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

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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
Oocyte utilization in ovarian stimulation

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...
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.

**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

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**Table 1** Impact of age and ovarian response on live birth rate.

| Age and ovarian response categories | Absolute difference in birth rate, % (95% confidence limits) | 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       |                                                             |         |
| 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  |

*aMultiple linear regression analysis with live birth rate as a dependent (outcome) variable, and age categories and ovarian response categories as independent (explanatory) variables. 
*bAbsolute difference in birth rate compared with the reference category. 
*cMII, mature (metaphase II) oocyte.

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**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.
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 (2009a) 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.

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