous population of cells, including some with regenerative capacity. It has been hypothesized that adult stem or progenitor cells are responsible for the cyclical regeneration of the endometrial functionalis.[13]

The first evidence of stem cells regenerating the endometrium was based on functional assays.[14–16] In 2004, using purified single cell suspensions obtained from hysterectomy tissues, it was shown that $0.22 \pm 0.07\%$ of endometrial epithelial cells and $1.25 \pm 0.18\%$ of stromal cells formed individual colonies within 15 days when seeded at clonal density.[14] Two types of colonies were generated by both epithelial and stromal cells—large and small colonies. Large putative stem/progenitor cell colonies were rare; occurring at 0.08% and 0.02% for epithelial and stromal cells, respectively. These colonies displayed significantly greater self-renewal capability compared with the small, loose colonies that failed to serially clone and displayed limited proliferation potential. These investigators hypothesized that the large colonies were derived from putative endometrial stem/progenitor cells with a greater potential for self-renewal. By contrast, the small colonies are presumably derived from TA cells that lack the ability for self-renewal and thus display a diminished proliferative potential.

Schwab *et al.* performed a similar analysis of clonogenicity using samples collected from proliferative, secretory, and inactive endometrium.[16] This work demonstrated that the frequency of clonogenic epithelial and stromal cells did not vary in different phases of the menstrual cycle or in inactive endometrium. Because inactive endometrium contains only a basalis layer and not an endometrium functionalis, these data would suggest that putative endometrial stem/progenitor cells reside in the basalis layer and persist beyond menopause.

There are no specific known markers for endometrial progenitor stem cells that distinguish them from their mature progeny. In fact, recent studies have been evaluating candidate markers and, until now, no specific markers have been identified. These studies, although valuable, require further