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PLACENTATION IN OOCYTE DONATION PREGNANCIES: EVALUATION OF UTERINE ARTERIES DOPPLER AND PLACENTAL HORMONES

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1. INTRODUCTION

1.1 Epidemiology and relevant aspects about oocyte donation pregnancies

Since the first successful use of donated oocytes in 1984 (Lutjen P., 1984), the number of oocyte donation (OD) cycles in Europe and United States has dramatically increased. In 2010 in Europe the total number of IVF/ICSI (In Vitro Fertilization/ Intracytosplasmic Sperm Injection) cycle was 550,296, corresponding to 1,000 cycles for one million people. The number of OD cycles was 25,187, representing 4.5% of all the IVF/ICSI cycles (de Mouzon J., 2012). The ICMART report on worldwide ART (Assisted Reproductive Techniques) data in 2006 is the most comprehensive global statistical report to date. The paper showed that one of the most remarkable trends is the steady increase in the number of OD cycles from 14,887 in 2000 to 36,272 in 2006 (http://www.icmartivf.org/toolbox/toolbox-main.html). Oocyte donation is associated with the highest success rate among assisted reproductive techniques. Centers for Disease Control and Prevention report publishing data about oocyte donation cycles in USA in 2012, reported an average of 56% of transfers resulted in live births, independent of recipient age, while 37% of transfers resulted in singleton live births showing that implantation rates are affected by oocyte age and not recipient age (CDC, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology, 2012 Assisted Reproductive Technology National Summary Report). In these circumstances, OD nowadays has become a common treatment option, especially to overcome infertility due to advanced age. The increase in maternal age is a social multifactorial phenomenon with a general trend all over the world, and in Europe the percentage of older mothers, defined as women giving birth at 35 years or older, ranged from 10.9 in Romania to 34.7% in Italy, with a Europe median of 19.7% in 2010 (European perinatal health report, 2010). This success has lead obstetricians to face more and more frequently the assistance to pregnant women who undergo oocyte donation with a consequence of an increasing interest in the impact of oocyte donation on

several aspects. On the other side, while oocyte donation is widely offered to achieve pregnancy, there is a paucity of information about several aspects, such as maternal and fetal outcomes, for counseling couples considering this option or with an ongoing pregnancy.

In this perspective, as nowadays first trimester maternal and fetal assessment has become a pivotal part of antenatal care, pregnant women from donated oocytes routinely undergo first trimester Down syndrome screening at $11\text{-}13^{+6}$ weeks. This screening is performed by combining background risk of maternal age to measurement of nuchal translucency (NT) and two fetoplacental markers in maternal serum, free β-hCG and pregnancy-associated plasma protein-A (PAPP-A). There are many aspects that lead to consider the possibility that OD pregnancies can show differences compared to spontaneous pregnancies or IVF/ICSI autologous pregnancies. In oocyte donation pregnancies in fact the distinction between the donated oocyte, fully allogeneic to the recipient, and the hormonal prepared uterine compartment of the mother, could potentially have an impact on placentation. In case of Down syndrome screening, while first trimester serum markers of aneuploidies is well established in spontaneous pregnancy, in pregnancy after IVF/ICSI treatment has shown to be different, requiring specific precautions (Gjerris et al 2012). On the other side, while some studies showed that pregnancies achieved with in vitro fertilization (IVF) of donor oocytes are at increased risk of obstetrical complications, especially pre-eclampsia (PE), in comparison to spontaneous and IVF with homologous oocytes (Helmerhorst FM., 2004; McDonald SD., 2009; van der Hoorn MPL et al., 2010), less is known about physiology and physiopathology of the interaction between the embryo and the mother, of which placentation is one of the more evident epiphenomenon.

1.2 Vascular regulation during placentation: highlights

Placental extravillous trophoblast (EVT) migration to, invasion and remodeling of the uterine spiral arteries during the first trimester of human and nonhuman primate pregnancy are fundamentally important processes thought to be essential in promoting blood flow to and development of the

fetus. In normal pregnancy, in fact the spiral arteries in the placental bed are invaded by trophoblast, which becomes incorporated into the vessel wall and replaces the endothelium, muscular layer and neural tissue (Brosens IA., 1972; Pijnenborg R., 2006). These physiological changes convert the spiral arteries from narrow muscular vessels to wide non-muscular channels independent of maternal vasomotor control, leading to an increase of 50-fold of the uterine blood flow (Rosenfeld CR, 1977). The disintegration of the wall of uterine vessels, therefore, is only insufficient to explain major changes in uterine resistence to blood flow (Jauniaux E., 1992). In several animal species (such as horses, pigs and all ruminants) the fall in uterine vascular resistance occurs without destruction or invasion of the maternal uterine tissue and therefore, the vascular transformation secondary to placentation may also result from variations in circulating steroid and protein hormones. At the same conclusion came Jurkovic and co-workers as they found no difference in the mean RI of uterine and spiral arteries between intrauterine and ectopic human pregnancies, suggesting that uterine vascular changes occur even if the placentation site is outside the uterine cavity (Jurkovic et al 1992).

Along with the anatomic changes, the dramatic increase in uterine blood flow during pregnancy is a complex event. The increases in de novo uterine vascular prostacyclin (PGI2) secretion and in the expression of uterine artery endothelial of cytosolic phospholipase A2 (cPLA2), cyclooxygenase-1/2 (COX-1), and either PGI2 synthase (PGIS) accompany the rise in the uterine blood flow. These changes are partly mediated by increase in the plasma levels and actions of estrogen via the classical estrogen receptors (ERs). Infusion of estradiol-17β (E2β) in sheep causes rise in uterine blood flow, increases the uterine artery endothelial expression of cPLA2, COX-1, and PGIS, which leads to increases of the stable PGI2 metabolite, 6-keto-Prostaglandin F1α (6-keto-PGF1α). In human umbilical vein endothelial cells (HUVECs), E2β has also been shown to selectively stimulate PGI2 production in vitro primarily via ER-α (Jobe SO. Et al, 2012). There is also a substantial body of literature showing that estrogen-induced and pregnancy-associated rises in

umbilical blood flow are, to a great extent, mediated by an upregulation of the endothelial production of the potent vasodilator NO, via increasing eNOS protein expression, and by increasing eNOS activity (Pastore MB. et al, 2012). The same authors conclude that although PGI2 is a potent vasodilator, its role in pregnancy is not as well understood as that of NO, which has been shown to have a definitive role in partly regulating uteroplacental blood flow during gestation.

In these circumstances, estrogen may be such modulator of uteroplacental vascular function. Several groups have documented the presence of ERs in uterine and other reproductive and non-reproductive arteries (Chang K., 2008). The vascular actions of estrogens are mediated via the classical estrogen receptors (ERs), ER-α and ER-β.1 Although these two ERs exhibit substantial homology, they are structurally and functionally distinct. Vascular endothelial estrogenic responses are classified as genomic (classical/nuclear) and nongenomic (nonclassical/membrane). Estrogen binding to the substantially more abundant nuclear receptors regulates gene transcription, whereas the less abundant membrane receptors (3 to 5% of the total ERs) control very rapid activation of endothelial nitricoxide synthase (eNOS) and thus nitricoxide (NO)-mediated vasodilatation (Pastore MB. Et al, 2012). Up to 90% of estrogens synthesized in the syncytium (estradiol, estrone, and estriol) enter preferentially the maternal circulation. The major reason for directional movement of newly formed steroid into the maternal circulation is the nature of the hemochorioendothelial form of placentation. In this system, steroids secreted from the syncytiotrophoblast can enter maternal blood directly. Steroids that leave the syncytium toward the fetal compartment, however, do not enter fetal blood directly. First, steroids traveling toward the fetus must traverse the cytotrophoblasts and then enter the connective tissue of the villous core. Steroids in this space can reenter the syncytium. Second, steroids that escape the villous core must then traverse the wall of the fetal capillaries to reach fetal blood. Steroids in the fetal capillaries of the villous core then can reenter the connective tissue of the villous core and then the syncytium. The net result of this

hemochorial arrangement is that there is substantially greater entry of steroids into the maternal circulation compared with the amount that enters the fetal blood.

While it has been demonstrated that estrogens has in fact an essential role in promoting placental villous blood vessel development through their impact on angiogenesis and expression of angioregulatory growth factors and successful placentation and embryonic/fetal growth are dependent upon optimal vascularization of the villous placenta (Albrecht ED & Pepe GJ, 2010), to our knowledge there are no data on estrogen maternal blood level in OD pregnancy compared to spontaneous or IVF pregnancy. In baboon pregnancies, it has been shown that prematurely elevating estrogen levels in the first trimester can suppress trophoblast remodeling of the uterine spiral arteries and have an impact on uterine and umbilical blood flow dynamics (Aberdeen GW et al., 2012). It is possible indeed that hormonal administration to prepare endometrial receptivity in recipients during early pregnancy can modify the normal endocrine milieu and may interfere with early events of placentation.

1.3 Uteroplacental blood flow assessment by ultrasound and its clinical significance

In a non gravid state and at the very beginning of pregnancy the flow in uterine artery is of high pulsatility with a high systolic flow and low diastolic flow, with the presence of a physiological early diastolic notch observed in the Doppler spectrum. Resistance to blood flow gradually drops during gestation as greater trophoblastic invasion of the myometrium takes place. During pregnancy there is a significant increase in uterine artery compliance between 8 and 16 weeks, which continued to a lesser extent until 26 weeks' gestation. This physiologic change in compliance resulted in the loss of the diastolic notch, indicator of increased uterine vascular resistance, between 20 and 26 weeks' gestation. A range of common complications of pregnancy, encompassing recurrent first and second trimester losses, fetal growth restriction (FGR), early-onset preeclampsia, spontaneous preterm labour and preterm premature rupture of the membranes, are

associated with varying degrees of failure to anatomically transform the spiral arteries of the placental bed (Burton GJ., 2009). While PE is not a homogeneous condition, as late-PE is thought to be a manifestation of an underlying metabolic disorder with increased insulin resistance, histological studies showed that early onset severe PE- requiring delivery before 34 weeks - is associated with impaired placentation because of defective or absent remodelling of the myometrial segment of the utero-placental arteries (Brosens et al., 1972 and 2010). Doppler studies have demonstrated that a high proportion of pregnancies developing early-PE have increase in the impedance to flow in the uterine arteries manifested as increased PI as a consequent defective or absent remodelling of the myometrial segment of the utero-placental arteries (Papageorghiou A., 2001 and 2004; Plasencia W., 2007) and meta-analyses show that uterine artery Doppler analysis can predict women at increased risk of preeclampsia (Cnossen et al, 2008).

This process of placentation is reflected in the measurement of impedance to flow in the uterine arteries which can be investigated by the measurement of uterine artery pulsatility index (PI) (Yu et al., 2005; Plasencia et al., 2007). The uterine artery can be evaluated by direct visualization and after the acquisition of the spectrum is possible to obtain the blood flow velocity at peak systole (maximal contraction of the heart) and peak diastole (maximal relaxation of the heart) and the medium value. These values are then computed to derive a ratio. The most common approach is to measure the PI, calculated by the following equation: PI = (peak systolic velocity - end diastolic velocity) / time averaged velocity = (PSV - EDV) / TAV in which the peak of systole is divided by the sum of systole and diastole. Two types of uterine artery Doppler waveform analysis techniques have emerged for prediction of preeclampsia, as well as other disorders associated with impaired placentation (eg, fetal growth restriction, preeclampsia): (1) presence or absence of diastolic notching (unilateral, bilateral) of the uterine arcuate vessels, and (2) flow waveform ratios (eg, high resistance or pulsatility index, systolic/diastolic ratio).

Few histological and immuno-histochemical studies investigate the placenta in the oocyte donation (OD) pregnancy and consider the hypothesis that placenta may work differently in OD pregnancy compared to spontaneous pregnancies or pregnancy form in vitro fertilization (IVF) and to our knowledge none studied no placental markers and Doppler velocimetry of uterine arteries in OD.

2. HYPOTHESIS AND AIMS OF THE STUDY

To investigate the hypothesis that placentation in oocyte donation pregnancies presents differences in comparison to pregnancies form IVF and spontaneous, as a result of alteration in normal placental and fetal-maternal interaction responsible of physiological placentation, due to genetic or hormonal factor and abnormal uterine and placental perfusion, through the evaluation of placental perfusion and placental markers, we tested the following:

1. Is there any difference in PI of uterine arteries in the three groups and in the three trimesters of pregnancy?
2. Is there any difference in maternal serum levels of free β-hCG and pregnancy-associated plasma protein-A (PAPP-A)?
3. Is there any difference in maternal serum levels of estrogens in OD pregnancies, in vitro fertilization/intracytoplasmic sperm injection pregnancies with autologous oocytes (IVF/ICSI) and spontaneous pregnancies?
4. Are there any anomalies of nuchal translucency?
5. Are there potential differences in Down syndrome screening between OD pregnancies, in vitro fertilization/intracytoplasmic sperm injection pregnancies with autologous oocytes (IVF/ICSI) and spontaneous pregnancies due to any of the above alterations?

3. METHODS

In order to test our hypothesis we performed two studies concurrently.

3.1 First study

The first study was a prospective longitudinal study in singleton pregnancies to analyze uterine arteries Doppler in 3 steps: 11+0-13+6 weeks, 19+0-22+0 weeks, 30+0-32+0weeks. The OD study group included 55 OD IVF/ICSI - oocyte recipients pregnancies compared to 122 singleton spontaneously conceived pregnancies (Controls) and 48 pregnancies obtained with autologous IVF/ICSI randomly selected from our database of patients attending our antenatal clinic during the same study period and fulfilling the following reported criteria. All patients were attending our center from January 2013 to January 2015 for routine assessment of risk for chromosomal abnormalities by measurement of fetal nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 to 13 + 6 weeks' gestation. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by the Institution Review Board. Recorded patient characteristics included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian or mixed), method of conception (spontaneous or assisted, requiring the use of ovulation drugs), smoking status during pregnancy, personal and obstetric history, including parity (parous or nulliparous if no previous pregnancies at or after 24weeks). Maternal weight and height were also measured and the body mass index calculated in kg/m^2. From this population we considered 55 consecutive singleton and nulliparous pregnancies obtained with donor oocyte (no=55). Further criteria of inclusion were 1) successful recordings of uterine Doppler velocity waveforms, 2) absence of pre-existing maternal diseases (diabetes, chronic hypertension, renal disease), 3) absence of fetal structural or chromosomal anomalies, 4) exhaustive follow-up (i.e. delivered in our unit allowing confirmation of the occurrence of maternal and fetal complications).

All ultrasound examinations were performed transabdominally using Voluson E8 and Voluson 730 Expert ultrasound machines (GE Medical Systems, Milwaukee, WI, USA). Transabdominal ultrasound examination was carried out for the measurement of fetal crown–rump length (CRL) and NT thickness, diagnosis of any major fetal defects and measurement of uterine artery pulsatility index (PI). For the Doppler studies a sagittal transabdominal section of the uterus was obtained and the cervical canal and internal cervical os were identified. Color Doppler was used to visualize in turn the left and right uterine arteries on either side of the cervix before 14 weeks' gestation. Subsequently, the transducer was gently tilted from side to side and color flow mapping was used to identify each uterine artery along the side of the cervix and uterus at the level of the internal os. After 14 weeks (at 19+0 to 22+0 weeks and at 30+0 to 34+0 weeks) Color Doppler was used to visualize in turn the left and right uterine arteries at the apparent crossover with the external iliac arteries. Pulsed wave Doppler was used with the sampling gate set at 2 mm to cover the whole vessel and care was taken to ensure that the angle of insonation was less than 30◦. When three similar consecutive waveforms were obtained the PI was measured and the mean PI of the left and right arteries was calculated. All ultrasound and Doppler studies were carried out by 2 sonographers (L.M. and P.I.C.) who had received the appropriate Certificate of Competence in the 11 + 0 to 13 + 6 weeks' scan and Doppler of The Fetal Medicine Foundation (www.fetalmedicine.com).

After this evaluation, in order to test differences in maternal serum concentration of 17 β – estradiol between the groups, we collected 10 ml of blood from forearm vein for 82 spontaneous, 19 homologous IVF/ICSI and 10 OD pregnancies, selected randomly from this cohort after the ultrasound examination at 11 + 0 to 13 + 6 weeks' gestation. Serum samples were suddenly processed and assayed for 17 β – estradiol using the Electro Chemo Luminescence in Immunoassay (ECLIA – Elecsys/Cobas®) method to calculate the pg/mL concentration. We analyzed then the relationship between gestational age, estrogen concentration and uterine arteries Doppler.

Statistical analysis was performed using the Statistical Package for Social Science version 17.0 (SPSS Inc., Chicago, IL, USA). Usual descriptive statistics were computed considering 3 main groups (spontaneously conceived pregnancies (Controls), autologous IVF/ICSI pregnancies (Autologous IVF/ICSI), oocyte donation pregnancies (OD IVF/ICSI). The significance of the differences between groups' means was tested using one-way analysis of variance (ANOVA) and following the Fisher's Least Square Difference Method. Means, standard deviations, medians and other quartiles, when appropriate, were used for description. The p values were reported, and a p less than 0.05 (two tails) was considered for the statistical significance. Finally, 95% confidence intervals were also computed.

3.2 Second study

The second study was a cohort study performed to test differences in first trimester Down Syndrome screening markers. We analyzed data of singleton pregnant women who underwent their first trimester screening for aneuploidies between January 2000 and June 2013 carried out in a single reference, quality controlled and accredited laboratory. We collected 13624 spontaneously conceived pregnancies (Controls), 171 oocyte donation pregnancies (OD IVF/ICSI), and 76 autologous IVF/ICSI pregnancies (Autologous IVF/ICSI). In order to evaluate how older uteri contributed to explain the change in markers' concentrations, we then selected a cohort of 802 spontaneously conceived pregnancies with maternal age matched to OD recipients' age (Age-matched Controls). To this aim we excluded, in the spontaneous group, all patients younger than the youngest OD recipient. The age range was 38-46 years and the mean age 39.7 (±1.53) years. All participants granted a written informed consent.

First trimester combined screening for Down syndrome was performed at 11-13+6 weeks of pregnancy. The screening was performed according to the recommendation of the Fetal Medicine Foundation for nuchal translucency measurement, and gestational age was calculated from the

crown-rump length (CRL) both in spontaneous and in assisted reproductive technique (ART) pregnancies. The measurement of the concentration of free β-hCG and PAPP-A by immunofluorescence was carried out in a single, quality controlled, accredited reference laboratory (Bi-tech Ltd, Milan, Italy) which employed the following fully automatic random access immunoassays analyzer systems: the COBAS 6000 e601 system of Roche Germany Holding GmbH and the KRYPTOR system of Brahms GmbH. As concentrations of free β-hCG and PAPP-A are influenced by the machine, reagents used, gestational age, maternal weight, ethnicity, gravidity and smoking statuses, the measured marker levels are expressed as multiples of the gestation-specific normal median value (multiples of the median - MoM) after adjusting for these characteristics. No corrections for the mode of conception were applied by the laboratory. We used the software program PRISCA for the routine calculation of Trisomy 21 (T 21) and Trisomy 18 (T 18) during the first trimester. We used the donor age to calculate the age related risk of Down syndrome. When risk was equal or greater than 1 in 350 for T 21 (the cut off was customized in the lab to obtain a 5% overall screen positive rate) or 1 in 150 for T 18, the pregnant women were routinely offered invasive diagnosis by chorionic villus sampling or amniocentesis for fetal karyotyping. We compared free β-hCG and PAPP-A MoM values among Controls, Age-matched Controls, OD IVF/ICSI and Autologous IVF/ICSI. Furthermore, to study the effect of oocyte donation on the first trimester screening test, we also compared the NT values normalized to crown-rump length among the four groups. Information about NT measurements and patients characteristics were collected from patients records in the same laboratory where serum markers measurements were obtained. To compare serum maternal analytes concentration between the groups we excluded multiple gestations, structural fetal malformations and chromosomal abnormalities. In OD pregnancies, all women were advised to take estradiol valerate 2 mg, 3 tablets daily, and 400/600 mg of micronized progesterone vaginally/daily during preparation of transfer and until the end of the first trimester. No patients in the oocyte donation group underwent a simultaneous ovarian stimulation. All the

embryos transferred were fresh. Information about days of embryo culture, culture medium and number of embryo transferred were not available. IVF/ICSI patients underwent hormonal stimulation to obtain controlled ovarian stimulation using recombinant gonadotropins preparations. Protocols of controlled ovarian stimulation were decided individually according to each patient's clinical characteristics.

Statistical analysis was performed using the Statistical Package for Social Science version 17.0 (SPSS Inc., Chicago, IL, USA). Usual descriptive statistics were computed considering 4 main groups (spontaneously conceived pregnancies (Controls), N=13624; oocyte donation pregnancies (OD IVF/ICSI), N=171; autologous IVF/ICSI pregnancies (Autologous IVF/ICSI), N=76; spontaneously conceived age-matched pregnancies (Age-matched Controls), N=802). The significance of the differences between groups' means was tested using one-way analysis of variance (ANOVA) and Student's t test for post-hoc comparisons, following the Fisher's Least Square Difference Method. The Kruskal-Wallis one-way non parametric ANOVA on medians was also applied to confirm the results, taking into account the skewedness of the distributions of the variables implied. Means, standard deviations, medians and other quartiles, when appropriate, were used for description. The p values were reported, and a p less than 0.05 (two tails) was considered for the statistical significance. Finally, 95% confidence intervals were also computed.

4. RESULTS

4.1 First study

Table 1 presents the baseline characteristics of the study population. As expected, mean maternal age varied among groups (Controls: 32,8 ± 5,02 years; Autologous IVF/ICSI: 36,6 ± 4,24 years; OD IVF/ICSI - oocyte recipients: 43,2(4,74) years). All women are no smokers and primigravida. Mean Body Mass Index is similar among all groups: Controls 22,4 ± 3,78; Autologous IVF/ICSI 23,0 ± 3,86; OD IVF/ICSI 22,4 ± 3,61. All IVF and OD and 99% of the totality of controls was of southern European Caucasian ancestry.

When mean uterine artery PI at 11-13+6 weeks of the 3 groups of pregnancies were compared a difference was found between Controls and Autologous IVF/ICSI [1,679 (DS 0,456) and 1,706 (DS 0,481)] and OD IVF/ICSI - oocyte recipients [1,415 (DS 0,486)], showing the latter a reduced value [IC95% - p 0.001 (OD vs. Spontaneous conceived pregnancies) and p 0.007 (OD vs. Autologous IVF/ICSI)] (Table 2). By weeks, between 11 and 13+6 weeks, uterine arteries PI decreased with mean level of 1.68 at 11,5 weeks, 1.68 at 12,5 weeks and 1.43 at 13,5 weeks. The same was observed at 20 weeks: Controls 0,96 (0,294), Autologous IVF/ICSI 1,15 (0,407), OD IVF/ICSI - oocyte recipients 0,80 (0,292) ($p < 0.05$). In the third trimester, at 30-32 weeks, only the analysis between OD and spontaneous conceived pregnancies showed the same trend (p 0.018) (Table 2). The analysis of the variation of mean uterine PI between third and first trimester (Delta PI) did not reach the significance (IC95% - p 0,166 between all groups) (Figure 1).

We performed then analysis of maternal serum level of 17 β – estradiol, expressed in pg/ml at 11-13+6 weeks. By groups the mean (IC 95%) and the Standard Deviation in brackets (DS) was: controls 2844,93 (1516,29), autologous IVF/ICSI 2121,50 (1387,62) and OD IVF/ICSI - oocyte recipients 1705,33 (380,61). For Control group a more detailed analysis could have performed due

to numerosity of cohort. We have found then: minimum value of 568, First Quartile 1650, Second Quartile 2216, Third Quartile 2972, Maximum 8365 media = 2578 s = 1408. Serum level of 17 β – estradiol grows between 11 and 13^{+6} weeks, with mean level of 2423 at 11,5 weeks, 2521 at 12,5 weeks and 4654 at 13,5 weeks, showing that gestational age was a significant contributor to 17 β – estradiol serum level in spontaneous pregnancies with a R^2 0.06 (Figure 3). We then correlated maternal serum level of 17 β – estradiol and mean uterine artery PI at 11-13+6 weeks, but we could not find a clear relation at any weeks (Figure 4).

4.2 Second Study

Table 3 presents the baseline characteristics of the study population. As expected, mean maternal age varied among groups (Controls: 30.4 ± 3.6 years; OD IVF/ICSI - oocyte recipients: 41.9 ± 4.2 years; OD IVF/ICSI - oocyte donors: 25.9 ± 3.7 years; Autologous IVF/ICSI: 36.0 ± 4.8 years; Age-matched Controls: 39.7 ± 1.5 years). Women in the OD IVF/ICSI group were more likely to be primigravida than Controls. The totality of patients was of southern European Caucasian ancestry.

Comparisons of free b-hCG and PAPP-A maternal serum levels between groups are shown in Table 4. Free b-hCG levels were significantly higher both in OD IVF/ICSI (1.44 ± 1.06 MoM) and Autologous IVF/ISCI (1.48 ± 1.02 MoM) compared to Controls (1.15 ± 0.84 MoM; $p < 0.05$) and Age matched Controls (1.18 ± 0.98 MoM; $p < 0.05$). Free b-hCG levels did not differ respectively between OD IVF/ICSI and Autologous IVF/ISCI and between Controls and Age-matched Controls. PAPP-A levels did not significantly differ among the four groups (Controls: 1.10 ± 0.89 MoM; Age-matched Controls: 1.12 ± 0.73 MoM; OD IVF/ICSI: 1.09 ± 0.80 MoM; Autologous IVF/ICSI: 1.09 ± 0.56 MoM).

Table 4 presents the comparison of NT values of 13624 spontaneously conceived pregnancies (Controls) versus either the 802 spontaneously conceived age-matched pregnancies (Age-matched Controls), the 171 oocyte donation pregnancies (OD IVF/ICSI) and the 76 autologous IVF/ICSI pregnancies (Autologous IVF/ICSI). Overall, significantly lower NT was detected in Controls (1.41 ± 0.36 mm) compared to Age-matched Controls (1.44 ± 0.42 mm; $p < 0.05$), OD IVF/ICSI (1.46 ± 0.44 mm; $p < 0.05$) and Autologous IVF/ICSI (1.51 ± 0.34 mm; $p < 0.05$). NT values were not significantly different between Age matched Controls, OD IVF/ICSI and Autologous IVF/ICSI.

5. DISCUSSION

5.1 Main findings

We found that oocyte donation has a significant impact on placentation and blood placental markers' concentrations in the first trimester of pregnancy. We observed a statistically significant lower uterine arteries doppler pulsatility index in OD pregnancies in the first, second and trimester, in the latter case exclusively between OD and spontaneous conceived pregnancies. The analysis of the variation of mean uterine PI between third and first trimester did not reach the significance. Furthermore we found a higher value of free bhCG MoM in OD IVF/ICSI pregnancies and autologous IVF/ICSI compared to spontaneous pregnancies. Although we are not in the position to verify or falsify biological reasons of the alteration, our data allows us to hypothesize that it might be due to the IVF technique. In fact the observed alterations in OD IVF/ICSI pregnancies are similar to autologous IVF/ICSI pregnancies. A marginal decrease in NT was observed in Controls. However, this very small difference, even if interesting to be investigated (due to different accumulation of fluid in the third space?) is not clinically relevant and it is likely due to the higher numbers of this group, as well as to operator effect, with ART pregnancies more likely to be scanned by a small subset of operators linked to ART clinics. We did not find any difference in maternal serum concentration of 17-β estradiol at 11-13+6 weeks between groups, neither a relation between uterine arteries Doppler and 17-β estradiol in spontaneous pregnancies.

5.2. Discussion of the main findings with other reported data

Only one paper have been published on uterine arteries Doppler evaluation in oocyte donation pregnancies (Rizzo G., 2015). Rizzo and co-workers studied uterine artery and did not find any differences in oocyte donation pregnancies compared to IVF or spontaneous conceived pregnancies. Since mean uterine artery PI and placental volume values change with gestational age, they converted PI value into a multiple of the expected median (MoM), calculated from Fetal Medicine

Foundation reference limits for singleton pregnancies, after correction for gestational age, body mass index and maternal ethnicity. It is possible that this represents a bias as converting the PI values in MoM, as MoM is made by the ratio of the expected median, but is not known which is the correct median value for oocyte donation as no reference curves have been built, but the use of the expected median of spontaneous pregnancy introduces a potential error.

Few data have been published on biochemical markers in oocyte donation pregnancies and we found no report contrasting our results. Our data are in agreement with Bellver et al. that showed a slight increase of free b-hCG in OD compared to those found in naturally conceived pregnancies (1.20 MoM vs1.02 MoM) ($p < 0.02$) and no difference in PAPP-A in OD compared to 200 singleton pregnancies achieved naturally .

To our knowledge the only paper that studied the effect of hormonal variables on Doppler parameters is the one by Jauniaux and co-workers, where the authors found a correlation between 17 β – estradiol and uterine RI ($p < 0.001$) (Jauniaux E., 1992). The study was performed on 44 women between 5 and 16 weeks of gestation, a wide range and a small sample size.

5.3. Limitations of the studies

A limit of our study is the lack of information on the specific medical causes driving infertile women to opt for oocyte donation. Oocyte donation pregnancies form indeed a heterogeneous group that includes either old women or women with infertility caused or complicated by different clinical aspects such as premature ovarian failure, idiopathic infertility, endometriosis, and more. Each of these aspects taken alone may have a different impact on markers. On the other side, to collect a significant sample of OD pregnancies is a big effort, due to the fact that they are not referred to unique or specific units, such as for complicated pregnancy, and women tend to maintain privacy on data about OD pregnancies.

A limit of the uterine arteries analysis can be the relative small sample size, for sure affecting the hypothetic difference in maternal serum estrogen concentration. Another limit was the impossibility to evaluate for maternal serum concentration of progesterone.

About Down syndrome screening markers, even if we do not know how many embryos were transferred, Spencer K. et al. demonstrated that vanishing twins have no impact on levels of the free b-hCG marker, as this marker is very rapidly cleared from maternal blood, but rather determine a large and significant increase in PAPP-A concentration, partially related to the time between fetal demise and blood sampling (Spencer K., 2010).

5.4 Interpretation of findings and speculations on the possible impacts on clinical use

It would be particularly interesting to understand the physio-pathological mechanism that allows these changes in OD pregnancies. We suggest a series of hypotheses. The observed biophysical and biochemical differences could reflect alterations in the first stages of placentation. (Gagnon A., 2008; Cetin I., 2011; Nicolaides K.H., 2011). Van der Hoorn et al. showed that OD pregnancies are associated with a higher incidence of pregnancy-induced hypertension and placental pathology (Van der Hoorn, 2010). Along with these observations, abnormal marker levels might be predictors of these complications in OD pregnancies. We do not have, to date, complete analysis of data about this issue, as we are still examining OD outcomes, but Amor et al. showed that, even analyzing data according to the presence or absence of pregnancy complications, biochemical marker levels are still altered in ART pregnancies without complications (Amor D.J., 2012).

In addition, we know that in OD pregnancies there is a unique aspect represented by the interaction between the uterus and the immunogenetically unrelated embryo. Significant histological and immunohistochemical differences have been reported at the maternal-fetal interface of OD placentas (Gundogan F., 2010). Gundogan et al. demonstrated that there is a diffuse severe chronic deciduitis (CD) with dense fibrinoid deposition in the basal plate of OD placentas (45 % of cases),

where extravillous cytotrophoblast of fetal origin interfaces with and invades the maternal decidua. In contrast, in the non-donor IVF group the CD was observed in 8% of cases and was focal and mild. This study demonstrated significantly increased numbers of CD4+ T helper cells and CD56+ NK cells in the basal plates of oocyte donor cases. They found also in the 90% of cases in donor placenta the present of syncytial knots that represent the final stage of the apoptosis cascade in the syncytiotrophoblast. Considering the increased incidence of gestational hypertension in the egg donor-IVF group, the observation of increased syncytial knots was not unexpected. Although the authors concluded that their findings could represent a host versus graft rejection phenomenon, it could also represent an effort to suppress rejection. Today it is not well known the mechanism of maternal immune response and its characterization could supply additional insights into reproductive immunology and possibly the egg donor selection process. Perni et al. reported that placentas from donor cycles were significantly more likely to demonstrate certain pathologic findings: chronic villitis ($P<0.001$), chronic deciduitis ($P=0.034$), increased perivillous fibrin ($P=0.001$), ischemic change/infarction ($P=0.001$), and intervillous thrombi ($P=0.008$) (Perni SC., 2005). There was no statistical significance with respect to decidual vasculopathy, increased syncytial knots, or retroplacental hematomas. Schonkeren and co-workers found in 10 out of 26 placentas (38.5%) a lesion in several locations in the chorionic plate, consisted of a diffuse inflammatory infiltrate involving the entire chorionic plate from the amniotic epithelium towards the intervillous space (Schonkeren D., 2012). They found an associated myxomatous change in the mesoderm of the plate and inflammatory cells within the plate and the subchorionic space. It was restricted to the chorionic plate and not on the chorion laeve. In the fetal membranes there were no signs of the lesion. A significant relationship was found between the presence of the lesion in the chorionic plate and intervillositis ($P=0.018$), chronic deciduitis ($P=0.003$), presence of plasma cells in the decidua ($P=0.002$) and fibrin deposition in the decidua ($P=0.007$). He observed a significantly lower incidence of preeclampsia in the group with the lesion: 0% versus 45.5%. Preeclampsia only

occurred in the group without immunological lesion in the chorionic plate, regardless of the maternal age. This is very interesting because it is possible that immunological activation might be a crucial factor in protection against preeclampsia.

In OD pregnancies the fetus is allogeneic to the gestational carrier. This creates an interesting immunological paradox. The mother accepts the fetus although being immunogenetically completely unrelated to the mother. In solid organ transplantation the same immunogenic dissimilarity is present; however there are some differences in medical consequences between solid organ transplantation and OD. In the organ transplantation the clinicians have to do a medical screening for the donor in order to match donor-recipient, have to use immunosuppressive drugs to maintain the graft, have to do a close medical follow up. Fetus specific immunological tolerance during pregnancy depends on a very complex network of cytokines, complement, hormones, immune and non-immune cells. Acceptance is not simply based on the consequence of a balance between the type 1 (associated with abortion) and type 2 (associated with successful pregnancy) cytokines, since many cytokines are pluripotent. However, in an uncomplicated pregnancy the child is able to survive in the semiallogeneic environment and the mother accepts the semi-allograft. In OD pregnancy, the mother is exposed to foreign cells and antigens, a situation that has some resemblance to blood transfusions and organ transplantation. It is to be conceived that the down regulation of the maternal alloimmune response to the fetus during OD pregnancies needs more adaptation compared to a spontaneously conceived pregnancy. The degree of antigenic dissimilarity (reflected by the number of HLA mismatches) is in general higher in OD pregnancies compared to spontaneously conceived pregnancies. In the transplantation setting the degree of HLA compatibility between the donor and recipient is relevant for graft survival. More mismatches will lead to poorer graft survival (Opelz G., 2007). Compared with spontaneously conceived pregnancies, there is a higher degree of antigenic dissimilarity in OD cases. If the five most immunogenic HLA antigens (HLA-A, -B, -C, -DR and -DQ) are taken into consideration, the

maximal number of mismatches in spontaneous conceived pregnancies would be 5. In OD pregnancies this could reach a maximum of 10 mismatches. Since OD pregnancies are characterized by more HLA mismatches, it is to be expected that a possible relationship between aspects of immune regulation and the number of HLA mismatches will become more apparent in OD pregnancies. There are different defense mechanisms adopted by the fetus. Fetal trophoblast cells are the crucial cell population in the placenta, which protects the fetus from destruction by the maternal immune system. Villous cytotrophoblast is the inner layer of the villous surface epithelium. Villous syncytiotrophoblast is the superficial layer facing the intervillous space. Villous trophoblasts lack HLA expression and do not provoke an allogeneic immune response by circulating maternal T cells. The remaining trophoblasts cells, the extravillous trophoblasts, are the dominant cell type needed for the development of all non-villous parts of the placenta. Extravillous trophoblast does not express HLA-A or -B, but does express HLA-E, -F, -G and -C (Hunt and Orr, 1992), which serve as ligands for leukocyte inhibitory receptors. The consequences of these interactions include activation of pathways in natural killer (NK) cells and macrophages that interfere with the killer functions of these cells (Hunt and Orr, 1992; Hunt JS., 2006; Le Bouteiller P., 1997). HLA-G has potent immunomodulatory functions (McIntire and Hunt, 2005) whereas HLA-C and –E have shown to elicit an allogeneic immunomodulatory response by maternal NK and T cells (Moffett-King, 2002). Th2 cytokines, produced at the maternal–fetal interface, can inhibit Th1 responses, improving fetal survival but impairing responses against some pathogens (Wegmann TG., 1993). The human placenta produces immunosuppressive molecules as progesterone, prostaglandin E2, and anti-inflammatory cytokines as IL-10 and IL-4 (Hunt, 2006; Denison FC., 1998). If we consider the maternal side we know that during early pregnancy the uterus seems to be immune compromised, as T and B cells are hardly present. Macrophages and NK cells interact with the trophoblast cells. Decidual NK cells are different compared to peripheral NK cells. Decidual NK cells may recognize fetus HLA-C1 and HLA-C2 by the expression of KIR.

Macrophages are antigen-presenting cells and are the second most numerous types of leukocytes in the human decidua (Vince et al., 1994).

The number of decidual T cells increases during pregnancy, starting with 5–20% of all CD45+ decidual lymphocytes in early pregnancy, till 40–80% at term (Tilburgs et al., 2010). T cells are in close contact with fetal trophoblast cells in the decidua; however they do not attack the non villous trophoblast cells, since trophoblast lack HLA class Ia expression. Fetus specific regulatory CD4+CD25bright T cells are present in human decidua in higher numbers compared to peripheral maternal blood (Tilburgs et al., 2006) suggesting an important role for these cells at the fetal–maternal interface. T cells are able to produce a variety of type 1 and type 2 cytokines and thereby may contribute to the local regulation of the fetus-specific responses within the decidua.

Chernyshov et al. show increased percentages of intracellular IFN-c and IL-4 positive CD4+ T lymphocytes in group of pregnant women after oocyte donation compared with pregnant women after normal conception and pregnant women after IVF, confirming that allogenicity of the fetus induces a different activation of some intracellular cytokines (Chernyshov et al., 2008). Inflammatory response can limit the damage at the maternal–fetal interface and TNF-a induces the production of IL-10 that suppresses inflammation response. It would be interesting to verify the relation between serum markers' concentration and histological and immunohistochemical findings. To our knowledge, no studies have been performed so far to explore this relationship.

About Down syndrome markers, as the same differences are observed either in autologous IVF/ICSI and OD IVF/ICSI group, the most suitable hypothesis to explain our results points to the fertilization technique itself and/or to the hormonal treatment used. The protocol to obtain a superovulation, or to establish endometrial receptivity and to support pregnancy during the first trimester, provides the use of high doses of gonadotropin and/or estrogen and progestin combinations. To our knowledge there are no data on human estrogen maternal blood levels in

autologous IVF/ICSI and OD IVF/ICSI, compared to data on spontaneously conceived pregnancies. We do however know that, in baboon pregnancy, it has been demonstrated that prematurely elevating estrogen levels in the first trimester can suppress trophoblastic remodeling of the uterine spiral arteries and have an impact on uterine and umbilical blood flow dynamics (Aberdeen G.W., 2012). Estrogens have an essential role in promoting placental villous blood vessel development through their impact on angiogenesis and expression of angioregulatory growth factors. Successful placentation and embryonic/fetal growth are dependent upon optimal vascularization of the villous placenta (Albrecht E.D., 2010). It is plausible indeed that hormonal administration during early pregnancy can modify the normal endocrine milieu and may interfere with early events of placentation. Bonagura and co-workers showed that estrogen normally controls the extent to which the uterine arteries are transformed by placental extravillous trophoblast EVT in primate pregnancy by regulating expression of VEGF and particular integrin extracellular remodeling molecules that mediate this process (Bonagura TW., 2012). In particular the authors found that extravillous placental expressions of VEGF and the α1β1 and α5β1 integrins were decreased in baboons in which EVT invasion and remodeling of the uterine spiral arteries were suppressed by prematurely elevating estradiol levels in the first trimester of pregnancy. The low levels of estradiol typical of early human and nonhuman pregnancy permit aggressive EVT migration and remodeling of the uterine spiral arteries, whereas the elevation in endogenous estradiol with advancing normal pregnancy is a physiologically important event which represses and thus controls the extent of uterine vessel transformation During the first postconception weeks, maintenance of human pregnancy is completely dependent on hormonal support by progestational compounds, as rising levels of human chorionic gonadotropin maintain production of estradiol in the maternal corpus luteum due to interaction between the luteinized theca and granulosa cells. The functional activity of maternal ovaries decreases significantly by the seventh week of pregnancy as after the seventh week of pregnancy the luteoplacental shift is well advanced (Prietl G., 1992). At this time there is a

luteal-placental transition such that more than 50% of estrogen entering the maternal circulation is produced in the placenta. In oocyte donation pregnancies, to create the endometrial receptivity and then maintain pregnancy in women without functional ovaries, and hence a corpus luteum, exogenous hormones need to be administered. Standard therapy is composed by oral administration of progesterone and estradiol valerate. Estradiol valerate is an esterified form of natural 17β-estradiol (estradiol), the most potent endogenous human ovarian estrogen. After oral administration, estradiol valerate is rapidly hydrolyzed to estradiol in the intestinal mucosa. After this passage, estradiol, like endogenous estrogens, is metabolized in the liver and the predominant metabolites in postmenopausal women are estrone, estrone sulphate and estradiol sulphate. Circulating estrogens, including exogenous estradiol, are reversibly converted to estrone and exist in a dynamic equilibrium; estrone and estradiol are converted to estriol, the main urinary metabolite. Estrogens undergo enterohepatic recirculation via sulphate and glucuronide conjugation; the sulphates are mainly hydrolysed and reabsorbed, whereas the glucuronides are excreted in the bile or urine within 48 hours of administration. Estrogens, including orally administered estradiol, passively diffuse through cellular membranes and bind to estrogen receptors present in the nucleus. Estradiol is the predominant source of estrogen in premenopausal women; after menopause, estrone is the main source of estrogen and is derived from peripheral conversion of androstenedione. In premenopausal women, serum levels of estradiol range from 0.18 to 1.1 nmol/L during different stages of the menstrual cycle, whereas serum estradiol levels drop to approximately 0.05 nmol/L in postmenopausal women (Wellington K, Perry CM, 2002). Usually oral therapy is maintained at the same dosage soon after the embryo transfer until the end of third trimester. Due to pharmocodynamic an pharmacokinetic profile, the steady state is rapidly obtained (Wellington K, Perry CM, 2002). In this regimen maternal serum concentration are more or less the same during the entire first trimester of pregnancy. Our finding that at 11-13+6 weeks of pregnancy the maternal serum concentration of 17-β estradiol is not different between groups means that in oocyte donation

recipients at the beginning of pregnancy, when the concentration of estradiol in spontaneous pregnancy is demonstrated to be significantly lower (Devroey P., 1990), the maternal serum concentration of estrogen should be higher. It would be necessary to design larger prospective studies to understand the real curve of maternal hormonal concentration in order to prescribe a replacement therapy for recipients of donated oocytes more similar to the normal biology of first trimester.

Two other aspects of our study can have an important clinical impact. Firstly, irrespectively of the identification of the underlying mechanism, the importance of first and second trimester evaluation of uterine arteries Doppler is the possibility to include this parameter into an algorithm that allow to individuate women at high risk for developing preeclampsia, since this condition is associated with an increased risk of perinatal mortality and morbidity and both short- and long- term maternal complications that deserve targeted antenatal care and surveillance. In this perspective it would be interesting to reference range specific for oocyte donation pregnancies. Secondly the markers' modification of our sample resemble the direction or pattern of fetuses affected by Down syndrome, and this can lead to a higher false positive rate in first-trimester Down syndrome screening in oocyte donation pregnancies. In our study we could not evaluate the statistical performance of the screening, as we did not observe affected fetuses in our cohort. Results obtained are probably due to the younger age of the oocyte donors and to the influence of “oocyte age” in the algorithm for risk, calculation.

6. TABLES AND FIGURES

Table 1. Characteristics of the study population

	Spontaneous			*Autologous IVF/ICSI*			*OD IVF/ICSI**	
	Min	**Media (DS)**	**Max**	**Min**	**Media (DS)**	**Max**	**Min**	**Media (DS)**	**Max**
Age	17	32,8(5,02)	44	27	36,6(4,24)	44	33	43,2(4,74)	52
Height	150	163,1(6,36)	180	150	164,0(5,65)	175	155	165,7(6,66)	184
Weight	40	59,6(10,88)	98	46	61,8(10,36)	89	46	61,1 (9,28)	98
BMI	16,8	22,4(3,78)	35,5	17,8	23,0(3,86)	34,8	17,6	22,4(3,61)	39,9

IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection, OD Oocyte Donation pregnancies

(* Oocyte recipients)

Table 2. Uterine Arteries Doppler Pulsatility Index (PI)

	Controls	*Autologous IVF/ICSI*	*OD IVF/ICSI**	
	Media (DS)	**Media (DS)**	**Media (DS)**	**P**
Uta PI 11-13+6 w	**1,679 (0,456)**	**1,706 (0,481)**	**1,415 (0,486)**	**p < 0.05 a,b**
Uta PI 20-22 w	**0,96 (0,294)**	**1,15 (0,407)**	**0,80 (0,292)**	**p < 0.05 c,d**
Uta PI 30-32 w	**0,64 (0,308)**	**0,52 (0,166)**	**0,53 (0,195)**	**p < 0.05 e**

IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; OD IVF/ICSI, Oocyte Donation (*****Oocyte recipients)

a when comparing OD and Controls

b when comparing OD and Autologous IVF/ICSI

c when comparing OD and Controls

d when comparing OD and Autologous IVF/ICSI

e when comparing OD and Controls

Table 3. Characteristics of the study population

	Controls (N= 13624)	*Age-matched Controls (N= 802)*	*OD IVF/ICSI (N= 171)*	*Autologous IVF/ICSI (N= 76)*
Maternal Age (years)	30.4 (3.6)	39.7 (1.5) [c,d,e]	41.9 (4.2)*[a] 25.9 (3.7)**	36.0 (4.8) [b,f]
Weight (kg)	62.2 (11,3)	62.5 (10.4) [d]	60.6 (8.7)	60.0 (8.9)
Primigravida (%)	48.5	24.4 [c,d]	73.6 [a]	-
Risk > 1/350 n/N (%)	376 (2.8)	159 (19.8) [c,d,e]	10 (4.9) [a]	8 (10.7) [b]

IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection

Results are expressed as mean (SD), %, n/N (%).

* Oocyte recipients; **Oocyte donors.

[a] $p < 0.05$ when comparing Controls and OD IVF/ICSI

[b] $p < 0.05$ when comparing Controls and Autologous IVF/ICSI

[c] $p < 0.05$ when comparing Controls and Age-matched Controls

[d] $p < 0.05$ when comparing OD IVF/ICSI and Age-matched Controls

[e] $p < 0.05$ when comparing Autologous IVF/ICSI and Age-matched Controls

[f] $p < 0.05$ when comparing OD IVF/ICSI and Autologous IVF/ICSI

Table 4. Comparison of NT, free β-hCG and PAPP-A median MoM levels in Controls versus Age-matched Controls, OD IVF/ICSI and Autologous IVF/ICSI

	Controls *(N= 13624)*	*Age-matched Controls* *(N= 802)*	*OD IVF/ICSI* *(N= 171)*	*Autologous IVF/ICSI* *(N= 76)*
NT (mm)	1.41 (0.36)	1.44 (0.42) [c]	1.46 (0.44) [a]	1.51 (0.34) [b]
CRL (mm)	60.6 (8.42)	61.1 (8.45)	62.2 (7.59) [a]	62.4 (6.83)
Free β-hCG MoM *Mean (SD)*	1.15 (0.84)	1.18 (0.98) [d, e]	1.44 (1.06) [a]	1.48 (1.02) [b]
PAPP-A MoM *Mean (SD)*	1.10 (0.89)	1.12 (0.73)	1.09 (0.80)	1.09 (0.56)

NT, nuchal translucency; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; PAPP-A, pregnancy-associated plasma protein-A; Free β-hCG, free beta-human chorionic gonadotropin; MoM, multiples of the median.

[a] $p < 0.05$ when comparing Controls and OD IVF/ICSI

[b] $p < 0.05$ when comparing Controls and Autologous IVF/ICSI

[c] $p < 0.05$ when comparing Controls and Age-matched Controls

[d] $p < 0.05$ when comparing OD IVF/ICSI and Age-matched Controls

[e] $p < 0.05$ when comparing Autologous IVF/ICSI and Age-matched Controls

Figure 1. Mean uterine arteries PI and its Δ between third and first trimester

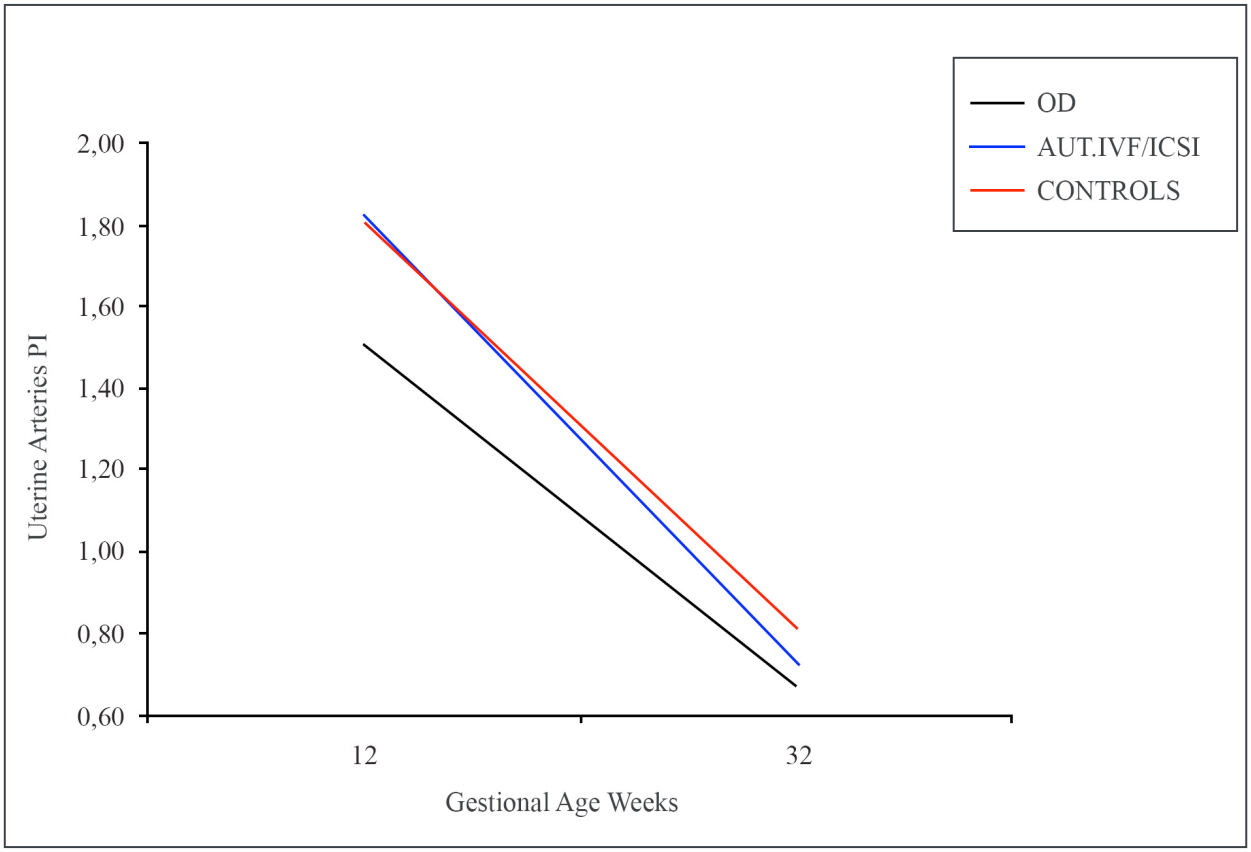


Figure 2. Mean uterine arteries PI at 11-13^{+6} and maternal serum estrogen by groups

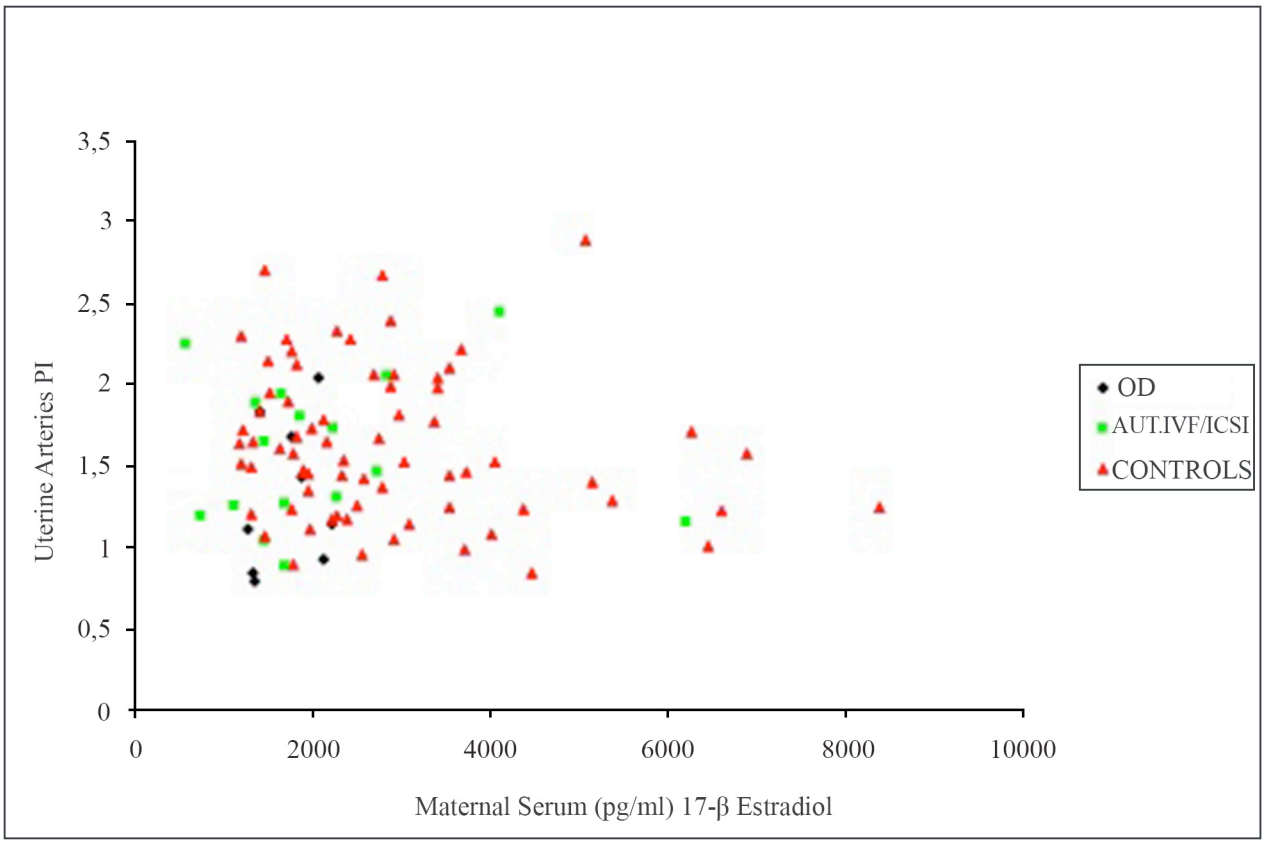


Figure 3. First trimester mean uterine arteries PI and maternal serum estrogen in spontaneous pregnancies by weeks

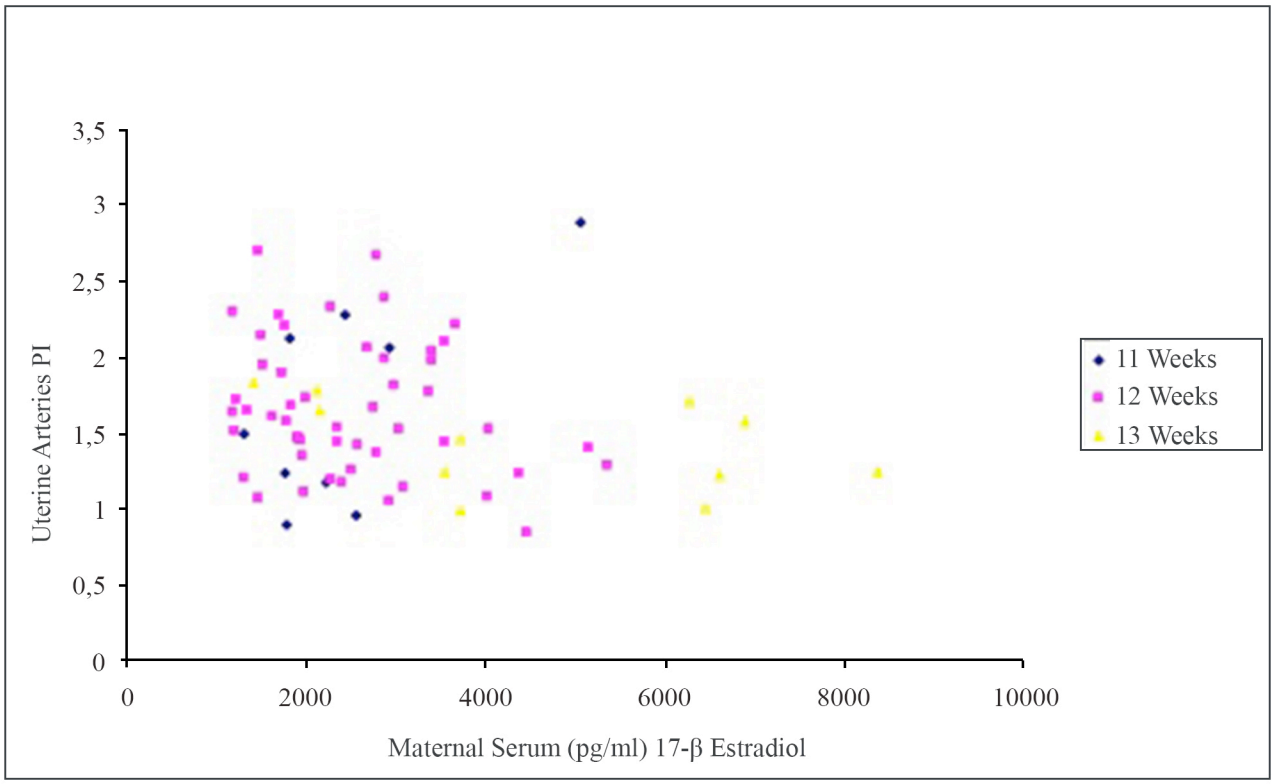
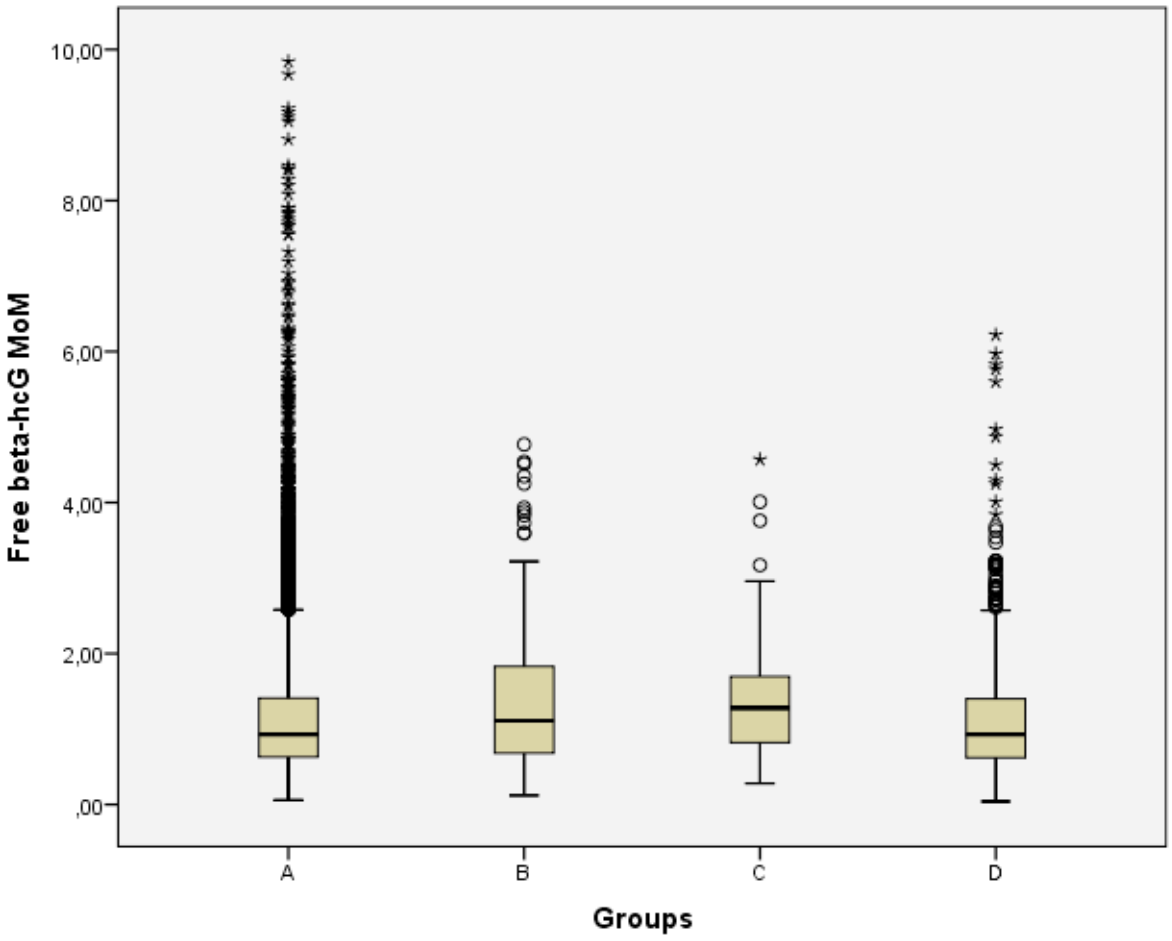


Figure 4. Free β-hCG MoM in spontaneous pregnancies versus oocyte donation pregnancies, IVF pregnancies and spontaneous age-comparable pregnancies



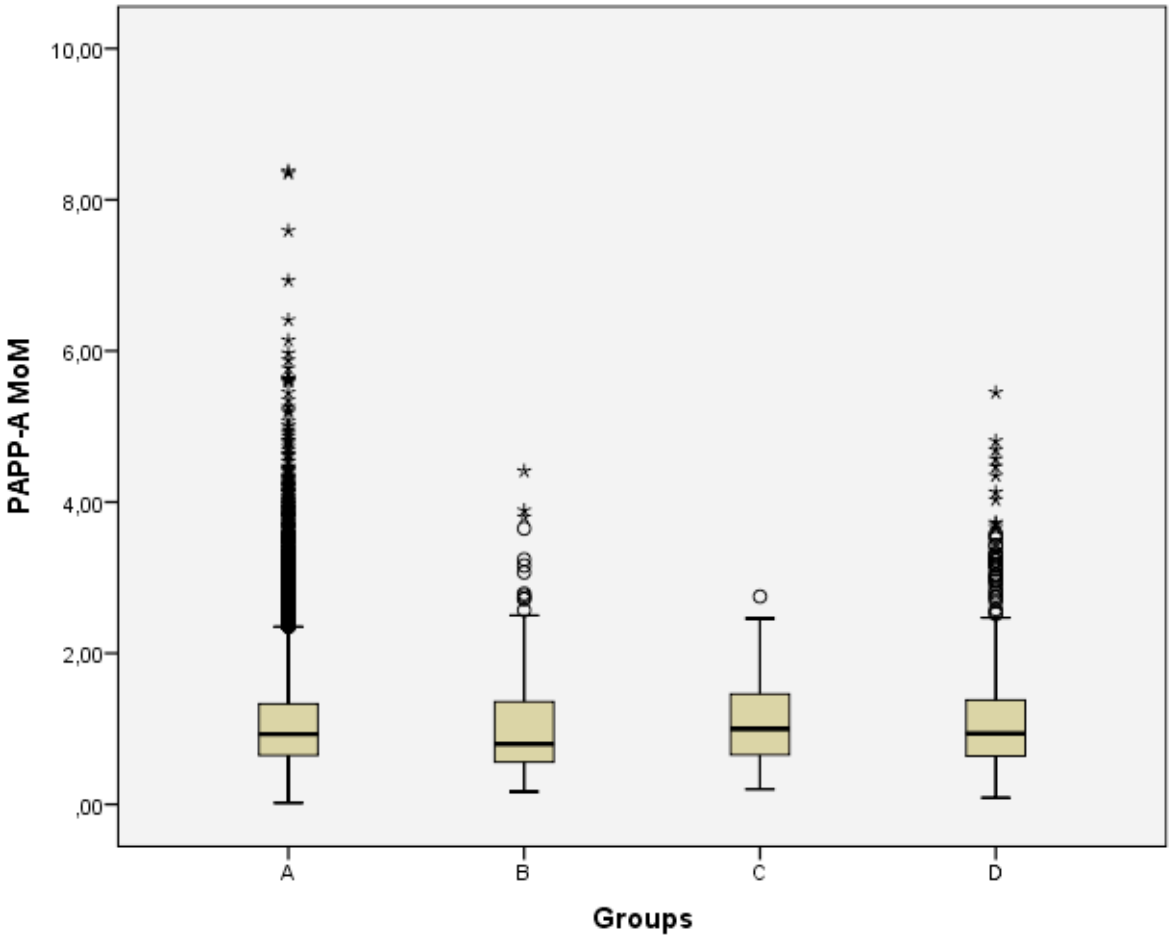
Group A: Spontaneous Pregnancies

Group B: Oocyte Donation Pregnancies

Group C: IVF/ICSI Pregnancies

Group D: Spontaneous age-comparable Pregnancies

Figure 5. PAPP-A MoM in spontaneous pregnancies versus oocyte donation pregnancies, IVF pregnancies and spontaneous age-comparable pregnancies



Group A: Spontaneous Pregnancies

Group B: Oocyte Donation Pregnancies

Group C: IVF/ICSI Pregnancies

Group D: Spontaneous age-comparable Pregnancies

7. BIBLIOGRAPHY

Aberdeen G.W., Bonagura T.W., Harman C.R., Pepe G.J., Albrecht E.D. Suppression of trophoblast uterine spiral artery remodeling by estrogen during baboon pregnancy: impact on uterine and fetal blood flow dynamics Am. J. Physiol. Heart Circ. Physiol., 302 (2012), pp. 936–944

Albrecht E.D., Pepe G.J. Estrogen regulation of placental angiogenesis and fetal ovarian development during primate pregnancy Int. J. Dev. Biol., 54 (2010), pp. 397–408

Amor D.J., Xu J.X., Halliday J.L., et al. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome Hum. Reprod., 24 (2009), pp. 1330–1338

Bonagura TW, Babischkin JS, Aberdeen GW, Pepe GJ, Albrecht ED. Prematurely elevating estradiol in early baboon pregnancy suppresses uterine artery remodeling and expression of extravillous placental vascular endothelial growth factor and α1β1 and α5β1 integrins. Endocrinology. 2012 ;153:2897-906.

Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Annu. 1972;1:177-91.

Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta. 2009;30:473-82.

Centers for Disease Control and Prevention. Assisted Reproductive Technology (ART). URL http://www.cdc.gov/art/ART2008/sect4_fig46-50.htm#f46

Cetin I, Huppertz B, Burton G, et al. Pregenesys pre-eclampsia markers consensus meeting: What do we require from markers, risk assessment and model systems to tailor preventive strategies? Placenta 2011; 32 Suppl:S4–S16.

Chang K, Zhang L. Review article: steroid hormones and uterine vascular adaptation to pregnancy. Reprod Sci. 2008;15(4):336–348

Chernyshov VP, Tumanova LE, Sudoma IA, Bannikov VI. Th1 and Th2 in human IVF pregnancy with allogenic fetus. Am J Reprod Immunol 2008:59;352-358.

Cnossen JS, Morris RK, ter Riet G, Mol BW, van der Post JA, Coomarasamy A, Zwinderman AH, Robson SC, Bindels PJ, Kleijnen J, Khan KS.. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. SOCMAJ. 2008;178:701

de Mouzon J., V. Goossens, S. Bhattacharya, et al., European IVF-Monitoring (EIM), Consortium for the European Society on Human Reproduction and Embryology (ESHRE), Assisted reproductive technology in Europe, 2007: results generated from European registers by ESHRE, Hum. Reprod. 27 (2012) 954e966.

Denison FC, Kelly RW, Calder AA, Riley SC. Cytokine secretion by human fetal membranes, decidua and placenta at term. Hum Reprod 1998:13;3560-5.

Devroey P, Camus M, Palermo G, Smitz J, Van Waesberghe L, Wisanto A, Wijbo I, Van Steirteghem AC Placental production of estradiol and progesterone after oocyte donation in patients with primary ovarian failure. Am J Obstet Gynecol. 1990;162:66-70.

Gagnon A., Wilson R.D., Audibert F., et al. Obstetrical complications associated with abnormal maternal serum markers analytes J. Obstet. Gynaecol. Can., 30 (2008), pp. 918–949

Gjerris AC, Tabor A, Loft A, Christiansen M, Pinborg A. First trimester prenatal screening among women pregnant after IVF/ICSI. Hum Reprod Update. 2012 Jul;18(4):350-9. Epub 2012 Apr 20.

Gundogan F., Bianchi D.W., Scherjon S.A., Roberts D.J.. Placental pathology in egg donor pregnancies Fertil. Steril., 93 (2010), pp. 397–404

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Review: Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. BMJ. 2004 ;32:261.

Hunt JS. Stranger in a strange land. Immunol Rev 2006:213;36-47.

Le Bouteiller P, Mallet V. HLA-G and pregnancy. Rev Reprod 1997:2;7-13.

Hunt JS, Orr HT. HLA and maternal–fetal recognition. FASEB J 1992:6;2344-8.

Jauniaux E, Jurkovic D, Delogne-Desnoek J, Meuris S. Influence of human chorionic gonadotrophin, oestradiol and progesterone on uteroplacental and corpus luteum blood flow in normal early pregnancy. Hum Reprod. 1992 Nov;7(10):1467-73.

Jobe SO, Ramadoss J, Wargin AJ, Magness RR. Estradiol-17β and its Cytochrome P450- and Catechol-O-Methyltransferase–Derived Metabolites Selectively Stimulate Production of Prostacyclin in Uterine Artery Endothelial Cells. Hypertension. 2013;61:509-518.

Jurkovic D, Bourne TH, Jauniaux E, Campbell S, Collins WP. Transvaginal color Doppler study of blood flow in ectopic pregnancies. Fertil Steril. 1992 Jan;57(1):68-73.

Lutjen P, Trounson A., Leeton J., Findlay J., Wood C., Renou P. The establishment and maintenance of pregnancy using in vitro fertilization and embryo donation in a patient with primary ovarian failure. Nature 1984;307:174-5

McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A; Knowledge Synthesis Group. Preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. Eur J Obstet Gynecol Reprod Biol. 2009;146:138-48.

McIntire RH, Hunt JS. Antigen presenting cells and HLA-G—a review. Placenta 2005:26;104-9.

Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol 2002:2;656-63.

Nicolaides K.H., Screening for fetal aneuploidies at 11 to 13 weeks, Prenat. Diagn 31 (2011) 7e15.

Opelz G, Dohler B. Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. Transplantation 2007:84;137-43.

Papageorghiou AT, To MS, Yu CK, Nicolaides KH. Repeatability of measurement of uterine artery pulsatility index using transvaginal color Doppler. Ultrasound Obstet Gynecol. 2001;18:456-9.

Papageorghiou AT1, Yu CK, Nicolaides KH. The role of uterine artery Doppler in predicting adverse pregnancy outcome. Best Pract Res Clin Obstet Gynaecol. 2004;18:383-96.

Pastore MB1, Jobe SO, Ramadoss J, Magness RR. Estrogen receptor-α and estrogen receptor-β in the uterine vascular endothelium during pregnancy: functional implications for regulating uterine blood flow. Semin Reprod Med. 2012;30(1):46-61.

Perni SC, Predanic M, Cho JE, Baergen RN. Placental pathology and pregnancy outcomes in donor and non-donor oocyte in vitro fertilization pregnancies. J Perinat Med 2005:33;27-32.

Pijnenborg R, Vercruysse L, Hanssens M. Review: The uterine spiral arteries in human pregnancy: facts and controversies. Placenta. 2006 ;27:939-58.

Plasencia W., Maiz N, Bonino S, Kaihura C, Nicolaides KH. Uterine artery Doppler at 11 + 0 to 13 + 6 weeks in the prediction of pre-eclampsia. Ultrasound Obstet Gynecol. 2007;30:742-9.

Prietl G, Diedrich K, van der Ven HH, Luckhaus J, Krebs D. The effect of 17 alpha-hydroxyprogesterone caproate/oestradiol valerate on the development and outcome of early pregnancies following in vitro fertilization and embryo transfer: a prospective and randomized controlled trial. Hum Reprod. 1992 Jun;7 Suppl 1:1-5.

Rosenfeld CR. Distribution of cardiac output in ovine pregnancy. Am J Physiol. 1977;232(3):H231–H235.

Schonkeren D., Swings G., Roberts D., Claas F., de Heer E., Scherjon S. Pregnancy close to the edge: an immunosuppressive infiltrate in the chorionic plate of placentas from uncomplicated egg cell donation PLoS One, 7 (2012), p. e32347

Schulman H, Fleischer A, Farmakides G, Bracero L, Grunfeld L. Development of uterine artery compliance in pregnancy as detected by Doppler ultrasound. Am J Obstet Gynecol 1986;155:1031-6.

Spencer K., Staboulidou I., Nicolaides K.H. First trimester aneuploidy screening in the presence of a vanishing twin: implications for maternal serum markers. Prenat. Diagn, 2010; 30 : 235–240

Tilburgs T, Claas FH, Scherjon SA. Elsevier trophoblast research award lecture: unique properties of decidual T cells and their role in immune regulation during human pregnancy. Placenta 2010:31;82-6.

Van der Hoorn M.L., Lashley E.E., Bianchi D.W., Claas F.H., Schonkeren C.M., Scherjon S.A.. Clinical and immunologic aspects of oocyte donation pregnancies: a systematic review Hum. Reprod. Update, 16 (2010), pp. 704–712

Vince GS, Starkey PM, Jackson MC, Sargent IL, Redman CW. Flow cytometric characterisation of cell populations in human pregnancy decidua and isolation of decidual macrophages. J Immunol Methods 1990:132;181-9.

Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993:14;353-6.

Wellington K, Perry CM. Estradiol valerate/dienogest. Drugs. 2002;62:491-504

Yu CK, Smith GC, Papageorghiou AT, Cacho AM, Nicolaides KH; Fetal Medicine Foundation Second Trimester Screening Group. An integrated model for the prediction of preeclampsia using maternal factors and uterine artery Doppler velocimetry in unselected low-risk women. Am J Obstet Gynecol. 2005; 193:429-36.