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# Determinants of pregnancy rate in the donor oocyte model: a multivariate analysis of 450 frozen-thawed embryo transfers

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**BACKGROUND:** Conflicting results have been published about the determinants of pregnancy after oocyte donation (OD). We used the OD model to determine predictive factors of pregnancy in the recipient after frozen-thawed embryo transfer (FTET) in a specific series where all the embryos were cryopreserved without any prior selection for fresh transfer.

**METHODS:** We report a retrospective study in a university tertiary care center. Multivariate analysis and logistic regression were used to identify predictive factors of pregnancy in a series of 450 OD FTET cycles in 198 infertile women between January 1992 and December 2006.

**RESULTS:** The mean ($\pm$ SD) recipient age was 35.7 ($\pm$ 4.5). Impaired ovarian function was the main indication for OD. The mean $\pm$ SD (range) number of embryos transferred was 1.65 $\pm$ 0.5 (1–3). Overall clinical pregnancy, implantation and delivery rates were 30, 18 and 23%, respectively. After univariate analysis, pregnancy rates were significantly higher in recipients under 35 years, in women with a body mass index (BMI) $<30$ kg/m$^2$, in women with an endometrial thickness of $\geq 8$ mm, in amenorrheic women and in women not receiving pituitary down-regulation before endometrial preparation. Using multivariate analysis, the BMI, endometrial thickness and the use of pituitary down-regulation were independent predictors of pregnancy, regardless of age.

**CONCLUSIONS:** This study supports that endometrial thickness of $<8$ mm, obesity and the use of GnRH analogue pituitary down-regulation before endometrial priming negatively impact pregnancy rates, independently of the recipient's age.

**Key words:** oocyte donation / frozen embryo transfer / pregnancy rate / implantation rate

## Introduction

For about two decades, oocyte donation (OD) has been used to treat infertile women presenting with premature ovarian failure, decreased ovarian function or repeated failure of *in vitro* fertilization (IVF). OD is also a solution to avoid the transmission of some genetic diseases. Despite ethical concerns, OD has enabled women to become pregnant after natural menopause (Sauer and Kavic, 2006).

While OD programs lead to the highest pregnancy rates (PR) in reproductive medicine (Budak *et al*., 2007), policies differ from one country to the next (Letur-Könirsch, 2004) and conflicting results have been published about the prognostic factors of pregnancy (Soares *et al*., 2008).

Frozen-thawed embryo transfer (FTET) has been associated with lower implantation and PRs than fresh embryo transfer (Check *et al*., 2001). Yet embryo cryopreservation has been increasing due to the general trend in IVF programs to reduce the number of embryos transferred to decrease multiple pregnancies and associated morbidity (The ESHRE Capri Workshop Group, 2000; Practice Committee of Society for Assisted Reproductive Technology; Practice Committee of American Society of Reproductive Medicine, 2008). Hence, better understanding of the prognostic factors would help to adapt IVF strategies to improve PRs after FTET.

Because OD is a good model to study and isolate key factors in the IVF process, we evaluated FTET in an OD program to determine specific predictive factors of pregnancy. Between 1996 and 2004,

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there was in France a specific context regarding OD. An amendment to the Bioethics Acts (that first established the conditions for OD in 1994) imposed embryos obtained after OD-IVF to be cryopreserved and quarantined for 6 months before transfer (Law 96-993 12 November 1996). This procedure was designed to ensure that the donor remained unaffected by any transmittable infectious disease. The Bioethics Acts were revised in 2004 and the quarantine was given up when the French biomedical community raised concern of the lower success rates with quarantined frozen embryos, whereas the risk of HIV transmission from donated oocytes was not established.

# Methods

We performed a retrospective study of 198 patients who had undergone 450 embryo transfer cycles after OD, *in vitro* fertilization (IVF) and embryo cryopreservation between January 1992 and December 2006 in our department. The local database and all medical and biological records were reviewed. All the women included in the donation program gave written informed consent for the procedures and for digital recording and the use of the data related to their medical history.

## Oocyte donation

In France, OD is anonymous, disinterested and restricted to medical indications in women of normal reproductive age. Potential donors are mothers under years 35, usually recruited in a patients' network of contacts.

## Donors

All the women included in the donation program were between the ages of 18 and 35 and gave written informed consent. These volunteers were informed of the conditions of OD and of the procedure and risks of ovarian stimulation and ovarian puncture during an inclusion visit. A psychological evaluation was performed and their complete medical history recorded. Women with current or past exposure to intravenous drugs, hazardous chemical substances, blood transfusions or radiation as well as subjects with a family history of hereditary or chromosomal disease were not retained. All the donors had normal physical and gynecological examinations, normal vaginal cultures and pap smears and a normal karyotype.

Donors were tested for HIV 1&2, HTLV 1&2, HBV, HCV, CMV and syphilis. Blood group, serum levels of FSH, LH and estradiol were determined. A pelvic ultrasound was performed. Specific genetic screening was performed according to family history and ethnic background.

## Ovarian stimulation

After 1 month of a combined oral contraceptive pill, donors were stimulated either by a long gonadotropin-releasing hormone (GnRH) agonist (50%), by a short GnRH agonist (25%) or by an antagonist protocol (25%). Ovarian stimulation was monitored by ultrasonography and hormonal dosages every 2–3 days. When the mean diameter of the leading follicle was $\geq$18 mm with two or three others $>$15 mm, stimulation was discontinued and 5000 IU human chorionic gonadotrophin (HCG) was injected. Follicular aspiration and oocyte retrieval were planned 35–36 h after HCG injection and performed by transvaginal ultrasonographically guided puncture under general anesthesia.

## Recipients

All recipients were compliant with the French law on OD, i.e. on medical indication and restricted to infertile couples of reproductive age who were married or had attested to 2 years of conjugal life.

Before treatment, a careful evaluation of the couples was performed, including a general physical and gynecologic examination, cervical smears and evaluation of the ovarian function by blood tests and pelvic ultrasounds. The same serologic checks were performed as in the donors. The uterine cavity was assessed by hysterosalpingography and/or hysteroscopy. The male partners underwent semen analysis and cultures and were checked for blood group, HIV 1&2, HTLV 1&2, HBV, HCV and syphilis.

All the cases were discussed at an interdisciplinary meeting. Two referents were responsible for matching each donor to one or several recipients on the basis of genetic criteria (to avoid cumulative risk factors), donor-recipient CMV concordance and phenotype matching to take into account the patient's wishes. Anonymity of the recipients and donors was strictly maintained.

Recipients with ovarian failure were treated by increasing doses (4–6 mg/day) of estradiol valerate for 15 days before embryo transfer. To avoid spontaneous ovulation, recipients with persisting ovarian function underwent pituitary down-regulation with a GnRH agonist before replacement therapy. A single injection of 3.75 mg depot triptorelin was administered in the mid-luteal phase of the previous cycle. Ovarian quiescence was checked before starting the estradiol on day 3 after menstruation. All the recipients were given micronized progesterone vaginally (600 mg/day) starting 3 days before embryo transfer and continued thereafter. In the event of pregnancy, estrogen and progesterone were continued until 12 weeks of gestation.

Endometrial thickness and pattern were monitored by ultrasonography. The endometrial thickness was measured in the sagittal plane, as the maximum anteroposterior distance between the hyperechogenic endometrial–myometrial interfaces. The endometrial pattern was graded according to the classification proposed by Gonen and Casper (1990) as follows: type A if entirely hyperechogenic, type B if isoechogenic to the surrounding myometrium with a poorly defined central echogenic line, type C in case of a typical multilayered triple line endometrium.

## IVF

The technique for IVF was chosen according to semen characteristics. For most of the cycles, banked sperm was used which was convenient for organization and anonymity purposes. Intracytoplasmic sperm injection (ICSI) of oocytes was performed mainly if less than 500 000 motile spermatozoa were obtained after selection.

Fertilization was assessed on day 1 to select zygotes with two normal pronuclei. High-quality (HQ) embryos according to morphological criteria were chosen for cryopreservation, taking into account the number and symmetry of the blastomeres, the kinetics of cell division and the degree of cytoplasmic fragmentation. Briefly, HQ embryos had three to four symmetric blastomeres on day 2, six to eight blastomeres on day 3 and less than 10% fragmentation.

## Cryopreservation

The embryos were frozen on days 2 or 3 in sealed plastic straws. Slow freezing in 1.5 M propanediol and 0.1 M sucrose was performed using a computer monitored cooler. Straws were stored for at least 6 months in liquid nitrogen. After checking that the oocyte donors had remained unaffected by any transmittable infectious disease and had confirmed donation consent, frozen embryos became eligible for transfer.

## Embryo transfer

Three to four embryos were thawed at each attempt.

After thawing, the embryos were selected for transfer if more than 50% of the blastomeres and the zona pellucida remained intact. One or two embryos (occasionally three) were transferred with a soft catheter under ultrasound guidance. Single embryo transfer was preferred in patients at risk for premature delivery or with a scarred uterus. The degree of transfer difficulty was recorded.

Serum βhCG was measured 12 days after transfer. If pregnancy occurred, ultrasonography was performed within a month.

## Statistical analysis

The main outcome measures were the clinical PR assessed by the presence of a gestational sac in the uterus at ultrasonography, the implantation rate defined by the number of pregnancies per embryo transferred and the live birth rate (LBR).

Univariate analysis was performed to determine the relevant predictive factors of pregnancy. Chi-square tests were used to compare PRs. *P*-values $\leq 0.05$ were considered to denote a significant difference and factors displaying *P*-values $< 0.2$ were considered for multivariate analysis.

Multivariate logistic regression analysis was used to test the association between clinical characteristics (including patient age, body mass index (BMI), tobacco use, gestity and parity, infertility characteristics, ovarian status, hormonal endometrial preparation, GnRH agonist use, endometrial thickness, transfer characteristics and the number and quality of the embryos transferred) and the probability of pregnancy. Backward variable selection was performed to determine independent covariates. Multivariate logistic regression analysis was used to predict the individual patient probability of pregnancy. Multiple imputation of missing data was performed to ensure that no important differences occurred between models with and without imputation.

The analyses were performed with the R statistical software available online at http://www.r-project.org.

# Results

## Characteristics of the population

The clinical characteristics of the 198 patients are detailed in Table I.

The indication for OD in 183 of the 198 women (95%) was advanced or relative premature ovarian failure (POF). These women were under 40, had elevated FSH levels (over 20 IU/L), low inhibin B and/or AMH and a poor follicle count at ultrasound. POF was idiopathic in 91 cases (50%), iatrogenic in 50 (27%) (40 post-surgery and 10 post-chemotherapy) and associated with a gonadal dysgenesis in 42 (23%). Overall, 26 women had an abnormal karyotype, 9 women had repeated failure of IVF with poor oocyte quality and 6 patients carried a genetic transmittable disease.

Detailed information on the transfer cycles are given in Table II. Of the embryos harvested, 82% survived the freezing and thawing procedures. There were 450 embryo transfers performed in 198 women (mean 2.3 transfers per patient, range 1–5) and 745 embryos were transferred. The mean ± SD (range) number of embryos transferred was 1.65 ± 0.5 (1–3). There was no difference in quality (as defined by the number and morphological aspect of the embryos) between first and second transfers from a single donor.

**Table I Clinical characteristics of the 198 recipients included in the OD program**

| | |
|---|---|
| Mean age ± SD (range) | 35.7 ± 4.5 years (25–46) |
| Mean BMI ± SD (range) | 23.3 ± 4.4 kg/m$^2$ (16–37) |
| Mean BMI ≥ 30 AND age ≥ 35 | 26/198 (13%), OR = 2.45 [1.13–5.3] |
| Mean BMI ≥ 30 AND age <35 | 7/198 (3.5%) |
| Smokers | 38/198 (19%) |
| Geographic origin | |
| Europe | 152/198 (76%) |
| Africa | 14/198 (7%) |
| West Indies | 12/198 (6%) |
| Asia | 9/198 (4.5%) |
| Northern Africa | 7/198 (3.5%) |
| Indian Ocean | 5/198 (2.5%) |
| South America | 1/198 (0.5%) |
| Nulligravidas | 145/198 (74%) |
| Primigravidas | 25/198 |
| Multigravidas | 28/198 |
| Spontaneous cycle | 86/198 (43.4%) |
| Primary amenorrhea | 33/198 (16.7%) |
| Secondary amenorrhea | 79/198 (39.9%) |
| Amenorrhea AND age ≥35 | OR = 0.29 [0.19–0.46], *P* < 0.001 |
| Duration of infertility: mean ± SD (range) | 7.8 ± 3.7 years (2–23) |
| Primary infertility | 147/198 (74.2%) |
| Associated infertility factors | 86/198 (43%) |
| Tubal | 38 |
| Uterine | 37 |
| More than one factor | 11 |

## Results of OD

As shown in Table III, 135 clinical pregnancies occurred. The clinical PR was 30% and the implantation rate was 18%. There were 102 babies born, including 9 pairs of twins. There was no high-order pregnancy. The delivery rate per transfer was 23%. There was no statistical difference in the clinical PRs between first and second transfers, thus all the analysis were performed on the 450 transfers.

## Univariate and multivariate analysis

The clinical PR was 32% in women younger than 35 and 20% in those older than 35 (OR = 1.8, $CI_{0.95}$ = [1.16–2.8], *P* = 0.006). A fairly linear relationship was observed between age and PR (Fig. 1).

Clinical PR was higher in the amenorrheic women (29%) than in the cycling women (18%, OR = 1.8, $CI_{0.95}$ = [1.1–2.8], *P* = 0.016).

Of the women receiving a GnRH agonist for ovarian suppression, 15% became pregnant compared with 28% without ovarian suppression (OR = 2.2, $CI_{0.95}$ = [1.3–3.7], *P* = 0.004).

The mean ± SD endometrial thickness was 8.8 ± 1.76 mm in cycling recipients and 9.2 ± 1.84 mm in amenorrheic recipients (not significant). Endometrial thickness was 9.5 ± 1.9 mm ($CI_{0.95}$ = [9.1–9.9])

**Table II Characteristics of 450 FTETs in 198 recipients included in an OD program**

| | |
|---|---|
| Number of donors | 125 |
| Number of oocyte retrievals | 139 |
| Number of recipients | 198 |
| Total number of transfers | 450 |
| Fertilization | |
| IVF | 271/450 (60.7%) |
| ICSI | 177/450 (39.3%) |
| Hormonal preparation of the recipients | |
| Estrogen + progesterone (EP) | 307/450 (68.2%) |
| GnRH agonist before EP | 131/450 (29.1%) |
| Gonadotropin stimulation | 4/450 |
| None (spontaneous cycle) | 8/450 |
| Endometrial thickness | |
| Mean ± SD (range) | 9 mm ± 1.9 (5–17) |
| Missing data | 57 (12.7%) |
| C type endometrium | 238/450 |
| B type | 67/450 |
| A type | 3/450 |
| Unknown pattern | 142/450 |

**Table III Fertility outcome in 198 recipients undergoing 450 FTETs in an OD program**

| | |
|---|---|
| Number of cycles | 450 |
| Number of women | 198 |
| Number of embryos transferred | 745 |
| Number of gestational sacs with fetal heart beat at ultrasound | 135 |
| Number of miscarriages | 31 |
| Number of ectopic pregnancies | 2 |
| Number of babies born | 102 |
| Twin | 9 pairs |
| Single | 84 |
| Implantation rate | 18.1% |
| Clinical PR per cycle | 30.0% |
| LBR per cycle | 22.7% |

in the women who became pregnant compared with 8.9 ± 1.9 mm ($CI_{0.95}$ = [8.7–9.1]) in those who did not ($P = 0.01$). Clinical PR in women with an endometrial thickness of <8 mm were 8/68 (11.7%) and 80/319 (25%) and 2/6 (33%) for women with an endometrial thickness comprised between 8 and <15 mm and ≥15 mm, respectively ($P = 0.1$). A cut-off value of 8 mm gave a group with a higher probability (endometrial thickness of ≥8 mm, PR = 82/325 (25%)) and a group with a significantly lower probability of pregnancy (8/68 (12%); OR = 2.5 [1.2–5.4], $P = 0.016$).

PRs were 28% in women with a BMI <30 and 12% in those with a BMI ≥30 (OR = 2.8 [1.1–7.2], $P = 0.03$).

The following criteria had no influence on PRs: indication for OD; infertility duration; the presence of associated infertility factors; tobacco use; the IVF technique used; the duration of cryopreservation and the characteristics of the embryos and embryo transfers.

The main results of the univariate analysis are summarized in Table IV.

Using multivariate analysis, after backward variable selection, only BMI, endometrial thickness and ovarian suppression were individualized as independent covariates for the prediction of pregnancy.

The results of multivariate analysis are presented in Table V. Using multiple imputation of missing data did not result in significant changes in the results of multivariate analysis.

## Discussion

We used a specific model to evaluate predictive factors of pregnancy in the recipients after FTET. Our results support that endometrial thickness of <8 mm, the use of GnRH agonist ovarian suppression and obesity negatively impact PRs.

To our knowledge, this is the largest single center series reporting the results of FTET in an OD program. Moreover, due to the specific context of the French law, the uniqueness of this study is the use of embryos that were not selected before cryopreservation because all had to be frozen. In all other medical literature, the data concerning frozen-thawed embryos is biased by the selection of the best looking embryos for fresh transfer. By using the donor oocyte model and less hearty frozen-thawed embryos, instead of fresh embyryo transfers, we expected to identify more subtle variations in the factors impacting prognosis in the recipient.

Using univariate analysis, we observed that an endometrial thickness <8 mm was associated with a significantly lower probability of pregnancy. Moreover, endometrial thickness was an independent predictive factor of pregnancy after multivariate analysis. Due to the small sample size of women with a very thin endometrium, no lower cut-off value could be determined under which pregnancy did not occur. Some large retrospective studies have not observed a significant difference in outcome according to endometrial thickness (Remohí *et al.*, 1997; Soares *et al.*, 2005) but several others have found it to be an important prognostic factor in OD (Borini *et al.*, 1996; Noyes *et al.*, 2001; Tesarik *et al.*, 2003). The results of case control studies are also controversial. In one series, endometrial thickness was found to be no different in matched women sharing oocytes from the same donors but with discordant outcomes (Garcia-Velasco *et al.*, 2003) whereas an endometrium <8 mm was found only in failed cycles in another study (Zenke *et al.*, 2004). Our data suggest that a less optimal endometrium might be more detrimental in the context of FTETs than in fresh embryo transfers. Nevertheless, additional investigations are required to clarify the relationship between endometrial thickness and receptivity. In the future, combining molecular tools with ultrasound could prove useful for better evaluating endometrial receptivity (Lédée *et al.*, 2008).

Recent reports (Metwally *et al.*, 2008; McClamrock *et al.*, 2008) have emphasized that excess weight is associated with reduced fertility, pregnancy and implantation rates, insufficient follicle development, lower oocyte numbers and increased gonadotropin requirements. The impact of obesity on the miscarriage rate is less clear (McClamrock *et al.*, 2008; Bellver *et al.*, 2003). Bellver *et al.*, in a retrospective

**Table IV Univariate analysis of 450 FTETs showing determinants of pregnancy in 198 recipients included in an OD program**

| Women | *n* | Pregnant | Non-pregnant | *P*-value | OR ($IC_{0.95}$) |
|---|---|---|---|---|---|
| Age < 35 | 158 | 50 (31.6%) | 108 | 0.006 | 0.538 (0.345–0.827) |
| Age ≥ 35 | 281 | 56 (19.9%) | 225 | | |
| Missing data | 11 | | | | |
| BMI < 30 | 208 | 58 (27.9%) | 150 | 0.03 | 0.36 (0.14–0.9) |
| BMI ≥ 30 | 41 | 5 (12.2%) | 36 | | |
| Missing data | 201 | | | | |
| No smoker | 342 | 86 (25.1%) | 256 | 0.56 | |
| Smoker | 86 | 19 (22.1%) | 67 | | |
| Missing data | 22 | | | | |
| Cycling | 173 | 32 (18.5%) | 141 | 0.016 | 0.56 (0.35–0.9) |
| Amenorrheic I | 89 | 28 (31.5%) | 61 | | |
| Amenorrheic II | 176 | 48 (27.3%) | 128 | | |
| Amenorrheic | 265 | 76 (28.7%) | 189 | | |
| Missing data | 12 | | | | |
| Ovarian suppr. | 131 | 20 (15.3%) | 111 | 0.004 | 0.5 (0.3–0.8) |
| No suppression | 313 | 88 (28%) | 225 | | |
| Missing data | 6 | | | | |
| Endometrium | | | | | |
| <8 mm | 68 | 8 (12%) | 60 | 0.016 | 0.39 (0.18–0.84) |
| ≥8 mm | 325 | 82 (25%) | 243 | | |
| Missing data | 57 | | | | |

Amenorrheic I (II): primary (secondary) amenorrhea, $IC_{0.95}$: 95% confidence interval, OR: odds ratio, Suppr: suppression.

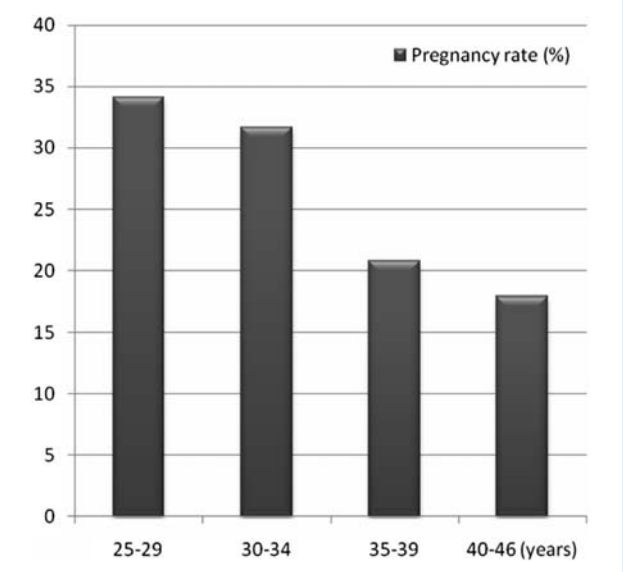


**Figure 1** Pregnancy rates according to age in 198 patients undergoing 450 FTET cycles after OD.

study including a total of 2656 first ovum donation cycles with good quality embryos, noted that women with a BMI < 25 kg/m$^2$ presented an ongoing PR per cycle of 45.5% compared with 38.3% for those with a BMI ≥ 25 kg/m$^2$ (OR: 0.85, 95% CI, 0.76–0.95; $P = 0.003$) (Bellver *et al*., 2007). In the present study of 450 FTET, we confirm that obesity (as defined internationally by a BMI > 30) was associated with lower PRs, independently of the recipient's age.

In the present study, we observed that ovarian suppression before priming appeared detrimental to the PR. GnRH agonists are used in cycling women to favor donor-recipient synchronization at the moment of donation by preventing the premature secretory changes in the endometrium consequent to the spontaneous rise in luteinizing hormone (LH) (Remohi *et al*., 1995). Yet, GnRH agonist use is thought to have a negative effect on endometrial development. Recent studies have shown that the use of GnRH agonists during in-vitro fertilization cycles leads to alterations in the hormonal profiles of the luteal phase as well as changes in endometrial histology (Saadat *et al*., 2004; Simon *et al*., 2005; Diluigi *et al*., 2007). Our data are in accordance with those of previous studies reporting higher PRs in recipients with ovarian failure compared with those with persistent ovarian function (Check *et al*., 1995; Noyes *et al*., 2001) suggesting a more favorable uterine environment in patients with ovarian failure. Alternatively, the use of GnRH agonists may cause the negative effect on implantation in patients with ovarian function (Check *et al*., 1995). The results of a randomized study, comparing the effects of mid-cycle administration of HCG or placebo on endometrial thickness and implantation rate in subgroups of menopausal and down-regulated cycling women receiving embryos from OD cycles, suggest that LH might have a direct action on the uterus to support endometrial growth and

**Table V Multivariate analysis of 450 FTETs showing determinants of pregnancy in 198 recipients included in an OD program.**

| Covariates | Estimate | CI lower | CI upper | Std. error | *P*-value |
|---|---|---|---|---|---|
| (Intercept) | −0.39037372 | −3.84399661 | 3.06324917 | 1.75202571 | 0.82368122 |
| With imputation | 0.57142446 | −1.8107995 | 2.95364848 | 1.21125671 | 0.63738971 |
| BMI | −0.09873069 | −0.17995246 | −0.01750893 | 0.04120387 | 0.01656825 |
| With imputation | −0.06979094 | −0.14452985 | 0.004947978 | 0.03623405 | 0.06587948 |
| Endom.thickness | 0.23699703 | 0.06255481 | 0.41143925 | 0.08849468 | 0.00740425 |
| With imputation | 0.17323150 | 0.02609345 | 0.320369559 | 0.07276161 | 0.02221555 |
| Ovarian suppr. | −0.9760091 | −2.02874561 | 0.07672742 | 0.53405409 | 0.06761752 |
| With imputation | −0.69385461 | −1.38493710 | −0.00277212 | 0.35150050 | 0.04909256 |
| Smoking | 0.69170987 | −0.21692814 | 1.60034787 | 0.46095280 | 0.13345675 |
| With imputation | 0.04147481 | −0.55421040 | 0.637160029 | 0.30297403 | 0.89118727 |
| Amenorrhea | 0.13277916 | −0.80249598 | 1.06805431 | 0.47446585 | 0.77959275 |
| With imputation | 0.15314068 | −0.49175175 | 0.79803311 | 0.32766434 | 0.64058453 |
| Age | −0.01369357 | −0.09182402 | 0.06443687 | 0.03963564 | 0.72972874 |
| With imputation | −0.04435683 | −0.09820225 | 0.00948859 | 0.02738297 | 0.10611069 |

Multivariate analysis was performed without (upper lines) and with multiple imputation of missing data.
CI: confidence interval, BMI: body mass index in $kg/m^2$, Suppr: suppression.
The following cut-offs were used: 30 $kg/m^2$ for BMI, 8 mm for endometrial thickness, and 35 years for age.

uterine receptivity in the implantation window (Tesarik *et al*., 2003). Thus menopausal women with high endogenous LH may have a better uterine environment than cycling women undergoing pituitary down-regulation. In accordance with this hypothesis is the observation by Borini *et al*. that implantation rates in cycling women undergoing OD are improved and comparable to those of a control group of menopausal women after longer term pituitary down-regulation (Borini *et al*., 1995). Moreover, as retrospective (Gelbaya *et al*., 2006) and randomized studies (Ghobara *et al*., 2008) have concluded that in FTETs both natural and down-regulated hormonally prepared cycles have similar implantation and PRs in women with regular menstrual cycles, the cost-effectiveness of GnRH agonist use should be better evaluated (Del Prato and Borini, 2006; Ghobara *et al*., 2008).

In the present study, multivariate analysis concluded that patient age was not an independent determinant factor of pregnancy in OD. Although conflicting data have been published concerning the influence of recipient age on PRs in OD (Flamigni *et al*., 1993; Legro *et al*., 1995; Sauer *et al*., 1996; Abdalla *et al*., 1997; Paulson *et al*., 1997; Yaron *et al*., 1998; Moomjy *et al*., 1999; Noyes *et al*., 2001; Mirkin *et al*., 2003), the results of the largest (Toner *et al*.) and most recent studies (Soares *et al*., 2005) with some analyzing outcome in shared oocyte programs (Garcia-Velasco *et al*., 2003; Bodri *et al*., 2007) support the concept that age does not impact PRs until the late 40's. Our data are concordant with these results. However, in our study population, there were not enough women over 40 years to evaluate if the use of frozen-thawed embryos has an additional impact on PRs in the older women.

The results of this study confirm that the potential positive impact of 6 months quarantine on consumer's safety was low (no donor was rejected secondarily), in comparison to the potential detrimental effect of freezing all the embryos. In our department, PRs after OD and fresh ET were 42% before quarantine cryopreservation was imposed by law in 1996 (Catteau-Jonard, 2007). The results of the present study are not better than those observed after FTET in OD programs where both fresh and FTET is performed from the same cycles (Pados *et al*., 1992; Toner *et al*., 2002; Mirkin *et al*., 2003; Söderström-Anttila and Vilska 2007; Gunby *et al*., 2008; Riggs *et al*., 2008). Moreover, pregnancy and delivery rates after OD and fresh ET have improved over the past 10 years both in Europe and in the USA (Noyes *et al*., 2001; Society for Assisted Reproductive Technology, American Society for Reproductive Medicine, 2004; Budak *et al*., 2007).

Vitrification is a rapid freezing procedure that prevents ice crystal formation, thus limiting the cellular damages associated with cryopreservation (Yavin and Arav, 2007). Experimental data suggest that it might have less impact on embryo and oocyte physiology than classical slow freezing (Gardner *et al*., 2007). Vitrification has recently given promising clinical results (Borini *et al*., 2008) and appears easy to perform (Dessolle *et al*., 2009). If the diffusion of this technique confirms that embryos cryopreserved by vitrification keep a potential that is very close to fresh embryos, then the quarantine cryopreservation after OD could possibly be reconsidered.

Our study has several weaknesses. It is retrospective and reports the data of a single center. However, we present quite a large original series of FTET without prior selection for fresh embryo transfer, which is unique in the literature. There were missing data regarding BMI and endometrial thickness (Table IV). We found no specific association between missing data and the outcome and multiple imputation supports that missing data might not have influenced our results. Our series is small in comparison to some studies that have evaluated comparable endpoints in fresh ET after OD. Yet, our data not only corroborate the impact of BMI and of the quality of endometrial priming on

the success rates, but also show that frozen ET is likely to have an additional negative impact. In our opinion, these data will be important for the transfer of the numerous embryos that are still cryopreserved with the slow freeze technique.

## Conclusion

We used a unique model to isolate predictors of pregnancy in the recipient after FTET in an OD program. Our results support that endometrial thickness of <8 mm, obesity and the use of GnRH analogue pituitary down-regulation before endometrial priming negatively impact PRs, independently of the recipient's age. In comparison to fresh embryo transfer, FTET appears to worsen the impact of these negative factors. Favoring weight loss and optimizing endometrial priming might improve PRs in FTET.

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