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ARTICLE

Intrafollicular soluble receptor for advanced glycation end products (sRAGE) and embryo quality in assisted reproduction

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Abstract The developmental potential of human embryos has important implications in assisted reproduction and depends, among other factors, on oocyte competency. The receptor for advanced glycation end products (RAGE) is a member of the superfamily of immunoglobulin cell-surface molecules that are constitutively expressed during embryonic development. RAGE is down-regulated in homeostasis in adult life. This study measured the concentration of soluble RAGE (sRAGE) in follicular fluid obtained from the leading follicle after ovarian stimulation of 54 women undergoing intracytoplasmic sperm injection. Corresponding embryos and sRAGE concentrations in follicular fluid were evaluated and correlations were investigated by multi-adjusted regression analysis. High intrafollicular sRAGE concentrations predicted poor-quality embryos ($n = 45$, OR = 0.986; $P = 0.026$), adjusted for patient age, body mass index and oocyte quality, showing an inverse association between intrafollicular sRAGE concentrations and embryo development.

KEYWORDS: embryo quality, follicular fluid, ICSI, sRAGE

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Introduction

Glycation is the non-enzymic addition of sugars to proteins and lipids. Advanced glycation end products (AGE) are a heterogeneous group of non-enzymically modified proteins. Protein glycation was originally thought to tag senescent proteins for degradation by macrophages. Also, defective clearance of AGE-modified proteins was believed to increase with ageing, and accelerated AGE formation occurs in diabetes and atherosclerosis (Vlassara et al., 1992).

The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily located in the membranes of immune, endothelial, epithelial and central nervous system cells (Schmidt et al., 2000). Binding of AGE to RAGE triggers signalling of the MAP kinase and NF-κB pathways (Bucciarelli et al., 2002), and glycation is responsible, via RAGE, for increased oxidative stress and inflammation caused by the formation of reactive oxygen species. RAGE is frequently associated with pro-inflammatory responses in which AGE, the ligands of RAGE, accumulate and may contribute to immune disorders, cardiovascular diseases, diabetes, Alzheimer's disease and cancer. In addition to activating pro-inflammatory responses, increased RAGE concentrations down-regulate cellular defence mechanisms (Bierhaus et al., 2005).

RAGE is constitutively expressed during embryonic development; however, it is down-regulated in adult life except in the skin and lungs (Brett et al., 1993). Thus, RAGE is usually expressed at low levels during homeostasis (Neeper et al., 1992), but its expression increases in diseases characterized by the up-regulation and accumulation of its ligands, namely AGE. In numerous cell types, the main mechanism of RAGE action is through changes in signal transduction induced after AGE binding (Ramasamy et al., 2009).

A circulating soluble isoform of RAGE (sRAGE) has been identified in humans. This isoform contains the extracellular domain of RAGE but is missing the cytosolic and transmembrane domains. sRAGE is believed to act as a decoy that binds to pro-inflammatory ligands and prevents their access to the cell membrane (Hudson et al., 2008). In addition to the relationship between sRAGE and pro-inflammatory diseases (Maillard-Lefebvre et al., 2009), sRAGE serum concentrations have been associated with both physiological and pathological states during pregnancy, such as preterm labour and pre-eclampsia (Germanova et al., 2010).

Both soluble and intact forms of RAGE have been studied in polycystic ovarian syndrome (PCOS). Higher serum concentrations of AGE and of full-length RAGE were found in young normoglycaemic PCOS patients relative to healthy controls (Diamanti-Kandarakis et al., 2005). The same group has demonstrated that AGE and RAGE expression was higher in PCOS than normal ovarian tissue; AGE was localized in the follicular cell layers (i.e. the granulosa and theca layers) and luteinized cells, and RAGE stains were more pronounced in granulosa cells, theca interna, endothelial and stromal cells on PCOS patients (Diamanti-Kandarakis et al., 2007).

sRAGE concentrations in the follicular fluid of assisted reproduction patients were higher in women who had successful pregnancies following IVF relative to those who did not conceive. However, serum sRAGE concentrations showed a negative correlation with the number of stimulated follicles and retrieved oocytes (Malickova et al., 2010). Fujii and Nakayama (2010) studied AGE, sRAGE and vascular endothelial growth factor (VEGF) in plasma and follicular fluid of patients undergoing IVF as a function of patient age. Follicular VEGF was higher in older patients, but follicular sRAGE was not significantly different in young or older women. The authors suggested a correlation between the regulation of RAGE—VEGF and reproductive dysfunction in ageing women (Fujii and Nakayama, 2010).

Recently, other investigators have evaluated AGE (e.g. toxic AGE, pentosidine and carboxymethyl lysine) in blood and follicular fluid of patients undergoing assisted reproduction treatment and observed that accumulation of intrafollicular pentosidine (a product of AGE) was highly correlated with poor follicular and embryonic development and a lower probability of a pregnancy (Jinno et al., 2011).

The aforementioned studies have established that sRAGE has a role in the follicular environment and that higher sRAGE but lower AGE concentrations could be associated with better IVF outcomes. There are no studies regarding intrafollicular sRAGE and its connection to embryo development. The aim of the present study was to measure sRAGE concentrations in follicular fluid samples obtained from leading follicles of women undergoing intracytoplasmic sperm injection (ICSI) cycles and test whether sRAGE concentrations correlated with the quality of the corresponding oocytes and embryos.

Materials and methods

Patients

This study was approved by the Ethics Committee of the Federal University of Sao Paulo (protocol 1699/06, 15 June 2007) and informed consent was obtained from all participants. Between January 2007 and December 2008, 132 patients undergoing ICSI cycles at the Fertility-Assisted Fertilization Centre, Sao Paulo, Brazil, were prospectively enrolled. Inclusion criteria were presence of both ovaries, regular menstrual cycles, body mass index (BMI) lower than 35 kg/m^2, no current infectious diseases, no uterine pathology, basal FSH less than 14 IU/l and basal oestradiol less than 70 pg/ml. Exclusion criteria were male partners presenting with severe oligozoospermia or azoospermia. Because some studies suggested that endometriosis (Sharma et al., 2010) and PCOS (Ramasamy et al., 2009) may affect sRAGE concentrations, this study also excluded patients diagnosed with those pathologies. After all the exclusion criteria had been applied, 54 patients qualified to participate in this study (**Figure 1**).

IVF procedure

Pituitary blockage was obtained with a gonadotrophin-releasing hormone (GnRH) agonist (Lupron; Abbot SA Societé Française des Laboratories, France) (67.6% of the participants) or a GnRH antagonist (Cetrotide; Serono, Switzerland) (32.4% of the participants). Ovarian stimulation was accomplished using recombinant FSH (Gonal-F; Serono, Switzerland). When at least two follicles reached a diameter of 16 mm, follicular maturation was triggered