

# HCG administration after endogenous LH rise negatively influences pregnancy rate in modified natural cycle for frozen–thawed euploid blastocyst transfer: a pilot study

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## Abstract

**Purpose** The aim of the present study was to evaluate whether in a modified natural cycle (modified-NC) for a frozen-thawed single euploid blastocyst transfer, a critical LH value, above which human chorionic gonadotropin (hCG) administration should be avoided, may be defined.

**Methods** One hundred and sixty-seven patients underwent modified natural cycle in order to transfer a single frozen-thawed euploid blastocyst. All embryos were obtained by intracytoplasmic sperm injection and were biopsied at the blastocyst stage and analyzed by means of array comparative genomic hybridization (aCGH). Ovulation was induced using 10,000 IU hCG when the mean follicle diameter was at least of 17 mm, independently from LH values. The primary end points were the hCG-positive test and clinical pregnancy. The interim analysis showed that LH value  $\geq 13$  mIU/ml on the day of hCG injection may negatively influence the clinical results, suggesting that in this condition, it should be advisable waiting for spontaneous ovulation.

**Results** Among patients who received hCG for ovulation induction, the hCG-positive test and clinical pregnancy rates in modified-NC were significantly lower in cycles with LH  $\geq 13$  mIU/ml in respect to those with LH  $< 13$  mIU/ml (45.4 vs 73.3 and 36.4 vs 65.9%, in LH  $\geq 13$  and LH  $< 13$  groups, respectively). In patients with LH value  $\geq 13$  mIU/ml, hCG administration led to significantly lower rates of hCG-positive test (45.4 vs 74.5% in hCG administration and spontaneous ovulation groups, respectively) and clinical pregnancy (36.4

vs 64.7% in hCG administration and spontaneous ovulation groups, respectively). The baseline patient characteristics were comparable in all groups.

**Conclusions** The findings of this study highlight that LH elevation  $\geq 13$  mIU/ml prior to hCG administration may negatively affect clinical pregnancy rates in modified-NC for single euploid blastocyst transfer. The LH determination should be routinely performed during follicular monitoring. In the presence of LH level  $\geq 13$  mIU/ml, hCG administration should be avoided, and the embryo transfer should be planned only after spontaneous follicular rupture.

**Keywords** Frozen-thawed transfer · Euploid blastocyst · Modified-natural cycle · Ovulation induction

## Introduction

Cryopreservation of supernumerary embryos increases the cumulative pregnancy rate per oocyte retrieval, encouraging the choice of a single embryo transfer for multiple pregnancies prevention. Recently, it has been suggested that a high estrogen level obtained during ovarian stimulation for in vitro fertilization (IVF) may impair endometrial receptivity [1–8]. Preimplantation genetic screening (PGS) requires blastocyst formation. Blastocyst stage, depending on embryo developmental rate, can be obtained late in the culture potentially leading to progesterone elevation and endometrial advancement, especially when it is reached out of the implantation window. This condition enhances the importance of embryo cryopreservation, allowing for a more efficient planning of embryo transfer [2, 9–11].

Many studies demonstrated that frozen-thawed embryo transfers (FTET) result in better clinical and obstetrical outcomes compared to fresh cycles. This evidence encourage a

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“freeze all” strategy in which the whole embryo cohort obtained in a cycle is cryopreserved waiting for later thawed embryo transfer in a natural cycle [1, 2, 10–14]. Therefore, the embryo storage following IVF has actually become an important feature in assisted reproduction.

Cycle regimens used for endometrial preparation in FTET are commonly classified into three types: (i) natural cycle, with or without ovulation induction using human chorionic gonadotropin (hCG); (ii) artificial cycle, in which the endometrium is prepared by exogenous estrogens, with or without GnRH-agonist administration; and (iii) stimulated cycles, in which follicular development is supported by follicle-stimulating drugs [12, 15–23]. Until now, no conclusion has been drawn on the superiority of any regimen in terms of ongoing pregnancy rate, especially when an euploid blastocyst is transferred [23].

In spontaneous cycle, LH surge and subsequent ovulation initiate an endocrine shift from estrogen to progesterone and the onset of a period of optimal fecundity in which embryos should be transferred. In clinical practice, the LH surge can be achieved by recognition of natural mid-cycle LH surge (NC) or, alternatively, triggering the ovulation with an injection of hCG (modified-NC) [18]. The administration of exogenous hCG in the follicular phase may prematurely induce a cascade of endometrial events which, based only on the endogenous LH rise, would have started several days later. This event could be the reason of the very low pregnancy rate reported in patients receiving hCG after LH surge [17].

Until now, little attention has been paid to the optimal LH level on the day of hCG injection for FTET. To address this issue, we tried to identify the LH threshold value that should be evaluated in a modified-NC for frozen-thawed single euploid blastocyst transfer over which the hCG administration should be avoided, waiting for spontaneous follicular disruption.

## Materials and methods

### Study design

In this study, 167 frozen-thawed single euploid blastocyst transfers were planned to be performed in modified-NCs between September 2015 and 2016. The Institutional Review Boards of European Hospital and Genoma Laboratory approved the study before initiation. All participants gave written consent after having been informed on all the aspects of the study. All the clinical and biological procedures were conducted at the Center for Reproductive Medicine of European Hospital, Rome (Italy), whereas the genetic screenings were performed at the Genoma Laboratory, Rome (Italy). All procedures were performed according to the Helsinki Declaration of 1975 and its further modifications. All women enrolled in

this study were at their first IVF attempt, and repeated cycles were not considered.

The primary end points were the hCG-positive test and clinical pregnancy rates defined respectively as the number of positive pregnancy tests per transfers performed and the number of pregnancies which completed 12 gestational weeks with embryo heart activity respect to the number of transfers performed. The secondary end point was the implantation rate defined as the percentage of embryos transferred that develops to the stage of ultrasound fetal heartbeat. The miscarriage rate was considered as the percentage of spontaneous pregnancy loss before 24 weeks of gestation respect to the number of transfers performed. Biochemical pregnancy was defined as evidence of conception based only on biochemical data in the serum without ultrasound evidence of a gestational sac.

### Patient population

All patients enrolled in the study satisfied the following inclusion criteria: maternal age < 42 years old, regular menstrual cycle, normal intrauterine cavity at the pretreatment assessment, and the presence of at least one vitrified euploid blastocyst obtained after intracytoplasmic sperm injection (ICSI) followed by preimplantation genetic diagnosis at blastocyst stage by means of aCGH and the consent to undergo to a frozen-thawed single transfer in a modified-NC. Exclusion criteria were as follows: ovulation disorders, body mass index > 29 kg/m<sup>2</sup>, endometriosis grade  $\geq$  III according to the American Fertility Society criteria, and the use of testicular sperm for ICSI.

### IVF-ICSI treatment and embryo culture

Controlled ovarian stimulation was performed using recombinant FSH (Gonal F, Merck Serono, London, UK) and gonadotropin-releasing hormone agonist in a long suppression protocol or GnRH-antagonist protocol according to ovarian reserve and AMH values. When at least three follicles reached 18 mm in diameter, hCG (Gonasi, 10.000 IU, IBSA, Lodi, Italy) was administered by intramuscular injection. Oocytes were retrieved 36–38 h later by ultrasound-guided transvaginal follicular puncture. The denudation procedure was completed between 38 and 40 h after hCG administration, and the oocytes were treated by ICSI immediately thereafter using previously described techniques and instrumentation [24]. Fertilization was considered successful when two clearly distinct pronuclei and two polar bodies were presented 16–18 h after ICSI, as described elsewhere [24]. Embryo culture was carried out in cleavage medium under mineral oil (Sage In-Vitro Fertilization, Inc., Trumbull, CT, USA) up to day 3 and then continued in blastocyst medium (Sage In-Vitro Fertilization, Inc., Trumbull, CT, USA) up to day 5, 6, or 7 at 37 °C and under 5% O<sub>2</sub> and 6% CO<sub>2</sub>. Embryo culture

was performed in a time-lapse incubator (EmbryoScope; Unisense FertiTech, Denmark) or in a mini-incubator (multigas incubator; Sanyo, Panasonic Biomedical, the Netherlands). Embryo culture was performed in Embryoscope or in a mini-incubator (SANYO), where all embryos from each patient were kept separately from other couples throughout the whole culture duration.

### **Biopsy procedure and cell preparation**

On day 3 of culture, when the embryos reached the 6–8-cell stage, a noncontact 1.48 diode laser was used to create a circular 6–9-diameter opening in the zona pellucida, in order to allow the biopsy of 5–10 herniated trophoblast (TE) cells on day 5, 6, or 7, depending on the speed of blastocyst development. On the day of the biopsy, TE cells were gently aspirated into the biopsy pipette (biopsy pipette; COOK Ireland Ltd., Limerick, Ireland), washed in sterile phosphate-buffered saline solution (PBS) and then placed in microcentrifuge tubes containing 2 mL of PBS, spun down for few seconds, and sent to GENOMA Laboratory for genetic analysis. TE cells were lysed and genomic DNA was amplified using the SurePlex DNA Amplification System (BlueGnome, Cambridge, UK). Whole Genome Amplification (WGA) products were fluorescently labeled and competitively hybridized to 24sure V3 arrays (BlueGnome, Cambridge, UK) with a matched control in an array CGH experiment format. A laser scanner was used to excite the hybridized fluorophores and read the resulting images of the hybridization. Scanned images were then analyzed and quantified by algorithm fixed settings in BlueFuse Multi Software (BlueGnome, Cambridge, UK), a software package that performed the steps of grid placement, quantification, normalization, and post-processing automatically.

### **Blastocyst vitrification and warming**

Vitrification procedure was used to cryopreserve all blastocysts, both euploid and aneuploid, accordingly to the Italian law that forbids any embryo destruction. Vitrification and warming were carried out with the use of the Kuwayama protocols [25]. Briefly, blastocysts were placed in equilibration solution (Kitazato Vitrification Kit, BioPharma, Shizouka, Japan) containing 7.5% ethylene glycol and 7.5% dimethyl sulfoxide for 15 min at room temperature and then moved to a vitrification solution composed of 15% ethylene glycol, 15% dimethyl sulfoxide, and 0.5 mol/L sucrose for 30 to 60 s. The blastocysts were singly loaded onto the polypropylene strip of the Cryotop in a volume of <math>0.1 \mu\text{L}</math> and stored in a liquid N<sub>2</sub> storage tank at  $-196 \text{ }^\circ\text{C}$ . Warming of a euploid blastocyst for transfer was performed by placing the Cryotop in a thawing solution (Kitazato Warming Kit, BioPharma, Shizouka, Japan) of 1 mol/L sucrose for 45 to 60 s at  $37 \text{ }^\circ\text{C}$ .

The blastocyst was then transferred to a dilution solution of 0.5 mol/L sucrose for 3 min and washed with medium without sucrose for 5 min. The surviving blastocysts were incubated for 2 h before their transfer to the uterus.

### **Modified natural cycle**

On cycle day 3, all patients underwent a transvaginal ultrasound control to assess the ovarian status showing the early follicular phase. Subsequently, serum estradiol and LH dosage and endometrial evaluation by transvaginal ultrasound were performed serially according to the physician's decision based on the dominant follicle growth. All hormonal parameters were evaluated in the same laboratory, using identical kits and validated measurement methods. Criteria for hCG administration included the following: dominant follicle mean diameter of at least 17 mm, endometrial thickness  $>7$  mm and serum progesterone  $<1.5$  ng/ml. Final oocyte maturation was induced using 10,000 IU of hCG (Gonasi, 10,000 IU, IBSA, Lodi, Italy), independently of LH values. Intramuscular administration of progesterone at dose of 50 mg/day (Prontogest, IBSA, Lodi, Italy) was started in all patients 2 days after hCG. The blastocyst transfer was performed on day 6 of progesterone treatment. In cases of pregnancy, the luteal phase support was continued until the 12th gestational week.

### **Embryo transfer technique**

All embryo transfers were performed under ultrasound control. For this reason, patients were asked to fill their bladder to provide an acoustic window for uterus visualization. The catheter tip (Wallace, Smits-Medical, Dublin, Ireland) was placed 1.0–2.0 cm below the apex of the uterus cavity. Care was taken to avoid contact of the transfer catheter with the uterine fundus, in order to prevent uterine contractions.

### **Sample size and statistical analysis**

Quantitative variables were compared between the groups by unpaired Student's *t* test. Chi-square test was used to compare categorical variables. Statistical analyses were performed with Stata software (StataCorp 2013; Stata Statistical Software: Release 13, College Station, TX, USA: StataCorp LP). The significance level was set at  $P < 0.05$ .

## **Results**

One hundred and sixty-seven patients were planned to receive a single frozen-thawed euploid blastocyst transfer in a modified-NC. After the first 80 patients had completed their FTET cycle, an interim analysis was performed revealing that LH

value  $\geq 13$  mIU/ml on the day of hCG injection (22 subjects) negatively influenced the clinical results. Therefore, to the remaining women showing this condition, it was proposed to continue the ultrasound and hormonal monitoring until spontaneous ovulation, avoiding hCG administration.

The demographic and clinical characteristics of the study population are presented in Table 1. There were no differences in the age, BMI, AMH, and infertility history between patients who received hCG for ovulation induction and those attending a spontaneous follicular disruption due to the endogenous LH surge.

The characteristics of FTET cycles are summarized in Table 2. The endometrial maximum thickness and the estradiol peak levels were similar between the two groups. However, as expected, LH level on the day of triggering was significantly lower in group receiving hCG. The ovulation induction in women undergoing a modified-NC was performed on mean cycle day  $12.6 \pm 2.6$  and mean follicular diameter of  $17.5 \pm 1.3$  mm. The spontaneous LH surge was observed at mean cycle day of  $13.8 \pm 2.8$  and mean follicular diameter of  $18.0 \pm 1.6$  mm. No significant differences were observed in the number of ultrasound controls and the number of laboratory dosages among patients who received hCG administration and those waiting for spontaneous ovulation. However, the number of venous samplings was significantly lower in patients receiving hCG (Table 2).

The blastocyst survival rate was 100%, and only one embryo was transferred per cycle. In addition, no significant differences in the blastocysts quality, biopsy-day, or incubator distribution were observed between the groups (data not shown).

The hCG-positive tests and clinical pregnancy rates per transfer in modified-NC (68.1 and 62.1%, respectively) were slightly lower than in spontaneous cycles (74.5 and 64.7%,

respectively) (Table 3). However, the stratification of clinical results, based on LH value on the day of hCG triggering, showed a significant decrease in the hCG-positive tests per transfer (45.4 vs 73.3% in  $LH \geq 13$  and  $LH < 13$ , respectively,  $p < .05$ ) and in the clinical pregnancy rate (36.4 vs 65.9%, in  $LH \geq 13$  and  $LH < 13$ , respectively,  $p < .05$ ), with a threshold value of  $LH = 13$  mIU/ml.

Moreover, among patients with a LH value  $\geq 13$  mIU/ml, significantly higher hCG-positive test and clinical pregnancy rates were observed in patients with spontaneous ovulation compared to those undergoing hCG administration (hCG-positive test 74.5 vs 45.4% and clinical pregnancy 64.7 vs 36.4%, in the spontaneous ovulation and in the hCG administration groups, respectively;  $p < .05$ ).

The biochemical pregnancy rates were comparable in all groups. The miscarriage rate was identical in the patients undergoing the modified-NC (12.5%) or the spontaneous ovulation (12.7%). No miscarriages occurred in the women who received hCG with  $LH \geq 13$  mIU/ml.

## Discussion

Under physiologic circumstances occurring during a natural cycle, an increase in the endogenous LH appears at the end of the follicular phase and the endometrium will not be exposed to hCG until embryo apposition has occurred. In modified-NC for FTET, the hCG administration for ovulation induction terminates the follicular phase, allowing to plan embryo transfer independently of the natural endogenous LH surge [16].

However, the administration of hCG in a natural cycle may have an impact on uterine receptivity. In fact, both LH and hCG act on the endometrium through the same receptor [26] that is present in its full-length form only from the

**Table 1** Demographic and clinical characteristics of women undergoing frozen-thawed euploid blastocyst transfer in natural cycle with hCG administration and spontaneous LH surge for ovulation induction

	HCG group ( $n = 116$ ) Mean $\pm$ SD (range)	LH surge group ( $n = 51$ ) Mean $\pm$ SD (range)
Age (years)	$35.5 \pm 3.5$ (25.0–43.0)	$36.1 \pm 3.3$ (29.0–43.0)
BMI ( $\text{kg}/\text{m}^2$ )	$21.6 \pm 2.6$ (17.2–30.8)	$22.6 \pm 4.2$ (17.9–36.1)
AMH (pmol/l)	$22.6 \pm 8.7$ (12.0–33.4)	$25.6 \pm 13.8$ (10.9–37.1)
Primary IVF indication (%):		
Female factor	26 (22.4%)	12 (23.6%)
Male factor	35 (30.2%)	19 (37.2%)
Couple factor	25 (21.5%)	7 (13.7%)
Unexplained	30 (25.9%)	13 (25.5%)
PGS indication (%):		
Advanced maternal age	21 (18.1%)	8 (15.7%)
Recurrent implantation failure	10 (8.6%)	7 (13.7%)
Recurrent pregnancy loss	8 (6.9%)	4 (7.8%)
Embryo chromosomal evaluation	59 (50.9%)	24 (47.1%)
Multiple factor	18 (15.5%)	8 (15.7%)

**Table 2** Characteristics of frozen-thawed euploid blastocyst transfer cycles in both study groups

	HCG group ( <i>n</i> = 116) Mean ± SD (range)	LH surge group ( <i>n</i> = 51) Mean ± SD (range)
Endometrium diameter (mm)	9.5 ± 1.4 (7.1–15.0)	9.6 ± 1.5 (6.5–14.0)
Peak E2 level (pg/ml)	309.4 ± 126.0 (99.0–1026.0)	346.0 ± 124.0 (103.0–613.0)
Peak LH level (mIU/ml)	9.0 ± 7.2 (2.1–55.0)	36.6 ± 18.4 (13.0–92.0)
Maximum follicle diameter (mm)	17.5 ± 1.3 (15.0–20.0)	18.0 ± 1.6 (15.0–22.0)
Cycle day on hCG injection or endogenous LH surge ( <i>n</i> )	12.6 ± 2.6 (7.0–28.0)	13.8 ± 2.8 (8.0–23.0)
Clinical or ultrasound controls ( <i>n</i> )	4.0 ± 1.1 (1.0–8.0)	4.8 ± 1.2 (3.0–8.0)
Laboratory dosages ( <i>n</i> )	5.5 ± 3.0 (2.0–18.0)	9.0 ± 3.5 (2.0–18.0)
Venous sampling ( <i>n</i> )	2.2 ± 1.2 (1.0–6.0)	3.4 ± 1.3* (1.0–7.0)

\**p* < .05 compared to the patients receiving hCG

proliferative to the mid-luteal phase. Thereafter, only the extracellular domain of the receptor is expressed [27] and the deletion of exon 10 of the receptor impairs the LH action without affecting the hCG action [28]. A differential effect of exogenous hCG and endogenous LH surge can also be explained by the shorter half-life of LH compared to that of hCG. In addition, hCG can also act by regulating the expression of the endometrial vascular endothelial growth factor (VEGF), which is a key regulator of neo-angiogenesis and vascular functions. Therefore, the use of exogenous hCG can increase the intrauterine VEGF concentrations, suggesting its involvement in the vascularization process essential for the formation of a functional placenta [27, 29].

Besides an intrinsic molecular difference between LH and hCG, no dose-dependent studies have been performed to determine the optimal hCG concentration and the ideal moment to replace the endogenous LH in concomitance with the natural LH progressive release process.

The study by Groenewoud et al. [18] evaluated whether there is a threshold serum LH concentration that could negatively affect pregnancy rates after FTET in modified-NC. Anyway, the ROC curve showed little discriminating capacity and no optimal cutoff in LH concentration could be

established. An LH surge, defined as its plasma concentration higher than 10 IU/l, occurred in 52.4% of cycles, but no significant difference in pregnancy rate was observed between cycles with or without LH surge. Based on these findings, the authors concluded that regular ultrasound evaluation of the dominant follicle in combination with hCG triggering of ovulation was an effective way of FTET planning and no benefit of a single routine LH evaluation prior to the ovulation was demonstrated [18]. Anyway, the stage of embryos was not exactly specified and PGS was not performed. Some other studies [30, 31] reported an LH surge in 35–50% patients undergoing FTET, without any significant effect on pregnancy rates.

The opposite conclusions can be inferred from Fatemi et al. [17]. In this clinical trial, patients underwent FTET both 5 days after the LH surge or after the administration of 5.000 IU of hCG when a follicle of at least 17 mm was present on ultrasound examination, therefore independently of LH level. The pregnancy rate was significantly higher in the spontaneous LH group (34.4%, 21/61) compared with the hCG group (20.6%, 13/63). The rise in LH levels occurred in 36.5% (23/63) of the patients who were planned to receive hCG. Of these patients, only 4.3% (1/23) became pregnant. Therefore, the pregnancy

**Table 3** Clinical outcomes from frozen-thawed euploid blastocyst transfer in the study groups

	HCG group ( <i>n</i> = 116)	LH surge group ( <i>n</i> = 51)	HCG group with LH < 13 ( <i>n</i> = 94)	HCG group with LH ≥ 13 ( <i>n</i> = 22)
HCG positive test (%)	79 (68.1%)	38 (74.5%)	69 (73.3%)	10 (45.4%)***
Clinical PR (%)	72 (62.1%)	33 (64.7%)	62 (65.9%)	8 (36.4%)***
Biochemical PR (%)	7 (6.0%)	5 (9.8%)	7 (7.4%)	2 (9.0%)
Miscarriage rate (%)	9 (12.5%)	4 (12.7%)	9 (14.5%)	0 (0.0%)
Implantation rate (%)	72 (62.1%)	33 (64.7%)	62 (65.9%)	10 (45.4%)
Ongoing PR (%)	63 (54.3%)	29 (56.8%)	53 (56.4%)	10 (45.4%)

\**p* < .05 compared to the patients with LH < 13; \*\**p* < .05 compared with patient following endogenous LH surge

rate in patient who received hCG in absence of a spontaneous LH surge could be estimated at 30% (12/40), comparable to patients following spontaneous ovulation. This observation suggests that LH evaluation before ovulation induction is of fundamental importance to optimize the clinical results in FTET performed in natural cycle. Nevertheless, neither in this study PGS was performed nor all embryos were frozen on day 3 of culture after ICSI. These findings confirm the observation that hCG administration in the late follicular phase induces an early cascade of events in the endometrium, which would have started several days later in the presence of LH rise [17].

In our study, the hCG-positive test and clinical pregnancy rates in modified-NC (68.1% and 62.1%, respectively) were comparable to the results obtained from the spontaneous cycles (74.5 and 64.7%, hCG-positive test and clinical pregnancy, respectively). However, the hCG-positive test and clinical pregnancy rates in modified-NC were significantly lower in cycles with  $LH \geq 13$  mIU/ml in respect to those with  $LH < 13$  mIU/ml (45.4 vs 73.3%, hCG-positive test and 36.4 vs 65.9%, clinical pregnancy, in  $LH \geq 13$  and  $LH < 13$ , respectively,  $p < .05$ ). These findings are in agreement with the study of Fatemi et al. [17], whereas they are in contrast with previous published papers showing no impact of LH surge associated with hCG administration on the clinical results [18, 30, 31]. The clinical results in patients receiving hCG with  $LH \geq 13$  mIU/ml were also significantly decreased compared to the women undergoing spontaneous ovulation. Therefore, spontaneous follicular rupture expectance in patients with  $LH \geq 13$  mIU/ml may be considered a strategy to maintain hCG-positive test and clinical pregnancy rates similar to those observed in women receiving hCG with  $LH < 13$  mIU/ml (hCG-positive test 74.5 vs 73.3% and clinical pregnancy 64.7 vs 65.9%, in  $LH \geq 13$  without hCG and  $LH < 13$  with hCG, respectively).

Undoubtedly, it would be interesting to evaluate histologically endometrium samples after exposure to exogenous hCG in presence of natural LH surge. This approach might dissolve the doubts about effective hCG endometrial impact and opportunity of an earlier embryo transfer planning rather than spontaneous ovulation attendance. However, endometrial biopsy performed in cycle in which embryo transfer is planned could negatively affect embryo implantation.

In addition, previously published papers reported a discordant opinion regarding the number of clinical and laboratory controls that should be performed in women undergoing natural cycle with spontaneous and hCG-induced LH surge [19, 23, 32]. In our study, patients following spontaneous ovulation due to the endogenous LH surge had a comparable number of ultrasound controls and laboratory parameters with patients receiving hCG administration. The number of venous samplings resulted significantly higher in natural compared to modified-NC cycles, but patient dropout was not observed,

suggesting once again an excellent compliance of natural cycle.

In conclusion, we suggest that LH elevation before hCG administration may negatively affect the clinical results in modified-NC and thus, a LH determination should be routinely performed during the regular ultrasonic evaluation of dominant follicle growth. The  $LH \geq 13$  mIU/ml imposes hCG avoidance for ovulation induction, planning the embryo transfer only after spontaneous follicular rupture. Undoubtedly, this evidence should be validated in a larger population in a prospective study prior to being applied broadly.

**Compliance with ethical standards** The Institutional Review Boards of European Hospital and Genoma Laboratory approved the study before initiation. All participants gave written consent after having been informed on all the aspects of the study. All the clinical and biological procedures were conducted at the Center for Reproductive Medicine of European Hospital, Rome (Italy), whereas the genetic screenings were performed at the Genoma Laboratory, Rome (Italy). All procedures were performed according to the Helsinki Declaration of 1975 and its further modifications.

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