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ARTICLE

Association between leukaemia inhibitory factor gene polymorphism and pregnancy outcomes after assisted reproduction techniques

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Abstract Certain gene polymorphisms are associated with implantation failure and pregnancy loss. Studies of leukaemia inhibitory factor (LIF) gene polymorphisms are scarce. The LIF single nucleotide polymorphism (SNP) thymine (T)/guanine (G) (rs929271) was studied in women to determine whether an association existed with pregnancy outcomes after intracytoplasmic sperm injection (ICSI); 411 women who underwent ICSI were recruited. DNA was extracted from the peripheral blood, and the *LIF* gene SNP T/G (rs929271) was genotyped using real-time polymerase chain reaction. Participants were divided into three groups according to their LIF genotype: T/T ($n = 168$), T/G ($n = 202$) and G/G ($n = 41$). All IVF and ICSI procedures were carried out under the same clinical and laboratory conditions. The ICSI cumulative results (from fresh plus frozen cycles) of each genotype group were analysed. The G/G genotype in women was associated with a higher implantation rate (T/T: 15.9%, T/G: 16.2%, G/G: 27.0%; $P < 0.05$), ongoing pregnancy rate/patient (T/T: 31.5%, T/G: 36.1%, G/G: 53.7%; $P < 0.05$) and ongoing pregnancy rate/transfer (T/T: 18.5%, T/G: 20.2%, G/G: 36.7%; $P < 0.05$). LIF SNP T/G (rs929271) seems to be a susceptibility biomarker capable of predicting implantation efficiency and pregnancy outcomes.

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Introduction

Most women undergoing IVF and intracytoplasmic sperm injection (IVF-ICSI) can achieve embryo transfer with good-quality embryos upon routine assessment. The embryo implantation rate, however, and therefore the pregnancy rate, is consistently low despite advances in ovarian stimulation and in-vitro embryo culture and development. It is estimated that more than two-thirds of transferred embryos do not implant (CDC Centers for Disease Control and Prevention, 2012; Simon and Laufer, 2012). In fact, considering the losses during pregnancy in addition to implantation failures, only a small percentage of all women undergoing IVF-ICSI cycles have a live birth.

The successful implantation of a good-quality human embryo in a receptive endometrium requires a remarkable and complex collaboration of factors (Koot et al., 2012; Sallam, 2005), including cytokines, growth factors and hormones. Recently, the involvement of genetic factors, including polymorphisms, in the implantation process has been highlighted, especially in patients who experience repeated IVF implantation failures and recurrent spontaneous abortions. The over- or underexpression of genes that encode the proteins required for embryo implantation may be associated with these clinical issues (Madon et al., 2005; Urman et al., 2005).

Leukaemia inhibitory factor (LIF) is a multifunctional pleiotropic cytokine member of the interleukin-6 family. It was first described as a factor that inhibits the proliferation of murine myeloid leukaemic cells and induces their differentiation into macrophages (Gearing et al., 1987; Hilton et al., 1988). In humans, LIF regulates various functions and is produced by several types of cells, such as fibroblasts, osteoblasts, hepatocytes, monocytes, macrophages and T cells (Mathieu et al., 2012; Metcalf, 2003). It plays a critical role in the reproductive process and is considered essential to successful pregnancy in humans (Franasiak et al., 2014; Fritz et al., 2014; Güney et al., 2007; Kimber, 2005). A higher concentration of LIF in the follicular fluid is correlated with embryo quality, which suggests that LIF has an important role in the physiology of ovulation and early embryonic development (Arici et al., 1997). In humans, LIF controls the uterine receptivity to blastocyst implantation and trophoblastic function by promoting proliferation, invasion and differentiation (Fitzgerald et al., 2008). In the endometrium, both glandular and luminal epithelial cells express LIF; however, LIF expression is more intense in the glandular epithelium with high expression during early implantation (Aghajanova, 2004; Aghajanova et al., 2009; Hu et al., 2011; Laird et al., 1997). Unlike LIF, the expression of LIF receptor (LIFR) is greater in the endometrial luminal epithelial cells than in the glandular epithelial cells. LIF binds to the LIFR and recruits its co-receptor, interleukin 6 signal transducer (IL6ST), forming a high-affinity signalling complex (Lass et al., 2001). Following the attachment of the blastocyst to the endometrium, the trophoblast also begins expressing LIF, which may have an autocrine effect on its physiological functions (Charnock-Jones et al., 1994; Conquet and Brûlet, 1990; Kojima et al., 1995). Villous and extravillous trophoblasts express LIF and its receptor during pregnancy (Sharkey et al., 1999).

Changes in the expression of the *LIF* gene, which encodes this cytokine that is critical for implantation, have been associated with infertility. Although the importance of *LIF* gene variants in human fertility has been investigated, few studies have analysed the correlation between *LIF* gene changes and reproductive capacity. A higher prevalence of mutations near the start codon of exon 1 and in exon 3, regions that are functionally important for controlling the biological activity of LIF, was observed in infertile women and has been associated with unexplained infertility and recurrent implantation failure after IVF and embryo transfer (Giess et al., 1999; Kralickova et al., 2006; Novotny et al., 2009; Steck et al., 2004).

The single nucleotide polymorphism (SNP) thymine (T)/guanine (G) located in the untranslated region 3′ (3′ UTR) (rs929271/ c.1414T > G) is a polymorphism of the *LIF* gene that has recently been investigated. In addition to its reproductive role, LIF acts on the differentiation of neurones. This polymorphism has been positively associated with schizophrenia; in fact, the T-allele and T-carrier genotypes (T/T and T/G) of rs929271 were found to be risk factors for hebephrenic schizophrenia (Okahisa et al., 2010). During the process of reproduction, Kang et al. (2009) observed that the G allele is significantly enriched in patients under the age of 35 years but not in older patients. Furthermore, the G allele was associated with a history of fertility medication use, indicating an association between the G allele and infertility, especially in patients under the age of 35 years (Kang et al., 2009). Ucisik-Akkaya et al. (2010) reported that human embryo survival can be influenced by the genotype LIF T/G (rs929271) in a sex-dependent fashion. In contrast, Fraga et al. (2014) did not observe a correlation between LIF SNP T/G (rs929271) and recurrent pregnancy loss. In the same context, Paskulin et al. (2013) found no correlation between LIF SNP T/G (rs929271) and endometriosis or IVF failure. Furthermore, Tagliani-Ribeiro et al. (2012) analysed the correlation between genetic polymorphism and the incidence of dizygotic and monozygotic twins, and found no difference between cases and controls in the allelic or genotypic frequencies of LIF T/G (rs929271).

On the basis of the small number of published studies, and the need for a better understanding of the action of the *LIF* gene in the human reproductive process, we aimed to determine whether the LIF SNP T/G (rs929271) in women is associated with pregnancy outcomes after ICSI.

Materials and methods

Study participants

A total of 411 women with infertility issues enrolled in the ICSI programme at the Center for Human Reproduction Prof Franco Jr were included in this study. All of the women in the infertile study group met the following inclusion criteria: age 39 years or younger at the time of oocyte retrieval, a normal