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ARTICLE

Down-regulation of the *CYP19A1* gene in cumulus cells of infertile women with endometriosis

Ionara Diniz E.S. Barcelos [a], Flávia Capello Donabella [a], Cristiana Padovan Ribas [a], Juliana Meola [a], Rui Alberto Ferriani [a], Cláudia Cristina Paro de Paz [b], Paula A. Navarro [a,*]

[a] *Department of Gynecology and Obstetrics, Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto, SP, Brazil;* [b] *Department of Genetics, Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto, SP, Brazil*

* Corresponding author. *E-mail address:* navarro@fmrp.usp.br, drapaulanavarro@gmail.com (PA Navarro).

Dr Ionara Barcelos holds a PhD in Obstetrics and Gynecology and Reproduction Biology, obtained in 2013 at the University of São Paulo, Ribeirão Preto, Brazil. She has worked as Professor at the Faculty of Medicine of University of West Parana and at a private reproductive medicine clinic. Her area of interest is reproductive medicine, endometriosis, assisted reproduction and recently epigenetics in reproduction.

Abstract Aromatase plays a fundamental role in the establishment of oocyte quality, which might be compromised in infertile women with endometriosis. The expression of the *CYP19A1* gene (that encodes aromatase) was compared in cumulus cells and oestradiol concentrations in the follicular fluid of infertile women with and without endometriosis submitted to ovarian stimulation for intracytoplasmic sperm injection. Cumulus cells were isolated and the expression of the *CYP19A1* was quantitated through real-time polymerase chain reaction. Oestradiol concentrations in follicular fluid were measured by chemiluminescence immunoassay. A lower expression of the *CYP19A1* in the cumulus cells of infertile women with endometriosis was observed compared with controls (0.17 ± 0.13 and 0.56 ± 0.12, respectively), and no significant difference in the follicular fluid oestradiol concentrations was observed between groups. Our results show reduced expression of the *CYP19A1* in cumulus cells of infertile women with endometriosis, which may play a role in the pathogenesis of endometriosis-related infertility.

KEYWORDS: cumulus cells, *CYP19A1* gene, endometriosis, follicular fluid, infertility

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Introduction

Endometriosis, one of the most common gynaecologic diseases, affects about 10–15% of reproductive-age women and is associated with subfertility (Donnez et al., 2002; Holoch and Lessey, 2010). The prevalence of this condition increases to 40% in women with subfertility, and about 30–50% of affected women are estimated to be infertile (Holoch and Lessey, 2010). The mechanisms involved in the aetiopathogenesis of infertility in patients with endometriosis are likely to be multifactorial, although this complexity has not yet been fully elucidated (De Ziegler et al., 2010).

New approaches to the treatment of endometriosis-related infertility have become available, with an emphasis on assisted reproduction techniques, which are becoming increasingly common. The effect of endometriosis on assisted reproductive outcomes has been questioned, and it is unclear whether a deleterious effect of the disease occurs in assisted reproduction technique results (Aboulghar et al., 2003; Al-Fadhli et al., 2006; Barbosa et al., 2014; Barnhart et al., 2002; Harb et al., 2013; Lin et al., 2012). Some studies have shown low fertilization, pregnancy, implantation rates, or both, in women with endometriosis submitted to ovarian stimulation followed by IVF (Aboulghar et al., 2003; Al-Fadhli et al., 2006; Barbosa et al., 2014; Barnhart et al., 2002; Harb et al., 2013; Lin et al., 2012), which may be due, at least in part, to lower oocyte quality (Díaz et al., 2000; Simón et al., 1994). A previous study by our group has suggested an impairment or delay of the completion of meiosis I in in-vitro matured oocytes obtained from stimulated cycles of patients with endometriosis, although the underlying mechanisms were not elucidated (Barcelos et al., 2009). Because human oocytes are extremely rare, however, and their use in invasive studies is usually not feasible because it prevents their use in assisted reproduction techniques, few studies have evaluated the quality of oocytes from endometriosis-infertile women submitted to ovarian stimulation for assisted reproduction techniques.

Oocyte competence depends on the quality of the follicular microenvironment, and the presence of adequate bi-directional cumulus cell-to-oocyte signalling is a prerequisite for the acquisition of both oocytes and cumulus cell competence (Assou et al., 2006, 2008, 2010; Gasca et al., 2007; Ouandaogo et al., 2011, 2012). Follicular cells obtained during oocyte recovery in regular IVF cycles can be divided into two subpopulations: cumulus cells and mural granulosa cells. The cumulus cells form a group of closely associated cells that surround the oocyte in the antral follicle, and the mural granulosa cells line the follicular wall. Granulosa cells play an essential role in follicular differentiation, leading to optimal conditions for oocyte development, ovulation, fertilization and subsequent implantation (Adashi, 1994). Moreover, the bi-directional communication between the oocyte and these cells occurs throughout follicular development (Buccione et al., 1990; Eppig et al., 2002; Gilchrist et al., 2004; Makabe et al., 2006; Senbon et al., 2003; Sirard et al., 2006), and is essential for the acquisition of developmental competence in mammalian oocytes (de Loos et al., 1991; Fair, 2003; Webb et al., 2002). Therefore, the analysis of the expression of genes involved in the acquisition of oocyte competence in the cumulus cells of mature human oocytes can be used as a form of non-invasive assessment of oocyte quality and for the prediction of assisted reproduction technique results (Assou et al., 2006, 2008; Cetica et al., 2001; Hamamah et al., 2006; Hamel et al., 2008; Haouzi and Hamamah, 2009; Tesfaye et al., 2009).

A rate-limiting enzyme for the synthesis of oestrogen from androgens, P450 aromatase, is involved in the conversion of androstenedione and testosterone into oestrone and oestradiol, respectively. Aromatase is present in granulosa cells and plays a fundamental role in follicle maturation and the establishment of oocyte quality (Erickson et al., 1989; Foldesi et al., 1998; Hamel et al., 2008).

An increased expression of aromatase has been reported in endometriotic tissue (Noble et al., 1996) and endometriomas (Smuc et al., 2009). Although not detectable, aromatase expression has been observed in the eutopic endometrium from healthy women (Bulun et al., 2004; Hatok et al., 2011; Maia et al., 2012). In contrast to the increased aromatase expression in endometriotic tissue, Harlow et al. (1996) and de Abreu et al. (2006), through in-vitro studies using luteinized granulosa cell culture from women with and without endometriosis submitted to ovarian stimulation for assisted reproduction techniques, reported decreased aromatase activity in the granulosa cells of women with endometriosis, which might lead to defects in granulosa cell steroidogenesis and abnormal oocyte functioning (Harlow et al., 1996). Data on the expression of the aromatase gene (gene *CYP19A1*) in luteinized mural granulosa cells are controversial (Abreu et al., 2011; Lu et al., 2012), and no studies have evaluated the expression of the *CYP19A1* gene in cumulus cells from infertile women with endometriosis.

The main objective of the present study was, therefore, to compare the expression of the *CYP19A1* gene in cumulus cells of infertile women with and without endometriosis submitted to ovarian stimulation for intracytoplasmic sperm injection (ICSI). As secondary objective, oestradiol concentrations in follicular fluid were compared between infertile women with and without endometriosis.

Materials and methods

This prospective case-control study was approved by the Research Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo (FMRP-USP), Brazil on 17 January 2008 (approval reference number HCRP 10187/2007).

Setting, patients and study size

No data have been published on the expression of the *CYP19A1* gene in cumulus cells from human cumulus-oocyte complex (COC). Therefore, a pilot study was conducted in which all the women submitted to ovarian stimulation for ICSI in the Sector of Human Reproduction, Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirão Preto, University of São Paulo (FMRP-USP, SP, Brazil), were analysed consecutively from February 2009 to October 2010, a period in which all infertile patients were submitted to diagnostic laparoscopy as part of the infertility investigation at our service. Of these, all the patients who met the eligibility criteria listed