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From fresh heterologous oocyte donation to autologous oocyte banking

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Abstract

Introduction: **Today, oocyte donation has become well established, giving rise to thousands of children born worldwide annually. The introduction of oocyte cryopreservation through vitrification allows the introduction of egg banking, improving the efficiency and comfort of oocyte donation. Moreover, the vitrification technique can now enable autologous donation of oocytes to prevent future infertility.**
Methods: **We evaluated fresh heterologous oocyte donation in terms of obstetrical and perinatal outcome as well as of the reproductive outcome of past donors. We then evaluated the efficiency of a closed vitrification device and its clinical applications within ART. Thirdly, we evaluated the opinion of women with regard to preventive egg freezing and the efficiency of a human oocyte in relation to age.**
Results: **Oocyte donation is associated with an increased risk of first trimester bleeding and pregnancy induced hypertension. Donating oocytes does not seem to increase the likelihood for a later need of fertility treatment. The chance of an oocyte to result in live birth (utilization rate) in women <37 years old remains constant with a mean**

of 4.47%. A significant proportion of young women would consider safeguarding their reproductive potential through egg freezing or are at least open to the idea.
Discussion and Conclusion: **The introduction of efficient oocyte cryopreservation has revolutionized oocyte donation through the establishment of eggbank donation. The technique also enables women to perform autologous donation after preventive oocyte storage in order to circumvent their biological clock.**

Key words: **Oocyte donation, vitrification, autologous donation, social freezing, age related fertility decline.**

Introduction

Oocyte donation has now become a well established method of treating infertility, especially in women with premature ovarian failure, those with repeated failed attempts at in vitro fertilisation (IVF) or those who are carriers of a genetic disorder (Abdalla et al., 1998). However, it is still the fertility treatment of last resort.

The continuing shift in age at childbearing and the introduction of egg banking are two important evolutions that have changed the face of oocyte donation since its introduction. In the USA, between 1980 and 2004, the proportion of births increased 3-fold in women aged ≥ 30 (from 8.6 to 25.4%), 6-fold in women aged ≥ 35 (from 1.3 to 8.3%) and 15 fold in women age ≥ 40 (from 0.1 to 1.5%) (Martin at al., 2006). This causes an increase in age-related infertility and has significantly increased the number oocyte donation cycles performed worldwide. When comparing the 11th European IVF monitoring report (2004) with the data from the 8th report (2007), a 50% increase in the number of egg donation cycles can be observed from 10.334 to 15.731 (Andersen et al., 2008; de Mouzon et al., 2012).

Furthermore, the introduction of oocyte cryopreservation through vitrification allows the introduction of eggbanks, improving the efficiency and comfort of oocyte donation.

Oocyte cryopreservation has become a routine procedure only since a few years even though the first successful pregnancies obtained after slow-rate freezing were pioneered more than two decades ago (Chen 1986; Al-Hasani et al., 1987). Slow controlled-rate freezing allowed oocytes to be cooled to very low temperatures while minimizing intracellular ice crystal formation and the detrimental influences of increased solute concentrations. In 1999, Kuleshova et al. (1999) reported the first birth from vitrified human oocytes after vitrification of 17 oocytes in open pulled straws. This technique represents ultra rapid cooling, using high concentrations of cryoprotectants that solidify without the formation of ice. The perceived inefficiency and theoretical safety concerns had, however, limited its adoption to clinical situations in which no alternative was available, e.g. gonadotoxic therapy in single women. In the last decade, consensus grew that oocytes can be successfully cryopreserved by either slow-rate freezing or by vitrification through direct liquid nitrogen contact (Oktay et al., 2006).

Recently, several reports have demonstrated excellent clinical outcome from cryopreserved oocytes, especially through the open vitrification technique (Kuwayama et al., 2005; Antinori et al., 2007; Nagy et al., 2009; Cobo et al., 2010a). Moreover, prospective randomized trials concluded that the outcome of vitrified-warmed oocytes was not inferior to fresh oocytes with regard to fertilization and embryo development and that comparable ongoing pregnancy rates were achieved (Cobo et al., 2010a; Rienzi et al., 2010). It is noteworthy that all studies concluding the non-inferiority of vitrified oocytes as compared to fresh oocytes have been performed with open devices.

Potential cross contamination through the direct contact of the vitrification sample with non-sterile liquid nitrogen in open devices remains a controversial issue (Tedder et al., 1995; Fountain et al., 1997; Bielanski et al., 2003; 2009). Therefore, methods have been developed to prevent any contamination. Sterilization of small amounts of liquid nitrogen during vitrification has been described as a feasible technique, while storage can be performed in the vapour phase of nitrogen (Parmegiani et al., 2009; Eum et al., 2009; Cobo et al., 2010b). Alternatively, open carriers can be placed in a hermetically sealed container before being plunged into the liquid nitrogen. This double bagging technique (straw in straw method) appears to result in good post warming embryo development after pronuclear oocyte or embryo vitrification (Kuleshova et al., 2000; Vajta et al., 2009).

More recently, closed carriers have been introduced to deal with the above-mentioned concerns, allowing both vitrification and storage in the same device. Data obtained with closed vitrification systems such as Cryo TIP are scarce (Smith et al., 2010). Many authors share the opinion that the decreased cooling rate observed in closed devices due to isolation of the vitrifying samples potentially reduces the efficiency of oocyte vitrification. These

assumptions are supported by data that suggest that the oocyte ultrastructure is better preserved with an open carrier (Bonetti et al., 2010). A recent randomized trial comparing an open (cryotop) versus a closed (cryotip) device found lower fertilization and cleavage rates with the closed system (Paffoni et al., 2011). However, no prospective randomized trial has been performed comparing clinical outcomes. Moreover, data on clinical outcome after closed oocyte vitrification are limited.

In 2010, Jaime Knopman demonstrated that cryopreserved oocytes could serve as the treatment for secondary infertility. The authors call it a novel model for egg donation. This case report illustrates that autologous donation of oocytes after preventive cryopreservation can be an alternative for the classical oocyte donation, which has always been heterologous.

This autologous form of oocyte donation has only become available since the introduction of efficient egg banking. In contrast to the established and successful treatment of heterologous oocyte donation byyoung oocyte donors, the success of autologous donation depends to a large extent on the age at the time of cryopreservation and the efficiency of the cryopreservation technique used. An advantage of autologous donation is the fact that it no longer depends on the efforts of other altruistic women and that women preserve the genetic bond with their offspring.

This novel method, commonly known as 'social egg freezing', has been offered to patients at the Centre for Reproductive Medicine since 2009 (Stoop, 2010a).

This thesis covers a wide range of issues related to oocyte donation and the clinical applications of oocyte storage. A crucial aspect that relates to all topics discussed in this thesis is the phenomenon of ovarian aging and its effect on the reproductive outcome. Three different research questions were studied in this thesis: (1) the evaluation of the established fresh oocyte donation, (2) evaluation of the introduction of oocyte banking into clinical practice and (3) the introduction of autologous oocyte banking.

Methods

1. Fresh heterologous donation

1a. Obstetric outcome after oocyte donation

A retrospective analysis was performed to investigate the impact of oocyte donation on the obstetric and perinatal outcome by comparing it with a strictly matched control group (Stoop et al., 2012a). On a one-to-one basis, the oocyte recipient cohort was matched for age, ethnicity, parity and plurality, with a cohort of women who underwent in-vitro fertilisation treatment. Using this methodology we were able to minimise confounding bias and to investigate the effect of both oocyte donation and the medical condition underlying ovarian insufficiency.

At the Dutch-speaking Brussels Free University, data collection with regard to the obstetrical and perinatal outcomes after oocyte donation and after ART have long been integrated into the clinical programme of the fertility centre (Bonduelle et al., 1995). All pregnancies between January 1999 and December 2008 that had been obtained after oocyte donation and resulted in offspring after more than 20 weeks of gestation were included in this study. Matched controls were selected from the patient population that underwent in-vitro fertilisation with autologous oocytes during the same period. All pregnancies in oocyte recipients and in controls were conceived after ICSI. The individually matched controls were selected from a total of 3707 ICSI pregnancies reaching 20 weeks of gestation. Pregnancies after PGD or after testicular sperm extraction (TESE) or use of donor sperm were not included in this study.

1b. Reproductive health in past oocyte donors

We investigated the reproductive performance of oocyte donors (Stoop et al., 2012b). A standardized telephone questionnaire was developed, aiming to evaluate fecundity before and after the donation procedure. The study was conducted with the approval of the institutional ethics committee. We were able to contact 205 of the 307 women who donated oocytes in our centre between 1999 and 2010. Respondents were informed about the purpose of the study and 194 consented orally to participate, representing a total of 343 donation cycles.

2. Oocyte banking

2a. Validation of a closed vitrification system

A prospective, observational study was performed aimed at evaluating the reproductive outcome after closed system oocyte vitrification in an egg donation program (Stoop et al., 2012c). All donors were healthy women under the age of 35 and were screened and tested according to the Belgian regulations. Twenty recipients were included in the study. All participants fulfilled the admittance criteria and couples signed an informed consent.

2b. Oocyte banking in intra-uterine insemination cycles

This study investigated the value of establishing a pool of cryopreserved excess oocytes after ovarian

stimulation for intra-uterine insemination and its potential to result in additional pregnancies through future IVF and embryo transfer (Stoop et al., 2010b). The pilot study was carried out at the Centre for Reproductive Medicine, UZ Brussels between February and July 2009. The primary endpoint was clinical pregnancy rate per patient undergoing an excess oocyte aspiration prior to IUI. Secondary outcomes were the multiple birth rate, IUI cancellation rate, premature luteinization rate and number of vitrified oocytes.

Eligibility inclusion criteria were: (i) female age ≤ 40 years undergoing ovarian stimulation (OI); (ii) FSH on day 3 of < 12 IU/mL (iii) more than 2 follicles of ≥ 16 mm diameter or more than one follicle ≥ 16 mm when in combination with 2 follicles ≥ 14 mm; (iii) no more than 8 previous OI cycles (iv) exclusion of tubal factor by hysterosalpingography or laparoscopic tubal patency test in the last 12 months (v) insemination motile count of $> 1 \times 10^6$ spermatozoa.

Ultrasound-guided transvaginal follicle aspiration was performed with a single lumen needle (Cook, K-OPS-1230-VUB, Limerick, Ireland) 36 hours after hCG administration. All patients had a paracervical infiltration with a local anaesthetic. The aspiration pressure was 110 mm Hg, as routinely used for mature oocyte aspiration in our centre. The two largest follicles, preferably one on each side, were left intact. IUI was only performed when the number of excess oocytes retrieved at least equalled the number of follicles of ≥ 16 mm that had been observed at pelvic ultrasound scan on the day of hCG administration minus two.

Excess oocyte aspiration was immediately followed by IUI with a flexible Frydman classic catheter (Laboratoire C.C.D; Paris, France). Immediately after cumulus cell removal, all metaphase II (MII) oocytes were vitrified one by one using a closed vitrification system.

3. Autologous oocyte banking

3a. Societal impact of autologous oocyte banking

In this study we investigated attitudes concerning social oocyte freezing among women of reproductive age in Belgium (Stoop et al., 2010c). The following research questions were addressed: (1) how many women would consider social oocyte freezing, (2)are there significant differences between women categorized according to their willingness to cryopreserve oocytes, (3) what is the profile of the women who are categorised as potential oocytes freezers regarding their awareness about fertility and aging, their desire for a child, attitudes towards motherhood and career and the use of donor material.

An electronic survey was completed by 1049 women between 21 and 40 years of age and living in Belgium. Email addresses of the women surveyed were retrieved from a large nationwide registry of people that consented to be contacted for the purpose of surveys. The questionnaire was e-mailed to women representative of all social classes and geographic locations within the country. The construction of the questionnaire was based on previous research on oocyte donation resulting in the 'Attitudes towards donation scale' (Skoog et al. 2003) and on the basis of the information gathered after explorative interviews with women who were candidate to freeze their oocytes and focus group interviews with students and health care professionals. During the administration of the questionnaire, women received an informative text on oocyte cryopreservation where the possible use of the technique for medical and social reasons, the success rates, the associated risks, the side effects and the experimental state of the technique were described.

3b. Oocyte utilization in ART

We analysed data of 23.888 IVF cycles performed in our Centre for Reproductive Medicine at the Free University Hospital, Brussels between 1992 up to 2009 (Stoop et al., 2012d). Only ICSI cycles were included in order to have the opportunity to examine mature oocytes. Two hundred and twelve thousand and nine (212.009) mature oocytes were retrieved through trans-vaginal pickup. Cycles with use of donated oocytes, surgically retrieved sperm or with use of pre-implantation genetic diagnosis or screening (PGD/PGS) 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 by number of 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 carried out for the whole population and for the three groups: (1) live birth rates per started cycle, (2) oocyte utilization rate (number of live births per mature oocyte), (3) 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 matched per age. This approach is justified by the "random way" of thawing embryos.

Table I. — Obstetrical outcome in pregnancies with donated oocytes versus individually matched pregnancies with autologous oocytes further adjusted for paternal age, oocyte age, number of embryos per transfer, and singleton/twin pregnancy.

Conditional logistic regression analysis		
Obstetrical outcome parameter	Matched OR	95% CI
Vaginal bleeding		
1st trimester	1.47	1.02 – 2.12
2nd trimester	1.27	0.52 – 3.12
3rd trimester	0.98	0.24 – 3.99
Nausea and vomiting		
1st trimester nausea	0.87	0.58 –1.29
Hospital admission because of hyperemesis	1.88	0.59 – 6.04
Hypertensive disorders		
Pregnancy induced hypertension	1.50	1.02 – 2.19
Pre-eclampsia	1.31	0.83 – 2.08
Preterm labour		
pPROM	0.78	0.29 – 2.13
Preterm labour	0.74	0.47 – 1.16
Gestational diabetes	1.59	0.85 – 2.98

Results

1. Fresh heterologous donation

1a. Obstetric outcome after oocyte donation

After testing all the parameters in the multivariate regression model, after adjusting for paternal age, donor age, the number of embryos transferred, and singleton/twin pregnancy, the only factors that remained statistically significant were first trimester vaginal bleeding and PIH, with matched odds ratios (95% confidence limits) of 1.49 (1.04-2.15) and 1.50 (1.02-2.21), respectively (Table I).

The study also evaluated the birth parameters after oocyte donation. Gestational age, birth weight, height and head circumference and the calculated standard deviations scores were comparable for both groups. The children of oocyte recipients were not at increased risk of low-birth-weight (LBW), very-low-birth weight (VLBW) or poor Apgar scores. A higher incidence of preterm birth was observed in singletons and twin pregnancies after oocyte donation: 14.2% vs. 12.2% (*P* value for McNemar test < 0.001) and 64.9% vs. 57.1% (*P* value for McNemar test < 0.001), respectively. However, the mean gestational age, as well as the delivery rate before 34 weeks was not different (Stoop et al., 2012a).

1b. Reproductive health in past oocyte donors

Sixty past oocyte donors attempted to conceive since the donation procedure. At the time of survey, 47 of these women (78.3%) had given birth while another seven women (11.7%) had an ongoing pregnancy. The remaining six women (10%) reported an as yet unfulfilled desire for a child of no longer than 12 months. Further follow-up of these six women found that all of these eventually conceived within a one-year period. The mean age at first donation was 29.7 years (SD +/-3.9) with an average follow-up since that first donation of 4.5 years (SD +/-2.3). The oocyte donors performed an average of 1.6 donations (SD +/- 0.8). Forty-one oocyte donors (75.9%) had given birth to 75 children in total before being involved as oocyte donors. Out of these 41 women, three (7.3%) had pursued fertility treatment prior to oocyte donation and all three had achieved a pregnancy. The indication for fertility treatment was male factor infertility in all three cases. On the other hand, unassisted pregnancy after oocyte donation occurred in 57 past oocyte donors (95%), mostly within a period of 12 months (54 women), while the other three donors reporting a maximum of 18 months interval between desire of pregnancy and conception. Three women needed fertility treatment (5.0%) after oocyte donation; two cases had primary male factor infertility and one had primary unexplained subfertility at the age of 38 years (Table II). It is noteworthy, however, that incidences of infertility among donors before their donation is biased by donor selection and therefore limits its value as a control group. Changes in the menstrual pattern following oocyte donation were reported by 16.3% of the respondents, although none of the women reporting these changes reported fertility problems (Stoop et al., 2012b).

Table II. — Active desire for children after oocyte donation: fertile versus subfertile ex-donors.

	Fertile	Subfertile	P value
Number of women	51	3	–
Mean age at first donation (SD)	29.7 (3.9)	29.0 (6.1)	0.78
Mean no. of donations	1.6 (0.8)	1.7 (0.6)	0.83
Mean age at time of survey (SD)	34.0 (4.5)	33.3 (4.0)	0.80
Proportion baring children before donation (%)	42/57 (73.7)	3/3 (100)	1.00
Proportion with fertility treatment before donation (%)	3/42 (7.1)	0/3 (0)	1.00
Proportion with menstrual changes after donation (%)	9/51* (17.6)	0/3	1.00

*6 women were excluded, as the menstrual pattern could not be evaluated due to an intra-uterine device.

2. Oocyte banking

2a. Validation of a closed vitrification system

A total of 123 metaphase II oocytes were vitrified of which 111 (90.2%) survived after warming and 86 survived oocytes were normally fertilized after ICSI (77.5%). Twenty warmed oocyte embryo transfer (WOET) cycles were performed. Thirty-six percent of embryos were scored as excellent. Twenty-two of the in total 36 embryos transferred were of excellent quality (61.1%). A total of 12 embryos were cryopreserved of which four at cleavage stage and eight at blastocyst stage. Three patients had a warmed oocyte frozen embryo transfer cycle (WOFRET), one double embryo transfer at blastocyst stage and two transfers (one single and one double) at cleavage stage.

WOET cycles resulted in a clinical pregnancy rate and ongoing clinical pregnancy rate of 50 and 45% respectively (Table III). One pregnancy ended in a miscarriage and two WOET cycles resulted in a twin pregnancy. One of the three WOFRET cycles with transfer at the blastocyst stage resulted in an ongoing twin pregnancy (Stoop et al., 2012c).

2b. Oocyte banking in intra-uterine insemination cycles

The overall clinical pregnancy rate was 23.5% per initiated cycle and 26.7% per IUI. All pregnancies were singletons. Clinical pregnancy rate in women with PCOS was 35.7% as compared to 18.7% in patients without PCOS. No statistically significant differences were detected.

PCOS patients under the age of 36 years had an average of 6.07 vitrified oocytes per cycle as compared to 1.5 in the non-PCOS group. The patients with an IUI not resulting in a pregnancy had a similar average number of vitrified oocytes as compared to the group as a whole, both in the PCOS and in the non-PCOS patients. The total number of vitrified oocytes among women under the age of 36 with a failed IUI was 70 (Stoop et al., 2010b).

3. Autologous oocyte banking

3a. Societal impact of autologous oocyte banking

A total of 1049 women out of 1914 filled out the questionnaire leading to a response rate of 55%.

Table III. — Clinical outcome after closed system oocyte vitrification.

	Warmed oocyte embryo transfer (WOET)	**Warmed oocyte - frozen embryo transfer (WO-FRET)**	**All transfers**
Number of warming cycles	20	3	23
Number of transfers	20	3	23
Clinical pregnancy rate (%)	10/20 (50.0)	1/3 (33.3)	11/23 (47.8)
Ongoing pregnancy rate (%)	9/20 (45.0)	1/3 (33.3)	10/23 (43.5)
Implantation rate (%)	12/36 (33.3)	2/5 (40)	14/41 (34.1)

Table IV. — Intentions to freeze oocytes among women aged 20 to 40 years old.

Would you consider to freeze oocytes for social reasons?	n	%	Group	%
Yes	32	3.1%	Potential freezers	31.5%
Maybe	291	28.4%		
I don't know	171	16.7%	Doubtful group	16.7%
No	530	51.8%	Non-freezers	51.8%

Another 25 cases were excluded because of an inconsistent or incomplete answering pattern. Of the 1024 responders 3,1% (n = 32) answered 'yes' as to the question whether they would consider freezing their oocytes in the future (Table IV). 28,4% (n = 291) answered 'maybe', 16,7% (n = 171) stated they 'do not know/have no idea' and half of the women (51,8%; n = 530) answered 'no' (Stoop et al., 2010c).

Potential freezers would be more likely to embark on social oocyte freezing, primarily if they were more reassured about risks to their future fertility related to the procedure (75.2%) and the health safety of the children resulting from cryopreserved oocytes (70.9%). Doubtful women report the same concerns, but in a different order. Non-freezers primordially indicate that if they would not have had children yet (36.2%) or would not have completed their desire for children (34.9%) they would be more likely to freeze oocytes (Table V).

3b. Oocyte utilization in ART

The number of oocytes per live birth is presented in Figure 1. This graph visualizes the steep increase in number of oocytes needed per live birth after the age of 38, while being fairly constant before that age. The mean number of live births per mature oocytes in the age group from 23 up to 37 years is 22.53 (SD: 1.55) compared to 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. Overall, 3.83% of aspirated mature oocytes resulted in a live birth. The oocyte utilization rate between the age of 23 and 37 years old remains constant with an average of 4.47 (95% CI; 4.32-4.61) (Stoop et al., 2012d).

Table V. — Factors that would make women more likely to freeze oocytes.

Potential freezers		Doubtful		Non-freezers	
1. Doesn't affect future fertility	75.2%	1. Health safety children	59.6%	1. If I wouldn't have children	36.2%
2. Health safety children	70.9%	2. Doesn't affect on future fertility	57.3%	2. If I had a desire for a child	34.9%
3. More reimbursement	65.9%	3. Treatment less complex	51.5%	3. Health safety children	29.4%
4. More guarantees for success	61.6%	4. More guarantees for success	45.6%	4. Doesn't affect future fertility	27.2%
5. Treatment less complex	58.2%	5. More reimbursement	43.9%	5. More guarantees for success	24.2%
6. If I wouldn't have children	57.3%	6. If I had a desire for a child	42.1%	6. Treatment less complex	20.0%
7. If I had a desire for a child	55.7%	7. If I wouldn't have children	40.4%	7. More reimbursement	16.2%
8. Treatment in nearby hospital	47.4%	8. Spoken to women that have undergone the treatment	34.5%	8. Spoken to women that have undergone the treatment	16.0%
9. Spoken to women that have undergone treatment	42.4%	9. Treatment in a nearby hospital	32.2%	9. Treatment in a nearby hospital	11.5%

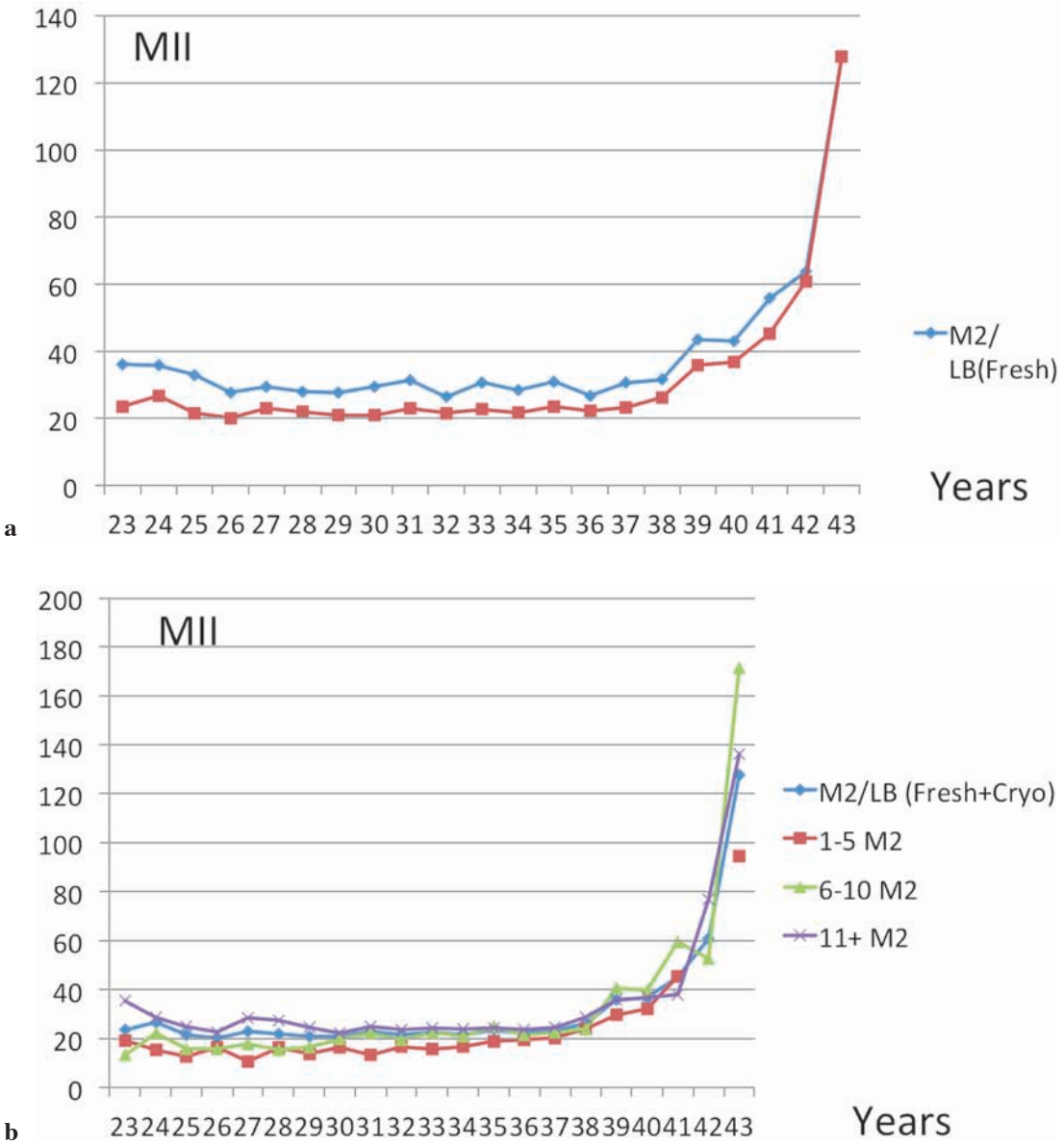

Fig. 1. — Mature oocytes per live birth: (a) Fresh cycles and cumulative outcome. (b) Cumulative outcome in relation to the oocyte yield.

Discussion

Assessment of fresh heterologous oocyte donation

The large cohort study examining the effect of oocyte donation on the obstetric and perinatal outcome aimed to answer the first research question. It includes more patients than all the previous controlled trials cumulatively (Klatsky et al., 2010). It concluded that the incidence of first trimester vaginal bleeding and PIH is significantly higher in pregnancies conceived with donated oocytes compared to matched controls with autologous oocytes: 20.6% vs. 10.3% and 19.1% vs. 8.3% respectively. However this did not affect the overall favourable outcome of oocyte donation pregnancies as compared to the control group.

In this study adequate matching was performed for confounding factors, which was facilitated by a large prospectively collected ART outcome database. Another strength of the study is that both cohorts of pregnancies deriving from donor and autologous oocytes were matched, not only for age, parity and plurality as previous trials did (Klatsky et al., 2010), but also for maternal ethnicity, which may be, a factor associated with the incidence of preeclampsia (Bodmar et al., 2007; Shental et al., 2010).

Caution is however mandatory as this study only evaluated the pure effect of oocyte donation on the pregnancy outcome. However, pregnancies after oocyte donation are often associated with other risk factors such as a more advanced age and oocyte recipient are also more often primiparous.

The second part of the first research question had its focus on the health of the oocyte donor. Two hundred and five ex-oocyte donors were contacted of which 194 consented to a standardized telephone questionnaire, aiming to evaluate fecundity before and after the donation procedure. Only three out of 60 women needed fertility treatment (5.0%) after oocyte donation; two cases had primary male factor infertility and one had primary unexplained subfertility at the age of 38 years. Changes in the menstrual pattern following oocyte donation were reported by 16.3% of the respondents, although none of the women reporting these changes reported fertility problems. The study suggests that short-term fertility problems after oocyte donation appear unaffected. As there is some literature evidence to suggest that ovarian trauma associated with ovarian puncture may induce the production of anti-ovary antibodies (AOA) (Barbarino-Monnier et al., 1991), further long-term studies are needed to assess the possibility of accelerated ovarian aging after oocyte donation.

Assessment of the introduction of oocyte vitrification

The second aim of this thesis was to evaluate oocyte cryopreservation. An observational study is presented with data that demonstrate that oocyte vitrification using a closed system can be an effective procedure for vitrification. Our study reports a 90.2% oocyte survival, 77.5% fertilization rate per survived oocyte and 69.9% fertilization rate per warmed oocyte. Moreover, this study also demonstrated efficient re-vitrification of embryos that originated from vitrified oocytes. The size of the data set is however limited.

The closed vitrification system cannot compete in terms of cooling rate with an open sample that is directly exposed to liquid nitrogen (Vajta et al., 2009). However the warming rates are minimally affected by closed system vitrification. Our data suggests that closed system vitrification is still likely to surpass the 1.3-warming/cooling, considered to be the most effective for successful vitrification (Isachenko et al., 1998; 1999).

Future randomized controlled trials need to further elucidate whether the present method of closed oocyte vitrification is equivalent to open vitrification combined with aseptical precautional measures.

Another clinical application assessed in this thesis was the aspiration and vitrification of excess oocytes in stimulated IUI cycles. Our findings indicate that additional microscopical confirmation of oocytes after aspiration of supernumerary follicles before IUI may further reduce the incidence of multiple pregnancies. Failure to detect the expected number of oocytes in the fluid of aspirated follicles should then result in the cancellation of insemination. The clinical efficiency of the excess oocyte vitrification technique was later confirmed by Akar et al. (2010) as they reported three ongoing pregnancies.

Assessment autologous oocyte banking

The third objective of this thesis is the assessment of autologous oocyte banking and the calculation of the efficiency of the individual oocyte retrieved after ovarian stimulation.

Only a small proportion (3.4%) of the women between 20 and 40 years of age contacted through an electronic questionnaire consider cryopreserving their oocytes for social reasons. However, another 28.4% of the women state that they would *maybe* consider undergoing such a procedure. In contrast to the few women currently undergoing preventive oocyte cryopreservation, only half of the responding women reported *not* to consider oocyte freezing.

Although desire to have children and the planned age for motherhood are important in women's attitude toward oocyte freezing, for most women, some important conditions still need to be fulfilled before embarking on this strategy. Although there are no indications to assume it would, it however still needs to be established that ovarian stimulation and oocyte retrieval does not negatively impacts future fertility in healthy women. A second important condition is the health safety of future children.

The oocyte utilization rate and oocytes per live birth rate remain remarkably stable between the age of 23 and 36 years old. Although a marked decline in live birth per cycle can already be observed from the early-thirties 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. 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.

Conclusion

Oocyte donation has been introduced in 1984 to allow women with ovarian insufficiency to become pregnant. The success of the technique led to a broadening of scope of the treatment to include indications of repeated IVF failure, advanced maternal age or inheritable disease. Today, OD has become well established with thousands of children born worldwide annually. As with any other

reproductive techniques, assessment of possible associated obstetric and perinatal risk remains of paramount importance. A matched-pair analysis has been performed including a total of 205 pregnancies after oocyte donation. The study concluded that oocyte donation is associated with an increased risk for PIH and first trimester vaginal bleeding independent of the recipients' age, parity and plurality and independent of the age of the donor or the partner. However oocyte donation has no impact on the overall perinatal outcome.

Oocyte cryopreservation is only now becoming a routine procedure, even though the first successful pregnancies obtained after slow freezing of eggs were pioneered more than two decades ago. Recently, several reports have demonstrated excellent clinical outcome from cryopreserved oocytes through the 'open' vitrification technique. In this thesis, a clinical validation study demonstrates that oocyte vitrification can be effectively performed with closed carriers. This straightforward method for aseptic vitrification appears to be a valuable alternative for other methods aimed at creating aseptic conditions.

Successful oocyte vitrification introduces multiple new applications in the clinical practice. This thesis describes that it enables the storage of excess oocytes aspirated prior to insemination to reduce the multiple pregnancy risk and to accumulate oocytes for later use.

The advent of oocyte vitrification also opens the door to a new medical and societal phenomenon of social oocyte freezing to avoid age-related subfertility. A study aimed at investigating the attitudes of woman of reproductive age towards this treatment option is presented. The electronic questionnaire completed by 1049 women showed that a significant proportion of young women would consider safeguarding their reproductive potential or are at least open to the idea of social freezing.

Potential oocyte freezers would be more likely to embark on social oocyte freezing, primarily if they were more reassured about risks to their future fertility related to the procedure. Therefore a cross sectional survey of 194 previous oocyte donors was performed by a standardized telephone questionnaire to evaluate the reproductive health after ovarian stimulation and oocyte retrieval. The data suggests that the procedures related to oocyte donation, or social egg freezing likewise, do not increase the likelihood for later need of fertility treatment.

An intriguing question on the mind of all women that choose to cryopreserve their oocytes is the average number of oocytes needed for a live birth. Therefore a retrospective analysis of existing data containing prospectively collected information on all consecutive patients was performed. 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 chance for an oocyte to result in live birth (utilisation rate) in women < 37 years old remains constant with a mean of 4.47% live birth per mature oocyte (95% CI, 4.32 to 4.61). Beyond 38 years, a significantly lower oocyte utilisation rate was noted, declining from 3.80% at the age of 38 to 0.78% at the age of 43 years ($P < 0.001$). In this 38-43 years age group, oocyte utilisation rate was no longer dependent on ovarian response.

References

Abdalla HI, Billett A, Kan AKS et al. Obstetric outcome in 232 ovum donation pregnancies. Br J Obstet Gynaecol. 1998; 105:332-7.

Akar ME, Johnston-MacAnanny EB, Carrillo AJ et al. The pregnancy outcome of retrieved excess eggs collected during selective follicular reduction from patients with three or more preovulatroy follicles undergoing controlled ovarian stimulation and IUI. Hum Reprod. 2010;25:2931.

Al-Hasani S, Dietrich K, van der Ven H et al. Cryopreservation of human oocytes. Hum Reprod. 1987;8:696-700.

Andersen AN, Goossens V, Ferraretti et al. Assisted reproductive technology in Europe, 2004: results generated from European registers by ESHRE. Hum Reprod. 2008;23:756–71.

Antinori M. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. Reprod Biomed Online. 2007;14,72-9.

Barbarino-Monnier P, Gobert B, Guillet-Rosso F et al. Antiovary antibodies, repeated attempts, and outcome of in vitro fertilization. Fertil Steril. 1991;56:928-32.

Bielanski A, Bergeron H, Lau P et al. Microbial contamination of embryos and semen during long term banking in liquid nitrogen. Cryobiology. 2003;46:146-52.

Bielanski A, Vajta G. Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units. Hum Reprod. 2009;24:2457-67.

Bodmar LM, Catov JM, Roberts JM. Racial/ethnic differences in the montly variation of preeclampsia incidences. Am J Obstet Gynecol. 2007;196(324):e1-5.

Bonduelle M, Legein J, Derde M-P et al. Comparative follow-up study of 130 children born after intracytoplasmic sperm injection and 130 children born after in-vitro fertilization. Hum Reprod. 1995;10:3327-31.

Bonetti A, Cervi M, Tomei F et al. Ultrastructural evaluation of human metaphase II oocytes after vitrification: closed versus open devices. Fertil Steril. 2011;95:928-35.

Chen C. Pregnancy after human oocyte cryopreservation. Lancet. 1986;1:884-6.

Cobo A, Meseguer M, Remohi J et al. Use of cryo-banked oocytes in an ovum donation programme: a prospective randomized, controlled, clinical trial. Hum Reprod. 2010a; 9:2239-46.

Cobo A, Romero J, Pérez S et al. Storage of human oocytes in the vapour phase of nitrogen. Fertil Steril. 2010b;94:1903-7.

de Mouzon J, Goossens V, Bhattacharya S et al. The European IVF-Monitoring (EIM)† Consortium for the European Society on Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2007: results generated from European registers by ESHRE. Hum Reprod. 2012;27:954–66.

Eum J, Park J, Lee W et al. Long-term liquid nitrogen vapor storage of mouse embryos cryopreserved using vitrification or slow cooling. Fertil Steril. 2009;91:1928-1932.

Evers JL. Female subfertility. Lancet. 2002;360:151-9.
Fountain D, Ralston M, Higgins N et al. Liquid nitrogen freezers: a potential source of microbial contamination of hematopoietic stem cell components. Transfusion. 1997;37:587-91.
Isachenko V, Perez-Sanchez F, Isachenko V et al. Vitrification of GV-porcine oocytes with intact intracellular lipids: effect of the cryoprotectant saturation/dilution stepping, elevated temperature and cytoskeletal inhibitor. Cryobiology. 1998;36:250-3.
Isachenko V, Gorbunov JL, Isachenko E et al. Some physical and technological aspects of GV porcine oocyte vitrification. Cryobiology. 1999;39:35.
Klatsky PC, Delaney S, Caughey AB et al. The role of embryonic origin in preeclampsia. Obstet Gynecol. 2010;116:1387-92.
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.
Kuleshova L, Gianaroli L, Magli C et al. Birth following vitrification of a small number of human oocytes. Hum Reprod. 1999;14:3077-9.
Kuleshova L, Shaw J. A strategy for rapid cooling of mouse embryos within a double straw to eliminate the risk of contamination during storage in liquid nitrogen. Hum Reprod. 2000;15:2604-9.
Kuwayama M, Vajta G, Kato O et al. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online. 2005;11:300-8.
Martin JA, Hamilton BE, Sutton PD et al. Births: final data for 2004. National Vital Statistics Reports. 2006;Vol.55:No. 1 National Center for Health Statistics, Hyattsville, MD.
Nagy ZP, Chang CC, Shapiro DB et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryobanking. Fertil Steril. 2009;92:520-6.
Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. Fertil Steril. 2006;86:70-80.
Paffoni A, Guarneri C, Ferrari S et al. Effects of two vitrification protocols on the developmental potential of human mature oocytes. Reprod Biomed Online. 2011;22:292-8.
Parmegiani L, Accorsi A, Cognigni G et al. Sterilization of liquid nitrogen with ultraviolet irradiation for safe vitrification of human oocytes and embryos. Fertil Steril. 2010;94:1525-8.
Rienzi L, Romano S, Albricci L et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod. 2010;25:66-73.
Shental O, Friger M, Sheiner E. Ethnic differences in the montly variation of preeclampsia among Bedouin and Jewish parturients in the Negav. Hypertens Pregnancy. 2010;29:342-9.
Skoog A, Lampic C, Bergh T et al. Public opinion regarding oocyte donation in Sweden. Hum Reprod. 2003;18:1107-14.
Stoop D. Social oocyte freezing. F, V & V in ObGyn. 2010a; 2:31-4.
Stoop D, Van Landuyt L, Paquay R, et al. Offering exess oocyte aspiration and vitrification to patients undergoing stimulated artificial insemination cycles can reduce the multiple pregnancy risk and accumulate oocyte for later use. Hum Reprod. 2010b;25:1213-8.
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. 2010c;26:655-61.
Stoop D, Baumgarten M, Haentjens P et al. Obstetric outcome in donor oocyte pregnancies: a matched-pair analysis. Reprod Biol Endocrinol. 2012a;10:42.
Stoop D, Vercammen L, Polyzos NP et al. Effect of ovarian stimulation and oocyte retrieval on the reproductive outcome in oocyte donors. Fertil Steril. 2012b;97:1328-30.
Stoop D, De Munck N, Jansen E et al. Clinical validation of a closed vitrification system in an oocyte-donation programme. Reprod Biomed Online. 2012c;24:180-5.
Stoop D, Ermin B, Polyzos NP et al. Reproductive potential of metaphase II oocyte retrieved after ovarian stimulation. An analysis of 23.354 ICSI cycles. Hum Reprod. 2012d; 27:2030-5.
Smith G, Serafini P, Fioravanti J et al. Prospective randomized comparison of human oocyte cryopreservation with slow-rate freezing or vitrification. Fertil Steril. 2010;94:2088-95.
Tedder R, Zuckermann M, Goldstone A et al. Hepatitis B transmission from contaminated cryopreservation tank. The Lancet. 1995;346:137-40.
Vajta G, Nagy ZP, Cobo A et al. Vitrification in assisted reproduction: myths mistakes, disbeliefs and confusion. Reprod Biomed Online. 2009;19:1-7

What is already known

This thesis centres on the place of oocyte donation in modern reproductive strategies. Diverse aspects of oocyte donation were addressed, such as the perinatal outcome of pregnancies obtained by oocyte donation, the effect of donation on the later fertility of donors, the feasibility of autologous oocyte donation and finally the attitude of women towards the novel concept of 'social egg freezing'.
Heterologous oocyte donation has been practiced since the last 30 years but it deprives the patient of its own genetic offspring. In a number of situations, however, autologous oocyte donation could circumvent this drawback. In contrast to sperm, oocyte cryopreservation, apart from the problem of the invasive character of obtaining oocytes, is more complex and remained elusive until recently. Therefore, cryopreservation of ovarian tissue was considered a better alternative, provided the right technique for in vitro maturation of oocytes could be developed. Ovarian tissue banks for young cancer patients have been created by many IVF centres over the last ten years but the recent development of reliable oocyte cryopreservation programmes by vitrification appears to render ovarian tissue banking obsolete. Although the best results have been obtained by vitrification of oocytes in an open system (direct contact with liquid nitrogen) the potential of contamination remains to be solved.

What is new from this research

Artificial Reproductive Technology abounds already with acronyms the majority of which are only understood by experts in the field. I now have to get acquainted with another two: WOET (warmed oocyte embryo transfer), WOFRET (warmed oocyte frozen embryo transfer cycle.

In a preliminary study, Dominic Stoop showed that vitrification of oocytes in a closed system, without the danger of contamination, results in very satisfactory fertilization and implantation rates.
Heterologous and by extension autologous oocyte donation does not seem to affect later fertility and is reassuring for young women who would consider preventive oocyte storage.
This thesis greatly contributes to the place of autologous egg freezing in artificial reproduction and the novel concept of social egg freezing.

Which questions will this new findings arise

Combining career success and motherhood was for many women a challenge for whom hitherto the only advice was the dictum from Egbert te Velde: 'een slimme meid krijgt haar kind op tijd'. Although we are still far away from the Brave New World as described by Aldous Huxley (1932!), we are getting nearer. More than twenty year ago, I prophesied that young men and women will store their gametes by cryopreservation for later use. This would not only safeguard their reproductive potential for later use but would also provide them with the possibility to dissociate sexual partnership from parenthood. Reliable cryopreservation of oocytes has now made this prospect a reality.
Egg banks are now being created worldwide and commercial advertisements and services can already be found on Internet, even if reproductive safety of oocyte freezing remains to be validated.
Randomised trials should learn us whether the reproductive outcome of oocyte freezing is comparable to that of embryo cryopreservation. In that case, shouldn't oocyte freezing be preferred to embryo freezing? It would anyway circumvent the moral and legal issues associated with embryo cryopreservation.

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