Mild-to-moderate foeto-maternal haemorrhage in the third trimester and at term of pregnancy: quantitative determination and clinical-diagnostic evaluation

Fausta Beneventi[1], Chiara Cavagnoli[1], Elena Locatelli[1], Silvia Bariselli[1], Margherita Simonetta[1], Gianluca Viarengo[2], Cesare Perotti[2], Arsenio Spinillo[1]

[1]*Obstetric and Gynaecology Department,* [2]*Immunotransfusion Department, "IRCCS Policlinico S. Matteo", Pavia, Italy*

Background. Foeto-maternal haemorrhage (FMH), a gestational event that occurs before or during delivery, consists of a loss of foetal blood into the maternal circulation. FMH occurs more frequently during the third trimester or labour both in normal and complicated pregnancies. In the case of alloimmunisation, the maternal immunological response and the severity of the resulting foetal or neonatal disease depend on the amount of foetal blood that passes into the maternal circulation. The aim of this study was to determine FMH in the third trimester and at term of pregnancy and to evaluate the role of clinical and ultrasound markers in the prediction of FMH.

Materials and methods. FMH was quantified by cytofluorimetric testing at 28 to 35 weeks of gestation in 223 women and at term in 465 women, all with risk factors. Foetal evaluation included foetal movement profile, middle cerebral artery peak velocity of systolic blood flow (MCA-PSV) and cardiotocographic monitoring.

Results. All women tested negative for FMH in the third trimester. Four patients (0.9%) tested positive at term, with estimated volumes of bleeding of 2.2, 8.1, 12.3 and 39.8 mL. Three FMH cases (75%) had a non-reassuring cardiotocography compared to 8.9% (42/461) of women without FMH (p=0.003) and two FMH cases reported a reduction in foetal movements reduction compared to four of those without FMH (p=0.001). Mean MCA-PSV was normal in both the groups with and without FMH (p=0.22).

Discussion. FMH is rare in pregnancy and at term. Cytofluorimetric testing is a specific method to detect mild-to-moderate FMH even when the MCA-PSV is not informative. Mild-to-moderate FMH is significantly associated with reduced foetal movements and non-reassuring cardiotocographic monitoring.

Keywords: foeto-maternal haemorrhage, cytofluorimetric assay, pregnancy, foetal anaemia.

Introduction

Foeto-maternal haemorrhage (FMH) is a gestational event that occurs before or during delivery and consists of a loss of foetal blood into the maternal circulation. Although the placenta is considered a barrier separating the maternal and foetal circulations, bidirectional trafficking of cells across the trophoblast is physiological. FMH occurs more frequently during the third trimester or labour both in normal and complicated pregnancies[1,2]. The passage of foetal blood into the maternal circulation can have obvious immunological consequences. Maternal alloimmunisation against foetal inherited paternal alloantigens can result in the formation of alloantibodies against foetal red blood cells or platelets. The subsequent potential foetal complications include foetal anaemia, haemorrhage, neonatal haemolytic disease and neonatal alloimmune thrombocytopenia[3]. In the case of alloimmunisation the maternal immunological response and the severity of the resulting foetal or neonatal disease depend on the amount of foetal blood that passes into the maternal circulation[4-7]. Passage of 25-30 mL of foetal blood into the maternal circulation is considered a massive FMH[8]. FMH occurs infrequently and in small volumes (from 0.05 to 0.5 mL in less than 5% of cases) during the first and second trimesters of pregnancy and well-known risk factors include threatened miscarriage, ectopic pregnancy, therapeutic abortion, chorion villus sampling and amniocentesis. Antepartum haemorrhage, placental abruption, abdominal trauma, or external cephalic version can cause a FMH of 0.2 mL in 98% of cases and >30 mL in 0.03% of cases. Caesarean section and/or manual removal of placenta can cause a FMH >10 mL in 0.3% of cases. Although risk factors for FMH are well known, in many cases a direct cause cannot be found[9]. The Kleihauer-Betke test has been the most widely performed, economic method for the diagnosis and

measurement of FMH, but has a poor reproducibility and is difficult to standardise[10]. The percentage of F cells (red blood cells carrying haemoglobin [Hb]F) can increase slightly physiologically during pregnancy. Maternal haemoglobinopathies (thalassaemia, sickle cell disease) produce a compensatory increase in HbF. The Kleihauer-Betke test cannot discriminate between maternal F cells and foetal HbF-positive red blood cells, which may lead to overestimation of the amount of FMH and false positive results[11]. The recent availability of routine duo-cytofluorimetric tests for the quantification of foetal cells in the maternal circulation allows quantitatively accurate, automated analysis of FMH with excellent reproducibility[12-14].

The aim of this study was to determine FMH by cytofluorimetry in women in the third trimester and at the term of pregnancy, with risk factors, and to evaluate the role of clinical and ultrasound markers in mild-to-moderate FMH.

Materials and methods

The pregnant women under study had antenatal care and were delivered at the Department of Obstetrics and Gynaecology of the Policlinico San Matteo of Pavia during the period June-December 2012. The study was undertaken in accordance with the regulations of the Institutional Ethics Committee on clinical research. Informed consent was obtained from all participants.

Maternal samples were obtained at 28 to 35 weeks of pregnancy from in 223 subjects, admitted to the Obstetrics Emergency Unit of our Department for different causes (Table I).

Table I - Demographic and obstetric characteristics of the pregnant women studied at 28 to 35 weeks of gestation.

Characteristics	Mean (±SD)
Maternal age, years	33 (±7.36)
Gestational age, weeks	31.2 (±1.98)
	Frequency (%)
Previous pregnancies	
0	93 (41.70)
1	66 (29.59)
>1	64 (28.69)
Causes of admission	
Premature rupture of membranes (<34 weeks)	30 (13.4)
3rd trimester bleeding	28 (12.5)
Reduced foetal movement	24 (10.76)
Threatened preterm delivery	45 (20.17)
Trauma	14 (6.3)
Pre-eclampsia	18 (8.07)
Foetal growth restriction	19 (8.52)
Oligo/anhydramnios	22 (9.86)
Polyhydramnios	23 (10.31)

SD: standard deviation.

Maternal venous blood samples were also obtained from 465 term pregnancies admitted to our hospital for various reasons (Table II). For each woman studied between 28 and 35 weeks we enrolled two women at term.

Samples of 3.5 mL of total venous blood were collected into ethylenediaminetetraacetate anticoagulant, and stored at 4 °C before processing (between day 0 and day 2).

The amount of foetal red blood cells in the maternal blood was quantified by flow cytometric assay using a Fetal Cell Count™ Kit (IQ Products, Groningen, The Netherlands; IVD-CE registered) according to the manufacturer's instructions. The analysis was performed by dual-colour cytogram with appropriate gating based on quality controls. Foetal red blood cells are identified by the strong expression of HbF marker combined with the absence of expression of the carbonic anhydrase (CA) marker (HbF+CA+). They are distinguished from maternal red blood cells, lacking any HbF signals but showing strong expression of the adult CA marker (HbF–CA+) and from maternal "F cells" presenting variably persistent HbF with lower expression and clear CA expression (HbF+CA+)[15,16]. The test result is expressed as the percentage of HbF+CA– red blood cells among the total of counted events of the sample. The volume of FMH is calculated using Mollison's formula[12]. An FMH of 0.1% was chosen as the cut-off for a positive

Table II - Demographic and obstetric characteristics of the pregnant women studied at 28 to 35 weeks of gestation.

Characteristic	Mean (±SD)
Maternal age, years	33.16 (±6.96)
Gestational age, weeks	39.25 (±1.2)
Birth weight, g	3,264.28 (±265.76)
Placental weight, g	544 (±44.29)
	Frequency (%)
Type of delivery	
Caesarean	124 (26.6)
Vaginal	321 (69)
Operative vaginal	20 (4.3)
Previous pregnancies	
0	187 (40.2)
1	140 (30.1)
>1	124 (26.5)
Information missing	14 (3)
Causes of admission	
Premature rupture of membranes	165 (35.48)
Uterine contractions	213 (45.80)
Reduced foetal movement	6 (1.30)
Trauma	10 (2.15)
External cephalic version	5 (1.07)
Bleeding	26 (5.6)
Pre-eclampsia	40 (8.60)

SD: standard deviation.

test. According to several authors this corresponds approximately to a volume of 2.2 mL of packed foetal red blood cells in the maternal circulation[12,17], which would be covered by a minimum standard dose of anti-RhD immunoglobulins (250 IU).

Clinical and socio-demographic data were collected and stored in a computer database. Ultrasound evaluation of foetal well-being, including assessment of foetal movement and amniotic fluid volume, and Doppler studies of umbilical artery, middle cerebral artery and ductus venosus blood flow velocity waveforms were performed in all women enrolled. The middle cerebral artery peak velocity of systolic blood flow (MCA-PSV) was measured using standardised methods[18].

Computerised cardiotocographic monitoring was also scheduled. Cardiotocographic results were classified as reassuring, non-reassuring and abnormal according to the classification of the Royal College of Obstetricians and Gynaecologists[19].

Statistical analyses were performed with Fisher's exact test and the Mann-Whitney U test to compare categorical and continuous variables, respectively.

Results

In the overall population studied the foetal red blood cell count in maternal blood ranged from 0.00 to 1.81% (median 0.37%).

The main characteristics of pregnant women from 28 to 35 weeks of gestation are reported in Table I. The mean value of foetal MCA-PSV was 49.4±4.8 cm/s. All the women examined tested negative for FMH and had an MCA-PSV within the 10th and 90th percentiles of Mari's range[18].

The main characteristics of the group of pregnant women at term are reported in Table II. The mean MCA-PSV value was 71±4.6 cm/s. All the subjects had a foetal MCA-PSV within the normal range. Out of the 465 women studied at term, only four patients (0.9%) tested positive for FMH. Their delivery details and foetal/neonatal outcomes are reported in Table III.

The estimated volumes of FMH were 2.2, 8.1, 12.3 and 39.8 mL. Among these four cases of FMH, three (75%) had a non-reassuring cardiotocograph compared to 8.9% (42/461) of women without FMH (p=0.003 by Fisher's exact test). Two women with FMH and four without FMH (p=0.001 by Fisher's exact test) reported a reduction of foetal movements at the time of admission. The mean MCA-PSV was 73.2±5.1 cm/s in FMH cases and 71±4.4 cm/s in women without FMH (p=0.22 by the Mann-Whitney test). The largest volume of FMH of 39.8 mL led to mild neonatal anaemia (haemoglobin 14.5 g/dL) and was associated with a higher MCA-PSV (80.78 cm/s). We found normal haemoglobin levels in the neonates of women without FMH.

The rate of Caesarean section was 25% (1/4) among FMH cases and 26.7% (123/461) among the controls without FMH (p=0.8).

Discussion

The clinical purpose of the present study was to evaluate the occurrence and significance of FMH in the third trimester of pregnancy and at term of pregnancy.

The basic principle of the test used, as well as the sensitivity and specificity of the method are essential for the evaluation of FMH. We used a double-staining cytofluorimetric method with anti-HbF and anti-CA. We used a detection threshold of 0,1% of foetal red blood cells which corresponds approximately to a volume of 2.2 mL[12], proposed by several authors as the optimal cut-off value to evaluate FMH accurately[18,20,21].

Traditionally, many complications of pregnancy, such as bleeding, threatened preterm delivery and abdominal trauma, have been considered significant risk factors for FMH[21]. For this reason, we systematically looked for FMH in a series of pregnant women admitted to hospital or seen at our Emergency Unit because of FMH risk factors in the third trimester or at term. None of the women in the third trimester of pregnancy had detectable FMH. These findings suggest that transplacental haemorrhage exceeding 0.1% during the

Table III - Delivery details and foetal/neonatal outcomes of four pregnant women with FMH detected at term.

Cause of admission	Type of delivery	CTG	Foetal movements	Gestational age at delivery (weeks)	Foetal MCA PSV (cm/s)	HbF+/CA− (%)	HbF+/CA− (mL)	Neonatal weight (g)	Neonatal Hb at birth (g/dL)	Umbilical cord blood pH
Uterine contractions	Vaginal	Reassuring	Normal	38	70.58	0.1	2.2	3,090	16.5	7.31
Bleeding	Caesarean section	Abnormal	Normal	39	69.87	0.37	8.1	3,760	17.6	7.16
Reduced foetal movements	Operative vaginal	Non-reassuring	Reduced	39	71.53	0.56	12.3	2,900	15	7.22
Reduced foetal movements	Operative vaginal	Non-reassuring	Reduced	41	80.78	1.81	39.8	3,270	14.5	7.12

CTG: cardiotocography; MCA-PSV: middle cerebral artery peak systolic flow velocity; HbF: haemoglobin F: CA: carbonic anhydrase; Hb: haemoglobin.

antenatal period is extremely improbable, confirming previous data obtained using identification of HbF by cytofluometry. Based on a cross-sectional study of 236 pregnancies, De Wit *et al.*[17] suggested that FMH is not correlated with gestational age in uncomplicated pregnancies and the reference range of foetal red blood cells during the second half of pregnancy is less than 0.125%[22].

Subsequent data on antepartum complications of pregnancy suggest that significant FMH is unlikely and associated with findings suggesting foetal hypoxia such as reduced foetal movements or non-reassuring cardiotocographic findings. It should be noted, however, that these findings are common among complicated pregnancies and are not specific to FMH. In our series of pregnancies at term three of the subjects with FMH had non-reassuring foetal heart rate traces and two had reduced foetal movements.

According to literature data[17,23-27], the risk of FMH increases in term pregnancies and delivery. In our series 0.9% of patients at term had a detectable FMH before delivery using a detection threshold of 0.1% and only two cases (0.4%) had an estimated FMH volume >12 mL. These results are comparable to those of Lubusky *et al*.[28] who reported rates of 1.4% and 0.23% for FMH >2.5 mL and >12.5 mL, respectively, in 3,457 examinations of pregnancies at term. Recent estimates of clinically significant consequences of FMH, such as foetal anaemia, suggest an incidence of one case per 10,000 pregnancies[25], indicating that the occurrence of severe FMH is rare compared to that of mild cases. Porra *et al.*[7], in a study of 455 term pregnancies evaluating the sensitivity of dual cytofluometry, reported a 9.7% prevalence of FMH when the detection threshold was set at 0.01% of foetal red blood cells in the maternal circulation.

Although the results of this study indicate that dual cytofluometry is a highly sensitive method for the detection of very small volumes of FMH, the clinical significance of a FMH <1 mL of packed red cells (<0.05%) is very limited and, according to De Wit *et al.*[17], the reference range during the second half of a normal pregnancy is less than 0.125%.

The obstetric and neonatal consequences of severe FMH are well known. Estimates derived from studies on RhD-negative women suggest an incidence of one to three cases per 1,000 live births, accounting for 14% of unexplained intrauterine foetal deaths[25]. Recent population studies[25,27] suggest that severe FMH causing foetal anaemia is associated with preterm birth, placental abruption, umbilical cord anomaly and foetal asphyxia. In a study of more than 200,000 births, Christensen *et al.*[29] identified 24 cases of severe foetal anaemia; non-reassuring foetal heart rate and reduced foetal movements were the most frequent obstetric correlates of anaemia. Doppler MCA-PSV has been used to detect foetal anaemia caused by RhD alloimmunisation and to evaluate the necessity of intrauterine transfusion in cases of foetal anaemia[30,31]. This measure has also been used to suspect FMH of other causes, and a direct correlation has been shown between this measure and abnormal foetal heart rate tracings.

Data on the obstetric and neonatal correlates of mild or moderate FMH are scant. Most of the recent studies[15,29] did not evaluate the association of mild-to-moderate FMH with parameters of foetal distress such as reduced foetal movements or a non-reassuring foetal heart rate pattern. In a study of 777 pregnant subjects at different gestational ages, Bakker-Jonges *et al*.[20] found that a reduction of foetal movements was a marker of mild-to-moderate FMH. Our data confirm this finding, suggesting that mild-to-moderate FMH can be associated with reduced foetal movements and a non-reassuring foetal heart rate pattern. Whether pregnant subjects at term reporting reduced foetal movements should be screened for FMH remains to be elucidated[25]. In obstetric practice, women reporting reduced foetal movements usually undergo evaluation of foetal heart rate tracing and biophysical profile[32,33]. These two tests should have a sufficient sensitivity to detect at least massive FMH. Doppler evaluation of the middle foetal cerebral artery has been proposed as a proxy for direct determination of the severity of foetal anaemia among subjects with suspected or proven FMH: an increase in MCA-PSV among women with anti-D alloimmunisation enables prediction of changes in foetal haemoglobin and haematocrit before the onset of hydrops[30,31]. Our data suggest that mild-to-moderate FMH, not causing a significant acute drop in foetal haemoglobin levels, did not influence the MCA-PSV. Thus, MCA-PSV evaluation should be used only in the setting of acute, massive FMH and not as a screening tool. After 35 weeks the sensitivity of MCA-PSV decreases and isolated detection of a pathological MCA-PSV >1.5 multiples of median, without other clinical signs of foetal anaemia, is not diagnostic and another evaluation could be performed after 12-24 hours[33].

Conclusions

Our study confirms that cytofluorimetric testing is a very specific method for detecting mild-to-moderate FMH early in pregnant women with risk factors for haemorrhage. The passage of foetal red blood cells into the maternal circulation is rare in the third trimester. The rates of FMH at term of pregnancy remain low and significantly associated with reduced foetal movements and a non-reassuring foetal heart rate tracing. Mild-to-moderate FMH is well detected by cytofluorimetry, even when the MCA-PSV is not informative.

Authorship contributions

This original article derives from close and interdisciplinary work. BF, CC, LE, BS, SM, SA enrolled patients and collected blood samples and data referring to pregnancy and delivery. VG, PC analysed blood samples performing cytofluorimetric tests. All data were collected in a dBase by contributors from both the Departments. SA performed the statistical analysis. All the Authors contributed by revising, interpreting and discussing the results. BF wrote the article.

The Authors declare no conflicts of interest.

References

1) Bianchi DW, Romero R. Biological implications of bi-directional fetomaternal cell traffic: a summary of a National Institute of Child Health and Human Development-sponsored conference. J Matern Fetal Neonatal Med 2003; **14**: 123.
2) Sebring ES, Polesky HF. Fetomaternal hemorrhage: incidence, risk factor, time of occurrence, and clinical effects. Transfusion 1990; **30**: 344-57.
3) Lee AI, Kaufman RM. Transfusion medicine and the pregnant patient. Hematol Oncol Clin N Am 2011; **25**: 393-413.
4) Kim YA, Makar RS. Detection of fetomaternal hemorrhage. Am J Hemat 2012; **87**: 417-23.
5) Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and the newborn. Blood Rev 2000; **14**: 44-61.
6) Finn R, Clarke CA, Donohoe WT, et al. Experimental studies on the prevention of Rh haemolytic disease. Br Med J 1961; **5238**: 1486-90.
7) Freda VJ, Gorman JG, Pollack W. Rh factor: prevention of isoimmunization and clinical trial on mothers. Science 1966; **151**: 828-30.
8) Porra V, Bernaud J, Gueret P, et al. Identification and quantification of fetal red blood cells in maternal blood by a dual-color flow cytometric method: valuation of the fetal cell count kit. Transfusion 2007; **47**: 1281-89.
9) Koelewijn JM, de Haas M, Vrijkotte TG, et al. Risk factors for RhD immunization despite antenatal and postnatal anti-D prophylaxis. Br J Obstet Gynaecol 2009; **116**: 1307-14.
10) M Rowley. Proposed changes to BCSH guidelines and NICE review of anti-D use. Anti-DDay, 6th Dec 2009, NHS Blood and Transplant.Available at: http://hospital.blood.co.uk/media/2293/7d9fb20f-e926-4bd9-9239-d2bbcf6fb80a.pdf. Accessed on 01/02/2017.
11) Roback JD, Grossman BJ, Harris T, et al. *Technical Manual*. 17th ed. Bethesda, MD: AABB; 2011.
12) Mollison PL. Quantitation of transplacental haemorrhage. Br Med J 1972; **3**: 31-4.
13) Patton WN, Nicholson GS, Sawers AH, et al. Assessment of fetal-maternal hemorrhage in mothers with hereditary persistence of fetal hemoglobin. J Clin Pathol 1990; **43**: 728-31.
14) Chambers E, Davies L, Evans S, et al. Comparison of haemoglobin F detection by the acid elution test, flow cytometry and high-performance liquid chromatography in maternal blood samples analysed for foetomaternal haemorrhage. Transfus Med 2012; **22**: 199-204.
15) Radel DJ, Penz CS, Dietz AB, Gastineau DA. A combined flow cytometry-based method for fetomaternal haemorrhage and maternal D. Transfusion 2008; **48**: 1886-91.
16) Sandler SG, Sathiyamoorthy S. Laboratory methods for Rh immunoprophylaxis: a review. Immunohaematology 2010; **26**: 92-103.
17) De Wit H, Nabbe KC, Kooren JA, et al. Reference values of fetal erythrocytes in maternal blood during pregnancy established using flow cytometry. Am J Clin Pathol 2011; **136**: 631-6.
18) Mari G, Deter RL, Carpenter RL, et al. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in anemic fetuses (Level II-1). N Engl J Med 2000; **342**: 9-14.
19) James DK, Arulkumaran S. *The use of electronic fetal monitoring: the use and interpretation of cardiotocography in intrapartum fetal surveillance. Evidenced-based Clinical Guideline No 8*. London: RCOG; 2001.
20) Bakker-Jonges L, Grootfaam D, Duvekot J. The detection of foetal red blood cells in maternal blood samples by dual flow cytometry. Available at: https://www.scribd.com/document/64820872/duvekot. Accessed on 01/02/2017.
21) Davis BH, Olsen S, Bigelow NC, Chen JC. Detection of fetal red cells in fetomaternal hemorrhage using a fetal hemoglobin monoclonal antibody by flow cytometry. Transfusion 1998; **38**: 749-56.
22) Chen JC, Davis BH, Wood B, Warzynski MJ. Multicenter clinical experience with flow cytometric method for fetomaternal hemorrhage detection. Cytometry 2002; **50**: 285-90.
23) Pelikan DM, Scherjon SA, Mesker WE, et al. Quantification of fetomaternal hemorrhage: a comparative study of the manual and automated microscopic Kleihauer-Betke tests and flow cytometry in clinical samples. Am J Obstet Gynaecol 2004; **191**: 551-7.
24) Kumpel BM, MacDonald AP, Bishop DR, et al. Quantification of fetomaternal hemorrhage and F cells in unusual maternal blood samples by flow cytometry using anti-D and anti-D. Transfus Med 2013; **23**: 175-86.
25) Stroustrup A, Trasande L. Demographic, clinical characteristics and outcomes of neonates diagnosed with fetomaternal haemorrhage. Arch Dis Child Fetal Neonatal 2012; **97**: F405-10.
26) MacKenzie IZ, Roseman F, Findaly J, et al. The kinectics of routine antenatal prophylactic intramuscular injections of polyclonal anti-D immunoglobulin. Br J Obstet Gynaecol 2006; **113**: 97-101.
27) Mollison PL, Barron SL, Bowley CC et al. Controlled trials of various anti-D dosages in suppression Rh sensitization following pregnancy. Report to the Medical Research Council by the working party on the use of anti-D immunoglobulin for the prevention of the isoimmunization of Rh-negative women during pregnancy. Br Med J 1974; **2**: 75-80.
28) Lubusky M, Simetka O, Studnickova M, et al. Fetomaternal hemorrhage in normal vaginal delivery and in delivery by Cesarean section. Transfusion 2012; **52**: 1977-82.
29) Christensen RD, Lambert DK, Baerl VL, et al. Severe neonatal anemia from fetomaternal hemorrhage: report from a multihospital health-care system. J Perinatol 2013; **33**: 429-34.
30) Imbar T, Lev-Sagie A, Cohen S, et al. Diagnosis, surveillance, and treatment of the anemic fetus using middle cerebral artery peak systolic velocity measurement. Prenat Diagn 2006; **26**: 45-51.
31) Shourbagy SE, Elsakhawy M. Prediction of fetal anemia by middle cerebral artery Doppler. Middle East Fertility Society Journal 2012; **17**: 275-82.
32) Olesen AG, Svare JA. Decreased fetal movements: background, assessment, and clinical management. Acta Obstet Gynecol Scand 2004; **83**: 818-26.
33) Zimmermann R, Durigb P, Carpenter RJ, Mari G. Longitudinal measurement of peak systolic velocity in the fetal middle cerebral artery for monitoring pregnancies complicated by red cell alloimmunization: a prospective multicentre trial with intention-to-treat. Br J Obstet Gynaecol 2002; **109**: 746-52.

Arrived: 14 November 2016 - Revision accepted: 2 February 2017
Correspondence: Chiara Cavagnoli
Gynaecological and Obstetrical Department
IRCCS Policlinico S. Matteo
Viale Golgi 19
27100 Pavia, Italy
e-mail: chiara1c@libero.it