Yield of stem cells from umbilical cord blood units collected in-utero versus ex-utero

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Abstract
Purpose: This study aims to compare yield of stem cells from umbilical cord blood units collected in-utero versus ex-utero. Methods: A descriptive comparative study was carried out at Labor and delivery room, operating room, and Research Center for Cord Stem Cells in Mansoura University, Egypt. A purposive sample of 124 pregnant mothers and their newborns were recruited, 62 umbilical cord blood units were collected in-utero and 62 units were collected ex-utero from vaginal and cesarean birth fields. Data were collected through an assessment sheet. Results: Average of umbilical cord blood volume obtained in-utero was significantly larger than that obtained ex-utero (155.8 ±21.2 vs. 147.1 ±23.7 ml respectively; p=0.033). The in-utero collected umbilical cord blood units yielded significantly higher total nucleated cell count (8.9 ±2.6 vs. 7.9 ±2.8 x10^6/ml respectively; p=0.040), and higher CD34+ counts (31.9 ±8.2 vs. 28.2 ±7.2 x10^6/ml respectively; p=0.009) equated to those collected ex-utero. Conclusion: The UCB units collected in-utero yielded larger blood volume and higher cellular content compared to the ex-utero collection.

Keywords: Umbilical cord blood units, in-utero collection, ex-utero collection, stem-cell therapy, blood volume, cellular content.

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I. INTRODUCTION

Stem cell is a dominant cell that almost can form any tissue in the human body. For many years bone marrow was the main source of stem cells, but extracting marrow from donor is a risky procedure. Umbilical cord blood (UCB) that is treated as a medical waste in the delivery rooms has been increasingly used as an alternative source to marrow for stem cells [1]. The UCB-derived stem cells have been used effectively for treatment of malignancies, immunologic deficiencies, hematologic disorders, marrow failure, and other genetic diseases [2, 3].

The UCB-derived stem cell is more advantageous over marrow stem cell; it is widely and more rapidly available, no donor attrition, safe collection without risk for the mother or newborn, carries better biologic characterization and lower possibility of transmitting infectious diseases to the recipient; due to immaturity of the newborn’s immune system [4]. However, the main factor limiting its use for transplantation is the lower content of stem cells; as reflected by Total Nucleated Cell count (TNC), and CD34 positive cells obtained from each UCB unit [5]. Accordingly, obstetricians, nurses and midwives who afford care in Labour and Delivery rooms and Operating Rooms of Obstetrics department and are the mainly responsible for UCB units collection create extensive efforts to obtain larger blood volumes and higher cellular content [6].

Aiming at maximizing the collected blood volume and cell amount derived from each UCB unit, studies have been conducted to identify optimal method of collection [7, 8]. Long time ago, Larry and colleagues 2002 [9] evaluated two different strategies for collecting blood from the umbilical vein. In-utero collection that refers to obtaining blood from the umbilical vein before separation of the placenta, and ex-utero collection which involves extracting the blood from the umbilical vein after placenta delivery. The same researchers [9], indicated no advantages of one method over the other. Conversely, Bassiony et al., [8] evaluated the two methods of collection and found positive outcomes of collecting UCB units in-utero. This contradiction between the existing findings signifies that advantageous method of UCB units' collection is still debatable issue and open for investigation and discussion. Therefore, the present study was carried out to compare yield of stem cells from umbilical cord blood units collected in-utero versus ex-utero.
Yield of stem cells from umbilical cord blood units collected in-utero versus ex-utero

1.1 Significance of the study
It was evidenced that more than half of the collected cord blood units (54.1%) were discarded before processing due to volume deficiency or inadequate stem cell content [10]. Investigating the proper means that may achieve higher yield of stem cells from umbilical cord blood units is inadequate research issue in Egyptian population. Recognizing such means can reduce the financial cost and time spent in collecting inefficient cord blood units. Among the means that may improve stem cells yield from UCB units is the method of UCB collection. Therefore, the present study was conducted to compare yield of stem cells from umbilical cord blood units collected in-utero versus ex-utero.

1.2 Operational definitions

1.2.1 In-utero and ex-utero cord blood collection
In-utero cord blood collection refers to collecting the cord blood while the placenta still in uterus; not yet delivered or even separated. Meanwhile, ex-utero cord blood collection refers to collecting the cord blood after complete expulsion of the placenta [9].

1.2.2 Umbilical cord blood units
It indicates to a unique bag contains the collected cord blood; this bag contains anticoagulant agent. The blood unit obtained by piercing vein of the umbilical cord; using a needle attached to the cord blood bag, for collecting blood left over in the umbilical cord after baby delivery. Umbilical cord blood is composed of white blood cells, red blood cells, platelets, plasma, as well as it contains fair count of hematopoietic stem cells.

1.2.3 The CD34+ cells
Membrane-bound cell surface antigen present on and identifying T cells, monocytes, and macrophages. It is found principally on endothelial cells and blood-forming stem cells, and regulates cell-to-cell adhesion [11].

1.3 Aim of the study
This study aims to compare yield of stem cells from umbilical cord blood units collected in-utero versus ex-utero.

1.4 Study question
To achieve aim of the present study, one question was formulated: “Is there a difference in blood volume and stem-cell content when umbilical cord blood units collected in-utero or ex-utero?”

II. SUBJECTS AND METHOD

2.1 Research design
A descriptive comparative design was followed to achieve aim of the present study. Two matched groups were assessed. The first group involved the in-utero collected UCB units; while the second group involved the ex-utero collected UCB units. The both group UCB units were compared for its yield of collected blood volume and stem-cell content.

2.2 Study setting
This study was carried out at three settings in Mansoura University, Egypt: Labor and delivery room, operating room, and Mansoura Research Center for Cord Stem Cells (MARC-CSC). Labor and delivery room and operating room, were the main sources that supply MARC-CSC with the UCB Units. MARC-CSC, is the first umbilical cord stem cell center established in Egypt at 2005.

2.3 Sampling
A non-probability purposive sampling technique was used to recruit 124 pairs (i.e., pregnant mothers and their newborns), in this study between July 2016 to June 2017.

2.3.1 Inclusion criteria of the mother
The pregnant mother was eligible to participate, if met the following inclusive criteria:
1. Expected to have uncomplicated vaginal delivery or planned to undergo elective cesarean section. As, neonates who were delivered by emergency cesarean section are more liable for experiencing stressful events of first stage of labor, which may adversely affect the UCB units' cellular content.
2. Free from medical or obstetric problems.

2.3.2 Inclusion criteria of the neonate
The neonate was suitable to enroll in this study, if fulfilled the following criteria:
1. Term, single, with normal Apgar scores at the 1st and 5th postpartum minutes (i.e., 7-10).
2. Birth weight ranges between 2500 to 4000 grams.
3. Had normal umbilical cord; free from abnormalities (i.e., false or true knot, thrombi, etc.).
4. Not known to have congenital anomalies.

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2.3.3 Sample size calculation

The required sample was calculated depending on the findings of a previous study [8]. Considering level of significance of 5% and study power of 80%, and by substitution in the following formula: \[ n = \frac{[(Z_{a/2} + Z_{b})^2 \times \{2SD\}^2]}{\text{mean difference between the two groups}} \]. Where SD = standard deviation, \( Z_{a/2} \): This depends on level of significance, for 5% this is 1.96, \( Z_{b} \): This depends on power, for 80% this is 0.84. The sample size \( n = [(1.96 + 0.84)^2 \times \{(2(1.6))^2\}] / (0.805)^2 = 61.9 \). Therefore, sample size required was 62 pairs; pregnant mothers and their neonates, for the in-utero group and the same number for the ex-utero group.

2.4 Tool of data collection

2.4.1 Assessment sheet

The required data were collected through an assessment sheet; such sheet was developed by the research team. It was consisted of three sections. Section I: concerned with maternal characteristics (e.g., maternal age, gestation age, parity, mode of delivery), and it was completed from the mother's medical charts. Section II: included the neonatal characteristics (e.g., neonatal gender, birth weight, placental weight), and it was completed by the researcher at the time of delivery. Section III: included data of the UCB units; in terms of blood volume in ml, and TNC and CD34 cells count. Content validity of the assessment sheet was proven by a panel of five experts in maternity nursing, obstetrics medicine and clinical pathology.

2.5 Ethical considerations

Approvals were obtained from the board of Obstetrics and Gynecology department and board of MARC-CSC of Mansoura University. The Ethical Committee of the Nursing Faculty accepted the study and the mothers gave their informed consents before enrolment. The collected UCB units were kept at MARC-CSC; for research purpose only, without any intend to donate it to any individual without taking owner’s permission.

2.6 Pilot study

Piloting was done on both vaginal and cesarean deliveries and units were collected in-utero and ex-utero. It was done on 12 UCB unit, aiming to test completeness of the assessment sheet and to identify the barriers that may face the researchers in collecting the blood units from the delivery room or operating theater. Result of the pilot indicated that the sheet’s statements were comprehensive and no modifications were needed. Pilot sample was excluded from the analyzed study sample.

2.7 Research process

Field work was done by preparation for the work, collection of the UCB units, and assessment of its yield to blood volume and cellular content of TNC and CD34+; as indicators for its content of the stem cells.

2.7.1 Preparation for the work

This was started by taking approval from the concerned authorities for conducting the study. Tool of data collection was designed. Thereafter, a nursing researcher was trained; by the team members of MARC-CSC on how to collect UCB units in-utero and ex-utero from both vaginal and cesarean deliveries fields. The study aim and procedure were explained to the nursing staff working at the study settings to get their cooperation. This phase took about one month.

2.7.2 Collection of the UCB units

To collect the UCB units, the researchers clarified the study’s aim to each eligible mother and got an informed written consent from each one. From vaginal deliveries, the UCB units were collected on Sunday, Tuesday, and Thursday. The UCB units were collected from cesarean deliveries field according to the operating theater schedule of elective cesarean deliveries; mostly on Monday. From both vaginal and cesarean deliveries, UCB units were collected in-utero and ex-utero. Collection of the UCB units consumed about eleven months.

In-utero cord blood collection

Immediately after baby delivery the umbilical cord was double clamped; as near from the baby’s side as possible to preserve the longest available part of the cord to placental site, and transected within 10 seconds according to standard protocol of vaginal delivery at Labor and Delivery room, Mansoura University Hospital. Immediately, after transferring the newborn from the delivery field to radiant heater, the distal end of umbilical cord from placental site was disinfected with Povidone-iodine swab and 70% alcohol swab; while the placenta still in uterus. The umbilical cord vein was once pierced using large needle connected to cord blood collection bag containing 30 ml anticoagulant (CPDA-1 U.S.P.) and cord blood was harvested by gravity until the vein is collapsed, then needle was removed. As more than one vein puncture allows air insertion and may affect the CD34 cell count, the researchers collected the UCB units by only one vein puncture.
Ex-utero cord blood collection

In this technique, after baby delivery the placenta was allowed to spontaneously and completely expel and taken in a stainless steel sterile dish to a side table; as early as possible usually within 2-3 minutes from expulsion. As in-utero technique, the umbilical cord was disinfected and punctured at its distal portion. By keeping the umbilical cord straight in a slight angle, cord blood was drained by gravity into a collection bag until blood flow blocked. Thereafter, each UCB unit identified by a sticky label with mother's name, file number, date and time of delivery and was subjected for assessing its volume and cellular content.

2.7.3 Assessment of the UCB units

In this study, each UCB unit was assessed for its yield of UCB volume and its content of TNC and CD34+ cells. The collected UCB volume was determined to the nearest gram by using a weighing scale at the Labour and Delivery room. The net volume of collected cord blood was calculated by deducting the empty unit weight (42 g) and the anticoagulant volume (30 ml) from the obtained weight, assuming that 1 gm. equals 0.95 ml. Meanwhile, nucleated cell count was assessed by extracting one ml blood from the collected cord blood sample and sending it to the hematology laboratory in the Oncology Center of Mansoura University Hospital. Using an automatic cell counter (CELL-DYN 3700, Abbott Laboratories, and Abbott Park, IL), nucleated cell count/ml was assessed and the TNC count per UCB unit was calculated by multiplying the given number with the net volume of cord blood. However, the CD34+ cells count was determined by flow cytometry (EPICS XL, Beckman Coulter, Hialeah, FL) at MARS-CSC. The collected UCB units were kept at 4 to 24°C in isothermal bag before conveyed to MARS-CSC, where it was stored in a refrigerator at 4°C ± 2 until processing within 48 hours from collection.

2.8 Strengths and limitations of the study

Studying enough sample size as specified by power analysis and including different level of parity and both modes of deliveries are factors that strengthen the existing study, while including normal mothers and neonates only and excluding high risk ones may limit generalizing the study findings.

2.9 Data analysis

All statistical analyses were performed using SPSS for windows version 20.0 (SPSS, Chicago, IL). Data were tested for normality of distribution prior to any calculations. Continuous data were normally distributed and were expressed in mean ± standard deviation (SD). Categorical data were expressed in number and percentage. The comparisons were determined using Student’s t test for variables with continuous data. Chi-square test was used for comparison of variables with categorical data. Statistical significance was set at p<0.05.

III. RESULTS

3.1 Descriptive data of the in-utero and ex-utero groups

In this study, blood samples were withdrawn from 124 umbilical cords, 62 samples were withdrawn in-utero and 62 were withdrawn ex-utero. The characteristics of donating mothers and newborns of the in-utero and ex-utero groups were demonstrated in Table 1. It is clear that mothers' age, parity, mode of delivery, gestational age, newborn gender, birth weight and the placental weight did not differ significantly between the two groups.

3.2 Comparison of the UCB unit's quality indicators according to method of blood collection

The average cord blood volume obtained in-utero was significantly larger than those obtained ex-utero (155.8 ±21.2 vs. 147.1 ±23.7 ml respectively, p=0.033). The TNC counts obtained in-utero was significantly higher compared to those obtained ex-utero (8.9 ±2.6 vs. 7.9 ±2.8 x10^6/ml respectively; p=0.040). Moreover, the umbilical cord blood units collected in-utero produced significantly higher CD34+ counts compared to that of the ex-utero collected units (31.9 ±8.2 and 28.2 ±7.2 x10^3/ml respectively; p=0.009) as shown in Table 2.

3.3 Comparison of the UCB unit's quality indicators according to mode of delivery

Table 3 compares the quality indicators of UCB units according to mode of delivery. The volume of umbilical cord blood was significantly larger in units obtained from cesarean deliveries than units obtained from vaginal deliveries by a mean difference of 9.5 ml and p= 0.019. The TNC counts obtained from cesarean births were significantly higher than that obtained from vaginal deliveries by a mean difference of 1.3 x10^6/ml and p=0.012. Similarly, UCB units withdrawn in cesarean births yielded significantly higher CD34+ cell count than those withdrawn in vaginal deliveries by a mean difference of 4.2 x10^3/ml and p=0.003.

3.4 Correlation of UCB unit's quality indicators with the mother’s age and parity

As shown in Figure 1, the collected blood volume, TNC and CD34+ counts per milliliter blood showed no significant correlation with the maternal age. The average cord blood volume and cellular counts of TNC and CD34+ obtained from primipara were significantly higher than that obtained from multipara by mean differences of 11.2 ml, p=0.016, 1.4 x10^6/ml, p=0.012 and 5.5 x10^3/ml, p<0.001 respectively as illustrated in Figure 2.
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Table 1. Descriptive data of the study subjects (n=124)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>In-utero group (n=62)</th>
<th>Ex-utero group (n=62)</th>
<th>Significance test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD or n, %</td>
<td>Mean ± SD or n, %</td>
<td>t or χ²</td>
</tr>
<tr>
<td>Mother's age (years)</td>
<td>25.9 ±4.7</td>
<td>24.7 ±4.3</td>
<td>1.49</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primipara</td>
<td>19, 30.6%</td>
<td>13, 21.0%</td>
<td>1.52*</td>
</tr>
<tr>
<td>Multipara</td>
<td>43, 69.4%</td>
<td>49, 79.0%</td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVD</td>
<td>31, 50%</td>
<td>31, 50%</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>31, 50%</td>
<td>31, 50%</td>
<td>0*</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>38.4 ±1.0</td>
<td>38.1 ±0.9</td>
<td>1.60</td>
</tr>
<tr>
<td>Newborn gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>33, 53.2%</td>
<td>26, 41.9%</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>29, 46.8%</td>
<td>36, 58.1%</td>
<td>1.58*</td>
</tr>
<tr>
<td>Newborn weight (Kg.)</td>
<td>3.144 ±0.286</td>
<td>3.176 ±0.297</td>
<td>0.62</td>
</tr>
<tr>
<td>Placenta weight (gm.)</td>
<td>460.8 ±75.2</td>
<td>476.1 ±75.8</td>
<td>1.13</td>
</tr>
</tbody>
</table>

* Means χ² chi square test

Table 2. Comparison of the UCB quality indicators according to method of blood collection (n = 124)

<table>
<thead>
<tr>
<th>Quality indicators</th>
<th>In-utero (n=62)</th>
<th>Ex-utero (n=62)</th>
<th>Mean Difference</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNC count (x10⁶/ml)</td>
<td>8.9 ±2.6</td>
<td>7.9 ±2.8</td>
<td>1.0</td>
<td>2.07</td>
<td>0.040*</td>
</tr>
<tr>
<td>Cord blood volume (ml)</td>
<td>155.8 ±21.2</td>
<td>147.1 ±23.7</td>
<td>8.7</td>
<td>2.16</td>
<td>0.033*</td>
</tr>
<tr>
<td>CD34+ cells (x10³/ml)</td>
<td>31.9 ±8.2</td>
<td>28.2 ±7.2</td>
<td>3.7</td>
<td>2.64</td>
<td>0.009*</td>
</tr>
</tbody>
</table>

* refers to significant

Table 3. Comparison of the UCB units quality indicators according to delivery mode (n=124)

<table>
<thead>
<tr>
<th>Quality indicators</th>
<th>Vaginal Delivery (n=62)</th>
<th>Cesarean Section (n=62)</th>
<th>Mean Difference</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNC count (x10⁶/ml)</td>
<td>7.8 ±2.6</td>
<td>9.1 ±2.8</td>
<td>1.3</td>
<td>2.54</td>
<td>0.012*</td>
</tr>
<tr>
<td>Cord blood volume (ml)</td>
<td>146.7 ±22.8</td>
<td>156.2 ±22.1</td>
<td>9.5</td>
<td>2.37</td>
<td>0.019*</td>
</tr>
<tr>
<td>CD34+ cells (x10³/ml)</td>
<td>27.9 ±7.0</td>
<td>32.1 ±8.3</td>
<td>4.2</td>
<td>3.02</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

* refers to significant

Figure 1. Correlation of the mean volume of collected cord blood, TNC counts per milliliter of cord blood, and CD34 count per milliliter of cord blood with the maternal age.

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Figure 2. Association of collected cord blood, TNC counts per milliliter of cord blood, and CD34 count per milliliter of cord blood with parity.

IV. DISCUSSION

This study aimed to compare yield of stem cells from umbilical cord blood units collected in-utero versus ex-utero. This aim was attained through the present study findings; in-utero collected UCB units produced larger blood volume and higher count of TNC and CD34 cells compared to those collected ex-utero. Thus, the study question “Is there a difference in blood volume and stem-cell content when umbilical cord blood units collected in-utero or ex-utero?” was positively answered.

In a national study, Bassiouny et al., 2015 [8] investigated the relation of cord blood collection strategy with the collected blood volume and cellular content of 100 UCB units from Egyptian mothers in which, 27 units were collected ex-utero and 73 units were collected in-utero. Bassiouny and colleagues; consistently with the present study findings, found the cord blood volume that was collected in-utero was larger than that was collected ex-utero by a mean difference of 24.97 ml, and cellular contents were higher in favor to in-utero technique by mean differences of 0.89 for TNC and 2 for CD34 cells.

To get the same goal; high quality UCB units, Munsell et al., [7] compared the two known strategies of cord blood collection with a new strategy; in-utero plus ex-utero collection. The investigators of such study [7], retrospectively analyzed the reports of 23,968 UCB units at Houston, Texas, and included in the findings that in-utero is superior to ex-utero collection by giving highly significant larger blood volume, higher TNC count, and CD34+ cell number. Authors of the current study, endorsed a reasoning for the lesser blood volume in UCB units collected ex-utero to that the cord blood was collected within 3-5 minutes after cord clamping, raising the possibility for placental vessels to form clots resulting in lower collected blood volume, and subsequently may explain the loss of cellular content in the collected volume.

Authors of the present study, investigated the relationship between mode of delivery and the quality parameters of the collected UCB units. Authors of the present study noticed larger blood volume and higher cell count in UCB units collected in cesarean deliveries compared to those obtained in vaginal deliveries. Supported the present study finding, Harris and followers [12], reported that cord blood units obtained from neonates born via cesarean section yielded higher TNC counts than those born by vaginal deliveries. Similarly, Bassiouny et al., [8] reported that the cord blood samples taken from cesarean deliveries were fairly larger than that taken from vaginal deliveries, and attributed such findings to that after cesarean deliveries the placenta is manually delivered faster than in vaginal deliveries, thus decreasing the risk of blood clot formation.

The same finding was given by Mancinelli and colleagues [13], and endorsed such finding to the act of the operating theater team: laying neonates of Cesarean deliveries on their mother’s abdomen; before umbilical cord clamping, which improves blood flow into the umbilical cord and placenta by gravity effect. However, authors of the present study, ascribed lower volume of collected UCB in vaginal delivery to use of oxytocics, which is causing regular uterine contractions with its associated recurrent fall in maternal blood supply, and consequently reduced utero-placental circulation [14].
The present study displayed a non-significant effect of maternal age on cell count of the collected UCB units. This finding supported that of Mohamed et al., [15], found no effect of maternal age on variables of UCB units suitability for stem cell therapy; count of CD34+ cell and TNC. On the other hand, previous studies found correlation between maternal age and cellular content of collected UCB units [3, 16, and 17]. The disagreement between present study finding and other studies finding, may be related to the notion that with advanced maternal age there is an increased risk for medical problems, which were excluded in the present study subjects.

It was marked before, that placental weight in multiparous is greater than that of nulliparous from mid-pregnancy onwards, and subsequently expecting to give higher blood volume and cell count [18]. However, it was surprisingly in the present study to find significant larger blood volume and higher cellular content among primipara equated to multipara. Current study finding, consistent with that obtained by Jan et al., [5]; demonstrated that low parity mothers donated cord blood with higher TNC count. Correspondingly, Keersmaekers et al., 2014 [19], considered parity as a maternal predictive variable. The authors [19] found a significant influence of parity on suitability of UCB units for stem cells extraction; in a large study assessed 7839 UCB units collected in-utero from two Hospitals in Michigan. Furthermore, the present study findings agreed with that of Mohamed et al., [15], demonstrated that parity less than four had a significant positive effect on cellular content of UCB units; in a study conducted on 100 UCB units collected in-utero during Cesarean deliveries at Augusta University Medical Center, USA. Other studies reported that order of birth showed a significant negative association with the cellular content are [20, 21]. Such disagreement between the present study finding and the report of Wallace [18], may be explained by difference in subjects’ criteria.

V. CONCLUSION AND RECOMMENDATIONS

Based on the present study findings, it can be concluded that collecting UCB in-utero was associated with larger blood volume and higher cellular content equated to the ex-utero collection. Such findings incite the following recommendations:

1. As an implication for nurses to be aware of collecting UCB units in-utero rather than ex-utero.
2. Future research addressing other means that may improving UCB yield of stem cells is recommended.

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