

## Amniotic Fluid Removal during Cell Salvage in the Cesarean Section Patient

Jonathan H. Waters M.D.,\* Charles Biscotti, M.D.,† Paul S. Potter M.D.,‡ Eliot Phillipson M.D.§

**Background:** Cell salvage has been used in obstetrics to a limited degree because of a fear of amniotic fluid embolism. In this study, cell salvage was combined with blood filtration using a leukocyte depletion filter. A comparison of this washed, filtered product was then made with maternal central venous blood.

**Methods:** The squamous cell concentration, lamellar body count, quantitative bacterial colonization, potassium level, and fetal hemoglobin concentration were measured in four sequential blood samples collected from 15 women undergoing elective cesarean section. The blood samples collected included (1) unwashed blood from the surgical field (prewash), (2) washed blood (postwash), (3) washed and filtered blood (postfiltration), and (4) maternal central venous blood drawn from a femoral catheter at the time of placental separation.

**Results:** Significant reductions in the following parameters were seen when the postfiltration samples were compared to the prewash samples (median [25th–75th percentile]): squamous cell concentration (0.0 [0.0–0.1 counts/high-powered field (HPF)] vs. 8.3 counts/HPF [4.0–10.5 counts/HPF],  $P < 0.05$ ); bacterial contamination (0.1 [0.0–0.2] vs. 3.0 [0.6–7.7] colony-forming units (CFU)/ml,  $P < 0.01$ ); and lamellar body concentration (0.0 [0.0–1.0] vs. 22.0 [18.5–29.5] thousands/ $\mu$ l,

$P < 0.01$ ). No significant differences existed between the postfiltration and maternal samples for each of these parameters. Fetal hemoglobin was in higher concentrations in the postfiltration sample when compared with maternal blood (1.9 [1.1–2.5] vs. 0.5% [0.3–0.7]). Potassium levels were significantly less in the postfiltration sample when compared with maternal (1.4 [1.0–1.5] vs. 3.8 mEq/l [3.7–4.0]).

**Conclusions:** Leukocyte depletion filtering of cell-salvaged blood obtained from cesarean section significantly reduces particulate contaminants to a concentration equivalent to maternal venous blood. (Key words: Autologous blood; autotransfusion; embolism; obstetrics.)

CELL-SALVAGE technology has been applied in numerous different clinical situations; however, it has not been extensively applied in obstetric hemorrhage. Justification for not applying cell-salvage technology in obstetrics is a theoretical fear of administering blood that contains amniotic fluid components, which could lead to an amniotic fluid embolism.

Before administering cell-salvaged blood in the obstetric setting, adequacy of the washing process should be assessed, with a focus on the components that are thought to be associated with amniotic fluid embolism. Bernstein *et al.*<sup>1</sup> demonstrated that amniotic fluid-derived tissue factor is eliminated from blood contaminated with amniotic fluid when the blood is processed through cell-salvage equipment. Amniotic fluid-derived tissue factor is thought to be an initiator of coagulation and may be responsible for the disseminated intravascular coagulopathy which is seen with amniotic fluid embolism.<sup>2</sup> Unfortunately, tissue factor may be only one of many components that lead to the syndrome of amniotic fluid embolism<sup>3,4</sup>; therefore, washing of this tissue factor would not guarantee that amniotic fluid embolism would not occur. Some investigators<sup>5,6</sup> think that particulate contaminants are responsible for amniotic fluid embolization. Durand *et al.*<sup>7</sup> showed that, despite washing, cell-salvaged blood still contained significant squamous cells.

Because the mechanism for amniotic fluid embolism is unclear, we evaluated the washout of several diverse components of amniotic fluid contamination. In addi-

This article is accompanied by an Editorial View. Please see: Weiskopf RB: Erythrocyte salvage during cesarean section. ANESTHESIOLOGY 2000; 92:1519–22.

\* Head, Section of Anesthesia for Obstetrics, Department of General Anesthesiology.

† Staff Pathologist, Department of Anatomic Pathology.

‡ Staff Anesthesiologist, Department of General Anesthesiology.

§ Head, Section of Obstetrics, Department of Obstetrics and Gynecology.

Received from the Departments of General Anesthesiology, Obstetrics and Gynecology, and Anatomic Pathology, Cleveland Clinic Foundation, Cleveland, Ohio. Submitted for publication October 12, 1999. Accepted for publication February 7, 2000. Support was provided by the Cleveland Clinic Foundation, Cleveland, Ohio; the Haemonetics Corporation, Braintree, Massachusetts, which kindly donated the cell-salvage disposable supplies; and Pall Medical Corporation, East Hills, New York, which donated the leukocyte depletion filters.

Address reprint requests to Dr. Waters: Department of General Anesthesiology, Cleveland Clinic Foundation, 9500 Euclid Avenue, E31, Cleveland, Ohio 44195. Address electronic mail to: watersj@ccf.org

tion, the influence of filtering the washed blood product with leukocyte reduction filters was assessed. The washed, filtered product was then compared to that of a maternal blood sample drawn from a catheter placed slightly above the uterine veins in the vena cava. The catheter tip placement was intended to capture any amniotic fluid contaminants as they occurred at the time of placental separation and should reflect levels of contaminants to which the mother normally is exposed.

### Materials and Methods

After institutional review board approval from the Cleveland Clinic Foundation, with written and verbal consent, 15 nonlaboring patients undergoing elective cesarean section were enrolled in the study. Exclusion criteria included maternal fever, infection, or antibiotic administration.

At arrival in the operating room, all patients received a spinal anesthetic using 1.5 ml hyperbaric bupivacaine, 0.75%, combined with 0.25 mg preservative-free morphine sulfate. At completion of the spinal anesthetic, all patients underwent placement of a 20-cm, 16-gauge femoral venous catheter during sterile conditions using a catheter-over-a-wire technique. To capture blood contaminated with amniotic fluid, the catheter was placed so that the catheter tip was above the level of the uterine veins as they flow into the common iliac vein. After placental separation, 45 ml maternal blood was aspirated through this line (maternal sample).

During the surgical procedure, all blood and amniotic fluid was suctioned directly into a cell-salvage reservoir that had a 120- $\mu$ m filter. In addition, all sponges were rinsed in normal saline, with the resultant bloody wash fluid aspirated into the reservoir. At completion of the surgical procedure, a sample of this blood (prewash sample) was taken for testing.

Blood was processed *via* a Haemonetics Cell Saver 5 (Haemonetics Corporation, Braintree, MA) machine during the operative period. Cell processing occurred using a 125-ml Latham bowl with fill rates of 300 ml/min, a wash rate of 300 ml/min, and a total wash volume of 1,000 ml. No red-cell-pack flexing or manipulation of the manufacturer-specified centrifuge speed was performed. After cell washing, a sample of the washed blood (postwash) was collected for analysis.

The washed blood was then filtered using a leukocyte reduction filter (LeukoGuard RS, Pall Biomedical Prod-

ucts Co., East Hills, NY). A sample of the filtered blood (postfiltration) was collected for analysis.

For each blood sample (maternal, prewash, postwash, and postfiltration), the following assays were performed: lamellar body count, quantitative fetal hemoglobin, potassium concentration, quantitative blood culture, and a visual count of squamous cells. Blood for each of these assays was collected and handled as follows:

1. Seven milliliters of blood for lamellar body count was placed in a plastic specimen tube at their acquisition and placed in an envelope to prevent light degradation of the lamellar bodies. The specimen tube in which the maternal blood sample was placed was rinsed with 1,000 U/ml heparin solution before the introduction of the blood sample to prevent clotting. Electronic resonance detection enhanced by hydrodynamic focusing (Sysmex SE9500; Roche Diagnostics, Indianapolis, IN) was used for lamellar body counting.
2. Five milliliters of blood was placed into a purple-top tube for quantitative fetal hemoglobin using the Kleihauer-Betke test.
3. Five milliliters of blood was placed into a red-top tube for potassium measurement. Potassium concentration was determined using ion-selective electrode technology (Beckman CX3; Beckman Instruments, Brea, CA).
4. Seven milliliters of blood was placed into a blood isolator tube for quantitative blood culture.
5. Ten milliliters of blood was placed into CytoLyt solution (Cytoc Corporation, Boxborough, MA) for evaluation of squamous cells. The vials of CytoLyt solution were centrifuged and the supernatant discarded. The sample was resuspended in Preservcylt (Cytoc Corporation) and processed on the ThinPrep 2000 Processor (Cytoc Corporation), yielding 1 alcohol-fixed slide/sample. Subsequently, the slide was stained using the Papanicolaou technique. Each slide was screened for the presence of squamous epithelial cells by a pathologist blinded to the origin of the sample. If squamous cells were present, counts were performed by starting on a representative high-magnification field, which contained squamous epithelial cells, and counting 10 consecutive fields. Counts were repeated four times and then averaged. Nucleated and anucleated squamous cells were counted. Anucleated squamous cells were defined as folded, eosinophilic, angular structures closely resembling their nucleated counterparts.

## CELL SALVAGE IN OBSTETRICS

**Table 1. Concentrations of Amniotic Fluid Markers**

Test	Maternal (n = 14)	Prewash (n = 14)	Postwash (n = 14)	Postfiltration (n = 14)
Potassium (mEq/l)	3.8 (3.7–4.0)	3.8 (2.4–4.0)	1.5 (1.1–1.5)*	1.4 (1.0–1.5)*
Lamellar body count (K/ $\mu$ l)	31.0 (24.0–49.9)	22.0 (18.5–29.5)	3.0 (2.0–3.5)*	0.0 (0.0–1.0)*
Squamous cell count (count/HPF)	0.0 (0.0–0.0)†	8.3 (4.0–10.5)	4.4 (3.0–7.6)	0.0 (0.0–0.1)†
Quantitative bacterial culture (CFU/ml)	0.0 (0.0–0.1)†	3.0 (0.6–7.7)	1.3 (0.4–6.1)	0.1 (0.0–0.2)†
Fetal hemoglobin (%)	0.5 (0.3–0.7)	1.1 (0.7–1.5)‡	1.7 (1.0–2.1)‡	1.9 (1.1–2.5)‡

Values expressed as median (twenty-fifth to seventy-fifth percentile).

\*  $P < 0.05$  compared with prewash and maternal.

†  $P < 0.05$  compared with prewash and postwash.

‡  $P < 0.05$  compared with maternal.

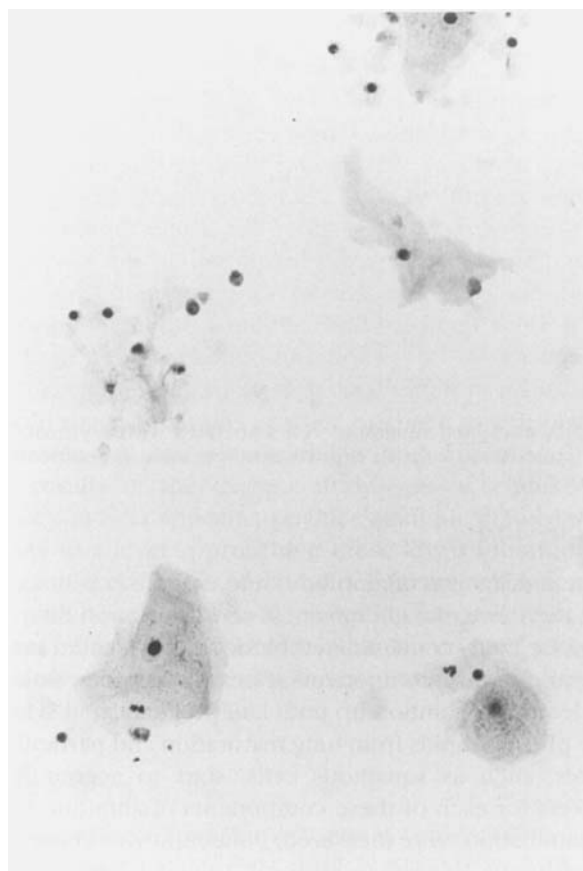
K = thousands; HPF = high powered field, CFU = colony-forming units.

### Statistical Analysis

The data were analyzed using standard descriptive techniques. Statistical significance between prewash, postwash, postfiltration, and maternal samples was assessed using a Kruskal-Wallis test with a Dunn *post hoc* test.  $P < 0.05$  was considered statistically significant.

### Results

Fifteen patients were enrolled in the study. The average patient age was  $33 \pm 6$  yr. The average gestational age of the fetus at cesarean section was  $38 \pm 2$  weeks. The average estimated blood loss was  $800 \pm 250$  ml. All cesarean sections were performed through a low transverse incision with manual removal of the placenta. One patient was excluded from the study after a cesarean section that resulted in such a small amount of blood loss that blood salvage and washing could not be performed. No patient showed evidence of amniotic fluid embolism during childbirth. Table 1 shows the results from the measured parameters. Figure 1 shows a typical Papanicolaou stain of blood from a prewash sample. Figure 2 shows a typical postwash sample. Figure 3 shows a typical postfiltration blood sample. Bacterial contamination was significantly less for the postfiltration and maternal samples when compared with the prewash sample. No statistical difference was found between these samples and the postwash, though the trend would suggest that with a larger sample size a difference would have been found. All prewash samples contained bacteria, primarily *Staphylococcus*. In addition, one sample contained gram-negative bacilli and one sample contained diphtheroid bacilli. In the postwash sample, 93%



**Fig. 1.** Squamous cells in a typical prewash sample are shown with numerous white blood cells. Red cells are visible but difficult to differentiate from the other contaminants (Papanicolaou stain).

of the samples grew only *Staphylococcus* species. The postfiltration sample grew *Staphylococcus* species in 50% of the samples. The maternal sample grew only staphylococci in 25% of the samples.

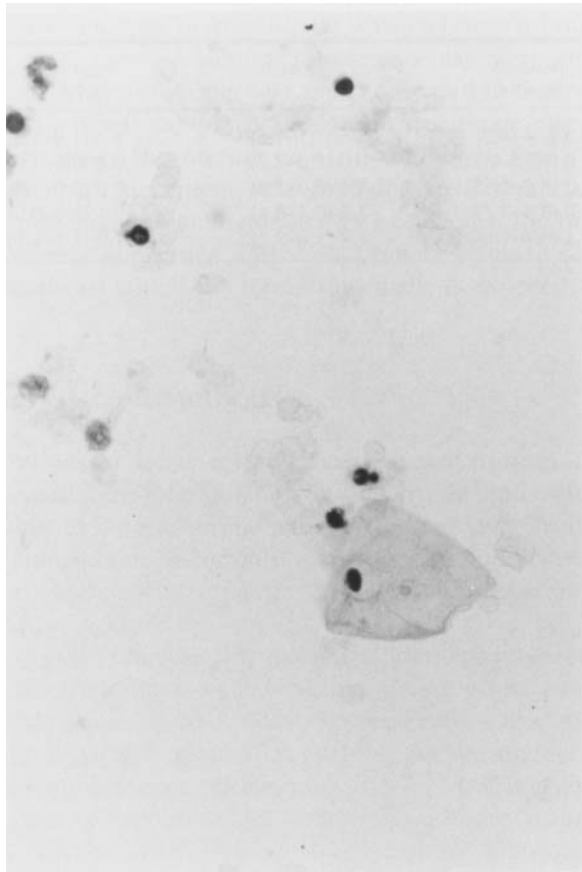


Fig. 2. A nucleated squamous cell associated with erythrocytes and white blood cells in a postwash specimen (Papanicolaou stain).

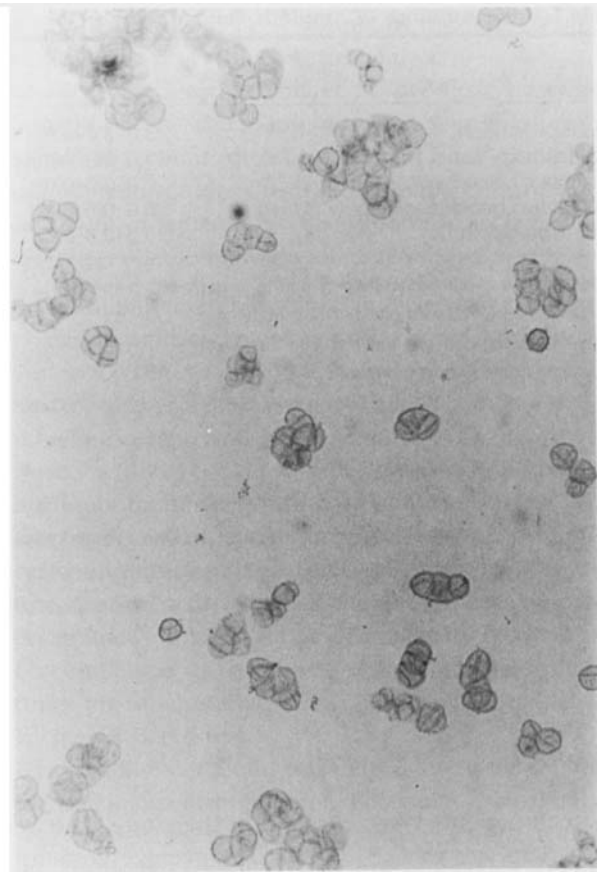


Fig. 3. A typical postfiltration sample is shown that is devoid of squamous cells and leukocytes. Only red cells are seen (Papanicolaou stain).

## Discussion

The mechanism of amniotic fluid embolism is not certain; therefore, the effectiveness of washing and filtering amniotic fluid-contaminated blood was evaluated using several different components. Because amniotic fluid is an electrolyte solution up until late pregnancy, at which time phospholipids from lung maturation and particulate matter, such as squamous cells, start to accumulate, markers for each of these components of amniotic fluid contamination were measured. Potassium was chosen as a marker for electrolyte removal. Lamellar bodies were chosen as a marker of phospholipids. (Lamellar bodies are composed of phospholipids, such as lecithin and sphingomyelin, and are derived from the type II epithelial cells of the fetal lung.<sup>8</sup>) Lastly, squamous cell contamination was chosen as a marker of particulate contamination. In addition, a previous investigator<sup>7</sup> found bacterial contamination and fetal hemoglobin in his cell-salvage product, so these markers were also measured.

Unlike all but one previous study, an additional step was added to the cell-salvage processing. This additional step was filtering the cell-salvage product with leukocyte reduction filters. Leukocyte reduction filters are used to remove leukocytes from donated units of blood. The filters work through the use of a small-pore microfiber web and a negative surface charge.<sup>9</sup> Several studies have shown that these filters are effective in filtration of tumor cells in cell salvage,<sup>10-13</sup> so it would be reasonable to postulate that these filters might be effective in removing the particulate components of amniotic fluid.

In the only other study of cell-salvaged blood contaminated with amniotic fluid in which leukocyte reduction filters were used, Catling *et al.*<sup>14</sup> found that half of their postfiltration samples were contaminated with fetal squamous cells. This result is significantly different than the findings of this study. This difference relates to a difference in the type of leukocyte reduction filters used among studies. Catling *et al.*<sup>14</sup> used the older generation,

Pall RC 100 filter; whereas, in this study, a Pall RS filter was used. According to the manufacturer, there are significant differences in the media (fiber, fiber diameter, spacing between fibers, composition, and charge) between the two filters, which would account for differences in filtering capability (personal oral communication, Girolomo Ortolano, Ph.D, Senior Scientist, Pall Biomedical Products Co., February 2000).

Because the process of washing and filtering was not expected to obtain a perfect product, a washed, filtered, salvaged blood product was compared with a maternal central venous blood sample. We found that after cell-salvage processing and filtration, squamous cell and bacterial contaminations were similar to those in maternal blood. Potassium and lamellar body concentrations were significantly less than maternal concentrations, whereas fetal hemoglobin concentrations were significantly higher. Thus, a combination of cell-salvage washing and filtration appears to produce a blood product comparable with maternal blood, with the exception of the fetal hemoglobin contamination.

The significance of fetal red cell contamination is unstudied. Fetal blood is routinely entrained into the maternal circulation during delivery; therefore, our supposition is that the fetal red cell contamination is insignificant, except when there is a red cell antigen incompatibility between mother and infant. Isoimmunization can occur from this exposure, leading to erythroblastosis in subsequent pregnancies. Blood group system (ABO) incompatibility tends to be a minor problem when compared with Rhesus factor (Rh) incompatibility. To prevent isoimmunization, anti-D immune globulin (Rhogam; Ortho-Clinical Diagnostics, Inc., Raritan, NJ) is administered to the mother, neutralizing this immune response. The immune globulin is administered based on a calculation of the volume of fetal red cells transferred to the mother (Kleihauer-Betke test).<sup>15</sup> If cell-salvaged blood was administered in the circumstances of an Rh incompatibility, it would seem logical to measure the maternal fetal hemoglobin concentration after cell-salvaged blood has been administered, so that the immune globulin dose is adequate to neutralize these additional cells.

Measurement of bacterial contamination was performed to determine the effectiveness of the leukocyte reduction filters in reducing bacterial contamination. Compared with the prewash sample, a 24-fold reduction of the bacterial colony counts was obtained through washing and filtering. Bacterial contamination of cell-salvaged blood has been recognized before and it has

been deemed to be clinically insignificant.<sup>16-19</sup> This insignificance relates to a number of factors. First, during the course of most operations, bacteremia is present. Second, homologous blood obtained from the blood bank is not bacteria free.<sup>20-23</sup> Lastly, postoperative infection rates are lower in patients receiving autologous blood when compared with homologous blood because of immunomodulation that occurs after homologous blood exposure.<sup>24</sup>

Unlike the methods of the current study, additional safety in the application of cell salvage might be achieved through the use of a double-suction setup. In this setup, one suction line should be connected to the cell-salvage reservoir and used for suctioning of maternal blood, whereas the other should be connected to the regular wall suction and used for aspiration of amniotic fluid.<sup>25-27</sup> By using separate suction devices, the contamination of the salvaged blood could be further minimized. The smaller the overall contamination of the salvaged erythrocytes, the lower the resultant concentration in the washed product. Although this recommendation seems logical and intuitive, it is unproven.

Support for the use of cell salvage in obstetric hemorrhage encompasses 390 reported cases in which blood contaminated with amniotic fluid has been washed and readministered without filtration.<sup>20,21,28-30</sup> Discussion with anesthesiologists from several different institutions would suggest that this practice has been established for a decade and has gone markedly underreported. From the results of the current study, use of a leukocyte reduction filter appears to reduce amniotic fluid contaminants to a level approaching those levels found in maternal blood. This study provides further evidence for the use of cell salvage in obstetrics. Because the incidence of amniotic fluid embolism is rare, the safety of cell salvage in obstetrics will most likely never be firmly established.

## References

1. Bernstein HH, Rosenblatt MA, Gettes M, Lockwood C: The ability of the Haemonetics 4 cell saver system to remove tissue factor from blood contaminated with amniotic fluid. *Anesth Analg* 1997; 85:831-3
2. Lockwood CJ, Bach R, Guha A, Zhou XD, Miller WA, Nemerson Y: Amniotic fluid contains tissue factor, a potent initiator of coagulation. *Am J Obstet Gynecol* 1991; 165:1335-41
3. Halmagyi DFJ, Starzecki B, Shearman RP: Experimental amniotic fluid embolism: Mechanism and treatment. *Am J Obstet Gynecol* 1962; 84:251-6
4. Morgan M: Amniotic fluid embolism. *Anaesthesia* 1979; 34:20-32
5. Maleck WH, Petroianu GA: Autologous blood transfusion. *Br J Anaesth* 1999; 82:154

6. Petroianu GA, Altmannberger SHG, Maleck WH, Assmus HP, Friedberg C, Bergler WF, Rufer R: Meconium and amniotic fluid embolism: Effects on coagulation in pregnant mini-pigs. *Crit Care Med* 1999; 27:348-55
7. Durand F, Duchesne-Gueguen M, Le Bervet JY, Marcorelles P, Tardivel R, Vovan JM, Le Goff MC, Genetet B: Rheologic and cytologic study of autologous blood collected with Cell Saver 4 during cesarean. *Rev Fr Transfusion Hemobiol* 1989; 32:179-91
8. van Kreel BK: Estimation of fetal lung maturity by assessment of lamellar body particle concentration. *Ann Clin Biochem* 1991; 28: 574-80
9. Dzik S: Leukodepletion blood filters: Filter design and mechanisms of leukocyte removal. *Transfusion Med Rev* 1993; 7:65-77
10. Perseghin P, Vigano M, Rocco G, Della Pona C, Buscemi A, Rizzi A: Effectiveness of leukocyte filters in reducing tumor cell contamination after intraoperative blood salvage in lung cancer patients. *Vox Sang* 1997; 72:221-4
11. Edelman MJ, Potter P, Mahaffey KG, Frink R, Leidich RB: The potential for reintroduction of tumor cells during intraoperative blood salvage: Reduction of risk with use of the RC-400 leukocyte depletion filter. *Urology* 1996; 47:179-81
12. Kongsgaard UE, Wang MY, Kvalheim G: Leucocyte depletion filter removes cancer cells in human blood. *Acta Anaesthesiol Scand* 1996; 40:118-20
13. Torre GC, Ferrari M, Favre A, Razzetta F, Borgonovo G: A new technique for intraoperative blood recovery in the cancer patient. *Eur J Surg Oncol* 1994; 20:565-70
14. Catling SJ, Williams S, Fielding AM: Cell salvage in obstetrics: An evaluation of the ability of cell salvage combined with leukocyte depletion filtration to remove amniotic fluid from operative blood loss at caesarean section. *Int J Obstet Anesth* 1999; 8:79-84
15. Cunningham FG, MacDonald PC, Gant NF, Leveno KJ, Gilstrap LC, Hankins GDV, Clark SL: Diseases and injuries of the fetus and newborn, Williams Obstetrics, 20th edition. Stamford, Appleton & Lange, 1997, pp 967-1008
16. Bland LA, Villarino ME, Arduino MJ: Bacteriologic and endotoxin analysis of salvage blood used in autologous transfusions during cardiac operations. *J Thorac Cardiovasc Surg* 1992; 103:582-8
17. Ozmen V, McSwain NE, Nichols RL, Smith J, Flint LM: Autotransfusion of potentially culture-positive blood (CPB) in abdominal trauma: Preliminary data from a prospective study. *J Trauma* 1992; 32:36-9
18. Andrews NJ, Bloor K: Autologous blood collection in abdominal vascular surgery: Assessment of a low pressure blood salvage system with particular reference to the preservation of cellular elements, triglyceride, complement and bacterial content in the collected blood. *Clin Lab Haematol* 1983; 5:361-70
19. Boudreaux JP, Bornside GH, Cohn I: Emergency autotransfusion: Partial cleansing of bacteria-laden blood by cell washing. *J Trauma* 1983; 23:31-5
20. Anonymous: Update: Yersenia enterocolitica bacteremia and endotoxin shock associated with red blood cell transfusions: United States, 1991. *Morb Mortal Wkly Rep MMWR* 1991; 40:176-8
21. Corash L: Inactivation of viruses, bacteria, protozoa, and leukocytes in platelet concentrates: Current research perspectives. *Transfusion Med Rev* 1999; 13:18-30
22. Soeterboek AM, Welle FH, Marcelis JH, van der Loop CM: Sterility testing of blood products in 1994/1995 by three cooperating blood banks in The Netherlands. *Vox Sang* 1997; 72:61-2
23. Wagner S: Transfusion-related bacterial sepsis. *Curr Opin Hematol* 1997; 4:464-9
24. Blajchman MA: Transfusion-associated immunomodulation and universal white cell reduction: Are we putting the cart before the horse? *Transfusion* 1999; 39:665-70
25. 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
26. Potter PS, Waters JH, Burger GA, Mraovic B: Application of cell-salvage during cesarean section. *ANESTHESIOLOGY* 1999; 90:619-21
27. 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
28. Rainaldi MP, Tazzari PL, Scagliarini G, Borghi B, Conte R: Blood salvage during caesarean section. *Br J Anaesthesia* 1998; 80:195-8
29. Grimes DA: A simplified device for intraoperative autotransfusion. *Obstet Gynecol* 1988; 72:947-50
30. Jackson SH, Lonser RE: Safety and effectiveness of intracesarean blood salvage (letter). *Transfusion* 1993; 33:181