Association Between Advanced Glycation End Products and Oocyte Developmental Abnormalities in Patients with Tubal Factor Infertility: Postprint

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Abstract

Objective: To investigate the effects of advanced glycation end products (AGEs) on oocyte quality and granulosa cell function.

Methods: Patients undergoing in vitro fertilization (IVF) due to tubal factors between February and June 2015, aged <35 years, with 8 retrieved oocytes, and stimulated using the long-acting long protocol were selected. All follicular fluid and granulosa cells were collected during oocyte retrieval and cryopreserved. A total of 19 cases were enrolled, with those having a high-quality embryo rate >35% assigned to Group A (n=10) and those with a high-quality embryo rate <35% assigned to Group B (n=9). Enzyme-linked immunosorbent assay (ELISA) sandwich antibody method was used to detect AGEs and reactive oxygen species (ROS) levels in follicular fluid on the day of oocyte retrieval, and Real-time PCR was used to detect RAGE m RNA expression in granulosa cells to analyze differences between the two groups.

Results: There were no statistically significant differences in general patient data between the two groups. Statistically significant differences were observed in the mean number of high-quality embryos (5.2±2.34 vs 1.50±1.08, P=0.001) and high-quality embryo rate (60.27%±12.48% vs 21.48%±14.02%, P=0.000). Follicular fluid reactive oxygen species levels (0.82±0.28 ng/m L vs 1.11±0.27 ng/m L, P=0.000) also showed statistically significant differences between the groups, while follicular fluid AGEs levels (0.33±0.13 g/m L vs 0.35±0.13 g/m L, P=0.462) showed no statistically significant difference. Granulosa cell RAGE levels (1.55±0.62 pg/m L vs 3.75±1.75 pg/m L, P=0.005) demonstrated statistically significant differences.

Conclusion: Oxidative stress status may be the cause of poor oocyte quality in young infertile patients with tubal factors, but the AGES-RAGE pathway may not be the mechanism of action.

Full Text

Preamble

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Relationship Between Advanced Glycation End Products and Oocyte Dysplasia in Patients with Tubal Factor Infertility

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Abstract

Objective: To investigate the effect of advanced glycation end products (AGEs) on oocyte quality and granulosa cell function in patients with tubal factor infertility.

Methods: Nineteen IVF patients with tubal factor infertility were enrolled between February and June 2015. All patients were under 35 years of age, retrieved 8 oocytes, and underwent controlled ovarian hyperstimulation using a long-acting long protocol. Follicular fluid and granulosa cells were collected during oocyte retrieval and cryopreserved. Patients were divided into two groups based on high-quality embryo rate: Group A (n=10) with >35% high-quality embryos and Group B (n=9) with <35% high-quality embryos. Follicular fluid AGEs and reactive oxygen species (ROS) levels were measured on oocyte retrieval day using sandwich ELISA, while granulosa cell RAGE mRNA expression was detected by real-time PCR. Inter-group differences were analyzed.

Results: No statistically significant differences were observed in general patient characteristics between the two groups. However, significant differences were found in average number of high-quality embryos (5.2±2.34 vs. 1.50±1.08, P=0.001) and high-quality embryo rate (60.27%±12.48% vs. 21.48%±14.02%, P=0.000). Follicular fluid ROS levels differed significantly between groups (0.82±0.28 ng/mL vs. 1.11±0.27 ng/mL, P=0.000), whereas follicular fluid AGEs levels showed no significant difference (0.33±0.13 g/mL vs. 0.35±0.13 g/mL, P=0.462). Granulosa cell RAGE levels differed significantly (1.55±0.62 pg/mL vs. 3.75±1.75 pg/mL, P=0.005).

Conclusion: Oxidative stress may contribute to poor oocyte quality in young infertile patients with tubal factor infertility, though the AGEs-RAGE pathway

may not be the underlying mechanism.

Keywords: advanced glycation end products; granulosa cells; follicular fluid; oxidative stress

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Since its clinical introduction in 1978, in vitro fertilization-embryo transfer (IVF-ET) technology has achieved remarkable progress, with clinical pregnancy rates increasing from approximately 5% to over 50% through continuous improvements in clinical and laboratory techniques. While advanced maternal age is widely recognized as a critical factor affecting oocyte quality and pregnancy outcomes, significant variations in oocyte and embryo quality persist even among young patients (<35 years) with normal ovarian function undergoing optimal stimulation protocols and standardized laboratory conditions. Notably, some young patients produce no transferable embryos, and even when transfer is possible, pregnancy rates remain disappointingly low.

Oxidative stress represents a major contributor to impaired oocyte development and subsequent embryonic dysfunction. Advanced glycation end products (AGEs) induce intracellular reactive oxygen species (ROS) production through interaction with their receptor (RAGE), potentially participating in the pathogenesis of oocyte developmental abnormalities. Previous investigations into the relationship between AGEs and oocyte quality have primarily focused on older women, patients with polycystic ovary syndrome (PCOS), and diabetic populations, with limited exploration of AGEs' impact on oocyte quality in young patients with tubal factor infertility. This study examines AGEs, RAGE, and ROS levels in follicular fluid and granulosa cells among this specific IVF population to elucidate their relationship with oocyte quality and subsequent embryonic development, thereby exploring the association between AGEs and unexplained oocyte dysplasia and identifying potential mechanisms underlying poor oocyte quality to inform novel therapeutic strategies for improving pregnancy outcomes.

This study was approved by the Institutional Medical Ethics Committee and Shenzhen Municipal Health and Family Planning Commission, with all participants providing informed consent prior to enrollment.

1. Materials and Methods

1.1 Patient Selection

The study enrolled IVF patients treated at our reproductive medicine center between February and June 2015. **Inclusion criteria:** tubal factor infertility, female age <35 years, retrieval of 8 oocytes, controlled ovarian hyperstimulation using a long-acting long protocol, and collection/cryopreservation of all follicular fluid and granulosa cells during oocyte retrieval. **Exclusion criteria:** endometriosis, endometrial polyps or intrauterine adhesions, uterine malformations, uterine fibroids, diminished ovarian reserve, chromosomal abnormalities

in either partner, systemic diseases, adverse obstetric history, or male factor infertility. Based on embryonic development outcomes, 19 patients were included and divided into Group A (n=10) with high-quality embryo rate >35% and Group B (n=9) with high-quality embryo rate <35%.

1.2 Sample Collection and Detection Methods

1.2.1 Follicular Fluid and Granulosa Cell Collection All collected follicular fluid was centrifuged over 45% Percoll at 1500 r/min for 15 minutes. The supernatant follicular fluid was aliquoted into EP tubes, labeled, and frozen at -70°C for subsequent analysis. Granulosa cells, which formed a white membrane at the Percoll interface, were aspirated, aliquoted into EP tubes, labeled, and frozen at -80°C until analysis.

1.2.2 Detection Methods Follicular fluid AGEs and ROS levels on oocyte retrieval day were measured using enzyme-linked immunosorbent assay (ELISA) with a sandwich antibody method. Granulosa cells were isolated using Percoll separation. Total RNA was extracted using Trizol reagent and reverse-transcribed into cDNA for real-time fluorescent quantitative PCR (Real-time PCR) of RAGE gene expression. Relative RAGE mRNA expression was calculated using the $\Delta\Delta$Ct method.

1.3 Statistical Analysis

Data were analyzed using SPSS 19.0 statistical software. Chi-square test was used for rate comparisons. Normality testing was performed on measurement data; normally distributed data between groups were compared using t-tests. Results are expressed as mean ± standard deviation. Two-sided tests were performed with $P<0.05$ considered statistically significant.

2. Results

2.1 Comparison of General Patient Characteristics

Comparison of basic patient characteristics between Groups A and B () revealed no statistically significant differences in age, duration of infertility, baseline sex hormone levels, hCG day hormone levels, number of oocytes retrieved, or 2PN fertilization rates. However, significant differences were observed in average number of high-quality embryos (5.2 ± 2.34 vs. 1.50 ± 1.08, $P=0.001$) and average high-quality embryo rate [$(60.27\pm12.48)\%$ vs. $(21.48\pm14.02)\%$, $P=0.000$].

2.2 Comparison of Follicular Fluid ROS, AGEs, and Granulosa Cell RAGE Levels

Follicular fluid ROS levels and RAGE levels in Group A were significantly lower than in Group B ($P<0.05$), whereas AGEs levels showed no significant difference between groups ($P>0.05$,).

3. Discussion

AGEs serve as a biological marker of human aging, present in numerous normal human tissues and cells, where they induce oxidative stress and inflammatory responses through direct effects or receptor binding, thereby causing tissue damage. Studies have demonstrated that elevated systemic and ovarian AGEs levels and high RAGE expression correlate closely with female infertility and adverse pregnancy outcomes, and that follicular fluid AGEs concentration correlates with ovarian response and oocyte quality in IVF cycles. It is hypothesized that AGEs may contribute to poor oocyte quality by participating in intrafollicular oxidative stress.

AGEs induce oxidative stress and inflammatory reactions through binding to RAGE, which is associated with cellular apoptosis and tissue damage. AGE-RAGE interactions are implicated in multi-organ system pathologies, including neurological, cardiovascular, immune, and musculoskeletal disorders. Prolonged AGEs exposure may cause oxidative damage to primordial follicles and ovarian stromal vasculature, promoting ROS elevation in the ovarian microenvironment during folliculogenesis. This subsequently compromises granulosa cell metabolism, antioxidant defense systems, and perifollicular vascularization, jeopardizing oocyte maturation, chromosomal integrity, and developmental competence. The decline in anti-glycation and antioxidant defense systems accompanying mitochondrial dysfunction may drive a positive feedback loop involving oxidative stress, carbonyl stress, and progressive AGEs accumulation.

Oocyte cytoplasmic maturation and meiotic competence are acquired during follicular development, with the follicular microenvironment determining oocyte developmental potential. An inappropriate follicular growth environment represents a critical factor influencing oocyte quality alterations. Studies have shown that excessive follicular fluid ROS levels may induce oocyte oxidative stress, adversely affecting oocyte maturation and subsequent embryonic development, and may represent one mechanism underlying age-related oocyte changes. Germ cells are rich in unsaturated fatty acids, making them particularly vulnerable to oxidative stress damage. ROS can directly affect intracellular calcium storage structures, disrupt calcium homeostasis, cause meiotic arrest and degradation, and subsequently trigger apoptosis.

Jinno et al. reported that AGEs concentration negatively correlates with estrogen levels on oocyte retrieval day, number of dominant follicles, oocytes retrieved, 2PN fertilization rate, and usable embryos. AGEs concentration >7.24 U/mL predicts low oocyte yield and poor pregnancy outcomes, serving as a predictor of ART outcomes beyond age and ovarian reserve. Our findings indicate that in PCOS patients, follicular fluid sRAGE and VEGF correlate with ovarian reserve capacity, and their interaction affects oocyte quality. Furthermore, PCOS patients exhibit significantly higher granulosa cell RAGE mRNA expression than age-matched non-PCOS patients, with a trend toward elevated follicular fluid AGEs levels, suggesting increased local ovarian oxidative stress

in PCOS. Pan Xiaoqin' s research demonstrated that elevated follicular fluid AGEs levels may contribute to poor ovarian response, and that increased follicular fluid AGEs upregulates granulosa cell RAGE mRNA expression, suggesting that AGEs-RAGE binding may be the primary mechanism through which AGEs affect ovarian function.

Our results show that patients with lower high-quality embryo rates had significantly higher follicular fluid ROS levels compared to those with higher rates, suggesting that excessive follicular fluid ROS-induced oocyte oxidative stress may underlie poor oocyte quality and subsequent embryonic developmental impairment. However, no significant difference in follicular fluid AGEs levels was observed between groups, while granulosa cell RAGE mRNA levels were higher in the poor-quality embryo group, suggesting that ROS elevation may not be mediated through the AGEs-RAGE pathway, though the mechanism remains unclear. These findings differ from previous reports, possibly due to differences in study populations. PCOS patients, particularly those with insulin resistance, exhibit metabolic abnormalities in C-reactive protein, sRAGE, and vascular endothelial growth factor, indicating that PCOS involves not only endocrine-metabolic dysfunction but also chronic inflammatory status, with insulin resistance potentially representing a key mechanism driving chronic inflammation and creating a vicious cycle. In contrast, our study population comprised young tubal factor infertility patients, among whom pelvic inflammatory status may represent the cause of oxidative stress, but not through the AGEs-RAGE pathway—a hypothesis requiring further investigation.

In conclusion, oxidative stress may contribute to poor oocyte quality in young patients with tubal factor infertility, though the AGEs-RAGE pathway may not be the underlying mechanism. Modulating oxidative stress status may help improve oocyte quality and achieve better pregnancy outcomes.

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Note: Figure translations are in progress. See original paper for figures.