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Original Research Article

# Expression of factors involved in the regulation of angiogenesis in the full-term human placenta: Effects of *in vitro* fertilization

*Chanjuan Li* [a,c,1], *Yuan Zhang* [a,1], *Lisha Tang* [b], *Haijun Zhao* [a], *Chao Gao* [a], *Li Gao* [a], *Yugui Cui* [a,*], *Jiayin Liu* [a,*]

[a] *State Key Laboratory of Reproductive Medicine, Clinical Center of Reproductive Medicine, First Affiliated Hospital, Nanjing Medical University, Nanjing 210029, China*

[b] *Maternal and Child Health Hospital in Lianyungang, Jiangsu 222006, China*

[c] *Maternal and Child Health Hospital in Nanjing, Nanjing Medical University, Nanjing 210005, China*

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ABSTRACT

The effects of assisted reproductive technologies (ARTs) on the safety of pregnancy and the resulting offspring remain controversial. Studies of placental functions, especially vasculogenesis and angiogenesis, in pregnancies established through ART are helpful for furthering our understanding of the safety of ART. This study compares the expression profiles of angiogenic factors in human term placentas obtained from natural (NAT) pregnancies *vs.* placentas obtained from pregnancies that resulted from ART. Term placentas were obtained from women who underwent an ART procedure ($n = 4$), and these were compared with term placentas that were obtained from women who had experienced a spontaneous pregnancy (controls, $n = 4$). An array analysis was performed using the Human Angiogenesis Antibody Array to detect 43 angiogenic factors and to identify which of these factors were differentially expressed between the two groups. The expression of six of these factors was greater in the ART group than in the NAT group. The levels of four of them, including vascular endothelial growth factor receptor-3 (VEGFR3), basic fibroblast growth factor (bFGF), interferon gamma (IFNG) and matrix metalloproteinase 1 (MMP1), were quantified using western blot analysis. These factors were examined using immunohistochemistry and microscopy in vascular endothelial cells or the cytoplasm and membranes of syncytiotrophoblast cells. Our finding that selected angiogenic factors exhibit altered expression profiles in ART placentas might be significant when evaluating ART safety.



* *Corresponding authors at*: State Key Laboratory of Reproductive Medicine, Clinical Center of Reproductive Medicine, First Affiliated Hospital, Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, China. Fax: +86 25 83674442.
E-mail addresses: cuiygnj@njmu.edu.cn (Y. Cui), jyliu_nj@126.com (J. Liu).

[1] These authors contributed equally to this work.

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## 1. Introduction

Assisted reproductive technology (ART) is a process that is widely used around the world. It has helped 85% of infertile couples to conceive babies, and it accounts for between 1% and 3% of all births in some countries [1]. The use of ART is growing exponentially, and 2–5% of all children are now born as a result of ART procedures [2]. As the number of ART-facilitated births has increased over the last few decades, researchers have become concerned about the safety of ART for the resulting offspring. Early studies suggested that ART is safe and leads to normal child development [3]. However, recent investigations have revealed that perinatal risks, including pregnancy-induced hypertension, gestational diabetes mellitus, preterm birth, low birth weight and long-term complications, such as congenital malformations and epigenetic disorders, can result from either ART or infertility treatments [4–8]. Ceelen et al. found that blood pressure levels were higher in IVF-conceived children than in naturally conceived children [9]. Recent evidence suggests that ART is an important cardiovascular risk factor, possibly because of epigenetic alterations [10], and that the adverse outcomes that are associated with ART can be attributed to the infertility or sub-fertility of the parents rather than the ART procedure itself [11]. However, our previous studies have shown that both ultrastructure and gene expression were altered in ART-derived placentas, suggesting that the placentas may be an important target for studies of ART safety [12,13].

The placenta is a temporary organ that forms a connection between the mother and fetus and plays a critical role in fetal growth, during which it acts as a barrier while also performing many major functions, including gas exchange, nutrient transport, and hormone secretion [14]. Placental angiogenesis is critical to the success of a human pregnancy. Normal angiogenesis establishes adequate placental perfusion, which provides the appropriate *in utero* environment for fetal development. Defective placental angiogenesis is associated with several pregnancy complications. Vascular dysfunction that is characterized by the overproduction of soluble fms-like tyrosine kinase-1 (sFLT1) in the placenta has been observed in women with preeclampsia [15,16]. In addition, alterations in placental angiogenesis, including the insufficient extension, branching, and dilatation of capillary loops during the formation of terminal villi, have been correlated with fetal intrauterine restriction [17]. In mouse placentas, angiogenesis was experimentally prevented by overexpressing placenta growth factor (PLGF), which led to gestational loss and growth restriction [18]. Lower maternal second trimester PLGF levels were correlated with narrower retinal arteriolar calibers in childhood, indicating that altered placental angiogenesis in a mother might affect the microvascular development of her offspring [19]. We hypothesize that many ART-related procedures could potentially disturb placental development and functions by inducing ovarian hyper-stimulation. Additionally, medications taken to sustain pregnancy, sperm preparation procedures, blastocyst culture, assisted hatching, intracytoplasmic sperm injection and embryonic biopsy can also disturb the placenta [20]. These procedures are thought to alter the placenta's vascular structure and function, and they are presumed to be the causes of the clinical and genetic problems that have been observed in ART offspring. A comprehensive screening of angiogenic factor expression in ART placentas would therefore be useful. In this study, we compared the expression profiles of angiogenic factors in placentas from women who underwent IVF procedures and placentas from women who had normal pregnancies.

## 2. Materials and methods

### 2.1. Subjects and ethics

Placentas were collected from women who underwent standard IVF or experienced normal pregnancies following cesarean deliveries that were performed from 2006 to 2009 in the Jiangsu Province Hospital Center of Clinical Reproductive Medicine (CCRM). Data were collected and organized in a database. The inclusion criteria for IVF subjects were the following: maternal age between 20 and 35 years old, full-term delivery, singleton pregnancy, childbirth weight between 2500 and 4000 g, and no pregnancy complications or birth defects. Data were obtained from couples who were referred for IVF solely because of oviductal factors, such as oviductal obstruction or resection. The sperm quality of all of the fathers was within the normal range of WHO parameters. These criteria were used to minimize the involvement of complications that resulted from variables relating to the parents and to place the focus of the study on the IVF process itself. The controls were chosen using the following criteria: maternal age, parity, gestational weeks, fetal weight and infant gender. To increase the similarity between this study and other studies [12,21–23], we used four samples from each group in the array analyses (Table 1). After the arrays were performed, eight additional samples from each group were used for western immunoblotting and immunohistochemistry to verify the results of the array analyses. The IVF and control groups were comparable with respect to the following parameters: maternal age ($p = 0.27$), gestational weeks ($p = 0.73$), and birth weight ($p = 0.20$). The clinical applications used to perform IVF were licensed by the Ministry of Health of the People's Republic of China. All of the subjects provided informed consent, and the research program was approved by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University.

### 2.2. Placenta sample collection

Each placenta was collected immediately following delivery. Fragments were dissected from the placental subchorial zone that corresponded to the umbilical cord insertion site. Areas of infarcted foci and hematomas were avoided. The placentas were then cut longitudinally from the maternal side to the fetal side. As described by Sood et al., the placental tissues were divided into three parts, each of which is known to have a different genetic profile: maternal, middle and fetal [24]. The villi of the middle section were collected to minimize contamination by maternal or fetal genes. All placental tissues were then floated in ice-cold PBS to avoid contamination by maternal and fetal blood. The tissues were then either stored in liquid nitrogen until total protein extraction or cut