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PTGS2 down-regulation in cumulus cells of infertile women with endometriosis

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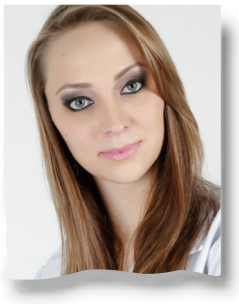

Caroline Mantovani da Luz has a master's degree in Medical Sciences, Gynaecology and Obstetrics, obtained from the University of São Paulo, Ribeirão Preto Medicine School, Human Reproduction Division, in 2016. Her project focused on endometriosis and oocyte quality. She has experience in experimental research and molecular biology, and an interest in reproductive medicine, endometriosis and infertility.

KEY MESSAGE

This study revealed decreased expression of *PTGS2* in cumulus cells of infertile women with endometriosis compared with controls. It is postulated that, based on *PTGS2* down-regulation, lower levels of cyclooxygenase 2 in cumulus cells might be involved in the impairment of oocyte development.

A B S T R A C T

A deleterious effect of endometriosis on oocyte quality has been proposed. Evidence suggests that cumulus cells could be used as indirect biomarkers of oocyte quality. The *PTGS2* gene, which encodes cyclooxygenase 2 (COX-2), is deregulated in endometriotic lesions and plays a crucial role in the acquisition of oocyte competence. To date, research evaluating *PTGS2* expression in cumulus cells of infertile patients with endometriosis has not been conducted. The aim this study was to compare the expression levels of *PTGS2* in cumulus cells of infertile women, with and without endometriosis, undergoing ovarian stimulation for intracytoplasmic sperm injection (ICSI). Therefore, a case–control study compared *PTGS2* gene expression in the cumulus cells of 38 infertile patients with endometriosis and 40 without, using real-time polymerase chain reaction. For the first time, decreased expression of *PTGS2* was found in cumulus cells of infertile women with endometriosis compared with controls (7.2 ± 10.5 versus 12.4 ± 15.7), which might be related to reduced levels of COX-2 in the cumulus cells of women with the disease. Consequently, we hypothesize that lower transcript levels of *PTGS2* in cumulus cells may be involved in the impairment of oocyte quality, suggesting a possible mechanism involved in disease-related infertility.

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Introduction

Endometriosis is a gynaecologic disease, characterized by the presence of functional endometrial-like tissue outside of the uterine cavity (Ozkan et al., 2008). About 10% of women of reproductive age are diagnosed with this oestrogen-dependent disorder (Ahn et al., 2015). They may display diverse clinical conditions, ranging from asymptomatic to symptoms such as dysmenorrhoea, chronic pelvic pain and subfertility, which significantly degrades the patient's quality of life (Bulun, 2009; Giudice and Kao, 2004). The incidence of the disease is increased in 40–60% of women with subfertility, and around 30–50% of the affected women are estimated to be infertile (Holoch and Lessey, 2010).

The mechanisms underlying endometriosis-related infertility remain to be elucidated, especially in the initial stages, when patients do not exhibit distortions and adhesions in the reproductive tract (Da Broi and Navarro, 2016). Several hypotheses have been proposed to justify the fertility impairment in these women, and some studies suggest a significant role of oocyte quality in this condition (Andrade et al., 2010; Barcelos et al., 2009, 2015; Da Broi and Navarro, 2016; Da Broi et al., 2014; Donabela et al., 2015; 2016; Garcia-Velasco and Arici, 1999; Simon et al., 1994; Sung et al., 1997). Oocyte analysis in these patients, however, is not routinely feasible, as human oocytes are rarely donated to research centres, and their application in invasive techniques precludes subsequent use in assisted reproduction technique procedures. Therefore, the indirect evaluation of oocyte quality (Assou et al., 2010; Ouandaogo et al., 2011, 2012), for follicle assessment, may contribute to the understanding of endometriosis-related infertility (McKenzie et al., 2004). In this context, evidence suggests that cumulus cells are intimately associated with oocyte development (McKenzie et al., 2004), contributing to cytoplasmic oocyte maturation (Furger et al., 1996; Tanghe et al., 2002). Moreover, it is believed that impaired cumulus cell functions may culminate in impaired fertility (Hizaki et al., 1999).

Endometriosis is known to be associated with several deregulated molecules related to the pathogenesis of the disease, such as cyclooxygenase-2 (COX-2) and aromatase (Banu et al., 2008; Gupta et al., 2008). The COX-2 enzyme, encoded by the *PTGS2* gene (prostaglandin–endoperoxide synthase 2), is naturally induced by aromatase and is involved in the conversion of arachidonic acid into prostaglandins (Sugimoto et al., 2007), which, in turn, regulate aromatase levels in endometriotic tissue (Bulun et al., 2001). In the endometrial tissue of patients with endometriosis, aberrant aromatase is induced via cyclooxygenase-2 prostaglandine-2 (COX-2–PGE_2) pathway deregulation, with a positive feedback cycle (Bukulmez et al., 2008; Bulun et al., 2002). It is also related to proliferative and inflammatory properties of ectopic implants (Bulun et al., 2001). Supporting these data, several studies have demonstrated COX-2 up-regulation in eutopic (Chen et al., 2012; Cho et al., 2010; Ota et al., 2001; Wang et al., 2012) and ectopic (Ota et al., 2001; Rakhila et al., 2013) endometrial tissues from women afflicted by the disease.

In contrast to what occurs in the endometrial tissue, aromatase activity is decreased in granulosa cells (Barcelos et al., 2015), and lower expression of the aromatase gene (*CYP19A1*), not only in luteinized mural granulosa cells (Lu et al., 2012) but also in cumulus cells of infertile women with endometriosis (Barcelos et al., 2015; Hosseini et al., 2016) was demonstrated. On this basis, decreased aromatase activity may reflect other disorders in the cumulus cells of these patients, such as reduced PGE_2 in response to low levels of COX-2; however, further investigation is required. Interestingly, it was verified that COX-2 levels in cumulus cells are associated with cumulus expansion and matured oocytes in cattle (Nuttinck et al., 2002), equine (Dell'Aquila et al., 2004) and mice (Calder et al., 2001) models. Moreover, the higher expression of *PTGS2* in cumulus cells was previously correlated to human oocyte competence acquisition and higher-grade embryos (McKenzie et al., 2004). Therefore, we wonder if this gene may be deregulated in the cumulus cells of infertile women with the disease, which might be related to the impairment of oocyte competence.

In cumulus cells, COX-2 plays a significant role during oocyte competence acquirement. Moreover, aromatase, and consequently COX-2, seem to be altered in the endometrial tissue of women with endometriosis; aromatase has also been reported to be decreased in granulosa and cumulus cells of endometriosis patients. On the basis of this, we hypothesize that the *PTGS2* gene may be deregulated in cumulus cells of women with endometriosis, consequently compromising oocyte quality. To date, however, published research evaluating *PTGS2* gene expression in cumulus cells of infertile patients with the disease is lacking. Therefore, the objective of the present study was to compare the transcript levels of the *PTGS2* gene in cumulus cells of infertile women, with and without endometriosis, undergoing ovarian stimulation for intracytoplasmic sperm injection (ICSI).

Materials and methods

Ethics

A prospective case-control study was conducted in the Human Reproduction Sector of the Department of Obstetrics and Gynecology, at the University Hospital of the Ribeirão Preto School of Medicine, at the University of São Paulo (FMRP-USP). The study was approved by the Research Ethics Committee of the University Hospital (HCRP 10187/2007) on 17 January 2008. All patients who met the eligibility criteria, and who agreed to participate in the study, provided written informed consent.

Settings and duration

Between February 2009 and October 2010, patients who participated in the Assisted Reproduction Program of the University Hospital, FMRP-USP, and underwent ovarian stimulation for ICSI, were evaluated according to eligibility criteria, and those who were considered eligible were interviewed. Cumulus cells from the human cumulus-oocyte complex (COC) were collected on the day of oocyte retrieval. The patients were then followed up until oocyte analysis to obtain the number of retrieved mature oocytes, included in the clinical data assessment. The samples were analysed at the Human Reproduction Core Laboratory of FMRP-USP. The cDNA processing and quantitative polymerase chain reaction (PCR) analysis were carried out at the beginning of 2011.

Participants and eligibility criteria

The inclusion criteria for the endometriosis group was the presence of infertility, exclusively associated with endometriosis, diagnosed by video laparoscopy according to ASRM criteria (Revised American Society for Reproductive Medicine classification of endometriosis: 1996,

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