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Contamination of salvaged maternal blood by amniotic fluid and fetal red cells during elective Caesarean section

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Background. Cell salvage in obstetrics is still a controversial subject and has yet to be fully embraced. The aim of this exploratory study was to measure amniotic fluid (AF), heparin, and fetal red cell contamination of washed filtered salvaged maternal blood and to investigate differences based on the number of suction devices used.

Methods. Patients undergoing elective Caesarean section were assigned alternately to one of two groups. In Group 1, all blood and AF was collected with one suction. In Group 2, AF was aspirated to waste with a second separate suction device before collection of any blood.

Results. In both groups, alpha-fetoprotein (AFP), squames cells, and heparin were significantly reduced ($P<0.001$) by the washing and filtering process. Mean AFP levels post-filtration were 2.58 IU ml^{-1} in Group 1 and 3.53 IU ml^{-1} in Group 2. Squames cells were completely removed in all but two cases. Fetal red blood cells were still present in the final product, range 0.13–4.35%. In Group 1, haemoglobin and haematocrit were higher than in Group 2, with lower white blood cell, AFP, and fetal red cell counts.

Conclusions. This study adds to the growing body of evidence that there is little or no possibility for AF contamination to enter the re-infusion system when used in conjunction with a leucodepletion filter.

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The use of cell salvage in obstetrics remains controversial. There is a concern of potential contamination of the salvaged blood with amniotic fluid (AF), which may cause AF embolus (anaphylactoid syndrome of pregnancy),[1] and the risk of alloimmunization of the mother with any fetal red blood cells aspirated.

Previous studies investigated the use of cell salvage in obstetrics and focused on the feasibility of the washing process in removing potential contaminants. After the use of a leucodepletion filter in cancer surgery, these filters were introduced into obstetric practice.[1] A later study using a newer advanced model demonstrated complete removal or a further reduction in contaminants by these filters.[2] Along with these results and later reports, the National Institute of Health and Clinical Excellence (NICE) published guidelines in 2005 suggesting that these filters can be considered for use in obstetrics.[3]

A common assumption was that two suction devices should be used, with as much AF aspirated to waste before any lost blood is collected.[1 4]

Although current evidence supports the use of cell salvage in obstetrics, a recent review suggested that more clinical data are required to add to this evidence and make the use of cell salvage more commonplace in obstetrics.[4]

The aim of the study was to add results to the current clinical evidence to support the use of cell salvage. We measured AF and fetal red cell contamination, and residual heparin levels of washed salvaged maternal blood when using either one or two suction devices, filtering the washed product through a leucodepletion filter for cell salvage (RS1 VAE PALL® Medical).

Methods

Information to perform power analysis and produce a well-designed prospective study is lacking due to the limited data available.[4] This study was set up as an exploratory study to investigate the safety and feasibility of cell salvage in obstetrics. Ethics Committee opinion was sought, but approval was not required. The sample size was dependent on the number of patients accessible over a set time period.

Written informed consent was obtained from 34 patients undergoing elective lower segment Caesarean section at the Royal Cornwall Hospital over a 4 month period. Patients were alternatively assigned to one of two groups before surgery. Group 1 (n=17) involved only one suction where all AF and blood was collected into the cell salvage machine, with Group 2 (n=17) using a second suction to aspirate as much AF to waste before any lost blood was collected.

All patients had a spinal anaesthetic apart from one who had an epidural top up; all spinals were performed using a Whitacre 25 gauge needle, with hyperbaric bupivacaine 0.5% and morphine 0.1 mg plus in some cases fentanyl 10–20 μg. In two cases, spinals had to be performed twice due to incomplete blocks and one was converted to a general anaesthetic.

During the surgical procedure, any blood lost was salvaged into a Dideco Electa Autotransfusion Cell Separator (Sorin Group, Milan, Italy). Aspirated blood was mixed with heparin 30 000 IU litre^{-1} of saline. Suction was set at 100–150 mm Hg with a prime at 100 ml min^{-1} and wash volumes of 900 ml on a continuing wash at 100 ml min^{-1}. The set was emptied at 150 ml min^{-1}. A 55 ml Latham bowl was used in all cases. In addition, any swabs from the surgical procedure were soaked in 1 litre of saline and 'compressed' at the end of case and solution aspirated, maximizing red cell collection. Processed washed blood was then gravity run through a Pall RS1 VAE leucodepletion filter (Pall Medical, Portsmouth, UK) into another re-infusion bag.

Pre-wash samples were taken from the collection reservoir, and post-wash and post-filtration samples were taken from the re-infusion packs, which were inverted gently several times to ensure even mixing before sampling.

The cell salvage machine use and sampling, in all cases, was performed by the same member of staff.

Pre-wash, post-wash, and post-filtration samples from both groups were tested for markers of AF contamination and heparin levels. Post-filtration samples were also tested to quantify any fetal red blood cells.

Six millilitres of blood were collected into a specimen tube and processed for alpha-fetoprotein (AFP) on an E170 Modular Analytics Analyser (Roche Diagnostics, Basel, Switzerland).

Four millilitres of blood were collected and centrifuged at 1600g for 5 min and processed for heparin levels on an Instrumentation Laboratory Futura Advance Coagulometer (Instrumentation Laboratory, MA, USA).

Six millilitres of blood were collected for fetal squames cell levels. After centrifugation at 1200g for 5 min, one part plasma was mixed with three parts Cytolyt Solution (Cytyc Corporation, West Sussex, UK). Vials were then centrifuged at 1200g for 5 min and supernatant discarded. Two to three drops of the cell bullet were then transferred to 20 ml PreservCyt Solution (Cytyc Corporation) and processed on a ThinPrep®2000 Processor (Cytyc Corporation) using blue-box non-gynae filters (Cytyc Corporation), generating one alcohol-fixed slide per sample. Slides were stained using the Papanicolaou technique. To avoid any potential carryover, pre-wash, post-wash, and post-filtration slides were stained separately using fresh reagents. Each slide was then examined under light microscopy for presence of fetal squames cells, with the whole slide being counted to exclude any variables, giving a result of number of cells per slide.

Two millilitres of blood were collected into EDTA for a full blood count to provide haemoglobin (Hb), haematocrit (HCT), and white blood cell (WBC) levels on a Bayer Advia 120 analyser (Bayer, Newbury, UK).

Post-filtration samples were also tested for fetal red blood cell quantification. Five millilitres of blood were collected into EDTA and processed with a monoclonal antibody to fetal Hb (IQProducts, Groningen, The Netherlands), with a Becton Dickinson FACSCalibur (NJ, USA) flow cytometer at 540 nm. Note: it was planed to perform all 34 cases for fetal red cells by flow cytometry, but due to unforeseen circumstances, the first seven cases were analysed using the Kleihauer–Betke technique.

Pre- and post-wash samples and post-wash and post-filtration samples were compared. Parametric data were analysed using a paired t-test, and non-parametric data analysed using a one-sample Wilcoxon test. $P<0.05$ was considered statistically significant. The observed power of the study was calculated after the collection of the data.

Results

Mean (SD) (range) age of patients in Group 1 was 32.1 (19–46) yr, and weight 82.7 (16.2) kg. In Group 2, the age was 32.1 (24–41) yr and body weight 82.4 (12.6) kg. Table 1 gives the reason for Caesarean section along with any coexisting diseases as shown in Table 2.

Table 1 Reasons for Caesarean section

Reason	Number
Previous section	23
Twins IVF	2
Back injury/pain	2
Breech	2
Failure to progress/Category 3	1
IUGR	1
Polyartetitisnodosa	1
Raised BP/pregnancy-induced hypertension	1
Placenta praevia	1

There were no surgical problems during the procedures. Four cases did not lose enough blood to obtain post-filtration samples (one from Group 1 and three from Group 2), with three further cases having partially filled bowls. A partial bowl was defined as a bowl that was not completely filled in the automatic mode of the cell salvage machine. These three bowls provided erroneous post-filtration results and, therefore, were excluded from analysis. The final blood product in these cases (one from Group 1 and two from Group 2) had a reduced Hb level of 7.1–10.3 g dl^{-1} and HCT of 21–31%, with a higher level of fetal red blood cells: 10.5% compared with 1.51%. Apart from fetal red blood cells in one case (Group 2), the values for Hb, HCT, and fetal red blood cells all were considerably above the upper 95% confidence limit for the full bowls.

The collected blood volume from Group 1 [1782 (354) ml] was slightly higher than that collected in Group 2 [1497 (562) ml], but the difference was not statistically significant (P=0.14). Similarly, on average, the red blood cell volume was higher in Group 1 [168 (77) ml] than in Group 2 [135 (94) ml], but again the difference was not statistically significant (P=0.30).

Table 3 shows the mean Hb, AFP, squames cells, and fetal red cell levels in the groups, along with statistical analysis.

Dependent on the manufacturer, the reference range for AFP is up to 10–15 IU ml^{-1}. The post-wash levels were significantly reduced (P<0.001) with no further reduction seen post-filtration (P=0.996). On average, there was a 98.7% reduction of pre-wash levels by the washing stage.

Mean heparin levels pre-wash were 1.33 IU ml^{-1} (95% CI 0.91–1.75 IU ml^{-1}) in Group 1 and 1.44 IU ml^{-1} (95% CI 0.82–2.05 IU ml^{-1}) in Group 2. Levels were reduced by 100% in both the groups (P<0.001) by the washing stage with no heparin being detected post-wash or post-filtration in all 34 cases.

Fetal squames cells were present in 12 out of 34 cases (six from each group) pre-wash, present in all post-wash samples, with a significant reduction post-filtration (P<0.001). Squames were present in two post-filtration samples, with two cells seen in each case. Both cases were from Group 1. Pre-wash samples were heavily diluted with AF and heparinized saline from surgery explaining why 22 samples were negative.

The mean percentage fetal red cells post-filtration from all cases (both groups) was 1.51% (95% CI 0.98–2.05), with a range of 0.13–4.35%. The first seven cases (three from Group 1 and four from Group 2) performed by the Kleihauer technique generated a mean 1.72% (95% CI −0.65 to 4.08), whereas the remaining cases performed by flow cytometry resulted in mean 1.47% (95% CI 0.92–2.01).

Table 2 Coexisting diseases

Co-morbidity	Number
Smoker	5
Diabetic	2
Asthma	2
Epilepsy	1
Protein S deficiency	1
Polyartetitisnodosa	1

Discussion

This study has shown the efficiency of the washing stage of the cell salvage machine, when used in combination with a leucodepletion filter, in significantly reducing levels of AF contaminants.

AFP was significantly reduced post-wash to levels well within the normal range for the general population, confirming the results from previous studies.[1 5 6]

During the study, the amount of heparin used was higher than with other surgical disciplines due to the hypercoagulable state of pregnancy. It would be expected that pre-wash samples were heavily contaminated with heparin, and this was confirmed during the study. It has been previously reported that residual levels of heparin remain in salvaged blood,[4] but we have now demonstrated the complete removal of heparin by the washing process in all 34 cases.

The role of fetal squames cells in AF embolus is still debated. In one study,[1] fetal squames cells were still present even with the additional step of filtering the washed blood through a leucodepletion filter, with only

Table 3 Analysis and comparison for Group 1 and Group 2. AFP, alpha-fetoprotein. Values are mean (95% CI). *We acknowledge that the observed power results are low; this, however, is one of the largest studies to date in the obstetric setting. †Mean HCT post-filtration was 45% in Group 1 and 42% in Group 2

	Group 1	Group 2	*P*-value	Observed power*
AFP pre-wash (IU ml^{-1})	253.72 (183.64–323.80)	381.60 (193.63–569.57)	0.19	0.26
AFP post-wash (IU ml^{-1})	2.71 (0.80–4.62)	3.14 (1.45–4.83)	0.72	0.06
AFP post-filtration (IU ml^{-1})	2.58 (0.54–4.62)	3.53 (1.29–5.76)	0.51	0.10
Hb pre-wash (g dl^{-1})	2.14 (1.52–2.75)	1.83 (1.17–2.50)	0.48	0.11
Hb post-wash (g dl^{-1})	16.95 (16.28–17.62)	16.20 (15.26–17.14)	0.18	0.27
Hb post-filtration† (g dl^{-1})	15.77 (15.07–16.47)	14.74 (13.70–15.78)	0.17	0.27
Squames cells pre-wash (cells per slide)	1.12 (−0.02 to 2.25)	0.94 (0.01–1.74)	0.80	0.06
Squames cells post-wash (cells per slide)	34.59 (10.89–58.28)	43.71 (14.42–73.00)	0.61	0.08
Squames cells post-filtration (cells per slide)	0.27 (−0.11 to 0.66)	0	0.18	0.26
Fetal red blood cells (%)	1.34 (0.56–2.12)	1.76 (0.95–2.57)	0.43	0.12

two out of 27 post-filtration cases being negative. Advanced filters may reduce this contamination.[2]

Of the 27 cases in our study, only two were positive post-filtration with two cells seen in each slide for each case. These were found when one suction was used. However, it is difficult to differentiate between fetal and adult squames cells. There is no evidence to show that fetal squames cells routinely enter the maternal circulation,[7] and so the significance of this contamination is difficult to quantify and in fact may have no clinical significance.

Fetal red blood cells were still present in the final product, consistent with previous studies,[2 6] and so could be significant in cases of red cell antigen incompatibilities between the mother and fetus. Rh(D) incompatibilities, although clinically significant, are generally avoided by routine prophylactic anti-D treatment throughout the pregnancy. However, fetal hyperbilirubinaemia and anaemia in future pregnancies can occur when antibodies have been formed to other red cell antigen incompatibilities. Examples of other clinically relevant antibodies that have been implicated in haemolytic disease of the newborn include anti-K, anti-c, anti-Fy(a), and anti-Jk(a).[8] Nevertheless, it must be appreciated that there is still a risk of alloimmunization of the mother either from transfusion of allogenic blood or a sensitizing event during pregnancy.

Current treatment of obstetric haemorrhage is with allogenic blood transfusion; however, there are concerns with allogenic blood. To date, there have been four confirmed cases of vCJD transmission via allogeneic blood, confirming that vCJD can be transmitted through blood transfusion.[9]

These potential risks of transfusion and reduction in availability of blood make it important to establish the use of cell salvage in obstetrics. It has been shown in more than 400 documented cases where cell salvaged blood has been returned to mothers with no significant adverse results.[4 10 11] Several key studies have commented on the use of cell salvage.[12–14]

This current study is one of the largest to date, with results that add to the growing body of evidence, showing there is little or no possibility for AF contamination to enter the re-infusion system, when used in conjunction with a leucodepletion filter. The role of the leucodepletion filter in this study has confirmed that it is required to remove AF contamination in cell salvage. WBC, platelets, and squames cells were still present post-wash but were significantly reduced by the filter. Whether the squames are of fetal or maternal origin is perhaps irrelevant as the filters have shown that they can remove both. As expected, AFP and heparin levels were not reduced by the filter, but heparin was completely removed in the washing process.

Regarding cell salvage in obstetrics, these results could be used to change the guidelines and allow routine re-transfusion of salvaged blood as opposed to overriding guidance in extreme emergency, and therefore allowing cell salvage to be used for elective and emergency cases. Even though it was not our intention to re-transfuse any salvaged blood in this study, ~500 ml was transfused to two cases. No changes in clinical state, heart rate, or clinical complications were noted, showing that in elective cases unexpected large blood loss can still occur and cell salvage can have a role to play in this situation.

This initial project acted as an exploratory study, and after the collection of these data, we have now implemented a comprehensive programme of cell salvage within our Trust to all women undergoing elective Caesarean sections, and further work is ongoing.

We conclude that one suction may be used in the obstetric setting, and washed filtered blood from partially filled bowls should not be re-transfused, regardless of the clinical situation. To obtain maximum washing efficiency in removing AF and fetal contaminants, only complete bowls in the automatic mode should be accepted.

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References

1 Catling SJ, Williams S, Fielding A. Cell salvage in obstetrics: an evaluation of the ability of cell salvage combined with leucocyte depletion filtration to remove amniotic fluid from operative blood loss at caesarean section. *Int J Obstet Anesth* 1999; **8**: 79–84

2 Waters JH, Biscotti C, Potter PS, Phillipson E. Amniotic fluid removal during cell salvage in the cesarean section patient. *Anesthesiology* 2000; **92**: 1531–6

3 UK National Institute for Health and Clinical Excellence. *Intraoperative Blood Cell Salvage in Obstetrics*. IP Guidance Number: IPG144. Available from URL: http://www.nice.org.uk/download.aspx?o=IPG144guidance (last updated November 2005)

4 Allam J, Cox M, Yentis SM. Cell salvage in obstetrics. *Int J Obstet Anesth* 2008; **17**: 37–45

5 Thornhill ML, O'Leary AJ, Lussos SA, Rutherford C, Johnson MD. An in-vitro assessment of amniotic fluid removal from human blood through cell saver processing. *Anesthesiology* 1991; **75**: A830

6 Fong J, Gurewitsch ED, Kump L, Klein R. Clearance of fetal products and subsequent immunoreactivity of blood salvaged at cesarean delivery. *Obstet Gynecol* 1999; **93**: 968–72

7 Davies S. What 'do' we know about AFE? *Int J Obstet Anesth* 2000; **9**: 142

8 Murphy M, Pamphilon D, Weatherall D. Prenatal and childhood transfusions. In: Murph M, Pamphilon D, eds. *Practical Transfusion Medicine*, 2nd Edn. Oxford: Blackwell Publishing, 2005; 97–118

9 Health Protection Agency Press Statement. *4th Case of Variant CJD Infection Associated with Blood Transfusion*. Available from URL:

http://www.hpa.org.uk/hpa/news/articles/press_releases/2007/070118_vCJD.htm (updated January 18, 2007)

10 Catling S, Joels L. Cell salvage in obstetrics: the time has come. *Int J Obstet Gynaecol* 2005; **112**: 131–2

11 Water JH. Indications and contraindications of cell salvage. *Transfusion* 2004; **44**: 40S–4S

12 Catling SJ, Freites O, Krishnan S, Gibbs R. Clinical experience with cell salvage in obstetrics: 4 cases from one UK centre. *Int J Obstet Anesth* 2002; **11**: 128–34

13 Rebarber A, Lonser R, Jackson S, Copel JA, Sipes S. The safety of intraoperative autologous blood collection and autotransfusion during cesarean section. *Am J Obstet Gynecol* 1998; **179**: 715–20

14 Potter PS, Waters JH, Burger GA, Mraovic B. Application of cell-salvage during cesarean section. *Anesthesiology* 1999; **90**: 619–21