A matched propensity score study of embryo morphokinetics following gonadotropin-releasing hormone agonist versus human chorionic gonadotropin trigger

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Abstract

Purpose To compare morphokinetic parameters and quality of embryos derived from GnRH antagonist ICSI cycles triggered either with GnRH agonist or standard hCG between matched groups of patients.

Methods Morphokinetic parameters of embryos derived from matched first GnRH antagonist ICSI cycles triggered by GnRH agonist or standard hCG between 2013 and 2016 were compared. Matching was performed for maternal age, peak estradiol levels, and number of oocytes retrieved. Outcome measures were: time to pronucleus fading (tPNf), cleavage timings (t2–t8), synchrony of the second and third cycles (S2 and S3), duration of the second and third cycle (CC2 and CC3), optimal cell cycle division parameters, and known implantation data (KID) scoring for embryo quality. Multivariate linear and logistic regression analyses were performed for confounding factors.

Results We analyzed 824 embryos from 84 GnRH agonist trigger cycles and 746 embryos from 84 matched hCG trigger cycles. Embryos derived from the cycles triggered with hCG triggering cleaved faster than those deriving from GnRH agonist trigger. The differences were significant throughout most stages of embryo development (t3–t6), and a shorter second cell cycle duration of the hCG trigger embryos was observed. There was no difference in synchrony of the second and third cell cycles and the optimal cell cycle division parameters between the two groups, but there was a higher percentage of embryos without multinucleation in the hCG trigger group (27.8% vs. 21.6%, $p < 0.001$).

Conclusion The type of trigger in matched antagonist ICSI cycles was found to affect early embryo cleavage times but not embryo quality.

Keywords GnRH agonist trigger · Time lapse · Morphokinetic parameters

Introduction

With the introduction of the time-lapse monitoring system (TMS), a continual assessment of embryo morphological changes could be performed. TMS allows embryo quality assessment by documenting timing of events and length of intervals in embryo development. Predictive software based on computerized algorithms is installed in current TMS,

assisting embryologists in embryo evaluation and providing an objective scoring of the embryos, for embryo selection. In addition, the continuous documentation of embryo development inside the incubator provides a stable culture environment by limiting exposure of the embryos to environmental changes [1–3].

Oocyte and embryo quality are important factors affecting the success of in vitro fertilization (IVF) and known to be associated with treatment protocol and ovulation triggering medications [4–6]. Over the last decade, gonadotropin-releasing hormone (GnRH) antagonist protocols have become the preferred pituitary downregulation as they are relatively short and simple and equally successful and generally require low dosages of gonadotropins compared with long agonist protocols [7]. Another advantage is the option to use GnRH agonist (GnRHa) triggering to prevent ovarian hyperstimulation

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syndrome (OHSS) in high responder patients [8–11]. It works by displacing the GnRH from the receptors that become activated and induce a release of gonadotrophins (flare up) eliciting an endogenous surge of LH and FSH that remain elevated for 24–36 hours, as opposed to the hCG-mediated LH activity that persists for several days [12–14]

Consequently, the two triggering agents affect oocyte maturation in different ways. GnRHa triggering was reported to be associated with retrieval of more metaphase II (MII) oocytes compared with hCG triggering [15]. This was related to the endogenous FSH surge elicited along with the LH surge after GnRHa triggering [15, 16]. The difference in LH surge kinetics and characteristics might alter not only oocyte quality but also embryo developmental kinetics. A previous study reported that embryos from cycles involving GnRH antagonist + GnRHa treatment cleaved faster than embryos derived from patients co-treated with a GnRHa + hCG [17]. Insufficient data have compared embryo morphokinetics between the two triggering agents in GnRH antagonist cycles.

Thus, the purpose of our study was to compare morphokinetic parameters of embryos derived from matched GnRH antagonist ICSI cycles triggered either with GnRHa or standard hCG using the TMS system.

Materials and methods

Study design

We conducted a retrospective cohort study evaluating embryo morphokinetic parameters and cycle outcome of embryos derived from fresh first GnRH antagonist ICSI cycles triggered either by GnRHa trigger (Decapeptyl 0.2mg) or standard hCG (Ovitrelle 250 mcg) performed between May 2014 and November 2019. Women ≤ 44 years were included only once, and the first antagonist ICSI cycle was matched for maternal age, peak estradiol levels, and number of oocytes retrieved according to the type of trigger, GnRH agonist, or standard hCG. The hospital's institutional review board approved the study [Study 0704-15-RMC]. Exclusion criteria were oocyte donor cycles and standard IVF cycles where oocyte maturation at insemination cannot be assessed, cycles with embryos cultured in a regular incubator, cycles with discarded embryos with early arrested development, and pre-implantation genetic testing cycles.

Ovarian stimulation protocol

All patients followed a flexible antagonist protocol consisting of daily gonadotropin stimulation started from day 2 to 4 of menstruation followed by daily injection of Cetrotide 0.25 mg (Merck, Merk KGaA, Darmstadt, Germany) or Orgalutran 0.25 mg (Organon, Oss, Netherlands) after the leading follicle

reached 14 mm and until the day of ovulation trigger. Gonadotropin stimulation consisted of recombinant FSH (Gonal F, Merk KGaA, Darmstadt, Germany; or Puregon (MSD, Kenilworth, NJ, USA), alone or in combination with urinary gonadotropins (hMG) (Menopur, Ferring, Saint-Prex Switzerland). The initial dose of gonadotropins was tailored according to patient characteristics or the previous stimulation response of the patient, when available. Ovarian response was monitored by transvaginal ultrasound measurements of follicular growth and serum estradiol and progesterone levels every 1–3 days. FSH and hMG dosages were adjusted accordingly. The choice of ovulation trigger was based on a clinical decision in attempt to prevent OHSS. Luteal support was provided by vaginal progesterone supplementation, and in GnRH agonist trigger cycles, the dose was doubled compared with hCG trigger cycles to counteract the luteolysis.

Transvaginal ultrasound-guided oocyte retrieval was performed under general anesthesia, 36–38 hours after the trigger. Oocytes were incubated individually in pre-equilibrated culture medium in EmbryoSlide® culture dishes (Vitrolife, Göteborg Sweden) covered with mineral oil in an atmosphere of 5.0% O₂ and 5.6% CO₂. Intracytoplasmic sperm injection was performed in all patients. Once injected, oocytes were placed in individual wells of a pre-equilibrated EmbryoSlide of the time-lapse system TMS (EmbryoScope™, Unisense Fertiltch, Vitrolife, Viby, Denmark). Embryos were cultured in regular culture media (LifeGlobal Media®, LifeGlobal, Brussels, Belgium or CSC™, Irvine Scientific, Santa Ana, CA, USA) and evaluated morphologically at 16–18, 48, and 72 h after sperm injection. Cell number, symmetry, degree of fragmentation, and the presence of multinucleated blastomeres at the 2-cell stage were evaluated [18]

Time-lapse embryo assessment

The timing of developmental parameters, including pronuclear fading in fertilized oocytes (tPNf) and cleavage timings from a zygote to 8 cell embryo (t2–t8), was determined using TMS, cleavage to a 2-blastomere (t2), 3-blastomere (t3), 4-blastomere (t4) and so forth until reaching an 8-blastomere embryo (t8). The time of all events is expressed as hours post ICSI microinjection. We also compared the synchrony of the second and third cleavages (s2 and s3 respectively) and the duration of the second and third cell cycles [CC2 = t3–t2 and CC3 = t5–t3]. Adequate CC2 was defined as CC2 > 5 hours and optimal S2 as less than 1 hour. Embryos were selected for transfer based on an integration of their morphological scoring on days 2 and 3, time-lapse embryo kinetics, and KID (Known Implantation Data) Score. The KID score time-lapse model is embedded in the annotation software and offered as a tool for deselection of embryos showing erratic morphokinetic patterns. The KID score model is a validated model based on the morphokinetic traits associated with the implantation

potential of embryos transferred on day 3 as determined by cumulative known clinical outcomes of transferred embryos as previously reported [19]. The KID score ranging from 1 to 5 is a relative scale of the embryo implantation potential.

Embryos with normal 2PN fertilization or 1PN embryos that continued to divide in culture conditions were included in the study as well as embryos that were either transferred or cryopreserved.

Statistical analysis

We used SAS Software, Version 9.4, for statistical analysis. We created a 1:1 propensity score matched data set with a maximum score difference of 0.5 using the PSMATCH SAS procedure. All statistical analysis was done on the matched data set. As the data are normally distributed, we used Student *t* test to compare continuous variables. Proportions were compared using Chi-Square or Fisher's exact when appropriate. *p* values of less than 0.05 were considered statistically significant. For continuous outcomes (tPB2, tPNf, t2-8, s2, s3, cc2, and cc3), a multivariate general linear model (GLM) was used. For binary outcomes of optimal cell cycle division parameters and embryo quality, a multivariate logistic regression model was used. Multivariate linear and logistic regression analyses were performed for selected variables controlling for confounders including type of gonadotropins (FSH or HMG), primary or secondary infertility, type of infertility, and trigger type (GnRH agonist vs hCG). Pregnancy rates were calculated only from cycles where embryo transfer was performed.

Results

Of the total 1570 embryos, 824 embryos derived from 84 cycles in the GnRHa trigger group and 746 embryos from 84 cycles in the hCG trigger group. The first antagonist ICSI cycle of each patient was matched for maternal age, peak estradiol levels, and number of oocytes retrieved. Demographic and treatment characteristics of the 2 groups were comparable. However, the cause of infertility varied between the groups, with a higher percentage of mechanical infertility and male infertility in the hCG trigger group and a higher rate of anovulation and unexplained infertility in the GnRHa trigger group. Pregnancy rates were lower in the GnRH agonist group yet did not reach a statistically significant difference (Table 1). Embryos deriving from hCG-triggered cycles cleaved faster than embryos deriving from GnRHa triggering throughout some of the early stages of embryo development (t3-t6) (Table 2). These findings remained significant after performing multivariate regression model adjusting for type of gonadotropins, primary or secondary infertility, cause of infertility, and trigger type (t3, *p* =

Table 1 Demographic parameters and treatment characteristics of women treated with GnRH antagonist ICSI cycles triggered either with GnRHa or standard hCG

# cycles	GnRH agonist trigger <i>n</i> = 84	hCG trigger <i>n</i> = 84	<i>p</i> value
Maternal age (years)	31.9 ± 5.1	32.1 ± 5.4	0.80
BMI	23.5 ± 4.0	25.9 ± 7.3	0.08
Primary Infertility	40 (47.6)	34 (40.8)	0.43
Gravidity	0.86 ± 0.9	1.1 ± 1.4	0.17
Parity	0.4 ± 0.6	0.5 ± 0.7	0.21
Cause of infertility (%)			
Mechanical Infertility	3 (3.5)	9 (10.7)	0.03
Male Infertility	46 (54.7)	56 (66.7)	
Unexplained	28 (33.3)	15 (17.8)	
Anovulation	7 (8.3)	4 (4.7)	
FSH dose (IU)	2690 ± 2,560	2231 ± 1074	0.41
HMG dose (IU)	2662 ± 1,685	3760 ± 2060	0.26
ICSI fertilization rate %	75	92	0.20
E2 level (pmol/L)	8276 ± 4,165	7643 ± 7,130	0.48
No of oocytes aspirated	17.3 ± 8.7	15.7 ± 6.9	0.17
Pregnancy rate % (n)	20 (23.8)	31 (36.9)	0.09

Data are represented as mean ± standard deviation or number (percentage), as appropriate.

0.0016; t4, *p* = 0.015; t5, *p* < 0.001; t6, *p* = 0.046; cc2, *p* = 0.002).

On univariate analysis, the percentage of embryos without multinucleation at the 2-cell stage was higher in the hCG group, compared with GnRHa group (Table 3). This difference was also significant on multivariate logistic regression analysis controlling for the type of gonadotropins, primary or secondary infertility, cause of infertility, and trigger type (*p* < 0.001). However, the percentages of embryos with symmetric blastomeres, adequate CC2, and optimal S2 durations, as well as the proportion of high (4/5) KID score embryos were similar between the groups (Table 3).

Discussion

Our study shows that embryos deriving from GnRH antagonist ICSI cycles triggered with hCG cleaved faster throughout some of the early stages of embryo development compared with those triggered with GnRH agonist in cycles matched for maternal age, peak estradiol levels, and number of oocytes retrieved. Since the proportion of high KID score embryos was similar in both groups, the clinical impact of the singular finding of a higher percentage of embryos without multinucleation in the hCG group remains unclear.

Oocyte quality has an effect on the developmental potential and quality of the embryos. Previous studies found that

Table 2 Embryo kinetics of embryo development after triggering with GnRH agonist or standard hCG

# embryos	GnRH agonist trigger <i>n</i> = 824	CI 95%	hCG trigger <i>n</i> = 746	CI 95%	<i>p</i> value
tPB2 (hrs)	4.00 ± 3.08	3.78–4.22	3.93 ± 2.88	3.72–4.15	0.67
tPNf (hrs)	24.96 ± 4.47	24.65–25.27	24.99 ± 4.69	24.64–25.33	0.89
t2 (hrs)	27.43 ± 4.61	27.11–27.75	27.22 ± 4.53	26.89–27.55	0.37
t3 (hrs)	37.67 ± 5.93	37.25–38.09	36.85 ± 5.76	36.42–37.28	0.007
t4 (hrs)	40.27 ± 6.28	39.83–40.72	39.51 ± 6.04	39.06–39.96	0.01
t5 (hrs)	50.24 ± 8.03	49.65–50.83	48.86 ± 8.24	48.22–49.50	0.002
t6 (hrs)	53.85 ± 8.08	53.25–54.46	52.93 ± 8.12	52.28–53.58	0.04
t7 (hrs)	56.83 ± 8.40	56.17–57.48	56.44 ± 8.57	55.73–57.15	0.43
t8 (hrs)	60.40 ± 9.21	59.60–61.20	60.42 ± 10.09	59.51–61.33	0.97
cc2 (hrs)	10.4 ± 4.92	10.05–10.74	9.78 ± 4.75	9.43–10.13	0.01
Cc3 (hrs)	13.02 ± 5.95	12.59–13.46	12.35 ± 5.9	11.89–12.81	0.037
S2 (hrs)	2.81 ± 4.55	2.49–3.14	2.79 ± 4.51	2.45–3.12	0.90
S3 (hrs)	23.62 ± 8.54	22.88–24.36	23.95 ± 8.9	23.14–24.75	0.563

Data are represented as mean ± standard deviation

treatment protocol and ovulation triggering medications may have an effect on oocyte development [20–22] and embryo kinetics. Munoz et al. were the first to report an association between the type of protocol and embryo kinetics. They compared embryo kinetics in cycles using long GnRH agonist protocol stimulation with hCG triggering to GnRH antagonist protocol stimulation with GnRHa triggering. They analyzed 2817 embryos from 400 couples undergoing oocyte donation and reported that embryos from cycles triggered with GnRHa cleaved faster in their first stages of development, with no difference in subsequent embryo development, embryo quality, or pregnancy rates between the 2 groups [17]. Since the GnRH analog for downregulation was different between the groups, and there was no standardization for treatment parameters or multivariate model adjusting for potential confounders, it is difficult to conclude that the difference in embryo kinetics is solely caused by the type of triggering. Another study, similar to ours, compared 252 embryos resulting from GnRH antagonist protocol and GnRHa triggering and 487 embryos from GnRH antagonist protocol and

hCG triggering. They found that embryos resulting from cycles triggered with GnRHa cleaved faster during early embryo development compared with hCG embryos. The authors also found a higher percentage of optimal embryos (according to S2 and CC2) in the GnRHa group [23]. By contrast, we found the opposite findings; embryos from cycles triggered with hCG cleaved faster during early embryo development. This finding remained significant after performing multivariate regression model adjusting for different confounders including type of gonadotropins, primary or secondary infertility, cause of infertility, and trigger type. Furthermore, we found no effect of the type of triggering on embryo quality.

In contrast to previous studies that demonstrated higher oocyte maturation rate and even higher embryo quality with GnRHa trigger [16], our study showed better morphokinetic parameters to the HCG embryos. The reason that embryos resulting from cycles triggered with hCG cleaved faster throughout some of the early stages of embryo development is unclear. It may reflect a different timing of oocyte maturation leading to an earlier activation pathway. Although the

Table 3 Proportions embryos with adequate grading in each of the study groups

No of embryos (%)	GnRH agonist trigger <i>n</i> = 824	hCG trigger <i>n</i> = 746	<i>p</i> value
Embryos with optimal S2 duration	416 (50.5)	372 (49.8)	0.79
Embryos with optimal cc2 duration	641 (77.7)	562 (75.3)	0.16
2 cell embryos with no multinucleated blastomeres	178 (21.6)	208 (27.8)	< 0.001
2 cell embryos with symmetric blastomeres	461 (55.9)	427 (57.2)	0.11
High KID Score (4–5)	70 (44.0)	47 (39.1)	0.25

Data are represented as number (percentage).

Optimal embryos; Optimal S2 duration defined as S2 < 1 hour; Optimal CC2 duration defined CC2 ≥ 5 hours; 120 embryos with arrested development (60 in each group) were excluded from the analysis

lower pregnancy rates with GnRHa trigger are usually related to its luteolytic effect and its negative effect on endometrial receptivity [24–26], the slightly lower pregnancy rate in the GnRHa trigger group in our study may be related to decreased embryo quality as well.

The main limitation of the study is its retrospective nature. The strengths of our study include a large sample size and a single center study with all embryos cultured in the same culture media using the same time lapse parameters under standardized laboratory conditions. Only GnRH antagonist protocol cycles were included in order to standardize the type of protocol, and only ICSI cycles were included to standardize fertilization time. Women were included only once, and only the first cycle was included minimizing the possible bias of a previous cycle outcome. Matching creates quasi-experimental samples, reducing potential bias caused by the imbalance between the two study groups. GnRHa triggering is usually given to prevent ovarian hyperstimulation syndrome (OHSS) in hyper-responder patients such as women with polycystic ovarian syndrome (PCOS) [24]. Matching of the cycles for maternal age, number of oocytes retrieved, and peak E2 levels was performed in attempt to isolate the effect of the triggering itself on the morphokinetic parameters of the embryos while reducing possible intrinsic maternal physiologic and metabolic influence on the kinetics of embryo development.

In conclusion, TMS allows embryo quality assessment by documenting timing of events and length of intervals in embryo development. It provides an objective scoring of the embryos for embryo selection. Standard hCG triggering in GnRH antagonist ICSI cycles has an effect on embryonic morphokinetic parameters resulting in faster cleavage of embryos compared with GnRHa triggering; however, embryo quality seems to be unaffected. The association between the type of oocyte maturation trigger and embryo morphokinetics should be considered to be taken into consideration in embryo selection models.

Compliance with ethical standards

Conflict of interest The authors report no conflict of interest

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