

Differential *proMMP-2* and *proMMP-9* secretion in human pre-implantation embryos at day 5 of development

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Morphological development is the most common non-invasive criterion used to select *in vitro* human embryos for implantation. With this criterion, however, embryos in cellular arrest go unnoticed. A more accurate criterion is needed to improve the success rate of implantation. Extracellular matrix metalloproteinases type 2 (MMP-2) and MMP-9 are key markers of embryonic development and the implantation process, according to various animal studies. The first objective of this study was to examine *proMMP-2* and *proMMP-9* activity in the culture media of human embryos with good morphological development. Secondly, the results of *proMMP-2* and *proMMP-9* activity in the culture medium were compared between pregnant and non-pregnant. Forty-two patients were approved by the Ethics and Research Committees of the Instituto Nacional de Perinatología in México City hospital, based on institutional inclusion criteria for *in vitro* fertilization. On day 5 of development, embryos were transferred to patients, and the culture media secretion profile of *proMMP-2* and *proMMP-9* activity was determined by substrate gel zymography. After analysis of embryo sac development, each patient was assigned to the pregnant (n=17) or non-pregnant (n=25) group. Our results demonstrate that *proMMP-2* was active in the culture media corresponding to all 17 women achieving full-term pregnancy and *proMMP-9* in the media corresponding to 11 of these women. Contrarily *proMMP-2* and *proMMP-9* were active in the culture media corresponding to 3 and 11 of the 25 non-pregnant patients, respectively. The clinical implications of this study suggest the activity evaluation of *proMMP-2* and *proMMP-9* in embryonic culture media on day 5 of development appears to be a reliable indicator of the quality of embryos and their capacity to establish a pregnancy.

Keywords: embryonic development, implantation, *in vitro* fertilization, matrix metalloproteinases, pregnancy

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Abbreviations: *proMMP*, matrix metalloproteinases; IL-1 β , interleukin-1beta; P4, progesterone; E2, estradiol; T4, testosterone

INTRODUCTION

Regarding the evaluation of embryo quality prior to implantation, there is as yet no quantitative method. Embryonic morphology is the criterion employed in clinical practice as a qualitative marker of the viability of embryos to be transferred to patients (Capalbo *et al.*, 2014; Minasi *et al.*, 2016).

The successful growth and implantation of blastocysts is a complex event involving maternal and embryonic signals (Fritz *et al.*, 2014; Matsumoto *et al.*, 2016). Related to such, cytokines (Krussel *et al.*, 1998; Prutsch *et al.*, 2012), growth factors (Paria *et al.*, 1999; Zeng *et al.*, 2016), and matrix metalloproteinases (MMPs) (Sternlicht & Werb, 2001; Nissinen & Kahari, 2014) are associated with an adequate interaction between the blastocyst and uterine endometrium after implantation (Tazuke & Giudice, 1996; Massimiani *et al.*, 2019). Taskin *et al.* (2012) detected the secretion of interleukin (IL)-1 β in the culture media of human embryos at distinct stages of development (Taskin *et al.*, 2012).

Among the signaling pathways regulated by inflammatory cytokines is that which activates MMPs (Chen *et al.*, 2013), a family of zinc-dependent endoproteases. MMPs participate in tissue remodeling and the degradation of various proteins in the extracellular matrix, including collagen (Shekhter *et al.*, 2019; Shin *et al.*, 2019), elastin (Yadav *et al.*, 2011; Van Doren, 2015), gelatin (Le *et al.*, 2007; Zitka *et al.*, 2010), matrix glycoproteins and proteoglycans (Pietraszek-Gremplewicz *et al.*, 2019; Theocharis *et al.*, 2019). The substrates degraded by MMPs determine the basis of classification of the latter. Commonly known MMPs are stromelysin-1 (MMP-3), -2 (MMP-10), and -3 (MMP-11), collagenase-1 (MMP-1), -2 (MMP-8), and -3 (MMP-13), gelatin-A (MMP-2) and B (MMP-9), matrilysin type I (MMP-7) and II (MMP-26) and membranal type I (MMP-14, -15, -16, and -24) and II (MMP-23) (Visse & Nagase, 2003; Brew & Nagase, 2010; Laronha & Caldeira, 2020). In addition to their role in pregnancy (Cohen & Bischof, 2007; Stojanovic *et al.*, 2010),

MMPs promote cell proliferation (Zhang *et al.*, 2016; Quintero-Fabian *et al.*, 2019), migration (Bischof *et al.*, 2002; Pollheimer *et al.*, 2014), and differentiation (Chan *et al.*, 2020; Gorter & Baron, 2020).

The first aim of the present study was to analyze the activity of *pro*MMP-2 and *pro*MMP-9 in the culture media of embryos with good morphology at day 5 of development. Secondly, the media were divided into two groups, corresponding to successfully and unsuccessfully implanted embryos, and compared to explore possible significant differences in the activity of *pro*MMP-2 and *pro*MMP-9.

MATERIALS AND METHODS

Ethics approval

The current protocol was reviewed and approved by the Ethics and Research Committees of the Instituto Nacional de Perinatología in México City (212250-22661). The purpose of the study was explained to all patients, and informed consent was signed by those who decided to participate.

Study design and patients

From May 2019 to May 2020, a cross-sectional study was carried out in the Department of Reproductive Biology of the Instituto Nacional de Perinatología in the Ciudad de México, México. A total of 42 patients diagnosed with infertility were admitted to the *in vitro* fertilization. None of the patients previously received therapeutic treatments.

Clinical definition and inclusion criteria

The following constituted the inclusion criteria: all participants were aged <37 years, had a regular menstrual cycle, a normal uterine cavity confirmed by hysteroscopy, absence of intrauterine adhesion or inflammation, an endometrial thickness in the late follicular phase ≥ 7 mm (measured by ultrasonography), a normal ovarian reserve (follicle-stimulating hormone <9.0 mIU/mL), a normal ovarian response to the stimulation protocols (>8 oocytes retrieved in a controlled ovary hyperstimulation cycle), and no hormone (estradiol/progesterone) treatment – during the endometrial cycle. The exclusion criterion: was the failure of the woman to undergo an ultrasound scan within 4 weeks after a positive pregnancy test. Non-inclusion criteria: were endometrial cancer or hyperplasia, endometriosis, and having a male partner with infertility.

Patients and hormonal stimulation

The patients received controlled ovarian stimulation with a conventional dose of 150–225 IU recombinant FSH (Gonadal-F; Merck Serono, Germany) according to the body weight of each patient (NyboeAndersen *et al.*, 2008). When the follicular diameter reached 18 mm, oocyte maturation was stimulated with 10,000 IU hCG (Ovitrelle; Merck), and follicular oocytes were obtained 36 h later with ultrasound guidance.

In vitro fertilization and developing embryos

Oocytes were fertilized *in vitro* by exposing them to 1×10^6 capacitated spermatozoa/mL for 18 h. This process was carried out in HTF-HEPES medium (Irvine Scientific, Santa Ana, CA, USA) supplemented

with 5% BSA (Sigma Carlsbad, CA, USA) under controlled conditions (37°C with a 5% CO₂ and 95% air). Fertilization was confirmed by the presence of a second polar corpuscle body (Vanderzwalmen *et al.*, 1997; Ziebe *et al.*, 1997). Each one of the fertilized oocytes was incubated in 50 μ L of G-1 PLUS culture medium (Vitrolife, Göteborg, Sweden) until day 3 of embryonic development, and later the embryos were transferred to 50 μ L of G-2 PLUS culture medium (Vitrolife). On day 5 blastocysts were evaluated according to the scoring system (type I, II, or III quality) (Cutting *et al.*, 2008) and transferred to women. The development embryos were cultured in an ASTEC incubator (EC6S-MD, PA, USA) at 37°C with a 5% O₂ and 6% CO₂ until being transferred to women on day 5.

Morphological development was monitored daily until day 5 at which time the culture media were retained for examination of MMPs, and two embryos were transferred to the uterine cavity of each patient with the Soft Cook technique by using a Flexible Pass intrauterine transfer cannula. The process was assisted by abdominal ultrasound guidance and a real-time, 5-MHz sector electronic array endovaginal test (Philips Epiq CVx; MO, USA).

Blood samples and quantification of sex hormone level

Peripheral blood samples (5 mL) were obtained from the patients by puncturing the cephalic vein on day 14 after the embryo transfer. Samples were placed in EDTA-K2 tubes (BD Vacutainer) and centrifuged at 14,000 rpm for 10 min. Serum was collected in Eppendorf tubes and stored at -70°C to await the hormone quantification assay, which was performed in the central laboratory of the Instituto Nacional de Perinatología on a cobas e411 modular analytical apparatus (Roche, USA). The serum levels of progesterone (P4), estradiol (E2), testosterone (T4), follicle-stimulating hormone (FSH), luteinizing hormone (LH), Anti-Müllerian hormone (AMH), and human chorionic gonadotropin (hCG) were measured with a commercial assay kit (Roche system, USA), according to the manufacturer's recommendations and as previously described (Acuña-González *et al.*, 2021). The lower limit of detection for these hormones was 0.4 ng/mL, 5.0 pg/mL, 0.025 pg/mL, 0.100 mIU/mL, 0.100 mIU/mL, 0.2 ng/mL, and 0.1 mIU/mL, respectively. The intra-assay coefficient of variation was 3%, 5%, 5%, 3%, 2%, 3%, and 5% respectively.

In the case of an apparently successful implantation of the embryo and good endometrial receptivity, embryo sac development was examined with an ultrasound probe. The results of this probe led to the final identification of pregnant (n=17) and non-pregnant patients (n=25).

Protein quantification and matrix metalloproteinase activity

The total concentration of proteins in the culture media of the embryo on the fifth day was determined by the colorimetric Bradford method (Bradford, 1976). The activity of the two MMPs was examined by using SDS-polyacrylamide gels with porcine gelatin (1 mg/mL), as described previously (Flores-Herrera *et al.*, 2012). A culture medium from U937 promyelocyte cells (ATCC, Manassas, VA, USA) served as the internal control of electrophoretic mobility. Each sample was loaded with 0.75 μ g of protein and the activity band was captured with the EpiChemi Darkroom gel

Table 1. Clinical data on the patients participating in the study.

Characteristics	Pregnant (n=17)	Non-pregnant (n=25)	p-value
Age (years)	35.7±2.4	36.8±3.1	0.23
BMI (Kg/mL)	27.2±3.7	26.3±3.2	0.43
Years of infertility	4.8±3.0	5.4±3.2	0.39
Number of embryos transferred			
1, n (%)	0 (0.0)	5 (20.0)	0.085
2, n (%)	17 (100)	20 (80.0)	
Quality of the transferred embryos			
I, n (%)	7 (22.0)	5 (11.1)	0.137
II, n (%)	25 (71.0)	26 (57.8)	0.04
III, n (%)	2 (7.0)	14 (31.1)	0.001
Serum hormone concentration			
hCG (mIU/mL)	2088.2±2300.4	1254.0±1149.2	0.70
E2 (pg/mL)	1496.7±793.9	1674±513.3	0.71
P4 (ng/mL)	0.65±0.4	0.55±0.3	0.90

BMI, body mass index; hCG, human chorionic gonadotropin; E2, estradiol; P4, progesterone. The criterion for assigning patients to the groups was embryo sac development (or lack thereof). Data are reported as the mean ± standard deviation.

documentation system (UVP, CA, USA). Optical densitometry was measured on the NIH ImageJ program.

Statistical analysis

The difference between the *pro*MMP-2 and *pro*MMP-9 optical density values detected in the culture media of the two groups of embryos (pregnant patients *vs.* non-pregnant patients) was examined with the Student's *t*-test. All values are expressed as the mean ± standard deviation (S.D.), and statistical significance was considered at $p \leq 0.05$. Statistical analysis was performed on GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). STATA software was used to plot the receiver operating characteristic (ROC) curves and calculate the area under the curve (AUC-ROC) (StataCorp LLC; v16, TX, USA). The cutoff value selected (based on the ROC curve) was that at which the sensitivity and specificity were best and the distance to the top-left corner of the ROC curve was the least. Sensitivity, specificity, and positive and negative predictive values were determined on STATA software.

RESULTS

Patient characteristics

The initial characteristics prior to hormonal stimulation of the 42 patients (pregnant, n=17 and non-pregnant, n=25) are compared in Table 1. No significant differences existed regarding to age ($p=0.23$), body mass index ($p=0.43$), years of infertility ($p=0.39$) and the concentration of hCG ($p=0.70$), E2 ($p=0.71$), and P4 ($p=0.90$) were determined, and we did not find statistically significant differences between both groups (Table 1).

Hormone profiling

Fourteen days after the embryos were transferred, the concentration of hormones was compared between the pregnant and non-pregnant patients (Table 2). No significant differences existed with respect to P4 on the day of final oocyte maturation ($p=0.664$), E2 in the non-follicular phase ($p=0.684$) or follicular phase ($p=0.326$), T4 ($p=0.336$), LH ($p=0.095$), or AMH ($p=0.263$). How-

Table 2. Comparison of the hormonal concentration between women with implanted versus non-implanted embryos.

Variable	Pregnancy (n=17)	Non-pregnancy (n=25)	p-value
P4 on day of final oocyte maturation (ng/mL)	0.49±0.20	0.45±0.28	0.664
E2, non-follicular phase (pg/mL)	60.7±16.4	63.7±2710	0.684
E2, follicular phase (pg/mL)	1368.0±582.1	1889.0±849.3	0.326
T4, follicular phase (pg/mL)	50.4±20.5	46.2±7.8	0.336
FSH, follicular phase (mIU/mL)	4.6±1.3	6.3±2.5	0.011
LH, follicular phase (mIU/mL)	4.9±1.2	5.8±1.6	0.095
AMH (ng/mL)	1.56±0.61	1.39± 0.37	0.263
hCG (mIU/mL)	61.8±32.7	2.7±1.4	<0.0001

P4, progesterone; E2, estradiol; T4, testosterone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, Anti-Müllerian hormone; hCG, human chorionic gonadotropin. Data are expressed as the mean ± standard deviation.

ever, significant differences were indeed found for FSH ($p=0.011$) and hCG ($p<0.0001$; Table 2).

Activity of proMMP-2 and proMMP-9 in the culture media of the embryos

On day 5 of development, the embryos were transferred to the patients and the secretion profile of *proMMP-2* and *proMMP-9* was later determined in the culture media (Fig. 1). The presence of *proMMP-2* was detected in the culture media corresponding to 82% of the pregnant patients (14 of 17; lane 1 to 17) and in 12% of the non-pregnant patients (3 of 25; lane 18 to 42; Fig. 1A). The latter group included three women who became pregnant and underwent a spontaneous abortion (P29, P30, and P38; Fig. 1A). The optical density of *proMMP-2* was quantified for each of the activity bands (Fig. 1B), showing a significant 1.4-fold lower value in the three non-pregnant patients with *proMMP-2* activity than in the pregnant women ($p=0.045$; Fig. 1B).

On the other hand, *proMMP-9* activity was found in the culture media corresponding to 11 of 17 (64.7%) pregnant and 11 of 25 (44%) non-pregnant patients. The optical density of the bands of *proMMP-9* displayed a significant 1.2-fold lower value in the culture media corresponding to the 11 non-pregnant patients with *proMMP-9* activity versus the media corresponding to the 11 pregnant women with *proMMP-9* activity ($p=0.002$; Fig. 1C).

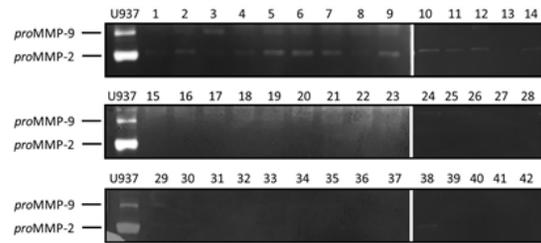
Predictive values of proMMP-2 and proMMP-9 in the culture media corresponding to embryos producing pregnancy

The ROC curve was used to evaluate whether the sensitivity and specificity of *proMMP-2* and *proMMP-9* are adequate for determining the capacity of transferred embryos to produce pregnancy (Fig. 2). For *proMMP-2*, the optical density of 423 was taken as the cutoff value, resulting in statistical significance ($p=0.0262$) with a sensitivity of 100% and a specificity of 100%. For *proMMP-9*, the optical density of 550 was adopted as the cutoff value, rendering statistical significance ($p=0.0035$) with a sensitivity of 81.8% and a specificity of 72.7%.

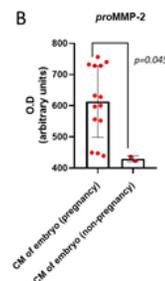
DISCUSSION

MMPs play an important role in the remodeling of different structural and support components during ovulation (Smith *et al.*, 2002; Rosewell *et al.*, 2015), decidualization (Jones *et al.*, 2006; Sharma *et al.*, 2016), and implantation (Wang *et al.*, 2003; Shokry *et al.*, 2009; Clark *et al.*, 2013). The main findings of the current study in relation to the activity of *proMMP-2* and *proMMP-9* in the culture media at day 5 of embryonic development can be summarized in five points. Firstly, the activity of *proMMP-2* was detected in 14 of 17 culture media corresponding to the patients who achieved a full-term pregnancy and in 3 of 25 culture media associated with women without this outcome. It was 1.4-fold greater in the former group. Secondly, the activity of *proMMP-9* was observed in 11 of 17 culture media corresponding to the patients who carried their pregnancy to term and in 11 of 25 culture media associated with women without this outcome. It was 1.2-fold greater in the former group (Fig. 1). Thirdly, in the three patients who did not carry their pregnancy to term, only *proMMP-9* activity was found. Fourthly, the activity of both *proMMP-2* and *proMMP-9* was identified in three non-pregnant patients

A



B



C

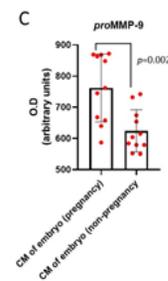


Figure 1. Activity of proMMP-2 and proMMP-9 in the culture media of human embryos on day 5 of development.

(A) The culture media from both implanted embryos (pregnant patients; lanes 1–17) and non-implanted embryos (non-pregnant patients; lanes 18–42) were analyzed using gel. The activity of *proMMP-2* (62 kDa) and *proMMP-9* (92 kDa) was identified with respect to the electrophoretic mobility of the culture medium of the promyelocyte cell line (ATCC, U937), as previously reported by our research group (Flores-Herrera *et al.*, 2012). Optical density (OD) of *proMMP-2* (B) and *proMMP-9* (C) in the culture media (CM). The points indicate the values of the activity band corresponding to the transferred embryos leading to pregnancy ($n=14$) and non-pregnancy ($n=28$). Optical density (O.D) was determined using the NIH ImageJ program (USA).

who had some pre-pregnancy complications (Fig. 1). Finally, there were no significant differences in the concentration of the hormones hCG, E2, or P4 between pregnant and non-pregnant patients (Table 1).

Gu and others (Gu *et al.*, 2015) reported the concentration of the active form of MMP-9 at 0.698 ± 0.022 ng/mL in the culture media of developing human embryos, which resulted in pregnancy for 77.0% of the participating patients (Woessner, 1991; Huang *et al.*, 1998; Gu *et al.*, 2015). According to the present study, the activity of *proMMP-2* and *proMMP-9* (Fig. 1B, C) was significantly more robust in the culture media of the embryos yield-

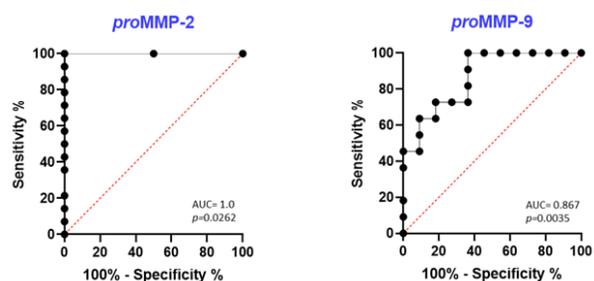


Figure 2. Predictive values of the ROC curves.

Based on the cutoff value that showed the best sensitivity and specificity for *proMMP-2* and *proMMP-9* as biomarkers of the success of implantation. We confirm that *proMMP-2* and *proMMP-9* are significantly elevated in pregnancy ($n=14$) and non-pregnancy ($n=28$), with an AUC=1 ($p=0.0262$) and AUC=0.867 ($p=0.0035$) respectively.

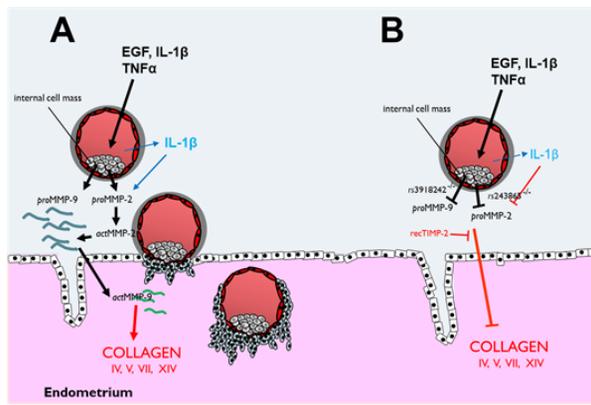


Figure 3. Model of active proMMP-2 and proMMP-9 secreted from embryos into the culture media.

(A) The developing embryos secrete interleukin type 1-beta (IL-1 β) (Librach *et al.*, 1994), tumor necrosis factor-alpha (TNF α) (Zhu *et al.*, 2012) and epidermal growth factor (EGF) (Maia-Filho *et al.*, 2015), promoting the expression of the collagenolytic protein extracellular matrix (Kitanaka *et al.*, 2019; Wang *et al.*, 2003). The actMMP-2 protein has been shown to remove propeptides associated with the catalytic site of proMMP-9, leading to activation of its degradative function (Kitanaka *et al.*, 2019; Maia-Filho *et al.*, 2015). (B) Although an embryo has the capacity to secrete IL-1 β into the culture medium (Librach *et al.*, 1991), there is a decrease in the signaling pathways for the activation of MMPs. Cytotrophoblast cells incubated with 5 nM recombinant tissue inhibitor of MMP (recTIMP-2) reduces the percentage of invasiveness (Basu *et al.*, 2018). Different polymorphisms found in the promoter regions have been associated with recurrent abortions (Bischof & Campana, 2000).

ing a full-term pregnancy than those that did not (Table 1). The culture media of embryos that generated a full-term pregnancy had a 1.4-fold greater proMMP-2 activity ($p=0.045$) than the media of the other cases (3/25) showing proMMP-2 activity. Similarly, the culture media displayed a 1.2-fold greater proMMP-9 activity ($p=0.002$) for the embryos engendering a full-term pregnancy (11/17) compared to the other cases (11/25) exhibiting proMMP-9 activity.

A conceptual model is herein provided (Fig. 3) to explain how MMPs are activated by epidermal growth factors (Gu *et al.*, 2015), interleukin (IL)-1 β , and tumor necrosis factor (TNF)- α (Basu *et al.*, 2018; Librach *et al.*, 1991). Sequeira and others (Sequeira *et al.*, 2015) reported a significant 15.4-fold greater concentration of IL-1 β in the culture media corresponding to developing human embryos successfully versus unsuccessfully implanted in patients (8.5 ± 1.4 vs 0.55 ± 0.25 pg/mL). Implantation was successful in 42.0% of the participants (Sequeira *et al.*, 2015).

One study found that syncytiotrophoblast cells secrete a 2.4- and 3.8-fold greater amount of IL-1 β in the first and second trimesters of pregnancy, respectively, compared to the pre-pregnancy level. The secretion of IL-1 β is associated with an increase in MMP-9 activity and invasion (Librach *et al.*, 1994). After interacting with its receptor, IL-1 β regulates the signaling pathway involved in the activation of the mitogen-activated protein kinase (MAPK), p38 MAPK, c-Jun N-terminal kinase (JNK), and the extracellular regulatory kinase (ERK) (Vincenti & Brinckerhoff, 2002; Sondergaard *et al.*, 2010). As a consequence, IL-1 β promotes the activation of nuclear factor kappa-beta (NF κ B) and the expression of MMP-13 (Liacini *et al.*, 2003).

The collagenolytic activity of MMPs is regulated by the specific tissue inhibitors of these proteinases (Woessner, 1991; Zhu *et al.*, 2012). Cytotrophoblast cells, treat-

ed with 50 nM of their tissue inhibitor, known as tissue inhibitor of metalloproteinase-2 (TIMP-2), exhibit a reduction (up to 40%) in invasiveness (Librach *et al.*, 1991; Bischof & Campana, 2000). The current results show a 1.4-fold and 1.2-fold decrease in proMMP-2 and proMMP-9 activity, respectively (Fig. 1B, C), in the culture media corresponding to the patients who were pregnant but did not carry to term. However, the expression of TIMPs was not presently evaluated in the culture media of developing embryos. It would be interesting to determine whether the MMP/TIMP relationship is involved in the mechanism responsible for regulating the progress of implantation and pregnancy.

Recently, polymorphisms localized in the promoter region of MMP-2 (-1306 C/T; rs 243865) and MMP-9 (-1562 C/T; rs 3918242) were found to induce changes in the levels of transcription and/or expression of the respective protein. These mutations have been proposed as a risk factor for spontaneous abortion (Barisic *et al.*, 2018; Basu *et al.*, 2018). Regarding the three patients of the present investigation that spontaneously aborted (P29, P30, and P38), the corresponding culture media displayed proMMP-2 and proMMP-9 activity (Fig. 1B, C). Future research should explore the possible relation of such pregnancy complications to mutations.

MMP-2 has the capability of degrading fibronectin, elastin, and collagen type IV, V, and VII. In contrast, MMP-9 degrades proteoglycans, elastin, and collagen I, IV, V, and XI (Kisalus *et al.*, 1987; Librach *et al.*, 1991; Mylona *et al.*, 1995; Iwahashi *et al.*, 1996), thus allowing the cytotrophoblast cells to invade the endometrium and prepare the way for implantation (Sharma *et al.*, 2003) (Fig. 3). During the implantation window, according to *in vivo* models (Liu *et al.*, 2006) and *in vitro* studies (Zhang *et al.*, 2013), the epithelial cadherin-like binding protein (E-cadherin) enables the embryo to adhere to the endometrial epithelium, which is degraded by MMP-9 (Maia-Filho *et al.*, 2015). Hence, previous reports evidence a key role played by MMP-2 and MMP-9 in embryonic development. The present results suggest that a successful implantation may be predicted by an assessment of the culture media of developing embryos based on the cutoff points of the ROC curve herein set for these two proteinases.

In conclusion, the current findings demonstrate the feasibility of detecting proMMP-2 and proMMP-9 activity in the culture media of embryos on day 5 of development by using *in gel* zymography. Additionally, such activity was associated with the implantation capacity of the embryos. Therefore, an evaluation of this activity could serve as a non-invasive method for determining the viability of human embryos developed *in vitro*.

Declarations

Conflicts of Interest. The authors declare no conflict of interest.

Authors contribution. FVM-H performed *in vitro* fertilization and obtained the culture medium for the developmental embryos of the embryos. FVM-H and GB-B conducted the tests to evaluate the activity of the MMPs. FVM-H, RJA-G, and MO-C participated in the analysis and discussion of the results. FVM-H, GB-B, RJA-G, MO-C, JSL-C, and JL-C participated in the discussion of the results. PV-C performed the statistical analysis of the ROC curve and participated in the discussion of results and writing of the manuscript. FVM-H and MO-C performed the analysis of MMP activity and participated in the writing of the manuscript. HF-H par-

participated in the design of the study, analysis of results, and writing of the manuscript, as well as obtaining financial and material support for the study.

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