The impact of fetal and maternal physiologic factors on umbilical cord blood quality as a source of stem cells in Egyptian population

Abeer Mohamed Abdelrazik,1 Manal Niazi El Said,1 Hossam Eldin M. Abdelaziz,1 Haithm Mohann Badran,2 and Eman Yousief Ali Abd Elal1

BACKGROUND: Umbilical cord blood (UCB) has rapidly become a clinically useful alternative stem cell source. Many variables have been used to evaluate a UCB unit and predict transplant outcomes. The objective of this study was to measure the expression of hematopoietic stem cells in UCB and its relation to certain maternal and neonatal physiologic factors to establish optimum criteria for UCB donor selection.

STUDY DESIGN AND METHODS: Two hundred UCB units were collected from normal uncomplicated vaginal and cesarean deliveries. Total volume was noted and immediately assessed for total nucleated cell (TNC) count and CD34+ cell concentration. Assessment of maternal and neonatal variables such as mode of delivery, placental weight, baby’s birthweight, and sex was made.

RESULTS: The volume of the donations ranged from 42.0 to 126 mL, the TNC count ranged from $5 \times 10^9$ to $28.7 \times 10^9$ cells/L, and CD34+ cells ranged from 0.03% to 0.62%. There was a significant positive correlation between cord blood volume and cesarean section (p < 0.01) and placental weight (p < 0.02). There was a significant positive correlation with a value of less than 0.05 between the number of CD34+ cells and UCB volume and TNC. There was no significant difference between the variables and the TNC count.

CONCLUSION: Our study concludes that cord units collected for banking should be obtained by selecting units of larger volumes, of higher TNCs, from female babies with heavy placenta, and from babies delivered via cesarean section.

Umbilical cord blood (UCB) is increasingly used as an alternative source of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) to marrow and mobilized peripheral blood for HSC transplantation in children and adults. More than 400,000 cord blood units are now stored for use in more than 100 quality-controlled public international cord blood banks. International networks, such as AABB, have agreed on standards for collection, processing, and handling of stored blood and they also established an accreditation of practices. There are two options of cord blood banks, private banks that store cord blood for individual use by families and public cord blood banks for unrelated-donor transplant. The donations are made on a volunteer basis. According to Ballen and colleagues, there is an estimated 0.04% to 0.005% chance that an individual will develop a disease that can be treated with their own stored cord blood by 21 years of age using a hematopoietic transplant approach.

The use of stem cells from cord blood has several clear advantages over marrow donation or collection of

ABBREVIATIONS: TNC(s) 5 total nucleated cell(s); UCB 5 umbilical cord blood.

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Received for publication May 14, 2015; revision received June 23, 2015; and accepted June 26, 2015.

doi:10.1111/trf.13258

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TRANSFUSION 2015;55:2882-2889
peripheral stem cells from a donor. Advantages are easy
extraction with no risk for the mother or baby, available on short
notice for transplant, no donor attribution com-pared with
marrow registry, ethnic diversity easier to achieve, painless
collection of stem cells, higher proli-fi-fer-tive capacity, and lower
rate of acute graft-versus-host disease.\textsuperscript{3,9}

One major limitation for successful outcomes of UCB
transplantation is the low cell dose of UCB since high CD341
cell count and total nucleated cell (TNC) number of UCB have
been found to associate with good engraft-ment results.\textsuperscript{10,11} The
main variables used in UCB banks include the TNC count,
percentage of CD34 cells, and the volume of blood.\textsuperscript{12}

Many variables that may improve the quality of UCB are
still under study and research, and these factors can lead to
lowering the cost and time consumed in processing and storage
of unsuitable UCB.\textsuperscript{13,14} Many fetal and maternal physiologic
factors such as birth-weight, weight of placenta, sex of newborn,
type of deliv-ery and mode of collection are correlated with
UCB volume and TNC count.\textsuperscript{3,15} New donor selection stra-
tegies to improve the quantity and quality of UCB units must be
proposed.

Our study aimed to identify fetal and maternal physiologic
variables that can affect HSC content in the UCB collected from
patients in Fayoum University Hos-pital, Egypt. These factors
may help to identify deliv-eries that are likely to yield UCB of
suitable volumes and quality.

MATERIALS AND METHODS

Recruitment

Two hundred UCB units were collected from normal
uncomplicated vaginal and cesarean deliveries performed in
Fayoum University Hos-pital over a period of 5 months (from
November 2014 to March 2015). Informed consent for cord
blood collection was obtained by attending obstetricians. All
donating mothers were from the Egyptian population with
Egyptian fathers and mothers. Women with hypertension or
diabetes mellitus and cases with neonatal congenital anomalies
were excluded. Cases with incomplete or abnormal placenta or
with clotted cord blood were also excluded.

Obstetric data

Patient information and medical history were collected during
the recruitment by attending obstetricians. These included
maternal age, parity, and presence of maternal disease. The
details of delivery including weight of placenta, sex of newborn,
birthweight, and gestational age were also collected after
delivery. A data
collection form was completed for each subject recruited.

Collection of cord blood

Under complete aseptic conditions, immediately after delivery
of the baby and before the delivery of the pla-centa the umbilical
cord was clamped from the baby side (as near as possible to
preserve the longest available part of the cord). The cord was
then washed with 70% ethanol swab, and the umbilical vein was
punctured using the wide-bore needle of the CB collection bag
containing CPDA-1 as anticoagulant. Blood was allowed to flow
by gravity, and the needle was removed when the blood flow
ceased. If the vein collapsed before reaching the adequate
amount, the cord was clamped proximal to the first puncture and
another puncture or more were performed until reaching the
adequate amount. After collection of the blood it was transferred
to the laboratory in a container and processed within 12 hours
postcollection.

Hematologic variables

UCB aliquot was collected in EDTA tube for TNC counting
with an automated hematology analyzer (Sysmex XS-800i,
Sysmex, Kobe, Japan) using a multiangle polarized scatter
separation technique.

Flow cytometric assessment of CD341 cells

According to the International Society of Hemato-therapy and
Graft Engineering, CD34 cells’ surface marker was assessed in
the mononuclear cells. The cells were double stained with
monoclonal antibodies that were anti-CD34 (HPC/ HSC)
conjugated with phycoerythrin (PE) and anti-CD45 (white blood
cell common anti-gen) conjugated with fluorescein
isothiocyanate (FITC). Flow cytometric analysis was performed
on a flow cytometer (Epics XL-MCL, Beckman Coulter,
Fullerton, CA). A gating strategy that uses light scat-tering
variables and CD34 and CD45 monoclonal fluo-
rescence had been used for accurate identification and enumeration of true
CD341 cells. First, in a CD45 (FITC) versus side scatter
histogram, gating was made on all the events except those that
are CD45 negative and dead cells. Second, on the previously
gated CD451 cells, a CD341 (PE) versus side scatter histo-
gram was made giving the percentage of CD341 cells from the
CD451 cells (CD451, CD341 cells). Utilizing the given
percentage, the absolute number of the tar-get cells was
material information

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including maternal age, obstetric history, number of parity, gestational age, family, and medical histories. Immediately after delivery neonatal data including birth-weight, neonatal sex, placental weight, and cord length were measured and recorded in the case report form. The UCB samples collected into blood bags were weighed and the volume was calculated according to the equation:

\[
\text{measured weight} - \text{weight of the empty bag \& containing CPDA-1 whole blood density}
\]

Collected data were stored in a computer program (Microsoft Excel, Microsoft Corp., Redmond, WA) day by day and later exported to a statistical package (SPSS, SPSS, Inc., Chicago, IL) for analysis.

**Statistical analysis**

Data were collected, coded, translated to English to facilitate data manipulation, and double entered into a computer program (Microsoft Access, Microsoft Corp.) and data analysis was performed using computer software (SPSS, Version 18, under Windows 7, SPSS, Inc.).

Simple descriptive analysis was in the form of numbers and percentages for qualitative data, arithmetic means as central tendency measurement, standard deviations as measure of dispersion for quantitative parametric data, and inferential statistic test. For quantitatively parametric data, independent t test was used to compare measures of two independent groups of quantiative data. For qualitative data, a chi-square test was used to compare two of more than two qualitative groups. Bivariate correlation test was used to test association between variables. The level p value of not more than 0.05 was considered the cutoff value for significance. The study design was a cross-sectional descriptive study.

**RESULTS**

**Characteristics of the study population**

A total of 200 UCB samples were analyzed; 150 were obtained from cesarean sections (75%) and 50 were obtained from normal vaginal deliveries (25%). Accord-ing to their newborn sex distribution, 85 infants (42.5%) were males and 115 (57.5%) were females. The gestational age ranged from 35 to 40 weeks with mean SD of 38.1 ± 1.3 weeks. The birthweight ranged from 2.2 to 4.3 kilograms with mean SD of 3.2 ± 0.43 kg. The age of the mothers involved in the study ranged from 19 to 35 years old with mean SD of 26.5 ± 4.3 years, and the number of parity of those mothers ranged from one to four with a median SD of 2 ± 3. The placental weight ranged from 300 to 780 g with a mean SD of 570.3 ± 103.1 g.

**Analysis of UCB samples**

The mean SD of the total volume collection and the TNC were 72.6 ± 18.7 mL and 10.9 ± 3 x 10^9 - 3 x 10^10 cells/L, respectively. The percentage of CD34 cells ranged from 0.03% to 0.62% with a mean SD of 0.24% ± 60.16%. The absolute number of CD34 cells per the UCB units collected ranged from 0.023 ± 10^3 to 2.4 ± 10^5 cells/mL with mean SD of 0.66 ± 3 x 10^6 - 0.52 ± 3 x 10^6 cells/mL.

**Correlation between physiologic maternal and neonatal factors and cord blood samples**

There were significant positive correlation between cord blood volume and each of cesarean section (p < 0.01) and placental weight (p < 0.02). Comparisons between maternal and fetal characteristics and cord blood volume are shown in Tables 1 and 2. The percentage of CD34 cells was positively affected in female babies (p < 0.001) and in cesarean section deliveries (p < 0.04) as shown in Table 3. There was no significant difference between the variables and the TNC count.

**Correlation between absolute number of CD341 cells and different maternal, neonatal, and cord blood variables among study group**

There was a significant positive correlation between absolute number of CD341 cells and each of UCB volume and TNC with p values of 0.002 and 0.005, respectively. There was no significant correlation between absolute number of CD341 cells and all other variables as shown in Table 4.

**DISCUSSION**

UCB is an effective alternative source of HSC for stem cell transplantation. The main disadvantages of cord blood are the small volume and the low number of cells in the unit, making it unsuitable for transplantation. Donor selection has been studied to improve the collection of UCB units. The cost of collecting, transferring, and storing unsuitable UCB can be avoided by appropriate selection of donors. Local data in our population are lacking; we thus estimated the expression of hematopoietic stem cells in UCB and its relation to certain maternal and
neonatal physiologic factors. This would help in establishing optimum criteria for UCB donors’ selection in our hospitals.

In this study, UCB volume was significantly higher in cesarean section delivery than in normal vaginal delivery. This was similar to the results of Chandra et al.16 who included 500 UCB samples and stated that higher volume was obtained from cesarean section. Also Wu et al.19 studied 4615 UCB units and revealed larger amounts of UCB with cesarean section but inferior cell count with vaginal delivery. In contrast, Al-Sweedan et al.20 stated that mode of the delivery has no impact on UCB volume collected. This may be attributed to different methods of collection.

Our study stated that placental weight positively affects the UCB volume. This was in agreement with several studies as Jones et al.,21 Nakagawa et al.,22 and Canabarro et al.,23 who found that larger placenta produce greater volume.

There was no significant difference between neonatal sex and the collected UCB volume. In agreement, Al-Sweedan et al.,20 who included 200 samples, found that

<table>
<thead>
<tr>
<th>TABLE 1. Correlation between UCB volume and different maternal, neonatal, and cord blood variables among the study group</th>
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<tbody>
<tr>
<td><strong>Variables</strong></td>
</tr>
<tr>
<td>Maternal variables</td>
</tr>
<tr>
<td>Maternal age (years)</td>
</tr>
<tr>
<td>Number of parity</td>
</tr>
<tr>
<td>Neonatal variables</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
</tr>
<tr>
<td>Birthweight (kg)</td>
</tr>
<tr>
<td>Placental weight (g)</td>
</tr>
<tr>
<td>Umbilical cord length (cm)</td>
</tr>
<tr>
<td>NS = nonsignificant; S = significant.</td>
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</tbody>
</table>

TABLE 3. Comparisons of CD34+ cells percentage between different sexes and mode of delivery among the study group*

<table>
<thead>
<tr>
<th><strong>Variables</strong></th>
<th><strong>CD34+ cells percentage</strong></th>
<th><strong>Mean</strong></th>
<th><strong>SD</strong></th>
<th><strong>p value</strong></th>
<th><strong>Significance</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td>Male</td>
<td>85</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>115</td>
<td>0.31</td>
<td>0.15</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td>Cesarean section</td>
<td>175</td>
<td>0.23</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal vaginal</td>
<td>25</td>
<td>0.38</td>
<td>0.07</td>
</tr>
<tr>
<td>HS* = highly significant; S = significant.</td>
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</table>

infant sex was not a significant factor regarding the UCB volume. On the other hand, Askari et al.13 suggested that female newborns were positively associated with greater volume, which is contradicted with a large cohort study by Cairo et al.24 that stated that a male baby was associated significantly with an increased UCB volume and still disagreed with ours.

No significant relation was found between maternal age and UCB volume. A similar finding was achieved by Jan et al.,25 Wu et al.,19 and Canabarro et al.,23 who found that maternal age had no effect on the CB volume.

In this work, there was no significant association between the collected UCB volume and the neonatal birthweight. Ballen et al.,26 who stated that bigger babies were more likely to produce cord blood units with larger volumes and higher cell counts and CD34+ cell counts, disagreed with the current results. Also Wen et al.,27 found that birthweight had a significant effect on the collected volume of the CB.

<table>
<thead>
<tr>
<th>TABLE 2. Comparisons of UCB volume between different sexes and mode of delivery among the study group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variables</strong></td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Mode of delivery</td>
</tr>
<tr>
<td>Cesarean section</td>
</tr>
<tr>
<td>Normal vaginal</td>
</tr>
<tr>
<td>NS = nonsignificant; S = significant.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 4. Correlation between absolute number of CD34+ cells and different maternal, neonatal, and cord blood variables among the study group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variables</strong></td>
</tr>
<tr>
<td>Maternal variables</td>
</tr>
<tr>
<td>Maternal age (years)</td>
</tr>
<tr>
<td>Number of parity</td>
</tr>
<tr>
<td>Neonatal variables</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
</tr>
<tr>
<td>Birthweight (kg)</td>
</tr>
<tr>
<td>Placental weight (g)</td>
</tr>
<tr>
<td>Umbilical cord length (cm)</td>
</tr>
<tr>
<td>Cord blood variables</td>
</tr>
<tr>
<td>UCB volume</td>
</tr>
<tr>
<td>TNCs</td>
</tr>
<tr>
<td>HS* = highly significant; NS = not significant.</td>
</tr>
</tbody>
</table>

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Some previous studies Al-Sweedan et al.,20 Wu et al.,27 and Ballen et al.26 have reported that gestational age has no impact on UCB volume. Our data are consist-ent with these findings; we did not find any correlation between gestational age and UCB volume. However, Jones et al.21 involved 9205 cases and found that factors like gestational age significantly influence the collected volume. This difference may be attributed to narrow range of ges-tational ages involved in this study (35-40 weeks).

In this study, higher CB volumes and TNCs were as expected associated with high absolute number of CD34 cells. This was in agreement with the findings of Wen et al.,27 who studied 1590 units of UCB and concluded that units with high volume will definitely have a greater chance to have higher final CD34 counts after volume concentration through the processing step. A similar rela-tionship was also found for CD34 counts and TNCs number. In a study by Eldaly et al.,28 which involved UCB samples from 90 mothers, it was observed that higher count of CD34 cells was associated with high number of TNCs. Jaime-Perez et al.29 who studied which is the best variable for the selection of CB units for cryopreservation and further transplantation, stated that TNC count was the best variable to identify CB content of CD34 cells and this greatly supported the recent findings.

In this work, it was found that mode of delivery has no effect on absolute number of CD34 cells in UCB. Similar findings were proved by Dimitriou et al.30 On the other hand, Eldaly et al.28 disagreed with this in that they found higher count of CD34 cells in the UCB of neo-nates delivered by normal vaginal deliveries. This differ-ence could be explained by Sparrow et al.,31 who considered that cesarean section results in volume increase while spontaneous delivery results in TNCs increase so that type of delivery does not influence the number of CD34 cells in UCB.

This study found no correlation between absolute number of CD34 cells in UCB and neonatal sex. In agreement, Eldaly et al.28 and Wen et al.27 found no corre-lation. In contrast, Jan et al.25 stated that male infants had higher CD34 cells in their CB.

In this work, it was found that maternal age has no impact on absolute number of CD34 cells in UCB. This agreed with the findings of Wu et al.26 and Jan et al.25 but Nakagawa et al.32 stated that younger maternal age was associated with a higher CD34 cell concentration.

Our study revealed that neonatal birthweight has no correlation with the absolute count of CD34 cells. Several studies opposed this finding. For example, Al-Sweedan et al.,20 Chandra et al.,18 and Jan et al.25 stated that bigger babies produce higher count of CD34 cells.

This work also stated that order of birth has no effect on absolute number of CD34 cells in UCB. Wen et al.27 supported this finding but Ballen et al.26 believed that birth order had significant effects on the number of CD34 cells in CB as each additional prior birth contrib-uted to a 17% decrease in CD34 cell count.

This work found no impact of gestational age on the absolute count of CD341 cells. In agreement with this, Ayad et al.22 stated that there was no significant correla-tion between gestational age of neonates included in the study and the CD34 cells in UCB. This also was in line with Surbek et al.33 who reported that the CD34 cell count per cord blood sample was independent of gesta-tional age. On the other hand, Omori et al.34 had found that total number of CD341 cells had a reverse correlation with the gestational age. Mancinelli et al.35 reported that gestational age of more than 39 weeks increases CD34 signifi-cantly. This was explained by the fact that with increased gestational age there is placental aging and the fetus encounters a progressive hypoxia resulting in defense mechanisms that tend to increase hematopoietic cells and circulating blood volume.

No correlation was found between placental weight and absolute count of CD341 cells. Al-Sweedan et al.20 supported this finding. In contrast, Wen et al.27 suggested that placental weight positively associated with total num-ber of CD341 cells in CB.

In this work, no significant correlation was detected between TNCs in CB and the mode of delivery. Jan et al.25 found lower TNCs with cesarean sections while Wen et al.27 found that vaginal delivery was more likely to pro-duce UCB with more TNCs numbers and both do not agree with our finding.

In our study, no significant correlation was detected between TNCs in CB and neonatal sex. Izu et al.36 stated the same finding but Askari et al.13 found that female newborns are associated with higher TNCs than males while Nakagawa et al.25 believed that male infants have higher TNCs in their CB and both opposed our result. In agreement with Wen et al.27 and Jan et al.25 no relation was found between maternal age, umbilical cord length, and TNCs in cord blood.

Order of birth was found to have no impact on the number of TNCs in CB. Izu et al.,36 who involved 40 preg-nant women, agreed with our finding. In contrast, Ballen et al.26 believed the presence of significant correlation between birth order and total cell counts. First babies had the most favorable values, with the results decreasing with each successive live birth. The etiology for this finding is unclear. Speculative hypotheses include a weakening of the placental vasculature. First babies are also associated with a longer labor; longer labor has been reported to be associated with higher TNC counts.37

No correlation was found between gestational age and TNCs in CB. Wu et al.19 and Ballen et al.26 believed that babies of longer gestational age had higher cell counts and this did not meet with our finding. Regarding neonatal birthweight, this study proved no effect of it on TNCs in CB that was different than the finding of Izu.
## TABLE 5. Comparison between our results and previous study results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation with UCB volume in our study</th>
<th>Previous studies agreed</th>
<th>Previous studies disagreed</th>
<th>Correlation with absolute number of CD341 cells</th>
<th>Previous studies agreed</th>
<th>Previous studies disagreed</th>
<th>Correlation with TNCs</th>
<th>Previous studies agreed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cesarean section</td>
<td>S</td>
<td>Chandra et al. 18</td>
<td>EF</td>
<td>Al-Sweedan et al. 6</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
</tr>
<tr>
<td>Placental weight</td>
<td>S</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
</tr>
<tr>
<td>Neonatal sex</td>
<td>NS</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
</tr>
<tr>
<td>Maternal age</td>
<td>NS</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
</tr>
<tr>
<td>Gestational age</td>
<td>NS</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
</tr>
<tr>
<td>Cord length</td>
<td>NS</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
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<tr>
<td>Birthweight</td>
<td>NS</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
</tr>
<tr>
<td>UCB volume</td>
<td>HS</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
</tr>
<tr>
<td>Absolute number of CD341</td>
<td>HS</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
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<td>EF</td>
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<tr>
<td>TNCs</td>
<td>NS</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
<td>EF</td>
</tr>
</tbody>
</table>

HS 5 highly significant; NS 5 non significant; S 5significant.
et al.\textsuperscript{36} and Al-Sweedan et al.\textsuperscript{20} found that there is a significant positive correlation between the initial cellularity with fetal weight.

Placental weight in this study was found to have no impact on the TNCs of the UCB. Solves et al.\textsuperscript{38} differed with this and stated that placental weight is a predictor variable for TNC count in CB.

In this study, no correlation was found between umbilical cord length and number of TNCs in CB. Wen et al.\textsuperscript{37} supported this finding. Comparison between our results and previous studies results are summarized in Table 5.

Conclusio

This research could help in the future regarding selection of the optimal UCB donors in our region, as at the present time there are no such records in Egypt. Moreover, there are only a few studies on the association between physiologic variables and UCB stem cells. This study may help to determine the best selection processes for donors of UCB (to improve quality) and to prevent storage of ineffective blood units, for processing of UCB units, to optimize the collection procedure, and to minimize the number of rejected UCB units.

In conclusion, we found that cord units collected for banking should be selected without regard to maternal age, number of parity, gestational age, birthweight, or umbilical cord length. Optimal results would be obtained by selecting units of larger volumes and higher TNCs, of female babies with heavy placenta, and of babies delivered via cesarean section.

Conflict of Interest

The authors have disclosed no conflicts of interest.

References


