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ORIGINAL ARTICLE *Infertility*

Reproductive potential of a metaphase II oocyte retrieved after ovarian stimulation: an analysis of 23 354 ICSI cycles

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BACKGROUND: Live birth per cycle and live birth per embryo transfer are commonly used outcome measures for IVF treatment. In contrast, the assessment of the reproductive efficiency of human oocytes fertilized *in vitro* is seldom performed on an egg-to-egg basis. This approach may however gain importance owing to the increasingly widespread use of oocyte cryopreservation, as the technique is becoming more established. The aim of the current study is to quantify the reproductive efficiency of the oocyte according to ovarian ageing and ovarian response.

METHODS: We performed a retrospective analysis of the outcome of all consecutive patients attending for treatment between 1992 and 2009. The outcome in terms of live birth after fresh and cryopreserved embryo transfer per mature oocyte was calculated for 207 267 oocytes retrieved in 23 354 ovarian stimulation cycles. The oocyte utilization rate (number of live births per mature oocyte) was further analysed in relation to the ovarian response.

RESULTS: The oocyte utilization rate in women in the age of $\leq$37 years remains constant with a mean of 4.47% live birth per mature oocyte [95% confidence interval (CI): 4.32–4.61]. From the age of 38 years onwards, a significantly lower oocyte utilization rate was noted, declining from 3.80% at the age of 38 years to 0.78% at 43 years ($P < 0.001$). In this 38–43 years age group, oocyte utilization rate was no longer dependent on ovarian response ($P = 0.87$).

CONCLUSIONS: The major strength of the study, which is also its weakness, is the fact that we included a large number of cycles performed over a long period of time. According to our results, the oocyte utilization rate between 23 and 37 years of age depends largely on ovarian response and to a much lesser extent on age. From the age of 38 years onwards, the utilization rate depends largely on age and to a much lesser extent on ovarian response. Considering the increased use of oocyte freezing for fertility preservation, these data are extremely valuable as they provide an estimate of the number of oocytes needed to achieve a live birth.

Key words: oocyte utilization / reproductive outcome / ICSI / fertility preservation

Introduction

Although live birth per cycle and per embryo transfer are commonly used outcome measures for IVF treatments (Sunkara *et al.*, 2011), live birth rates per retrieved mature oocyte may yield a more relevant outcome index for assisted reproduction techniques (ARTs) and the reproductive process itself. Nonetheless, the evaluation of the reproductive efficiency of human oocytes fertilized *in vitro* is seldom performed on an egg-to-egg basis.

Only recently, data using the concept of live birth per oocyte retrieved have been reported to assess the overall oocyte utilization rate during IVF procedures (Inge *et al.*, 2005; Patrizio *et al.*, 2007; Patrizio and Sakkas, 2009).

One may undeniably claim that the clinical impact of reporting live births per oocyte retrieval may be superfluous for all women undergoing ovarian stimulation. Although this may be a fact for the general population, cumulative pregnancy rates per oocyte may provide additional information, such as the rate of biological material loss (Meniru

and Craft, 1997; Edgar, 2007; Patrizio *et al*., 2007). In this regard, such an outcome may be of substantial importance for the clinical decision-making of specific groups of patients. The main advantage of reporting cumulative pregnancy rate per oocyte retrieval is that this different approach enables a genuine evaluation of cryopreservation procedures, as it clearly shows how a rigorous embryo selection may result in impressively high implantation rates (Gerris *et al*., 1999; Gardner *et al*., 2000). Furthermore, it appears to provide a new and really accurate method for evaluation of the oocyte cryopreservation procedure itself (Edgar, 2007).

Oocyte cryopreservation is a technique that allows fertility preservation in cancer patients, who are at risk of losing gonadal function because of surgery and chemo- and/or radiotherapy (Gidoni *et al*., 2008; Elizur *et al*., 2009; Redig *et al*., 2011). Furthermore, it may represent the most realistic approach for women who desire to prolong their reproductive life for family planning reasons or the absence of a partner (Knopman *et al*., 2010; Stoop *et al*., 2010). Given the excellent outcome after oocyte cryopreservation, the number of centres performing oocyte cryopreservation steadily increases (Rudick *et al*. 2010). Therefore, the actual potential of each individual oocyte retrieved after ovarian stimulation to result in a live birth might be crucial both for patients and clinicians.

Recent studies have reported pregnancy rates following oocyte cryopreservation that are comparable to those achieved following fresh IVF cycles (Cobo *et al*., 2008, 2010; Nagy *et al*., 2009a; Grifo and Noyes, 2010; Rienzi *et al*., 2010; Noyes *et al*., 2011). However, considering the time delay between oocyte harvest and the effective use of cryopreserved oocytes in clinical practice, the real impact of age-related decline in the oocyte quality for women undergoing oocyte cryopreservation remains unclear. The dramatic decrease in pregnancy rates with increasing maternal age is a well-known phenomenon, also present in oocytes that are cryopreserved (Ubaldi *et al*., 2010). Consequently, it appears that a good estimation of the number of oocytes needed to achieve a live birth among different patient age groups may be of paramount importance for the proper counselling of these patients.

Taking into account all the above, we attempted through an extensive set of retrospective data of ICSI cycles, carried out in the Centre for Reproductive Medicine at the Free University Hospital, Brussels, to provide information on IVF clinical outcomes expressed on an oocyte basis.

Materials and Methods

We analysed data of 23 354 IVF cycles performed in our Centre for Reproductive Medicine at the Free University Hospital, Brussels, Belgium, between 1992 and 2009. Only ICSI cycles were included in order to have the opportunity to examine mature oocytes. Mature oocytes (212 009) were retrieved through transvaginal pickup. Cycles with the use of donated oocytes, surgically retrieved sperm or with the use of preimplantation genetic diagnosis or screening were excluded. Oocyte retrievals without prior ovarian stimulation or after which all oocytes or embryos were cryopreserved were not included in the analysis. Cycles were subdivided into three groups according to mature oocyte yield at oocyte retrieval (Group 1, 1–5 oocytes; Group 2, 6–10 oocytes; Group 3, 11 + oocytes) and the following three measures were calculated for the whole population and for the three groups: (i) live birth rates per started cycle, (ii) oocyte utilization rate (number of live births per mature oocyte) and (iii) number of mature oocytes per live birth. The additional potential live birth yield from unused, still cryopreserved, embryos was extrapolated from the outcome achieved in embryos thawed in women who were matched by age. We calculated the potential total number of live births from cryopreserved embryos at a given age based on the performance of embryos that were thawed in women of that age. This approach is justified by the 'random way' of thawing embryos where the order of embryo thawing was independent of embryo quality.

Statistical analyses were performed using IBM Statistical Package for the Social Sciences 19 software (IBM SPSS, Inc., Chicago, IL, USA). Each outcome of interest was calculated by age, starting at 23 years and ending at 43 years of age, and by ovarian response, categorized into three mutually exclusive groups (1–5, 6–10 or 11 or more mature oocytes). Next, multiple linear regression models were fitted to quantify the independent impact of age and ovarian response on each outcome of interest.

Results

Population characteristics

Oocytes (207 267) originating from 23 354 cycles were considered for analysis. Oocytes originating from cycles in women under 23 years ($n = 3293$ oocytes; 265 cycles) and above 43 years ($n = 1449$ oocytes; 269 cycles) of age were excluded because of the low numbers. A total of 7944 live births were recorded in the 23–43-year age group. Six thousand eight hundred and five live births originated from fresh embryo transfers, the other 812 live births from frozen embryo transfers. For 327 cycles, an ongoing pregnancy was recorded but without registered data on live birth. As the pregnancy outcome could not be confirmed in these cycles, they were not considered as live births in the analysis. Significant differences in the mean age were observed between the three subgroups subdivided according to the oocyte yield [1–5 oocytes group: 35.18 years; 6–10 oocytes group: 22.22 years; 11 + oocytes group: 31.6 years ($P < 0.001$); Fig. 1a]. A steady decline in the mean number of retrieved metaphase II (MII) oocytes is observed in relation to age, ranging from 12.44 at the age of 23 years to 5.54 at the age of 43 years (Fig. 1b).

Live birth rate according to age and ovarian response in fresh cycles

Live birth rates following ovarian stimulation appear to correlate both with age and the level of ovarian response to stimulation (Fig. 2). However, the effect of age and ovarian response on live birth rates show completely different patterns. Although age inversely affects live birth rate among age groups beyond 38 years of age, no major differences in effect appear to exist among the age groups of 23–37 years (Table I, upper panel). On the contrary, live birth rates are strongly dependent on the number of mature oocytes retrieved, with higher numbers of mature oocytes retrieved resulting in significantly higher live birth rates (Table I, lower panel).

Number of mature oocytes per live birth and oocyte utilization rate

The number of oocytes per live birth is presented in Fig. 3a and b. This graph visualizes the steep increase in the number of oocytes needed

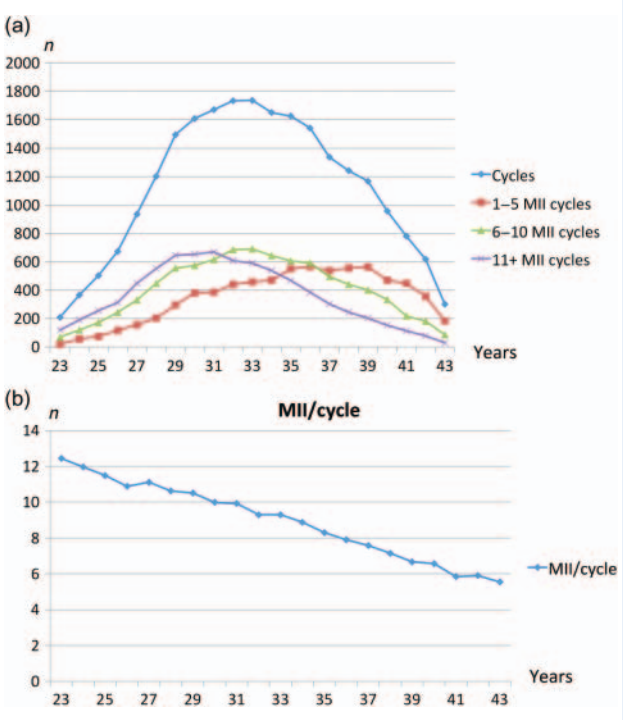

Figure 1 (**a**) Distribution of cycles and (**b**) number of mature [metaphase II (MII)] oocytes, by age.

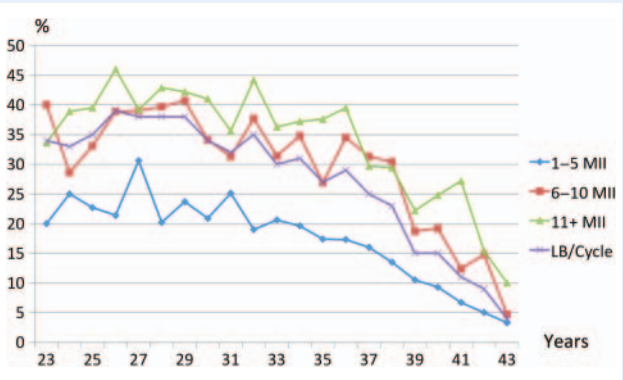

Figure 2 Live births (LB) per fresh cycle.

per live birth after the age of 38 years, while being fairly constant before that age. The mean number of live births per mature oocyte in the age group from 23 to 37 years is 22.53 (SD: 1.55) compared with 55.5 (SD: 34.0) in the age group of 38 years or more ($P < 0.001$).

These findings are further reflected in the oocyte utilization rate (Fig. 4a and b). Overall, 3.83% of aspirated mature oocytes resulted in a live birth. The oocyte utilization rate between the age of 23 and 37 years remains constant with an average of 4.47 [95% confidence interval (CI): 4.32–4.61]. In this 23–37 years age group, the oocyte utilization rate was highly dependent on ovarian response: compared with the referent ovarian response group with 11 or more mature oocytes, the oocyte utilization rate was 1.7% higher in the response group with 6–10 mature oocytes and 2.8% higher in the response group with only 1–5 mature oocytes, with absolute rate differences adjusted for age of 1.6% (95% CI: 0.9–2.4%; $P < 0.0001$) and 2.8% (95% CI: 2.1–3.5%; $P < 0.001$), respectively. From the age of 38 years onwards, a significantly lower oocyte utilization rate was noted, declining from 3.80% at the age of 38 years to 0.78% at 43 years ($P < 0.001$). In this 38–43 years age group, the oocyte utilization rate was no longer dependent on ovarian response rate ($P = 0.87$). Analysis of the oocyte efficiency over the 18-year period did not reveal a significant variation over the long period of study.

Table I Impact of age and ovarian response on live birth rate[a].

Age and ovarian response categories	Absolute difference in birth rate, % (95% confidence limits)[b]	*P*-value
Age categories (years)		
23–25 (reference category)		
26–28	4.1 (0.3 to 7.8)	0.036
29–31	1.5 (2.3 to 5.3)	0.441
32–34	−0.1 (−3.9 to 3.7)	0.972
35–37	−3.5 (−3.7 to 0.3)	0.072
38–40	−11.5 (−15.3 to −7.7)	<0.001
41–43	−20.2 (−24.0 to −16.4)	<0.001
Ovarian response categories[c]		
11 or more MII cycles (reference category)		
6–10 MII cycles	−4.3 (−6.8 to −1.8)	0.001
1–5 MII cycles	−16.4 (−18.9 to −13.9)	<0.001

[a]Multiple linear regression analysis with live birth rate as a dependent (outcome) variable, and age categories and ovarian response categories as independent (explanatory) variables.
[b]Absolute difference in birth rate compared with the reference category.
[c]MII, mature (metaphase II) oocyte.

Discussion

The data presented in this study show that oocytes retrieved after controlled ovarian stimulation are of a significantly lower quality after the age of 38 years. This finding is a clinical confirmation of the mathematical model developed by Faddy and Gosden (1995). The calculations based on follicle counts in histological sections observed a significant increase in atresia of small and resting follicles after the age of 38 years. On the other hand, the oocyte quality expressed as a number of oocytes per live birth and the oocyte utilization rate remain remarkably stable between the age of 23 and 37 years. After the age of 37 years oocyte utilization begins to decrease gradually but from 40 years of age it drops dramatically. Our data confirm previous reports on IVF per oocyte outcomes and moreover, with its larger size, this study provides precise data on oocyte quality per

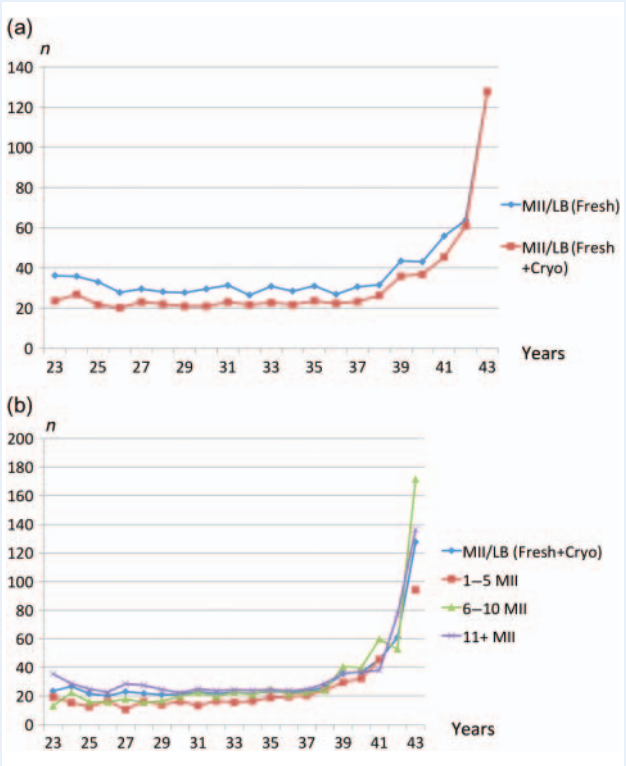

Figure 3 Mature oocytes per live birth: (**a**) fresh cycles and cumulative outcome and (**b**) cumulative outcome in relation to the oocyte yield. Cryo, cryopreserved.

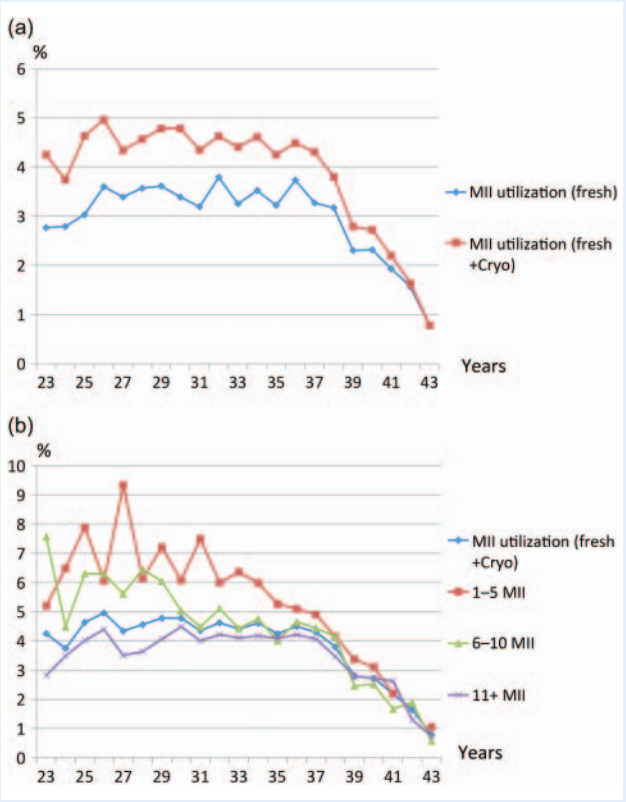

Figure 4 Oocyte utilization rate: (**a**) fresh cycles and cumulative outcome and (**b**) cumulative outcome in relation to the oocyte yield.

age group, ranging between the age of 23 and 43 years (Inge *et al.*, 2005; Patrizio *et al.*, 2007; Patrizio and Sakkas, 2009).

A second interesting finding is the influence of the number of retrieved oocytes on the reproductive potential of the individual oocyte. Meniru and Craft (1997) were the first to describe that although the pregnancy rate per cycle increased with the number of retrieved oocytes, the proportion of oocytes that produced embryos of quality suitable for transfer or cryopreservation fell. The authors suggested that either the production of many oocytes depresses the quality of resulting embryos or that there is a greater tendency to be more selective for embryos to transfer or for cryopreservation (Meniru and Craft, 1997). Arguments for the relation between a stricter embryo selection and the number of aspirated oocytes can be found in commonly used embryo transfer policy, in which blastocyst transfer depends on a Day 3 embryo evaluation and count (Kolibianakis *et al.*, 2004; Papanikolaou *et al.*, 2005). The former argument (related to the oocyte quality) has been confirmed after the introduction of fluorescence *in situ* hybridization on interphase nuclei. Several authors reported a higher incidence of chromosomal aneuploidies in relation to ovarian stimulation or to embryo culture conditions (Munné *et al.*, 1997, 2006; Katz-Jaffe *et al.*, 2005; Baart *et al.*, 2007).

In the absence of ovarian stimulation, the chance of achieving a pregnancy after timed intercourse in a non-selected population is 20–30% (Evers, 2002; Taylor, 2003). Wang *et al.* (2003) observed a 30% clinical pregnancy rate per cycle in a prospective observational cohort study of women with an average age of 24.8 ± 1.7 years. Our study reports a much lower live birth rate per oocyte of only 4.63%, among the same age groups who underwent stimulated cycles. These data are comparable to previously reported ones on stimulated cycles (Inge *et al.*, 2005). Natural cycle IVF appears to yield higher oocyte utilization rates as presented by Edwards *et al.* (1980) in the early days of ART, with live birth rates of 6.67% per oocyte. Recent data based on embryos derived from modified natural cycle IVF reported ongoing pregnancy rates of 22.0–23.2% per oocyte (Pelinck *et al.*, 2010). We can therefore conclude that ovarian stimulation either has a detrimental affect on oocyte quality or that ovarian stimulation rescues follicles from atresia, already destined to produce abnormal embryos (Kovalevsky and Patrizio, 2005). This, however, may not entirely explain the higher live birth per oocyte in women with low number of oocytes as this may be a reflection of poor ovarian reserve rather than mild ovarian stimulation. This finding is supported by the significantly higher average age of women in the 1–5 oocyte group (Fig. 1). Probably, the significantly higher proportion of cryopreserved embryos in high responders dilutes the effect of the better fresh embryo transfer rates, resulting in a lower overall live birth rate per oocyte (Fig. 4).

The oocyte utilization rate and oocytes per live birth rate remain remarkably stable between the age of 23 and 36 years. Although a marked decline in live birth per cycle with age can already be observed from the early-30s onwards, the per-oocyte outcome is stable. This should be attributed to the fact that a higher number of oocytes are

retrieved and a lower number of embryos transferred in the fresh cycle among patients of younger age. Therefore, a relatively higher proportion of embryos will be transferred in cryo-cycles in these women, embryos that eventually increase the overall pregnancy rates among them but at a relatively lower implantation rate per embryo. However, it is clear that the overall live birth per oocyte remains stable given that a higher number of oocytes are cumulatively utilized in women of younger age.

The clinical implication of our findings is that they may be of interest for the counselling of women that desire oocyte cryopreservation. Given that our results provide evidence of the actual oocyte potential for the achievement of a live birth, they may be utilized in order to tailor the management of those patients. Recently published studies comparing the ICSI outcome of fresh versus cryopreserved oocytes (Nagy *et al.*, 2009a; Cobo *et al.*, 2010; Grifo and Noyes, 2010; Rienzi *et al.*, 2010) concluded that the outcome of the cryopreserved oocytes was not significantly low to those achieved with fresh oocytes. Many of the available data are based on relatively 'young' oocytes as the studies have been performed in an oocyte donation setting (Nagy *et al.*, 2009a; Cobo *et al.*, 2010). However, a sibling oocyte study performed by Nagy (2009b) demonstrated high survival and fertilization as well as a good embryo development in women up to 39 years of age after oocyte vitrification. These findings were confirmed in a non-inferiority trial by Rienzi *et al.* (2010) in women with a mean age of 35.5 years (SD: 4.8). Although oocyte survival and embryo development did not seem to be affected by the maternal age, a recent study found that the maternal age was the only characteristic to influence the cumulative ongoing pregnancy rate (Ubaldi *et al.*, 2010).

In conclusion, this study shows that the live birth rate per oocyte and the number of oocytes per live birth remain remarkably stable between the age of 23 and 37 years. A marked decrease in the oocyte utilization rate is observed from the age of 38 onwards and a dramatic drop is seen after the age of 40 years. In view of recent studies reporting pregnancy rates from cryopreserved oocytes comparable to those achieved following fresh IVF, these data provide information on the expected outcome in women undergoing fertility preservation. Moreover, considering the time delay between oocyte harvest and the effective use of cryopreserved oocytes in clinical practice, it is crucial to provide precise estimates for the number of oocytes needed to achieve a live birth.

Authors' roles

D.S. was involved in conception and design and the interpretation of data. B.E. was involved in the acquisition and interpretation of data. N.P. and P.H. involved in the statistical analysis and interpretation of data. M.D, G.V. and P.D. were involved in the critical revising.

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Conflict of interest

None declared.

References

Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NGM, Verhoef A, Macklon NS, Fauser BCJM. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. *Hum Reprod* 2007;**22**:980–988.

Cobo A, Kuwayama M, Perez S, Ruiz A, Pellicer A, Remohi J. Comparison of concomitant outcome achieved with fresh and cryopreseved donor oocytes vitrified by the cryotop method. *Fertil Steril* 2008; **89**:1657–1664.

Cobo A, Meseguer M, Remohi J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. *Hum Reprod* 2010;**25**:2239–2246.

Edgar D. How should the clinical efficiency of oocyte cryopreservation be measured? *Reprod BioMed Online* 2007;**14**:430–435.

Edwards RG, Steptoe PC, Purdy JM. Establishing full-term human pregnancies using cleaving embryo grown *in vitro*. *Br J Obstet Gynaecol* 1980;**87**:737–755.

Elizur SE, Chian R-C, Holzer HEG, Gidoni Y, Tulandi T, Tan SL. Cryopreservation of oocytes in a young woman with severe and symptomatic endometriosis: a new indication for fertility preservation. *Fertil Steril* 2009;**91**:293e3.

Evers JL. Female subfertility. *Lancet* 2002;**360**:151–159.

Faddy MJ, Gosden RG. A mathematical model of follicle dynamics in the human ovary. *Hum Reprod* 1995;**10**:770–775.

Gardner DK, Lane M, Stevens J. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril* 2000;**73**:1155–1158.

Gerris J, De Neubourg D, Mangelschots K. Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial. *Hum Reprod* 1999;**14**:2581–2587.

Gidoni Y, Holzer H, Tulandi T, Tan SL. Fertility preservation in patients with non-oncological conditions. *Reprod BioMed Online* 2008; **16**:792–800.

Grifo J, Noyes N. Delivery rate using cryopreserved oocytes is comparable to conventional *in vitro* fertilization using fresh oocytes: potential fertility preservation for female cancer patients. *Fertil Steril* 2010;**93**:391–396.

Inge GB, Brinsden PR, Elder KT. Oocyte number per live birth in IVF: were Steptoe and Edwards less wasteful? *Hum Reprod* 2005;**20**:588–592.

Katz-Jaffe MG, Trounson AO, Cram DS. Chromosome 21 mosaic human preimplantation embryos predominantly arise from diploid conceptions. *Fertil Steril* 2005;**84**:634–643.

Knopman J, Noyes N, Grifo J. Cryopreserved oocytes can serve as the treatment for secondary infertility: a novel model for egg donation. *Fertil Steril* 2010;**93**:2413.e7–9.

Kolibianakis E, Zikopoulos K, Verpoest W, Camus M, Joris H, Van Steirteghem A, Devroey P. Should we advise patients undergoing IVF to start a cycle leading to a day 3 or a day 5 transfer? *Hum Reprod* 2004;**19**:2550–2554.

Kovalevsky G, Patrizio P. High rates of embryo wastage with the use of assisted reproductive technology: a look at the trends between 1995 and 2001 in the United States. *Fertil Steril* 2005;**84**:325–330.

Meniru GI, Craft IL. Utilization of retrived oocytes as an index of the efficiency of superovulation strategies for in-vitro fertilization treatment. *Hum Reprod* 1997;**12**:2129–2132.

Munné S, Magli C, Adler A, Wright G, de Boer K, Mortimer D, Tucker M, Cohen J, Gianaroli L. Treatment related chromosome abnormalities in human embryos. *Hum Reprod* 1997;**12**:780–784.

Munné S, Ary J, Zouves C, Escudero T, Barnes F, Cinioglu C, Ary B, Cohen J. Wide range of chromosome abnormalities in the embryos of young egg donors. *Rep Biomed Online* 2006;**12**:340–346.

Nagy ZP, Chang CC, Shapiro D, Bernal D, Elsner C, Mitchell-Leef D, Toledo A, Kort H. Clinical evaluation of the efficiency of an oocyte donation program using cryo-banking. *Fertil Steril* 2009a; **92**:520–526.

Nagy ZP, Chang C, Bernal DP, Shapiro DB, Mitchell-Leef D, Kort HI. Comparison of laboratory and clinical outcomes between fresh and vitrified/warmed sibling oocytes obtained from 30–39 year old IVF patients. *Fertil Steril* 2009b;**92**:S67.

Noyes N, Knopman J, Labella P, McCaffrey C, Clark-Williams M, Grifo J. Oocyte cryopreservation outcomes including pre-cryo and post-thaw meiotic spindle evaluation following slow cooling and vitrification of human oocytes. *Fertil Steril* 2011;**94**:2078–2082.

Papanikolaou EG, D'haeseleer E, Verheyen G, Van de Velde G, Camus M, Van Steirteghem A, Devroey P, Tournaye H. Live birth rate is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day 3 of embryo culture. A randomized prospective study. *Hum Reprod* 2005; **11**:3198–3203.

Patrizio P, Sakkas D. From oocyte to baby: a clinical evaluation of the biology efficiency of *in vitro* fertilization. *Fertil Steril* 2009;**91**:1061–1066.

Patrizio P, Bianchi V, Lalioti MD, Gerasimova T, Sakkas D. High rate of biological loss in assisted reproduction: it is in the seed, not in the soil. *Rep Biomed Online* 2007;**14**:92–95.

Pelinck MJ, Hoek A, Simons AHM, Heineman MJ, van Echten-Arends J, Arts EGJM. Embryo quality and impact of specific embryo characteristics on ongoing implantation in unselected embryos from modified natural cycle *in vitro* fertilization. *Fertil Steril* 2010;**94**:527–534.

Redig AJ, Brannigan R, Styker SJ, Woodruff TK, Jeruss JS. Incorporating fertility preservation into the care of young oncology patients. *Cancer* 2011;**117**:4–10.

Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, Colamaria S, Sapienza F, Ubaldi F. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. *Hum Reprod* 2010;**25**:66–73.

Rudick B, Opper N, Paulson R, Bendikson K, Chung K. The status of oocyte cryopreservation in the United States. *Fertil Steril* 2010; **94**:2642–2646.

Stoop D, Nekkebroeck J, Devroey P. A survey on the intentions and attitudes towards oocyte cryopreservation for non-medical reasons among women of reproductive age. *Hum Reprod* 2010;**26**:655–661.

Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. *Hum Reprod* 2011;**26**:1768–1774.

Taylor A. ABC of subfertility. *BMJ* 2003;**327**:434–436.

Ubaldi F, Anniballo R, Romano S, Baroni E, Albricci L, Colamaria S, Capalbo A, Sapienza F, Vajta G, Rienzi L. Cumulative ongoing pregnancy rate achieved with oocyte vitrification and cleavage stage transfer without embryo selection in a standard infertility program. *Hum Reprod* 2010;**25**:1199–1205.

Wang X, Chen C, Wang L, Chen D, Guang W, French J, for the Reproductive Health Study Group. Conception, early pregnancy loss, and time to clinical pregnancy: a population based prospective study. *Fertil Steril* 2003;**79**:577–584.