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Analysis of Placental Vascularization by Means of 3D Power Doppler in Women Pregnant Following Oocyte Donation

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Abstract

Objective: To analyze the differences in placental perfusion in pregnancies following oocyte donation compared to spontaneous pregnancies. **Participants and Methods**: A total of 33 patients who became pregnant after oocyte donation are studied and compared to 42 spontaneously pregnant participants. A 3-dimensional Power Doppler is used to obtain 3 placental volumes. Of each volume, 3 portions are analyzed in the form of a spherical "placental biopsy." Four vascularization rates are applied, using the virtual organ computer-aided analysis (VOCAL) method: grey index (GI), vascularization index (VI), flow index (FI), and vascularization-flow index (VFI). **Results**: Vascular alterations are demonstrated, manifested by a lower IG and a significantly higher IV in the group of spontaneous pregnancies (GI $P = .001$; (VI) $P = .039$) and is almost statistically significant in comparison to the IVF ($P = .051$). **Conclusions**: Placental perfusion in pregnancies obtained following oocyte donation is deficient in comparison to spontaneous pregnancies.

Keywords

Oocyte donation, recipient, placental vascularization, 3D Power Doppler

Introduction

The process of oocyte donation was first adopted in 1983.[1] It has evolved from being an unusual treatment indicated in very specific cases of gonadal dysgenesis, hereditary diseases, or early ovarian failure to experiencing explosive development in recent years due to various sociosanitary conditioning factors.

An increasingly greater number of women decide or are compelled to delay motherhood. As we know, women have an ovarian reserve that is limited to the production of oocytes in the antenatal period and therefore, her fertility is limited and decreases with time, with a higher risk of miscarriage and chromosomal alterations. Moreover, Spanish legislation, unlike other countries, allows the anonymous donation of oocytes to unmarried and homosexual women. All of the aforementioned factors together mean that the number of treatments involving oocyte donation is increasing.

Traditionally, this technique has been blamed for a high rate of miscarriages[2-5] and lower perinatal results, which were justified by the higher rate of multiple pregnancies[6] and advanced maternity age, as the oocytes are always donated by young women.

We should highlight that unlike spontaneous pregnancies, in which at least half of the genetic material of the embryo and the placental tissue is identical to that of the mother, a unique circumstance occurs in these pregnancies: the embryo is a graft that is not rejected, which is demonstrated by the higher rates of pregnancy, higher than in vitro fertilization (IVF) with the person's own oocytes. Therefore, the donation of oocytes constitutes an exemplary and unique experimental model, as no immunological characteristics are shared with the recipient. However, other factors may influence pregnancy rates and a major reason for increased success is that the age of donors is younger than that of recipients.

Once the embryo is implanted, we know that the main function of the placenta is the exchange of nutrients with adequate perfusion. It has been proven that the majority of the placental volume consists of the circulation of maternal and fetal blood.[7] A reduction in this volume, with a reduction in its vascular tree and a deficiency in its functioning, and in early stages, is related to fetal pathology, alterations in growth, and

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hypertensive states.[8,9] This fact, as well as the high incidence of hypertensive illness observed in these pregnancies, brings us to the possibility of these placentas having different vascular architectures, which we have tried to illustrate by using the three-dimensional (3D) Power Doppler study, which is the most accurate technique for providing detailed information about microvascularization.

Up to now, the only technique for quantifying placental flow is through the study of a single artery, the umbilical artery, using a conventional Doppler and assessing its flow wave, and it has not been possible to know what is really happening in the rest of the smaller arteries. The recent Power Doppler technology combined with 3D ultrasound, with reproducibility and clinical application already approved both in gynecology[10-13] and in obstetrics,[14-19] offers us the possibility of obtaining a more objective knowledge of the entire vascular network of the placenta.

It is sufficiently sensitive to detect the smallest blood vessels with a lower flow speed and obtain blood flow maps of the whole placental parenchyma, with subsequent analysis using the color histogram included in the virtual organ computer-aided analysis (VOCAL) system, which has also been approved for clinical application.[20-23]

Material and Methods

We designed a descriptive, transversal, observational study. A full ultrasound examination was carried out on pregnant women with single fetuses, discarding women with previous or current pathology (diabetes, hypertension, cardiopathy, systemic vascular disorders) that might influence placental vascular development. Cases with evident placental pathology were also excluded (signs of placental abruption, placenta previa, recent bleeding episodes).

A total of 33 women, with pregnancies resulting from an oocyte donation program, were compared with 42 other women with spontaneous pregnancies and no background of previous sterility. All of the fetuses examined were monitored until birth.

The ultrasound exploration was carried out between the 18th and 22nd week, by a single observer, skilled in Doppler and 3D technology, using the Voluson Expert (General Electric Medical Systems, S. A. Madrid, Spain) equipment with a multifrequency abdominal probe. Prior to this, the corresponding 2-dimensional (2D) study was carried out on fetal and placental position, assessment of amniotic fluid, biometrics, and a fetal morphology, which was normal in all cases. The flow curve of the umbilical artery was measured near the umbilical cord insertion.

Then, by means of 2D, the whole insertion of the placental tissue is again located on the maternal abdomen and at this moment the Power Doppler and 3D function is enabled. To streamline the results, the parameters used in the equipment are not varied, the pulse repetition frequency is not too low (0.9 kHz) to avoid heat artifacts, and sweep angle is small (40°) to make acquisition as fast as possible. Once this window is enabled, its size and shape are adapted to the image that appears on the screen, aiming to span the whole placental thickness from the chorial plate to the base plate and to make it as small as possible so that the sweep time can be as short as possible. In the seconds that coincide with the absence of maternal and fetal movements and even with the absence of maternal breathing, the volume acquisition is enabled while keeping the probe firm and still (Figure 1). Once this has been adequately obtained, it is saved in the archive software (Sonoview II) which is integrated in the information technology (IT) components of the apparatus. Then the probe is moved to locate a further 2 portions of the placenta and the acquisition of a second and third volume is repeated, with the intention of ensuring that it is as representative as possible of the total placental tissue studied in each case.

At a later stage, the volumes saved are analyzed, using the IT program VOCAL incorporated in the equipment. Each volume retrieved is represented on the screen on a multiplane basis. Using only plane A, obtained in the longitudinal section, the limits of a hypothetical axis of rotation are placed exactly on the chorial and base plates. Thus, by rotating on this axis, a sphere of tissue of a different volume is generated and it is considered valid for the study if it includes the whole thickness and does not include the large arteries that are present both in the base and chorial plates (Figure 2). For each volume of placenta that is acquired and saved, 3 adjacent spheres of tissue are obtained and subsequently analyzed, meaning that for each patient, there are 9 spheres of a further 9 "placental biopsies."

The quantitative analysis of vascularization is carried our by the application of the color histogram incorporated in the VOCAL system. The 3D color histogram measures the percentage of color and the flow amplitudes in the volume being studied, thus allowing to quantify the vascularization and the blood flow in a block of tissue. This system automatically calculates the gray and color scale values on the sphere obtained. All of the volumes obtained in 3 dimensions are constituted by units known as "voxels," the smallest unit of volume. The voxels on the gray scale contain all of the information, from black to white, from 0 to 100 in intensity. A similar scale is used for color. The parameters included in this system are 1 gray index and 3 color indices:

- **GI**: Gray index. Represents the gray average of the volume studied.
- **VI**: Vascularization index. Expressed as a percentage and measures the way in which blood is distributed in a tissue, that is vascular density.
- **FI**: Flow index: measures the average rate of flow.
- **VFI**: Vascular Flow Index: estimates both vascularization and flow, that is perfusion.

A sample size was calculated to be able to detect a minimum difference of 3.2%, in parameter IV, with a typical deviation of 4.7, risk $\alpha = .05$, $\beta = .80$, bilateral hypothesis, obtaining a minimum size of 33 patients in each group.

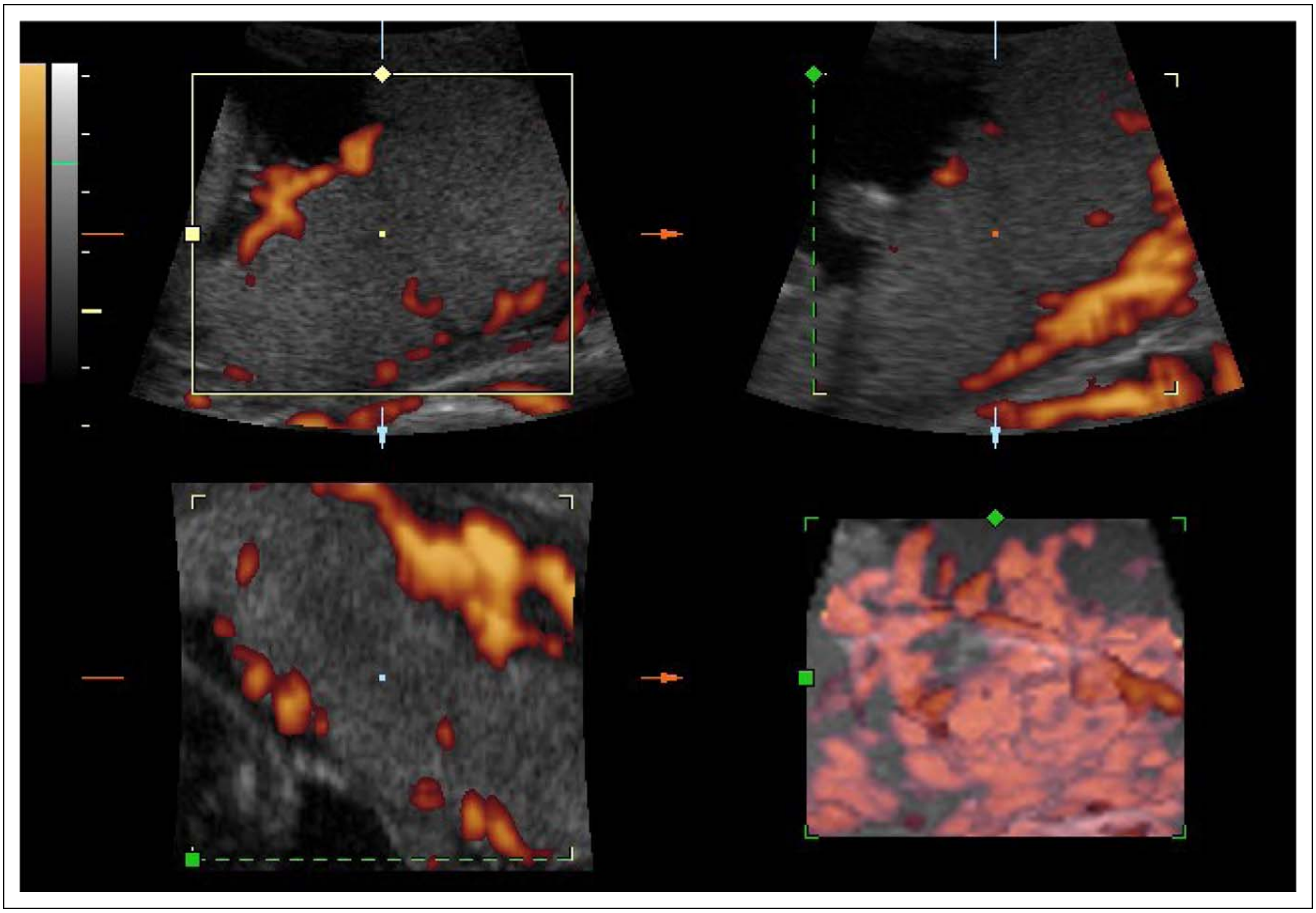

Figure 1. Acquisition of a placentary volume with 3-Dimensional Power Doppler between base plate and chorial plate.

The statistical analysis was carried out using the SPSS program, version 10.0. The differences were interpreted and estimated using the Student *t*, chi-square, and Mann-Whitney *U* tests for the bivariant analysis, as well as the Pearson correlation coefficient and multivariate analysis. The differences that displayed values $P < .05$ were considered statistically significant.

Results

A total number of 33 single pregnancies resulting from oocyte donation were examined. Of them, 8 followed IVF, 17 used intracytoplasmic sperm injection (ICSI) from the partner, and 8 used ICSI with donor sperm. These are compared to 42 spontaneous, nonsterile pregnancies. None of the pregnant women had any organic or functional gynecological pathology.

The average maternal age in the group of recipients was 39.1 ± 4.1 years, with a range between 32 and 47, whereas in the group of spontaneous pregnancies, the average age was 30.9 ± 3.2 years, with a range between 24 and 38 years ($P = .077$). The pregnancies in the group of recipients ended in week 37.6 ± 3.1, whereas 15 (45.4%) by vaginal birth and 18 (54.6%) by caesarean section. In the control group, pregnancies in the group of recipients ended in week 39.2 ± 1.3, with 32 (76.2 %) by vaginal birth and 10 (23.8 %) by caesarean section ($P = .024$). In the group of recipients, the average birth weight of the baby was 3.095 ± 629 g and in the control group it was 3.200 ± 479 g ($P = .020$).

Regarding the vascularization study, 3 volumes and 3 measures of each one were obtained in each case. The mean values obtained with the 9 measures collected from each placenta corresponding to the 4 indexes: GI, VI, FI, and VFI are obtained. When performing the bivariant analysis, as reflected in Table 1, a lower GI is obtained (25.2 vs 32.2; $P = .001$) and a significantly higher IV is obtained in the spontaneous pregnancies group (6.9 vs. 4.7; $P = .039$) and is almost statistically significant in the cases of the VFI (2.6 vs 1.6; $P = .051$). There are no differences regarding the FI (31.4 vs 30.6; P = .454). These results were independent of the age and no correlation was obtained between the age variable and the vascularization parameters VI ($P = .113$), FI ($P = .605$), and VFI ($P = .231$).

Discussion

As the placenta originates from the embryo, it contains genetic material from both progenitors. Therefore, it may be considered a semi-graft to the mother. Nevertheless, there are still loopholes regarding the fact that the embryonic trophoblast is

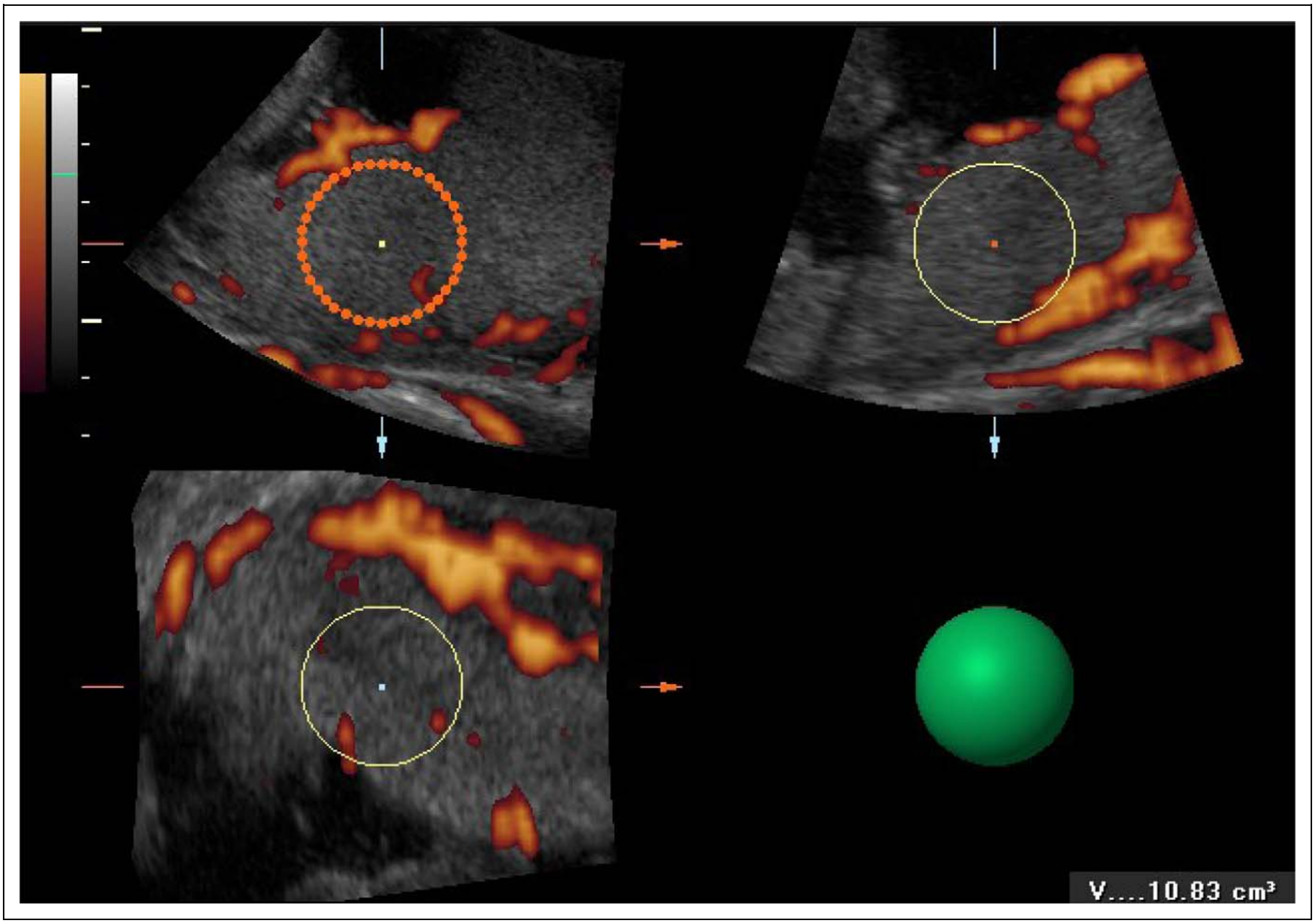

Figure 2. Using plane A, a spherical "placenta biopsy" is obtained in the thickness of the stored placentary volume. Then the vascular rates are calculated using the VOCAL method. VOCAL indicates virtual organ computer-aided analysis.

Table 1. Parameters of Placentary Vascular Study by Means of VOCAL Method in Women Pregnant Following Oocyte Donation and in Spontaneous Pregnancies[a]

	GI (units)	VI (%)	FI (Units)	VFI (Units)
Pregnancies following oocyte donation, n = 33	32.2 ± 10.3	4.7 ± 3.0	30.6 ± 3.8	1.6 ± 1.1
Spontaneous pregnancies, n = 42	25.2 ± 4.7	6.9 ± 5.7	31.4 ± 4.4	2.6 ± 2.8
P	*P* = .001	*P* = .039	*P* = .454	*P* = .051

Abbreviations: GI, gray index; VI, vascularisation index; FI, flow index; VFI, vascularisation flow index; VOCAL, virtual organ computer-aided analysis.
[a] Means and typical deviation.

not rejected by the mother's immune system. It is believed that the most important factor preventing the rejection is the lack of normal human leukocyte antigen system (HLA) antigenic expression by the trophoblast, as well as the suppressive T-cells, the blocking antibodies, the restriction of the passage of the maternal cytotoxic cells to the fetus, as well as possibly other less well-known mechanisms.[24]

Therefore, the donation of oocytes represents a unique, accessible experimental model, in which the fetus and its placenta behave like a real graft, as all of the antigens are foreign to the mother and despite this, the pregnancy proceeds to term.

The placenta separates the fetus and the uterus histologically, modifies the maternal endocrine and immune systems, and establishes the vascular connections between the mother and the embryo. There is no doubt that the placental perfusion is the primordial function in understanding the physiology of the development and nutrition of the fetus. It is very possible that many obstetrics pathologies may be underlying the anomalies of the placental tree: hypertension, oligohydramnios, stunted growth, placental thrombosis, twin-to-twin transfusion syndrome, and stroke or placental abruption. Up to now, only two methods were available for early detection of anomalies in placental perfusion: the assessment of the appearance of the placenta in 2-D images or analysis of the umbilical artery flow by conventional Doppler. However, alterations detected in this artery reflect late vascular changes in the villi tree. Moreover, anomalies are not detected in the small vessels, from which the aforementioned pathology probably originates.

The Power Doppler technique is the most sensitive and useful technique for vascular study in parenchymatous organs, as it is capable of viewing smaller blood vessels with a smaller diameter and a lower flow rate with specific characteristic of the placental vessels.[25-27] The flow signals are independent of the angle of insonation and also all of the vessels appear on the screen, including those that are analyzed in a transverse section. It is not capable of detecting the direction or rate of flow, but it does detect the density as blood flow maps may be used. If 3D is also used, we may quantify the vascularization and the blood flow in a whole block of chosen tissue, that is to view the multiple vascular overlapping, in savable and reproducible information. It is even capable of capturing villi branches including the third degree.[28]

The irregular shape of the placenta and its large extension means that it has only been possible to carry out a full study and assessment in the first trimester.[29] In the second and third trimester, the study of a "virtual biopsy" has been used, which might be representative for the whole placental vascular tree, with a methodology that has already been established and which is reproducible.[30]

In our study, we chose the second trimester, because it is a known fact that following the second trophoblastic migration, in the 14th week, the entire villi structure is established and its final structure is completed within the 16th week. Thus, we avoid any eventualities (bleeding, placental abruption, and hematomas) during the first trimester. Moreover, by avoiding the later weeks, the possible primary or secondary repercussions of maternal pathologies such as diabetes, hypertension, bleeding, and so on are avoided. We have also excluded cases where there is previous maternal pathology and multiple pregnancies.

The perinatal results were as expected: the number of caesarean births is significantly higher in the group of pregnant recipients, given the special characteristics of this population, which is being older and previously sterile, and a lower birth weight for their babies. It must be also pointed out that the maternal age of the egg recipients (39.1) was older than that of spontaneous pregnancies (30.9), but the bivariate analysis did not show differences between groups VI ($P = .077$), FI ($P = .134$), and VFI ($P = .174$). Only GI ($P = 0.001$) showed statistical difference that disappeared once the multivariate analysis was applied. Although this difference may not be statistically significant, it may still affect the results.

Parity was quite different between groups and weight was slightly different between groups, and both may influence placental size and vascularization, but in the multivariate analysis differences were not statistically significant.

Regarding the application of the VOCAL program, we should highlight the difference detected in the GI, much higher in the group of recipients. This result, by measuring the average gray of all of the gray voxels of volume obtained, represents a higher density of vascular tissue exempt of color. This combined with the higher VI found in the control cases, which reflects a higher number of vessels, demonstrates a higher vascular density in spontaneous pregnancies compared to pregnancies obtained following oocyte donation.

In this study, we confirmed that there is a different vascular pattern in pregnancies obtained following the donation of oocytes. This fact would justify the worse perinatal results, associated to impaired vascular patterns regardless of other factors already known such as maternal age and multiple births. Therefore, they should be considered for high-risk pregnancies.

We also believe that this innovative research methodology, which allows the study of small vessels, is very useful in obtaining a better knowledge of placental functioning. This is to try to solve the aforementioned major clinical findings originating in the latter: repeated miscarriages, anomalies in embryo development, hypertensive illnesses, stunted growth, premature placental abruption, and so on.

Finally, it could contribute to the study of the various complex mechanisms of the placenta that have yet to be solved: its immunological characteristics for adaptation, the contribution of the maternal and paternal genomes, the fetal−maternal biochemical and endocrine exchanges, the placenta as a graft, and so on.

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