Article

Age, oestradiol and blastocysts can predict success in natural cycle IVF–embryo transfer

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Abstract

The aim of this study was to evaluate the influence of maternal age and oestradiol concentrations on blastocyst development and live birth rates in natural cycle IVF–embryo transfer. This observational study included 397 natural cycles with IVF–embryo transfer for female infertility with embryo transfer on day 5. The cycles were divided into two groups according to the woman's age (<39 and ≥39 years of age), and into two groups according to oestradiol concentrations on the day of human chorionic gonadotrophin (HCG) administration (0.4–0.49 nmol/l and 0.5–1.2 nmol/l). Comparison between the cycles in younger versus older age groups showed significant differences in blastocyst development rate, live birth rate per embryo transfer and live birth rate per cycle (55 versus 29%, 23 versus 3% and 13 versus 2% respectively) ($P < 0.001$). Comparison between cycles with lower versus higher oestradiol concentrations showed no significant differences in blastocyst development rate, live birth rate per embryo transfer and live birth rate per cycle (47 versus 49%, 18 versus 18%, and 11 versus 10% respectively). Advanced maternal age negatively predicts the success of natural cycle IVF, while low oestradiol concentrations on the day of HCG administration (ultrasound criteria fulfilled) do not negatively predict blastocyst development and success of natural cycle IVF.

Keywords: blastocyst, maternal age, IVF–embryo transfer, natural cycle, oestradiol, predicting success

Introduction

The first successful IVF treatment was performed by Steptoe and Edwards in a natural cycle (Steptoe and Edwards, 1978). Because of poor results, this approach was later abandoned and the use of ovarian stimulation became standard practice (Jones *et al.*, 1982). There are numerous arguments for renewing interest in natural cycle IVF–embryo transfer: lower costs due to elimination of medication (Nargund *et al.*, 2001), little discomfort for patients, no need for anaesthetics, no risk of multiple births, low risk of preterm delivery, no ethical problems due to cryopreservation and/or fetal reduction, no risk of ovarian hyperstimulation and no risk of carcinogenesis (Pelinck *et al.*, 2002). In fact, the most important argument is the prevention of multiple pregnancies (Olivennes *et al.*, 1998; Hamberger *et al.*, 2005). In addition, cryopreserved embryos can be transferred in a natural cycle (Tomazevic *et al.*, 1999), and intracytoplasmic sperm injection (ICSI) can also be

performed in the natural cycle (Norman *et al.*, 1995; Lukassen *et al.*, 2001). Despite these arguments, most centres still remain reluctant to offer natural cycle IVF–embryo transfer.

However, some groups have demonstrated that acceptable results could be obtained in natural cycle IVF–embryo transfer. According to these reports, high oestradiol concentrations were a prerequisite for the success of natural cycle IVF (Ranoux *et al.*, 1988; Foulot *et al.*, 1989; Paulson *et al.*, 1990; Lenton *et al.*, 1992; Claman *et al.*, 1993; Daya *et al.*, 1995; Fahy *et al.*, 1995; Seibel *et al.*, 1995; Zayed *et al.*, 1997; Lindheim *et al.*, 1998). Consequently, the cancellation rates were high. It was noted that the problem of cancellation could be diminished and good results could be achieved in natural IVF–embryo transfer cycles by triggering ovulation with human chorionic gonadotrophin (HCG) at lower oestradiol concentrations (0.4–0.6 nmol/l),

provided that ultrasound criteria were fulfilled. In these cases, mature oocytes were obtained and embryos successfully transferred on day 4 after oocyte retrieval (Tomazevic *et al.*, 1999).

The present study analyses the influence of maternal age and different oestradiol concentrations on the day of HCG administration on blastocyst development and on the success of natural cycle IVF–embryo transfer for overcoming female infertility.

Materials and methods

This observational study included data on 397 consecutive classical natural IVF–embryo transfer cycles for female infertility and idiopathic infertility (297 cycles for tubal infertility, 79 cycles for endometriosis, 21 cycles for idiopathic infertility) in 210 normogonadotrophic normally ovulating women (basal FSH <12 IU), performed in the years 2000–2003. To exclude the impact of sperm quality, natural cycles with IVF–embryo transfer for male infertility were excluded from the analysis. No data were available on whether patients had primary or secondary infertility.

Evaluation of follicular maturation included oestradiol serum determinations (Delfia; Pharmacia, LKB, Uppsala, Sweden), ultrasound measurements of follicles and the endometrium using a 7-MHz vaginal probe (Bruel and Kjaer A/S, Gentofte, Denmark; model 8538), quick urinary LH determinations (Epignost; US patent 4.943.522) and information on the length of the menstrual cycle.

Daily oestradiol determinations started on day 9 of the menstrual cycle. Quality of oestradiol determinations was approved by the Central Laboratory Bonn, Germany. Daily vaginal ultrasound monitoring and daily urinary LH determinations were started when oestradiol was >0.39 nmol/l (>104 pg/ml).

The parameters oestradiol concentration >0.39 nmol/l, dominant follicle >16 mm and maximal endometrial thickness >5 mm were used as the minimal criteria of follicular maturity. When the minimal hormone and ultrasound criteria were fulfilled, and if the urinary LH test 17–18 days before expected menstrual bleeding was negative, ovulation was induced with 5000 IU of HCG (Profasi; Serono, Geneva, Switzerland). Following this individualized monitoring protocol, 5000 IU of HCG was given at follicular diameters ranging from 17 to 22 mm and at oestradiol concentrations ranging from 0.39 to 1.2 nmol/l. If the LH urinary test was positive, the cycle was cancelled because the timing of oocyte retrieval could not be properly determined. This occurred in 7 (2%) of the 397 analysed natural cycles with IVF–embryo transfer (**Table 1**).

Vaginal ultrasound-guided oocyte retrieval was performed 31–32 h after HCG administration using a single lumen needle with an outer diameter of 1.6 mm and an inner diameter of 1.0 mm (TIK, Kobarid, Slovenia) using negative pressure of 130 mmHg, without flushing. No anaesthetics were used during the procedure.

The oocyte was incubated in the test tube with 1 ml culture medium (IVF–ET Medium prep. 1031; Medi-Cult, Jyllinge, Denmark) in 5% CO_2 in air at 37°C and fully saturated humidity. Swim-up technique was used for semen preparation. After 1–3 h, the oocyte was inseminated with 50,000 progressively motile spermatozoa. Only the partner's semen was used. The presence of two pronuclei was confirmed 18–24 h post-insemination, and the zygote was transferred into a centre-well organ tissue

Table 1. Influence of maternal age on embryo development, clinical pregnancy rate and live birth rate.

Parameter	Group A	Group B	P-value
Maternal age (years)	<39	≥39	–
No. of natural cycles	286	111	–
Cancellation due to positive LH test/cycle (%)	4/286 (1)	3/111 (3)	NS
Follicle disappearance/cycle (%)	16/286 (6)	6/111 (5)	NS
Empty follicle/cycle (%)	46/286 (16)	19/111 (17)	NS
Oocyte retrieved/cycle (%)	220/286 (77)	83/111 (75)	NS
Immature oocyte/cycle (%)	9/286 (3)	5/111 (5)	NS
Embryo transfer/cycle (%)	163/ 286 (57)	58/111 (52)	NS
Blastocyst development rate (%)	89/163 (55)	17/58 (29)	<0.001
Pregnancy/blastocyst embryo transfer (%)	35/89 (39)	4/17 (24)	NS
Pregnancy/morula embryo transfer (%)	8/61 (13)	1/31 (3)	NS
Pregnancy/lower stage embryo transfer (%)	0/13 (0)	0/10 (0)	NS
Pregnancy/embryo transfer (%)	43/163 (26)	5/58 (9)	<0.001
Live birth/embryo transfer (%)	37/163 (23)	2/58 (3)	<0.001
Spontaneous abortion/clinical pregnancy (%)	6/43 (14)	3/5 (60)	<0.05
Live birth/cycle (%)	37/286 (13)	2/111 (2)	<0.001

NS = not statistically significant.

culture dish (Becton Dickinson, New Jersey, USA) with 0.5 ml fresh equilibrated culture medium. On each day of extended culture the embryo was transferred into a fresh equilibrated culture medium (IVF–ET Blast Assist Medium; Medi-Cult). All embryo transfers were performed on day 5 after oocyte retrieval with a Frydman catheter, using the double-channel technique.

Luteal phase was supported by 1500 IU HCG on day 9 after the first HCG injection. All clinical pregnancies were diagnosed by β-HCG serum determinations 10 days after the last HCG injection and by vaginal ultrasound examination demonstrating a gestational sac, 4 weeks after embryo transfer.

In order to investigate the influence of maternal age on the outcome of natural cycle IVF, the cycles were divided into two groups: group A with cycles in women aged <39 years, and group B with cycles in women aged ≥39 years.

In order to investigate the influence of oestradiol concentration on the day of HCG administration on the outcome of natural cycle IVF, the cycles were divided into two groups: a group of cycles with low oestradiol concentrations (0.4–0.49 nmol/l) and a group of cycles with higher oestradiol concentrations (0.5–1.25 nmol/l) on the day of HCG administration. Blastocyst development, clinical pregnancies and live birth rates were the main outcome parameters.

Statistical analysis was performed using Statistics Package for Social Sciences for Windows (SPSS, Inc., Chicago, USA). Chi-squared test and logistic regression analysis models were used for statistical analysis. Statistical significance was set at $P < 0.05$.

Results

The mean maternal age was 35.9 ± 3.7 (range 25–41) years. The average oestradiol concentration on the day of HCG administration was 0.56 ± 0.20 (range 0.38–1.2) nmol/l or 160 ± 35 (range 108–318) pg/ml. The mean number of previous stimulated IVF–embryo transfer cycles was 4.5 ± 2.4 (range 1–9).

Oocytes were obtained in 303 (76%) of the 397 natural cycles IVF. Seven cycles (2%) were cancelled because of a positive LH test. The disappearance of follicles was observed in 22 cycles (5%). If a follicle was present at the time planned for oocyte retrieval, oocyte retrieval by aspiration without flushing was positive in 82% (303/368) of cases. A total of 318 oocytes were retrieved; 303 from dominant and 15 from co-dominant follicles.

There was no evidence of fertilization in 46 (15%) of the 289 mature oocytes and in 14 immature oocytes from dominant follicles. Among the 243 fertilized oocytes, there were 13 triploidies, 14 embryos degenerated, and 216 (89%) embryos cleaved and were transferred. There was no evidence of fertilization in four (31%) out of 13 mature oocytes and one immature oocyte from co-dominant follicles. Among the nine fertilized oocytes, there was one triploidy, one embryo degenerated and seven embryos cleaved and were transferred.

Four embryos from co-dominant follicles cleaved to the morula stage and three to lower embryo stage.

A total of 210 embryo transfers of one embryo and seven of two embryos were performed on day 5, and were followed by 39 singleton deliveries and nine spontaneous abortions. The clinical pregnancy rate was 12% per cycle and 22% per embryo transfer.

The live birth rate was 10% per cycle (39/397 cycles) 18% per embryo transfer (39/217 embryo transfers), and 24% per woman (39/161 women).

Natural cycle IVF was offered once to 92 women (16% clinical pregnancy rate per cycle), twice to 38 women (20% clinical pregnancy rate per cycle), 3–5 times to 47 women (6% clinical pregnancy rate per cycle) and 6–10 times to six women. In this last group of women there was only one pregnancy, which ended in a spontaneous abortion. There were no significant differences in clinical pregnancy rates comparing tubal factor, endometriosis and idiopathic infertility patients (13, 14 and 10% respectively).

Overall, there were 106 embryo transfers with one blastocyst, three embryo transfers with one blastocyst and one morula, three embryo transfers with one blastocyst and one lower stage embryo, 94 embryo transfers with one morula, one embryo transfer with two morulae, and 25 embryo transfers with one early developmental stage embryo.

Blastocyst transfers were followed by significantly higher clinical pregnancy rates compared with morula stage embryo transfers: 37 versus 10% ($P < 0.001$). No pregnancies resulted from the transfer of an early stage embryo on day 5 ($P < 0.001$).

Embryo development and clinical pregnancy rates in the two different age groups are presented in **Table 1**. The live birth rate per cycle and live birth rate per embryo transfer in the younger group were significantly higher compared with those in the older age group of women: 13 versus 2% and 23 versus 3% respectively ($P < 0.001$). In the younger group, significantly more embryos developed to the blastocyst stage ($P < 0.001$) and there was a significantly lower spontaneous abortion rate ($P < 0.05$). There were more pregnancies/blastocyst transfers in the younger age group (39 versus 24%). This difference was not statistically significant, probably due low numbers.

Logistic regression analysis confirmed the woman's age to be the most significant negative predictor of clinical pregnancy in the natural cycle IVF. Contrary to expectations (Paulson *et al.*, 1994), oestradiol concentrations on the day of HCG administration were shown to be an insignificant predictor of clinical pregnancy in natural cycle IVF (**Table 2**).

Embryo development and clinical pregnancy rates in the two groups according to oestradiol concentrations (0.4–0.49 and 0.5–1.2 nmol/l) on the day of HCG administration (ultrasound criteria and cycle length criteria fulfilled) are presented in **Table 3**. There were no significant differences regarding blastocyst development and live birth rates between the two groups (**Table 3**).

Table 2. Logistic regression: woman's age and oestradiol concentration on the day of human chorionic gonadotrophin administration as independent variables to predict clinical pregnancy in the natural cycle IVF–embryo transfer as dependent variable.

Variable	Adjusted odds ratio	95% confidence interval	P-value
Woman's age	0.894	0.818–0.976	0.012
Oestradiol concentration	1.009	0.994–1.024	NS

NS = not statistically significant.

Table 3. Influence of oestradiol concentration on the day of HCG administration on embryo development, clinical pregnancy rate and live birth rate.

Parameter	Oestradiol concentration on day of HCG (nmol/l)	
	Lower group (0.4–0.49)	Higher group (0.5–1.25)
No. of natural cycles	180	217
Diameter of follicle (mm) on day of HCG (mean ± SD, range)	18.7 ± 1.53 (16–22)	18.5 ± 1.4 (16–22)
Cancellation due to positive LH test (%)	3/180 (2)	4/217 (2)
Follicle disappearance/cycle (%)	9/180 (5)	13/217 (6)
Empty follicle/cycle (%)	30/180 (17)	35/217 (16)
Oocyte retrieved/cycle (%)	138/180 (77)	165/217 (76)
Immature oocyte/cycle (%)	6/180 (3)	8/217 (4)
Embryo transfer/cycle (%)	105/180 (58)	117/217 (54)
Blastocyst development rate (%)	49/105 (47)	57/117 (49)
Pregnancy/blastocyst embryo transfer (%)	18/49 (37)	21/57 (37)
Pregnancy/morula embryo transfer (%)	6/45 (13)	3/47 (6)
Pregnancy/lower stage embryo transfer (%)	0/12 (0)	0/13 (0)
Pregnancy/embryo transfer (%)	24/105 (23)	24/117 (21)
Spontaneous abortion/pregnancy (%)	5/24 (21)	4/24 (17)
Live birth rate/embryo transfer (%)	19/105 (18)	21/117 (18)
Live birth rate/cycle (%)	19/180 (11)	21/217 (10)

There were no statistically significant differences between the two groups.

Discussion

The study was performed to analyse whether maternal age and serum oestradiol concentrations on the day of HCG administration, provided that ultrasound parameters are fulfilled, predict blastocyst development and successful outcome of natural cycle IVF–embryo transfer. The results in a group of negatively selected patients with normal FSH, who failed to become pregnant in previous repeated stimulated IVF cycles were evaluated. Logistic regression and chi-squared analysis confirmed maternal age as a significant negative predictor of clinical pregnancy in natural cycle IVF–embryo transfer.

Contrary to previously published reports (see above for references), logistic regression analysis showed that oestradiol concentrations on the day of HCG administration, with ultrasound parameters fulfilled, were not a significant predictor of blastocyst development and clinical pregnancy in natural cycle IVF.

The developmental stage of the embryo on day 5 significantly predicted the clinical outcome of the natural cycle IVF ($P < 0.001$).

Comparison between the cycles in women aged <39 years and those aged ≥39 years showed a significantly higher blastocyst

rate (55 versus 29%, *P* <0.001) and higher pregnancy rate per blastocyst transfer (39 versus 24%) in the younger women. Consequently, higher live birth rates per embryo transfer and per cycle were achieved (23 versus 3% and 13 versus 2% respectively). On the other hand, the abortion rate was significantly higher than in younger women (60 versus 14%, *P* <0.05). These results deserve to be discussed in greater detail.

Influence of maternal age on the outcome of natural cycle IVF–embryo transfer

The results of this study are in agreement with previous recommendations that women over 40 should not be advised to enter natural cycle IVF (Foulot *et al.*, 1989; Lenton *et al.*, 1992; Paulson *et al.*, 1994; Fahy *et al.*, 1995). Age is well known to be more accurate than any other predictor of conception in IVF. Despite a relatively high oocyte retrieval rate in this study, poor oocyte quality in women aged 39 years and over was clearly reflected in a poor blastocyst development rate and lower pregnancy rate per blastocyst transfer, as well as in a significantly higher abortion rate (*P* < 0.05), and consequently in an extremely low live birth rate per cycle.

The outcomes of natural cycle IVF in women aged ≥39 years in this study are similar to those of Kolibianakis *et al.* (2004), who observed no pregnancies in the modified natural cycle in patients with elevated (>12 IU) basal FSH values. Consequently, age-related poor responders are not good candidates for natural cycle IVF. According to the results of this study and contrary to some published data (Ubaldi *et al.*, 2005; Papaleo *et al.*, 2006), poor responders in advanced age with only a few follicles and poor quality oocytes can only occasionally benefit from natural cycle IVF–embryo transfer.

Unlike the women aged ≥39 years, ovulating normogonadotrophic women aged <39 years with female infertility problems, i.e. normal responders, are good candidates for natural cycle IVF. Good oocyte quality in this group of women is reflected in a high blastocyst development rate.

The present results, showing a 23% live birth rate per embryo transfer and a 13% live birth rate per cycle in women <39 years of age in whom IVF in stimulated cycles had failed 4–6 times, encourage a wider acceptance of IVF–embryo transfer in normogonadotrophic women <39 years of age. Natural cycle IVF in a non-negatively selected group of patients might possibly provide better results. The question is whether IVF–embryo transfer in the stimulated or in the natural cycle should be offered first (Paulson *et al.*, 1994). In patients under 35 years of age, the choice of natural IVF reduces the cost and risk to the patient, permitting her to have multiple, consecutive attempts, and cumulatively offers a clinical pregnancy rate which approaches that of stimulated IVF, with a multiple pregnancy rate significantly reduced compared with stimulated IVF treatment cycles. In patients over 35 years of age, the benefits of natural IVF are much less evident and the opportunity to transfer multiple embryos in these patients seems to be advantageous (Phillips *et al.*, 2007). In the authors' experience, if natural cycle IVF is offered more than five times, the chances of success are very low.

Individualized monitoring of IVF–embryo transfer in the natural cycle to avoid cancellation of cycles with LH surge at very low oestradiol concentrations

To avoid repeated daily LH measurements and still retrieve the oocyte, Foulot *et al.* (1989) and Paulson *et al.* (1990) proposed to trigger ovulation with HCG just before the spontaneous LH surge. The criteria to assess follicular maturation were developed incorporating both follicle diameter and oestradiol concentration.

According to Foulot *et al.*, the follicle was mature when oestradiol concentrations exceeded 180 pg/ml and the follicle diameter was >18 mm (Foulot *et al.*, 1989). Oocyte retrieval was planned for 36 h after 3000 IU of HCG. Paulson *et al.* (1990) proposed the following combination of follicle diameter and serum oestradiol concentration: 20 mm and 200 pg/ml, 18 mm and 250 pg/ml, or 16 mm and 300 pg/ml.

Our use of IVF in the natural cycle was started in 1992, when follicular growth monitoring was based solely on ultrasound measurements of the follicles; the follicle had to measure >17 mm in diameter. In 92 natural cycles with IVF, only three live births (3%) were achieved, so it became evident that more individualized follicle growth monitoring was required. Thus, Paulson's criteria for follicular maturity were introduced. However, due to increased cancellation rates, the natural cycle IVF was almost abandoned.

In 1994, the criteria for follicular maturity were changed by introducing lower oestradiol threshold values (0.4 nmol/l = 112 pg/ml) (Tomazevic *et al.*, 1999). Paulson and Foulot's idea of individualized monitoring combining ultrasound, oestradiol parameters, and excluding the LH surge using the urinary LH quick test was still respected. In this revised monitoring protocol, daily oestradiol determinations started on day 9 of the menstrual cycle. Daily vaginal ultrasound monitoring and urinary LH determinations were started when oestradiol was >0.39 nmol/l (>104 pg/ml). This concentration was also a threshold value for HCG administration, provided the ultrasound parameters were fulfilled, the urinary LH test was negative, and the woman was 18–17 days before the expected menstrual bleeding.

In the authors' experience, the disappearance of the follicle at the time planned for oocyte retrieval was also reduced if oocyte retrieval was performed earlier than recommended, 31–32 h and not 36 h after HCG (Tomazevic *et al.*, 1999).

On average, three oestradiol measurements and one to two ultrasound measurements per cycle were needed; in women with longer menstrual cycles, however, the number of oestradiol and ultrasound measurements was higher.

Using individualized monitoring in a previous study, no significant difference was observed between non-conceptual and conceptual cycles regardless of the follicle size (17–22 mm), oestradiol concentrations (0.4–1.2 nmol/l), endometrium thickness (6–12 mm) and the day of HCG administration (8–20 day) (Tomazevic *et al.*, 1999).

Despite triggering ovulation at very low oestradiol concentrations, provided that ultrasound criteria and cycle length criteria were fulfilled, and despite earlier oocyte retrieval, mature oocytes were obtained. Thus, there was no need for in-vitro maturation (Chian *et al.*, 2004; Mikkelsen, 2005) in the series of normally ovulating women.

After the introduction of lower oestradiol threshold values, provided that ultrasound criteria were fulfilled, the positive LH was rarely the cause of cancellation: only 2% in this series of patients. Disappearance of follicle in 6% of natural cycles with IVF in the present study is low, compared with the cancellation rate of 45% reported by Claman *et al.* (1993).

The average oestradiol concentration on the day of HCG in the present study was 0.57 ± 0.13 nmol/l. This value agrees exactly with the average oestradiol concentration 1 day before spontaneous LH surge of 159 ± 14 pg/ml, reported by Lindheim *et al.* (1998).

In the present study, in 180 of the 397 cycles (46%) ultrasound criteria and cycle length criteria were fulfilled and ovulation was triggered at very low oestradiol concentrations (0.4–0.49 nmol/l). These data could partly explain the 45% cancellation rate reported by Claman *et al.* (1993).

In the present study, similar results were achieved in cycles with very low oestradiol concentrations (0.4–0.49 nmol/l) compared with cycles with higher oestradiol concentrations (0.5–1.2 nmol/l), provided that ultrasound criteria, cycle length criteria and also minimal oestradiol criteria were fulfilled. This finding is important for cancellation prevention.

Embryo transfer on day 5 and IVF–embryo transfer in the natural cycle

Transfer of a blastocyst reflects high oocyte and sperm quality and can be considered to be a good predictor of success. The transfer of a morula on day 5 was followed by a significantly lower clinical pregnancy rate ($P < 0.001$), but not by higher spontaneous abortion rates. Retrieval of 220 oocytes from dominant follicles in cycles of women aged <39 years of age resulted in 43 clinical pregnancies, and the transfer of 89 blastocysts resulted in 81% of these clinical pregnancies.

Contrary to the results of the previous study where transfers of lower-stage embryos on day 4 were still followed by clinical pregnancies (Tomazevic *et al.*, 1999), the results of this study show that the transfer of the lower-stage embryo on day 5 will not be justified in the future. Fortunately, only 11% of all embryos did not reach the morula or blastocyst stage on day 5.

In the period 1996–1999, day 4 embryos were transferred, achieving a 14% live birth rate per cycle (Tomazevic *et al.*, 1999); embryo transfer on day 5 did not improve the results of natural cycle IVF, but it did improve insight into the factors that predict success.

The ability of a fertilized oocyte to develop to the blastocyst stage seems to be crucial in the prediction of success of natural cycle IVF, and is not influenced by very low oestradiol concentrations on the day of HCG (0.4–0.49 nmol/l). This is an important finding indicating no need for cancellation in women close to LH surge at such a low oestradiol concentration. By studying the factors that influence blastocyst development, the results of the natural cycle IVF might be improved in the future.

The transfer of a single blastocyst on day 5 in woman <39 years of age was followed by a high clinical pregnancy rate (39%), which is similar to that achieved by elective single blastocyst transfer in stimulated cycles (Virant-Klun *et al.*, 2003). Natural cycle IVF–embryo transfer could therefore be successfully used to perform a single embryo transfer in a selected group of normal responders. Trokoudes *et al.* (2005) obtained similar results using a modified natural cycle.

Based on the results achieved, this protocol of monitoring the natural cycle in normogonadotrophic women aged <39 years, i.e. in normal responders with tubal infertility factor, endometriosis or idiopathic infertility problems, may be considered an efficient infertility treatment option.

In the future, monitoring of the natural cycle could be made simpler by using a quick urinary oestradiol–glucuronide test, provided that it could be adjusted to serum oestradiol concentrations in the range of 0.35–0.4 nmol/l. The small portion of cancellations can further be reduced by using a semi-natural or a modified natural cycle (Castelo-Branco *et al.*, 2004; Trokoudes *et al.*, 2005).

In conclusion, maternal age ≥39 years unfavourably influences blastocyst development and clinical pregnancy rates in natural IVF–embryo transfer cycles. Low oestradiol concentrations on the day of HCG (0.4–0.49 nmol/l), provided that ultrasound criteria and cycle length criteria are fulfilled, do not unfavourably influence the blastocyst development and the clinical pregnancy rates in the natural cycles with IVF–embryo transfer for female infertility in women <39 years of age.

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