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Advanced glycation end product concentrations in follicular fluid of women undergoing IVF/ICSI with a GnRH agonist protocol

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KEY MESSAGE

Follicular fluid advanced glycation end-product concentrations were inversely associated with the number of oocytes retrieved, number of fertilized oocytes, number of high-quality embryos, fertilization rate and high-quality embryo rate, adjusted for potential confounders.

A B S T R A C T

The accumulation of advanced glycation end products (AGE) is associated with ovarian dysfunction. This study examines whether the accumulation of AGE in follicular fluid affects ovarian responsiveness and embryo quality during IVF/intracytoplasmic sperm injection (IVF/ICSI) with a gonadotrophin-releasing hormone (GnRH) agonist protocol. The levels of AGE in follicular fluid were measured in 127 women undergoing IVF/ICSI in GnRH agonist cycles. Plasma hormones were also measured. Embryos were graded using standard approaches. There were inverse associations between follicular fluid AGE concentration and number of oocytes retrieved, number of fertilized oocytes, number of high-quality embryos, fertilization rate and high-quality embryo rate, adjusted for potential confounders. AGE concentration in follicular fluid was significantly higher in women with an ovarian response below the target (<7 oocytes) compared with those reaching the target (7–15 oocytes) or above the target (>15 oocytes). The cut-off value of 15.3 μg/ml for follicular fluid AGE showed 84.6% sensitivity and 55.5% specificity in evaluating the response to ovarian stimulation as below the target. The results suggest that ovarian responsiveness and embryo quality are related to intraovarian exposure to AGE.

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Introduction

A key element in achieving successful pregnancy is to obtain a number of developmentally competent oocytes during assisted reproductive technologies. The development of competent follicles and oocytes intimately depends on the follicular microenvironment (Dumesic et al., 2015; Meldrum et al., 2016; Merhi, 2014). Several investigations have shown that advanced glycation end products (AGE) can accumulate in the ovary (Diamanti-Kandarakis et al., 2007; Hansen et al., 2003; Matsumine et al., 2008). AGE are toxic products of the non-enzymatic modification of proteins, lipids and nucleic acids by glucose (Thomas et al., 2005). They are usually formed slowly under physiological conditions. However, ageing, oxidative stress, hyperglycaemia, insulin resistance, obesity and hypoxia may accelerate the formation of AGE (Yamagishi et al., 2005). Besides endogenous AGE, food preparation at high temperatures facilitates the formation of AGE (Goldberg et al., 2004). The dietary intake of these glycotoxins also contributes to the accumulation of AGE in tissues and body fluids (Diamanti-Kandarakis et al., 2007). AGE act by binding to the extracellular matrix (receptor independent) or to an AGE receptor, RAGE (receptor dependent) (Merhi, 2014). AGE induce the crosslinking of key molecules in the extracellular matrix, causing malfunction of key molecules (Merhi, 2014). The interaction of AGE–RAGE triggers signalling pathways that in turn activate the MAP kinases and NF-κB, promoting the development of a pro-inflammatory state and generation of reactive oxygen species (ROS) (Huang et al., 2013; Merhi, 2014; Nejabati et al., 2017; Sharma et al., 2010). Thus, the accumulation of AGE in ovaries may change the ovarian follicular microenvironment and affect the response to ovarian stimulation and embryo quality during assisted reproductive technologies.

The present study examined whether the accumulation of AGE in follicular fluid affects ovarian responsiveness and embryo quality during IVF/intracytoplasmic sperm injection (IVF/ICSI) with a gonadotrophin-releasing hormone (GnRH) agonist protocol.

Materials and methods

Subjects

This study included 127 women undergoing IVF/ICSI treatment at The Centre of Reproductive Medicine, Jinling Hospital, from February 2015 to December 2016. The study was approved by the Ethics Committee of Jinling Hospital on 7 April 2015 (reference number 2015NZKY-003–01), and informed consent was obtained from all participants. Inclusion criteria consisted of women with the following infertility diagnoses: ovarian dysfunction, tubal factors, idiopathic factors, male factors, and both male and female factors. We excluded women with polycystic ovary syndrome (PCOS) as diagnosed by the Rotterdam criteria (Balen et al., 2003), because PCOS women are known to have abnormally elevated levels of systemic and tissue AGE (Diamanti-Kandarakis et al., 2005, 2007). Other exclusion criteria included liver, kidney, heart or blood vessel diseases, and untreated or insufficiently corrected endocrinopathies. On day 3 of the spontaneous menstrual cycle, clinical examinations including body mass index (BMI), blood parameters and hormone measurements were carried out between 08:00 h and 12.00 h, after 12 h of fasting. BMI was calculated as body weight in kilograms (kg) divided by body height in metres squared (m^2). After pituitary down-regulation, all patients underwent a transvaginal ultrasound examination to assess the number of antral follicles on the day before stimulation.

Cycle monitoring, ovarian stimulation and collection of follicular fluid

Patients underwent IVF/ICSI using a short-acting long protocol for ovarian stimulation by a GnRH agonist (Duan et al., 2017; Mao et al., 2014). In the mid-luteal phase, down-regulation was performed with triptorelin (0.05 mg, subcutaneous injection once a day, Ferring GmbH, Germany) until administration of recombinant human chorionic gonadotrophin (rHCG). Down-regulation was achieved when serum LH was <5 IU/l, serum oestradiol was <183.5 pmol/l and endometrial thickness was <5 mm. Then, ovarian stimulation was accomplished using recombinant FSHα (rFSHα, Gonal-F®; MerckSerono, Geneva, Switzerland). When at least two follicles reached a diameter of 18 mm, follicular maturation was triggered with an injection of 5000–10,000 IU of rHCG. After 35–36 h, transvaginal ultrasound-guided oocyte retrieval was performed. After selection of oocytes, pooled follicular fluid from mature follicles >18 mm was centrifuged for 10 min at 800*g*. The supernatant was collected and stored at –80°C until analysis.

IVF or ICSI was performed 40–42 h after rHCG administration. ICSI was performed when the partner had severe male infertility with a sperm count $<5 \times 10^6$ per ml and/or motility <10%, normal morphology <1%. Oocytes were considered to be fertilized when two pronuclei were observed at 17–18 h following insemination or ICSI. Morphological high-quality embryos were identified on day 3 as those having at least six regular blastomeres and <20% anucleated fragments (Van Royen *et al.*, 1999).

Measurements of AGE

The level of AGE in follicular fluid was measured in duplicate by enzyme-linked immunosorbent assay (ELISA) kits (Cusabio Biotech Co. Ltd, Wilmington, DE, USA) according to the manufacturer's protocol. The within- and between-assay coefficients of variation were 9.9% and 10.9%, respectively.

Statistical analysis

Continuous variables were expressed as means ± SD. Data distribution was assessed using the normal Quintal plot. Variables not normally distributed were reported as medians with interquartile ranges. The unpaired t-test was used to assess statistical significance. Correlations between variables of interest were analysed using Spearman's test. Univariate linear regression was used to estimate the value of independent variable AGE in follicular fluid. Multiple regression analysis was then applied to estimate the independent relationship between the level of AGE in follicular fluids and the number of oocytes retrieved, fertilization rate and embryo quality, with an adjustment for potential confounders. The comparison of continuous variables of the three responder subgroups was performed using the one-way ANOVA model. Receiver operating characteristic (ROC) curves were used to evaluate the feasibility of using the level of AGE in follicular fluid to assess the outcomes of stimulation. ROC curves were made based on a simple logistic regression model using the level of AGE in follicular fluid as a covariate. A *P*-value <0.05 was considered to be statistically significant. All analyses were performed using Empower (R) (www.empowerstats.com, X&Y Solutions, Inc., Boston, MA, USA) and R (www.R-project.org).

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