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La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-S1-003
S1-003

Evaluation of Serum and Follicular Fluid Levels of Advanced Glycation End products (AGEs) on ICSI Outcome

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Abstract

Background: Advanced Glycation End products (AGEs) are produced by normal body physiology through non-enzymatic change of nucleic acid, protein, and lipid in hyperglycemic situation. However, thermal processing and modifications can lead to increased formation of AGEs, mainly long-term or high temperature cooking methods such as frying, roasting, and grilling. Elevated AGEs level in the body have an adverse effect on the general body health and females fertility, probably by a rise in oxidative stress and apoptosis.

Method: Sixty-six infertile females undergoing controlled ovarian hyperstimulation, their age range between 19-44 years old were included in this prospective comparative study. In the day of oocyte retrieval, collect follicular fluid and serum for subsequent measurement of AGEs by ELISA kites.

Result: Significant negative relationship was observed between the oocytes retrieved number, percentage of embryo G1, and cleavage rate with AGEs concentration in follicular fluid and serum. While significant positive correlation was observed between the numbers of GV oocytes (immature oocyte) with AGEs concentration in follicular fluid and serum.

Conclusion: There are negative effects of Advanced Glycation End products (AGEs) on the oocytes number and embryo G1, while there is positive effect of AGEs on the number of GV oocytes.

Keywords: AGEs; Glycotoxins; ICSI; Follicular fluid; Controlled Ovarian Hyperstimulation

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Citation: Ali AA, Jawad MA, Al-Hilli N (2020) Evaluation of Serum and Follicular Fluid Levels of Advanced Glycation End products (AGEs) on ICSI Outcome. Prensa Med Argent, S1-003. DOI: https://doi.org/10.47275/0032-745X-S1-003

Received: May 09, 2020; **Accepted:** May 18, 2020; **Published:** May 20, 2020

Introduction

Tow essential events in all human's life are firmly linked with the final goals of pleasure, completeness, and family integration that are obtaining of children and childbearing. It is most commonly recognized that human life reaches completeness through a child and achieve the person's need for reproduction [1]. The World Health Organization (WHO) clear the word of infertility clinically as "a reproductive system illness which is a failure to accomplish a clinical pregnancy after two years or more of unprotected regular sexual relationships" [2]. Women fecundity decrease after 30 years old, and increased infertility after 35 years [3]. Primary infertility: refers to female incapability to pass pregnancy to live birth due to recurrent spontaneous abortion and still birth. Secondary infertility describes as a state when female has previous successful pregnancy and livebirth child and now is unable to get pregnancy, either due to incapability to pass a pregnancy to a live birth or failure to become pregnant [4]. Follicular fluid (FF) is a crucial microenvironment for thegrowth and maturation of both somatic and ovarian cells. Follicular fluid substances related to cell differentiation, oocyte and embryo quality and follicle wallrupturing [5,6]. The accumulation Follicular fluid is occurred through folliculogenesis, which starts at the recruitment of primordial follicles in the cortical region of the ovary and ends in ovulation or atresia [7]. In IVF/ICSI procedures, human folliculr fluid can be obtained easily during oocytes aspiration from follicles, and considerd as a perfect bases for non-invasive testing of many biomarkers for oocytequality, fertilization ratio success, IVF/ICSI result, pregnancy percentage, and disorders of the ovary [8]. Advanced glycation end products are damaging composites that are produced through non-enzymatic alteration of nucleic acid, proteins, and lipids by sugars generating Schiff bases and Amadori product, which then after a period of weeks produce AGEs. This consequence is known as the Maillard reaction, which is a reaction between the carbonyl group of reducing sugar and the amine group of amino acids, lead to the development of dark-brown melanoidins at the end of the reaction. Flavor, dye, and smell of the food are result from this reaction [9]. Carboxymethyl-lysine and Pentosidine are well described glycotoxins (AGEs) and have been used as signs of their accumulation in different tissues [10]. There are two major sources of AGEs (Glycotoxins) for humans. The first is exogenous by dietary

intake and the second source is endogenous formation via many biologic pathways. The exogenous AGEs occur in foods under high temperature processing and the endogenous AGEs are formed under hyperglycemic condition in the body during normal metabolism [11]. AGEs actions divided in to receptor-independent (causing transformation in protein construction cause them more resistant to degradation and consequently cross-linked outcomes accumulated in cells and body tissues) or receptor-dependent AGEs, which are an immunoglobulin superfamily present in many tissues predominantly in the heart, lung, skeletal muscle, vessel wall and ovaries [12,13]. Usually throughout normal life receptor-dependent AGEs level is depressed in most organs but RAGE look augmented another time with aging, possibly due to the receptor-dependent AGEs ligands accumulation result to rise in receptor appearance [14].Attachment of receptor-dependent AGEs to ligand activating more up-regulation of the receptor causes encouragement several signaling ways which lead to the transcript of genes responsible for inflammation and apoptosis [15,16]. However, various hostile pathogenic pathways result from extra AGEs compounds levels in the tissues and circulation [17,18]. In addition to the ordinary receptor, there are other forms of receptor-dependent AGEs named soluble receptor-dependent AGEs (s-RAGE) which are external receptors found in circulating blood and can bind to their ligands (AGEs) in the circulation, thus inhibiting the negative intracellular activities of the AGE-RAGE axis. In comparison to normal AGEs receptor, soluble AGEs receptor s-RAGE is regarded as 'good' receptor because their intensities have been shown to be down regulated in hyperglycemia and the severity of some vascular complications are in reversely associated with its levels [19,20]. The objective of the present study was to evaluate the correlation between advanced glycation end products and various ICSI outcome parameters.

Patients and Methods

This prospective comparative analysis involved sixty-six in fertile females who underwent ICSI at the infertility clinic of High Institute of Infertility treatment and assisted reproductive technologies / Al-Nahrain University- Iraq from July 2019 to May 2020. All patients were exposed to complete history, general and gynecological examination, hormonal analysis, and ultra sound in cycle day two, seminal fluid analysis for male partner; these steps were clarified to the couple and established their accord. Inclusion conditions consist of: 19 - 44 years old age females, primary and secondary types of infertility, normal ovarian reserve females confirmed by normal anti Müllerian hormone and normal antral follicle count, which are done normally as preparatory steps before each protocol, male factor infertility excluding non-obstructive azoospermia and unexplained infertility. While exclusion conditions consist of: Females have low ovarian reserve, male with non-obstructive azoospermia, and patient with endocrine dysfunction for instance thyroid dysfunction and diabetes mellitus [21].

Key Steps in the ICSI

ICSI procedure is a multiple steps process that contains controlled ovarian hyper stimulation, ovulation Triggering, semen collection, oocytes retrieval and grading, fertilization by ICSI, embryos grading, embryo transfer and lacteal phase support [22].

Controlled Ovarian Hyper Stimulation (COH)

Two types of COH protocols are involved in this study:

GnRH antagonist protocol: The antagonist protocol (flexible type) was used in 62 infertile females. (rFSH) (Merck Sereno, Switzerland) was started from 2nd or 3rd days of the menstrual cycle at different dosages according to female's age, previous response to ovarian stimulation drugs and BMI. When leading follicle reach 12-14 mm in diameter, then pituitary down regulation was done by Cetrotide subcutaneous injection (Cetrorelix acetate injection 0.25 mg: Cetrotide®, Merk, Switzerland) / day was added and continue to the day of ovulation triggering [23]. Short and early follicular antagonist protocol (sandwich protocol):In selected cases such as poor responder or old age females, specific GnRH antagonist protocol used in which Cetrotide was given 0.25 mg / day from cycle day 2 for 3 days in order to elongate the follicular phase and increase recruitment. When leading follicle reach 12-14 mm take Cetrotide again 0.25 mg /day until ovulation triggering day. This protocol was used for four patients [24].

Both E2 concentration measurement and vaginal ultrasound are used to observe the COH.

Ovulation Triggering

Ovulation triggered by either intramuscular injection of hCG 10000 IU (Pregnyl®, Organon, Holland) or by subcutaneous route (Ovitrelle®, 250 microgram Merk, Switzerland), when at least 2 or more follicles reach 18mm were found [25]. Decapeptyl® was used in case of Antagonist protocol for patient with high risk of OHSS [26]. The co-administration of GnRH agonist (Decapeptyl®) and hCG (Pregnyl®, Organon, Holland) to induce ultimate oocyte growth (dual trigger) was used for females with high percentage of immature oocytes and to improve ICSI outcome were applied 34-36 h prior to oocytes retrieval [27].

Semen Collection

Before Oocytes retrieval, fresh semen sample was collected and prepared for sperm extraction. The semen was collected after a period of 3-5 days of sexual abstinence by masturbation [28]. Testicular sperm extraction (TESE) is a surgical sperm retrieval technique used in infertility treatment for men with obstructive azoospermia [29].

Oocytes Retrieval and Grading

Oocytes retrieval under ultrasound control was performed 34-36 hours later to the ovulation triggered day just prior the rupture of follicles. The oocytes and follicular fluid were aspirated by transvaginal ultrasound guided oocyte retrieval using a very fine double lumen aspiration needle. Oocytes at retrieval were categorized according to its maturity into Metaphase II, Metaphase I and Germinal Vesicle and this was done after denudation [30].

Fertilization (ICSI)

The aspirated follicles were kept 1-2 hours in CO_2 incubator at 37°C. Latter, denudation and grading at Laminar Flow Cabinet was done to all oocytes. The mature eggs (MII) were selected and held with a specialized pipette. A very fine, sharp, and hollow needle was used to immobilize and pick up a single sperm. Subsequently the needle was carefully inserted through the shell of the egg into its cytoplasm at the 3 o'clock position. This procedure was done with the aid of Nikon ICSI Microscope. Then it was kept in the CO_2 incubator at 37° C waiting for result of cell division and fertilization [31]. The proportion of fertilized oocytes to the total number of MII oocytes that undergoing ICSI then the result multiplied to 100was estimated as fertilization rate [32].

Embryo Grading

After ICSI the fertilization and embryo quality were assessed by the

embryologistdepend on morphological character such as the quantity and quality of the blastomere, blastomere symmetry and fragmentation degree). On third day after fertilization embryo quality was assessed as in the following:

- Grade I: Top-quality embryos have equally sized blastomeres and less than 10% fragmentation.
- Grade II: Embryos with unequally sized blastomeric and less than 20% fragmentation.
- Grade III: Embryos with unequally sized blastomere, more than 20% fragmentation, and without cleavage arrest (i.e. cleavage must have occurred within the last 24 h) [33].

The proportion of cleaved embryo to the total number of fertilized oocytes (2PN) then the result multiplied to 100 was estimated as Cleavage rate [34].

Embryo grade was estimated as the proportion of the total number of embryos in each grade (GI, GII, and GIII) to the total number of embryos ×100.

Embryo Transfer

The fertilized embryo put into the uterine cavity under pelvic ultrasound guidance and by an intrauterine catheter. The quantity of transferred embryos was determined according to the embryo quality, patient age, numbers of previous attempt, and the patient's clinical history [35].

Luteal Phase Support

Maintenance of the luteal phase by either progesterone suppository (Cyclogest® 400 mg two times daily: Cox Pharmaceuticals, Barn staple, UK), or progesterone gel (Crinone® 8%, MERK) was started from day of oocyte retrieval until a pregnancy test. Pregnancy test was done 14 days after embryo transfer [36].

Collection of Serum and Follicular Fluid

Blood samples were obtained on day of ova pick up and left in a gel tube. The blood was centrifuged for about 10 minutes at 5000 rpm to separate the serum. Follicular fluid was obtained from the first retrieved follicle to avoid contamination of blood and media used during aspiration, collected in a plane tube, and then centrifuged for 10 min at 3000 rpm. Both serum and follicular fluid were stored in a sterile Eppendorf tube labeled with patient's number and name. Samples then refrigerated at (-40°C) until the time of examination. ELISA test was used to measure the advanced glycation end products in follicular fluid and serum [37].

Statistical Analysis

Statistical analysis was performed by using statistical package of science (SPSS); version 25.0 and Microsoft excel worksheet 2010. Numeric variables were expressed as mean ± standard deviation while nominal variables were expressed as number and percentage. The significance of the differences between values was considered statistically significant at the level of $p < 0.05$. The numerical data analysis was done by using correlation test ''correlation coefficient'' (r) which ranges from ((-1) to (+1)). If r = - ve (inverse association), if r = +ve (positive association) and if r = 0.00 (no association).

Results

The demographic features and Basal hormone level of infertile females enrolled in the present study are shown in Table 1, which expressed as either Mean ± Standard Deviation (SD) or percentage.

The correlation of AGEs concentration in follicular fluid and serum with ICSI outcome parameters illustrates in Table 2.

Table 1: Patients' demographic data and hormonal level.

Variable		Results
Age (years)	Mean ± SD	29.4± 5.9
BMI (kg/m²)	Mean ± SD	28.2±5.3
Duration of infertility (years)	Mean ± SD	6.4±3.8
Infertility types %	Primary infertility	63%
	Secondary infertility	37%
NO. of IVF trials %	First trial	73%
	Repeated IVF trials	27%
D2 FSH level(IU/ml)	Mean ± SD	7.3±3.1
D2 LH level (IU/ml)	Mean ± SD	4.6±2.1
D2 TSH level (m IU/ml)	Mean ± SD	1.7±0.7
D2 E2 level(pg./ml)	Mean ± SD	37.2 ± 17.3
D2 PRL level (ng/ml)	Mean ± SD	20.6 ± 10.9
E2 level at the triggering day (pg./ml)	Mean ± SD	1522.6 ± 323.74

Table 2: Correlation between the advanced glycation end products with ICSI outcome parameters.

Parameters	AGEs-F		AGEs-S	
	R	P-value	R	P-value
*Total oocyte No.	-0.8	0.03*	-0.7	0.04*
MII	0.01	0.5	0.01	0.3
MI	0	0.8	0.01	0.8
*GV	0.3	0.01*	0.2	0.01*
*G1	-0.7	0.003*	-0.2	0.02*
G2	0	0.9	-0.2	0.7
G3	0.2	0.3	0.1	0.9
Fertilization rate	-0.06	0.3	-0.08	0.2
*Cleavage rate	-0.5	0.002*	-0.6	0.005*
Pregnancy rate	-0.05	0.2	-0.03	0.3

- There was significant negative correlation was observed between the total quantity of oocytes retrieved and AGEs level in follicular fluid (p value=0.03, r=-0.8) and serum (p value=0.04, r=-0.7).
- There was Significant positive correlation was observed between the t number of GV and AGEs concentration in follicular fluid (p value=0.01, r=0.3) and serum (p value=0.01, r=0.2).
- There was Significant negative correlation was observed between the number of G1 embryo and AGEs level in follicular fluid (p value=0.003, r=-0.7) and serum (p value=0.02, r=-0.2).
- There was Significant negative correlation was observed between the cleavage rate and AGEs level in follicular fluid (p value=0.002, r=-0.5) and serum (p value=0.005, r=-0.6).
- There was no significant correlation was detected between the MII, MI, G2 embryo, G3 embryo, Fertilization ratio, and pregnancy percentage.

Discussion

In the latest years, novel actors have been implicated in the pathogenesis of infertility, the advanced glycation end products. Glycation is a natural non-enzymatic reaction of reducing sugars with free amino groups of nucleic acid, proteins, and lipids that produce Amadori products. The Amadori products submit to a multiple permanent dehydration and rearrangement reactions that produce

AGEs. Increased levels of AGEs have been noticed in the serum and ovary of women affected by infertility [38]. Moreover, serum AGEs associate positively with hyperglycemia, aging and issues associated with obesity such as dyslipidemia, hyperglycemia and insulin resistance in patients undertaking in vitro fertilization (IVF). Many researches exposed that a risen in the AGEs quantity, could disturb the ovary microenvironment possibly compromising oocyte quality and quantity, formation of healthy embryos and ultimately cleavage rate [39]. These finding agree with the result of the present study, which conclude that there was significant negative correlation (inverse relationship) between the percentage of Embryo G1, Cleavage rate and the total number of retrieved oocyte with AGEs concentration in follicular fluid and serum. While there was significant positive correlation between the percentage of Germinal Vesicle oocytes (immature oocyte) and AGEs amount in follicular fluid and serum. There was no significant correlation between the MII, MI, embryo G2, embryo G3, the percentage of fertilization rate, and pregnancy rate with AGEs amount in follicular fluid and serum. These findings disagree with previous study, which stated that AGEs accumulation in serum and follicular fluid have adverse effect on IVF outcomes such as (poor oocytes quality, lower fertilization percentage, poor embryos quality, and lower pregnancy ratio) [35].

Conclusion

Higher levels of AGEs, which are a reflection of unhealthy life style & dietary habits, have significant correlation with certain IVF parameters.

Acknowledgment

We would like to acknowledge the High Institution of Infertility Diagnosis and Assisted Reproductive Technologies/ Al- Nahrain University/ Baghdad, Iraq.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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