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Protocol

Plasma Rich in Growth Factors Treatment for Patients with Refractory Endometrium Undergoing Embryo Criotransfer. Phase Iii Trial Protocol

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Abstract

Background: According to several authors, thin endometrium is associated with lower pregnancy rates. Endometrial fibroblasts have shown very good response to plasma rich in growth factor (PRGF) in vitro. PRGF has proved great efficacy in promoting tissue growth and wound healing in other fields of application. **Methods:** This study is a single-arm multicenter pilot clinical trial. 12 patients between 18 and 43 years old with endometrium thickness under 7mm after endometrium preparation for transfer of vitrified embryos will be recruited. Patients and offspring will be followed during the whole pregnancy and the puerperium. A 9 ml blood sample will be obtained and centrifuged to obtain platelet rich plasma (PRP). PRP will be activated with calcium chloride to obtain the PRGF. 0.51 mL of the PRGF will be used for the uterine infusion and the remaining volume will be stored at -20°C. Endometrium response will be evaluated by transvaginal ultrasound. Treatment will be repeated if the appropriate thickness is not achieved 3 days after treatment. Each participant will be received a maximum of 4 doses (2 cycles). Biochemical and clinical pregnancy will be evaluated. **Discussion:** Nowadays there is no treatment for thin endometrium. This problem reduces pregnancy rate in these patients with cryopreserved embryos. PRGF could be a safe and a very cost-effective solution for patients failing in their endometrium preparation.

Keywords: Plasma rich in growth factors, thin endometrium, embryo transfer, infertility

Introduction

DImplantation is the last step of a successful in vitro fertilization (IVF) treatment. A receptive endometrium with a proper thickness is one of the main factors involved in implantation [1]. Although there is not a clear consensus about the minimum thickness required for a successful implantation, most of the studies agree that the pregnancy rate decreases under 7mm [2-4] .

Some women fail in their endometrium preparation for cryopreserved embryo transfer. Reproduction specialists have tried different approaches to help these women such as: additional estrogen, acetilsalicylic acid, sildenafil, vitamin E, granulocyte colony-stimulating factor or bone marrow stem cells. Most of these attempts have not proved to be effective in all cases and

some of them have shown discrepant results [5-11].

Platelet rich plasma (PRP) is defined as the plasma fraction containing five times greater platelets concentration than blood [12-14] . After activation of PRP, platelets create a clot and liberate growth factors. PRGF can be obtained by clot manipulation. PRGF has lower volume than PRP and has no cellular debris that could cause any interference.

Effectiveness and safety of PRGF have already been demonstrated in many different areas like orthopedic surgery, maxillo-facial surgery, ophthalmology or improvement in wound healing [15-19]. PRGF contains endothelial growth factor, transforming growth factor, plateletderived growth factors, epidermal growth

factor, cytokines and other modulator factors which are responsible for its regenerative effects. These molecules are located in α -granules and play an important role in the cell migration, proliferation and differentiation in vivo [20,21].

The use of PRGF or PRP in gynecology has been very limited. Only few groups have used PRP in the treatment of thin endometrium in reduced populations but with very promising results [22,23].

Several studies have demonstrated the safety and the absence of side effects in the use of PRGF in different fields [19,24,25]. Only minor side effects were reported in some cases and in some of them this effects appeared also in the control group [26,-29]. Some authors also proved these effects to be less frequent when using PRGF than when using PRP.

Objetives

To assess the endometrium growth in patients with thin endometrium after the PRGF uterine infusion in cryotransfer cycles. The effect in the implantation, pregnancy and newborn rates will also be evaluate.

Methods

Study design

This study is unicenter single-arm pilot clinical trial.

Patients

We will recruit 12 consecutive patients with an endometrial preparation failure in a previous cycle and that in the second endometrial don't reach a minimum endometrial thickness of 7mm.

Patients with factors contraindicating or influencing treatment like thrombocytopenia, uterine malformations, ovarian or uterine tumors, acute inflammatory diseases or having drugs influencing implantation or pregnancy will be excluded.

Hormone replacement protocol

Endometrium preparation will be performed with oral estradiol valerate or transdermal patches beginning the second day of the cycle. On the 10-13th day of the cycle endometrium thickness will be measured.

PRGF preparation

One 9 mL blood sample will be obtained in tubes containing 3.8% (wt/v) sodium citrate. Blood samples will be centrifuged at 580 g for 8 minutes at room temperature and PRP will be obtained using a closed method in accordance with the Spanish Agency for Medicines and Health Products' regulation. PRP will be isolated after centrifugation and activated using calcium chloride (PRGF®-Endoret® Activator). PRGF will be obtained by mechanical disruption of the resulting clot. 0.5-1mL of PRGF will be used for uterus infusion and the remaining volume will be frozen at -20°C for further treatments of the same patient if needed.

PGRF production using PRGF®-Endoret® kit have not been not approved for uterine infusion.

Patient's treatment

0.5-1 ml of the PRGF will be loaded in a syringe connected to an intrauterine insemination catheter and administered by ultrasound-guided transcervical intrauterine infusion. Endometri-

um thickness will be assessed by transvaginal ultrasonography 3 days after infusion and if the thickness is 7mm or greater the embryo transfer will be programmed. On the contrary, if endometrium thickness is under 7mm treatment repetition will be programmed. When a second infusion will be advised, infusion will be performed the day of ultrasonography and endometrium thickness will be reassessed 3 days after. In this case either embryo transfer or cancelation will be decided according to endometrium thickness.

Only two infusions will be performed per treatment. Embryo transfer will be cancelled if satisfactory endometrium growth is not detected after two PRGF infusions.

Ethics and informed consent

This study has been positively evaluated by the ethics and clinical research Committee of our institution. All participating women will sign informed consent prior to participation. Any protocol modifications will be reviewed by the Ethics Committee and the Spanish Agency for Medicinal Products and Medical Devices (AEMPS).

Outcomes

The main variable of this trial will be the increase in the endometrial thickness. We will measure the distance between the two external outlines of the echogenic area from one basal endometrial surface across the endometrial canal to the other basal surface in a sagittal plane. It will be assessed using transvaginal ultrasonography.

We will also evaluate the effects of the treatment in the outcome of the IVF treatment: pregnancy rate, newborn rate, abortion rate, ectopic rate, etc. The safety of PRGF will be carefully investigated.

Statistical Analyses

First of all, a descriptive analysis will be performed. Qualitative variables will be expressed as a percentage. Quantitative variables will be expressed as their mean, standard deviation and range.

Discussion

In the gynecological field the use of PRGF or PRP has been very limited. Specifically, the use for thin endometrium treatment is restricted to very few reports and only reduced populations. The first report was published by Chang in 2015 with a cohort of 5 patients. They used PRP and observed positive endometrium growth in all patients after 1 or 2 doses with a pregnancy rate of 100%²². In 2017 Zadehmodarres et al.³⁰also used PRP to treat 10 patients with 2 doses, also with 100% response rate, and 40% clinical pregnancy rate. These promising results encouraged some other trials with increasing sample size. Kim et al and Frantz et al. recruited 24 patients each and both groups obtained good endometrium response and pregnancy rates between 30% and 54% [32,33]. Eftekhari et al published in 2018 the first randomized clinical trial with 83 patients, divided into two groups. Response rate, endometrium thickness increase and pregnancy rate were higher in PRP treated women vs control group [34]. Two more randomized trials have been published and both also found also greater endometrium growth and higher pregnancy rate in treated patients^{35,36}. There are variations in

the PRP preparation protocols and the administration regimen between groups.

However, no studies about the use of PRGF for endometrium treatment have been published until this point, although the response of endometrium fibroblast to PRGF has been proven in vitro. The group of Anitua demonstrated the positive effect in proliferation, migration and secretion of active molecules [37].

Several studies have demonstrated the efficacy and safety and also the absence of side effects in the use of PRGF in different fields [19,24,38]. Some authors also proved these effects to be less frequent when using PRGF than when using PRP27. PRGF shows great advantages compared to other treatments for patients with refractory endometrium. It is a standardized recovery method, with minimal manipulation, which reduces inter-manipulator differences. Additionally it is easy to obtain, from a blood sample with minimal intervention.

Declarations

Ethics approval and consent to participate

This study has been positively evaluated by the ethics and clinical research Committee of our institution. All participating women signed informed consent in advance of participation. Any protocol modifications will be reviewed by the Ethics Committee and the Spanish Agency for Medicinal Products and Medical Devices (AEMPS).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Author Contribution

Conceptualization: Marta Calvo, Funding acquisition: Sara Rafael, Methodology: Marta Vidaurreta, Teresa Gastañaga, Silvia Veganzones, María Isabel Calventus, Maria Dolores Cuquerella, Ignacio Cristobal. Project administration: Sara Rafael and Marta Calvo. Writing – original draft: Sara Rafael and Amanda Lopez. Writing – review & editing: Marta Vidaurreta, Teresa Gastañaga, Silvia Veganzones, Maria Dolores Cuquerella, Ignacio Cristobal.

Abbreviations

AEMPS: Spanish Agency for Medicinal Products and Medical Devices; IVF: in vitro fertilization; PRGF: plasma rich in growth factors; PRP: platelet-rich plasma

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