Comparisons of the effects of long-acting and short-acting GnRH agonists on embryo quality, endometrial thickness and pregnancy rate in human *in vitro* fertilization

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Submitted: 2 March 2012
Accepted: 4 September 2012

Arch Med Sci 2014; 10, 1: 161–166
DOI: 10.5114/aoms.2014.40743
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Abstract

Introduction: The aim was to compare the efficacy of long-acting and short-acting gonadotropin-releasing hormone (GnRH) agonists by long protocol on embryo quality, endometrial thickness and pregnancy rate in *in vitro* fertilization.

Material and methods: In this retrospective study, long-term pituitary down-regulation, achieved with long- and short-acting GnRH agonists (GnRHa), was performed for patients undergoing *in vitro* fertilization (n = 175).

Results: There were no significant differences between the long and short-acting GnRH group (63.16% vs. 66.26%, $p > 0.05$), and the secondary and primary infertility group (63.47% vs. 66.86%, $p > 0.05$) in embryo quality. Logistic regression analysis showed that type of infertility and endometrial thickness were significantly associated with pregnancy outcome. Patients in the long-acting GnRHa group had a thicker endometrium on the day of human chorionic gonadotrophin (hCG) administration (10.79 ±2.62 mm vs. 9.64 ±1.97 mm, $p < 0.01$), lower serum luteinizing hormone (LH) concentration (1.21 ±1.13 vs. 2.53 ±3.39) and a higher pregnancy rate (59.60% vs. 43.42%, $p < 0.05$) than those of patients in the short-acting GnRHa group.

Conclusions: This work suggests that types of agonist protocol and infertility may not affect embryo quality. Type of infertility and endometrial thickness may be positive predictors for clinical pregnancy, but the key finding is that the long-acting GnRHa protocol may be an effective method of improving endometrial thickness, endometrial receptivity and pregnancy rate in *in vitro* fertilization.

Key words: embryo quality, endometrial thickness, fragmentation, GnRH agonist, pregnancy.

Introduction

Embryo fragmentation is a common phenomenon, and is used in most embryo scoring systems for selecting embryos for transfer in *in vitro* fertilization (IVF). Several studies have shown that increased embryonic fragmentation in human *in vitro* fertilization might have a relationship with endotoxin contamination [1], high reactive oxygen species [2] or low total antioxidant levels in culture media [3]. Other factors that

might influence embryonic fragmentation include apoptosis [4], serum nitric oxide [5], human follicular fluid levels of high-density lipoprotein cholesterol and apolipoprotein AI levels [6], follicle size [7], absence of a cytoplasmic halo [8], telomere length in human eggs [9], embryos derived from the andrological infertility group [10], polyspermic embryos [11], number of oocytes and embryos and age [12].

A recent study showed that endometrial thickness was associated with clinical pregnancy only in the long GnRH agonist protocol compared with the short GnRH agonist protocol and GnRH antagonist protocol [13]. However, the effect of long-acting and short-acting GnRH agonists in IVF-ET by long protocol on endometrial thickness is still not known in detail. In addition, recent studies found that there was no significant difference in terms of clinical outcomes between long-acting and short-acting GnRH agonist protocols [14, 15].

Our objective of this study was to compare the efficacy of a long-acting GnRH agonist protocol with that of the short-acting GnRH agonist protocol with a view to embryo quality, endometrial thickness and IVF treatment outcome in human *in vitro* fertilization.

Material and methods

Subjects

We performed a retrospective comparative study of 175 fresh IVF cycles in our hospital between December 2008 and April 2011. Indications for IVF were unexplained or female factor infertility. The patients, younger than 40 years, underwent standard downregulation protocols for ovarian stimulation (99 long-acting GnRH agonist (GnRHa) administration and 76 short-acting GnRH-agonist administration). Informed consent was obtained from all patients before the fertilization treatment and the study was approved by the Clinical Ethics Committee of Zhengzhou University.

Protocol

Basal (cycle day 3) serum levels of follicle-stimulating hormone (FSH), LH, estradiol (E2), and testosterone (T) were determined before entering an IVF cycle, and the long protocol was used in all patients. In group I (n = 99), a long-acting GnRHa triptorelin (Diphereline, Ipsen Pharma Biotech) was given as a single dose for pituitary desensitization. Long-term pituitary downregulation by starting the administration of GnRHa on day 21 (1.25 mg single-dose triptorelin) or day 2 (3.75 mg single-dose triptorelin) of the preceding IVF-ET cycle was performed for patients. In group II (n = 76), downregulation was started on day 21 of the preceding IVF-ET cycle, and achieved with the short-acting GnRH agonist Decapeptyl (Ferring, Kiel, Germany) at a dose of 0.1 mg/day before human chorionic gonadotrophin (hCG) administration.

For both groups, pituitary downregulation was evaluated by both ultrasound scan (absence of ovarian cyst formation and endometrial thickness < 5 mm) and serum E2 levels (≤ 40 pg/ml) before ovarian stimulation. Human recombinant follicle-stimulating hormone (Gonal-F, Serono, Geneva, Switzerland) was administered daily with a fixed dose of 150–225 IU from day 2 or 3 of the menstrual cycle for ovarian stimulation and continued on the day of hCG injection in both groups.

Ovarian follicular growth was monitored by serial transvaginal ultrasonography, LH peak and E2 levels. Ovulation was induced with 5,000–10,000 IU of HCG when at least two leading follicles were ≥ 18 mm in maximum diameter and serum E2 was rising. On the day of HCG injection, serum LH, estradiol (E2) and progesterone (P) levels were measured. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 h after hCG administration. Embryos were graded by two professional embryologists according to the number of blastomeres and degree of fragmentation and transferred on day 2 and 3. Embryos of good quality were defined as having at least 2–4 cells on day 2, 6–8 cells on day 3, and the amount of fragmentation was less than 20%. Serum hCG was measured 14 days after embryo transfer.

Embryologic scoring was performed by one or more embryologists.

Statistical analysis

All data were analyzed using statistical package SPSS version 11.0. Continuous data are presented as mean ± standard deviation and categorical data as percentage (%).

The differences were analyzed with independent-samples t test and χ^2 test. Furthermore, multiple logistic regression was used to determine the relation between pregnancy outcomes and those factors that might potentially influence outcome. Value of $p < 0.05$ was considered to be significant.

Results

There were no significant differences in age and proportion of primary infertility between the two groups. In addition, there were no significant differences between the two groups in numbers of retrieved oocytes and metaphase II oocytes, fertilization rate, and numbers of 2PN embryos and good quality embryos. There were also no significant differences between the two groups in basal levels of serum FSH, LH, E2, and T on menstrual cycle day 3. On the day of hCG injection, there

were no significance differences in serum E2 and progesterone levels. However, serum LH concentration was significantly lower in patients treated with long-acting GnRHa compared to patients treated with the short-acting GnRHa. Long-term pituitary downregulation by starting the administration of GnRHa on day 21 (1.25 mg single-dose triptorelin) or day 2 (3.75 mg single-dose triptorelin) of the preceding IVF-ET cycle was performed for patients, and there was no significant difference in endometrial thickness between the two protocols of long GnRH agonist ($p > 0.05$). Patients in the long-acting GnRHa group had a thicker endometrium on the day of hCG prior to oocyte retrieval and higher pregnancy rates (59.60% vs. 43.42%, $p < 0.05$) than those of patients in the short-acting GnRHa group (Table I).

The percentages of high quality embryo in the long-acting GnRHa group (group 1) and short-acting GnRH group (group 2) were 63.16% and 66.26%, respectively, and there was no significant difference between the two groups ($p > 0.05$). In addition, the percentages of high quality embryos in the secondary infertility group and primary infertility group were 63.47% and 66.86%, respectively, and there was also no significant difference between the two groups ($p > 0.05$). At 16–18 h after insemination, the zygote distribution was 3.07% 0PN, 5.49% 1PN, 85.15% 2PN and 6.29% 3PN. For the pronuclei of fertilized oocytes, the rate of high quality embryos of the 3PN group was significantly higher than those of the 1PN and 0PN groups. Furthermore, there was a significant difference between the rates of high quality embryos of the 2PN and 3PN groups. The rate of high quality embryos of 2PN fertilized oocytes colliding with the bottom surface of petri dishes and teased apart using fine needles in the degranulation process was lower than that of the rest of 2PN fertilized oocytes (Table II).

In the logistic regression model, type of infertility and endometrial thickness were significantly associated with pregnancy outcome. The pregnancy rate was significantly improved in secondary infertility versus primary infertility. In addition, the pregnancy rate improved as endometrial thickness increased. Estimated odds ratio (OR) for successful pregnancy with each additional millimeter of endometrial thickness was 1.493. However, age was not negatively associated with pregnancy outcome. Furthermore, the number of transferred good quality embryos per cycle and long-acting GnRH agonist did not independently increase the pregnancy rate (Table III).

Table I. General characteristics of patients treated with the short- and long-acting GnRH agonist ovarian stimulation protocols

Variable	Short-acting	Long-acting	Value of *p*
Female age [years]	31.62 ±4.48	30.92 ±3.81	NS
Proportion of primary infertility (%)	25 (19/76)	35.35 (35/99)	NS
Retrieved oocytes (*n*)	14.54 ±8.89	16.22 ±7.52	NS
Fertilization rate (%)	67.33	65.75	NS
Metaphase II oocytes (*n*)	12.82 ±8.23	14.72 ±6.72	NS
2PN (*n*)	9.79 ±6.59	10.67 ±5.55	NS
Good quality embryos (*n*)	6.49 ±4.52	6.74 ±4.50	NS
Number of good quality embryos transferred (*n*)	2.09 ±0.59	2.02 ±0.54	NS
Cycle day 3			
FSH [mIU/ml]	5.89 ±2.94	5.95 ±3.15	NS
LH [mIU/ml]	5.21 ±3.90	4.53 ±3.78	NS
E2 [pg/ml]	65.86 ±39.96	58.94 ±32.05	NS
PRL [ng/ml]	31.35 ±66.78	32.25 ±72.38	NS
T [ng/ml]	0.98 ±3.98	0.57 ±0.95	NS
On the day of HCG administration			
E2 [pg/ml]	3446.85 ±1508.13	3427.64 ±1542.26	NS
P [ng/ml]	1.94 ±1.53	1.68 ±0.54	NS
LH [mIU/ml]	2.53 ±3.39	1.21 ±1.13	< 0.01
Endometrium thickness [mm]	9.64 ±1.97	10.79 ±2.62	< 0.01

Table II. Association of IVF outcome predictors and embryo quality

Variable	Good quality (*n*)	Not-good quality (*n*)	Rates of good quality (%)
Controlled ovarian stimulation			
Long-acting GnRH-a	667	389	63.16
Short-acting GnRH-a	493	251	66.26
Infertility			
Secondary (2PN)	813	468	63.47
Primary (2PN)	347	172	66.86
Abnormal embryos			
0PN	39	26	60.00
1PN	39	77	33.62
3PN	104	29	78.20*
Normal embryos			
2PN	1160	640	64.44*
a + b	11	21	34.38

**p < 0.05 was considered statistically significant, a – 2PN fertilized oocytes colliding with bottom surface of Petri dishes in degranulation process, b – 2PN fertilized oocytes teased apart using fine needles in degranulation process*

Table III. Results of the final logistic regression model

Variable	*B*	SE	Wald	d*f*	OR	95% CI	Value of *p*
Female age	–0.051	0.046	1.224	1	0.950	0.868–1.040	0.269
Infertility							
Secondary versus primary	1.013	0.461	5.929	1	2.753	1.218–6.222	0.015
Endometrail thickness	0.391	0.086	20.550	1	1.478	1.248–1.750	0
Number of transferred good quality embryos per cycle	0.236	0.459	0.264	1	1.226	0.515–3.111	0.607
Protocol							
Long acting vs. short-acting	0.414	0.351	1.391	1	1.512	0.760–3.008	0.238

Discussion

Embryo quality is known to be associated with pregnancy outcomes. Clinical data suggested that female age affected embryo quality [16] and was one of the most important predictive factors of success in IVF [17]. A recent systematic review and meta-analysis also showed female age to be a predictor of pregnancy and they also suggested that other predictive factors, especially embryo factors, should also be focused on [18]. In this study, we studied factors relative to embryo quality and pregnancy rate. Unlike the previous study which showed that female age had a positive association with increased proportion of good quality embryos [16], our data showed that there was no significant difference in the percentage of good quality embryos between lower (less than 35 years) and higher (35 to 39 years old) age groups. In addition, our data showed that age did not independently increase the pregnancy rate in the logistic regression model, which was different from the previous study [18]. Not including patients aged more than 40 years in our study might be a possible reason for the variation.

The number of oocytes retrieved is considered to be an important prognostic variable. A recent large national database involving 400,135 IVF treatment cycles was analyzed. The data showed a non-linear relationship between the number of eggs and live birth rate, and suggested that the number of eggs was a robust surrogate outcome for clinical success [19]. Basal follicle number was proven to be associated with the numbers of oo-

cytes collected [20]. In addition, a recent study showed that maintenance or replacement of the type of GnRH analogue (agonist or antagonist) in repeated ovarian stimulation cycles also could influence the numbers of oocytes collected [21]. However, out data showed that there was no significant difference in numbers of retrieved oocytes between the long-acting GnRHa group and short-acting GnRH group ($p > 0.05$).

A previous study showed that the pregnancy rate increased significantly with the number of good quality embryos that were transferred [22]. However, our data showed that the number of transferred good quality embryos per cycle did not independently increase the pregnancy rate in the logistic regression model, and it was similar to a previous study which showed that there was no significant difference in pregnancy rate between groups with 2 good quality embryos transferred and 3 good quality embryos transferred [23].

Most early studies showed that there was no significant effect of endometrial thickness in the outcome of IVF/ICSI [24–27]. However, some recent studies suggested that endometrial thickness was associated with clinical pregnancy [28–30]. Our data showed that the pregnancy rate improved as endometrial thickness increased and the long-acting GnRH agonist did not independently increase the pregnancy rate in the logistic regression model, which also supported endometrial thickness as a positive predictor of clinical pregnancy. In addition, a recent study showed that endometrial thickness was associated with clinical pregnancy only in the long GnRH agonist protocol compared with the short GnRH agonist protocol and GnRH antagonist protocol [13]. However, the effect of long-acting and short-acting GnRH agonist in IVF-ET by long protocol on endometrial thickness is still not known in detail. Our data revealed no significant difference between the long-acting GnRHa group (group 1) and short-acting GnRH group (group 2) in embryo quality. Furthermore, endometrial thickness and clinical pregnancy rate were significantly lower in the short-acting GnRHa group compared with the long-acting GnRHa group, which suggested that the long-acting GnRHa group showed significantly higher endometrial thickness. To our knowledge, few studies have evaluated the beneficial effects of long- and short-acting GnRHa on endometrial thickness.

Previous data suggested that LH might act directly on the uterus by binding to the LH/HCG receptor [31]. However, little is known regarding the potential effects of LH on endometrial functional activation in *in vitro* fertilization [32]. Our data showed that serum LH concentration on the day of HCG injection was significantly lower in patients treated with long-acting GnRHa compared to patients treated with short-acting GnRHa, which suggested that lower serum LH levels in the long-acting GnRHa group might play a beneficial role for endometrial receptivity.

Meiotic and fertilization errors can result in 0PN, 1PN and 3PN zygotes which should not be transferred because the resultant embryos may be aneuploid [33]. Although there were genetic disorders in each of 0PN, 1PN and 3PN zygotes, the rate of high quality embryos of the 3PN group was higher than those of 0PN and 1PN zygotes, and we firstly found that the type of abnormal fertilization might also affect human embryo quality. A recent study showed that the frequency of fragmentation was increased in polyspermic porcine embryos. However, the rate of high quality embryos of the 3PN group was even higher than that of the 2PN group in our study, which indicated that variation between two different species might be the possible reason for different results. In addition, gentle manipulation and avoidance of teasing apart using needles in the degranulation process were also important because rough and/or excess handling might significantly increase embryonic fragmentation in human *in vitro* fertilization.

In conclusion, this work suggests that types of agonist protocol and infertility may not affect embryo quality. Endometrial thickness and type of infertility may be positive predictors for clinical pregnancy, but the key finding is that the long-acting GnRHa protocol may be an effective method of improving endometrial thickness, endometrial receptivity and the pregnancy rate in human *in vitro* fertilization.

Acknowledgments

The present study was supported in part by the Health Department of Henan Province, China (Grant No. 4117) and Foundation of He'nan Educational Committee (Grant No. 12B320025).

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