



**TED ANKARA COLLEGE  
IB PROGRAM  
BIOLOGY EXTENDED ESSAY**

Utilizing the number of the useful stem cell colonies by investigating the effect of time on stem cells in cord blood.

**TALYA POLAT  
IB NUMBER: 001129-0011  
SUPERVISOR: LEYLA YAĞUŞ**

## **CONTENTS**

<b>ABSTRACT.....</b>	<b>3</b>
<b>INTRODUCTION TO STEM CELLS.....</b>	<b>4</b>
<b>THE UMBILICAL CORD BLOOD.....</b>	<b>4</b>
<b>METHOD.....</b>	<b>5</b>
<b>DATA PROCESSING.....</b>	<b>8</b>
<b>ANOVA TEST.....</b>	<b>10</b>
<b>CONCLUSION.....</b>	<b>13</b>
<b>EVALUATION.....</b>	<b>14</b>

### **Abstract**

Nowadays, cord blood cells have a huge demand in medicine. The reason under this is that it can transform to every cell type. By that, it is possible to cure unhealthy and disordered cells in human body. This is basically called a cell therapy. Cord blood is not only a rich source for hematopoietic stem cells that give rise to different types of blood cells (hematogenic stem cells) but also for mesenchymal cells which produce cell types from other tissues – e.g. nerve, bone, muscle tissue including heart muscle cells. Basically, stem cells are important for lots of treatments.

For understanding the importance of the stem cells, a total of 40 females were recruited with the same age intervals of 20-35 in this experiment. Cord blood cells have been taken with the Cord Blood Collection Procedure and the number of the stem cells have been calculated with the Colony-Forming Unit Assay Procedure. This experiment evaluates the effect of time on the number of stem cell colonies, which have been taken at birth from cord blood cells. Personally, I've always been interested in stem cells because of their magnificent and complicated structure. I wondered, what if we wanted to utilize the number of the useful stem cell colonies, which time interval is the best for them to form as much as colonies they can. Because of that, my research question is **“How does the time affect the number of the useful stem cell colonies in cord blood?”**

## INTRODUCTION

Stem cells are cells that have the potential to replace dead cells with new ones. They are primitive, non-specialized cells with limitless potential to grow. They have capacity to differentiate into specialized cells which form different tissues of the body.\*<sup>1</sup>

The first known source of the stem cells was bone marrow. Stem cells derived from the bone marrow were utilized for the procedure called bone marrow transplantation. Currently the umbilical cord blood stem cells are commonly used for therapeutic purposes. The peripheral blood, skin, fatty tissue, and cord tissue are other sources of stem cells. The peripheral blood stem cells are obtained from the venous blood of the donor by the help of a cell separator. There is extremely less number of stem cells in pharmacologically immobilized peripheral blood. Growth factors of appropriate cell lines should be administered to the donor either by intravenous or subcutaneous route. Thus, obtaining stem cells from peripheral venous blood is expensive and takes a considerable time.

After the delivery of a baby the umbilical cord is clamped and cut. However, there is still some blood left in the blood vessels of the placenta and the part of the umbilical cord attached to it. This placental or umbilical blood is shortly called "cord blood". Collection of cord blood is the only noninvasive technique of obtaining stem cells. Compared to getting stem cells from bone marrow or peripheral blood, it is supposed to be less complicated. Obtaining hematopoietic stem cells from the bone marrow is only possible through an incision of the iliac crest. This procedure is done under local or general anesthesia. The collected specimen is routinely purified in a special cell separator. That's why the cord blood stem cells are used in this experiment. The umbilical cord blood that constitutes a rich source for stem cells is the blood that remains in the umbilical cord and the placenta after the delivery of the baby. Until recently the umbilical cord blood was considered "waste" and disposed together with placenta and the cord. Now, it is collected to obtain the stem cells for storage and later use for therapeutic purposes. The umbilical cord blood is a rich source for hematopoietic stem cells, similar to bone marrow cells that can easily be transplanted to treat haematopoietic and immune system disorders. Moreover these cells have been recognized as more effective in comparison to stem cells obtained from adult donors. It has been suggested that stem cells, originating from umbilical cord blood, have a huge capacity to proliferate and multiply after transplantation. This important property is what lies behind autologous transplantations (the donor is the recipient of the collected stem cells) as well as allogeneic transplants (transplantation between different individuals e.g. family members).

Hematopoietic stem cells that give rise to different types of blood cells (hematogenic stem cells) and also mesenchymal cells which produce cell types from other tissues like bone, nerve, muscle tissue such as heart muscle cells can be collected from cord blood. Treatment of the diseases in the following list requires the use of hematopoietic stem cells. The list has been compiled on the basis of the guidelines specified by the organization in charge of stem cell transplantation in Europe – EBMT\*<sup>2</sup>: Acute leukemias, Chronic leukemias, Myelodysplastic syndrome, Diseases caused by stem cell defect, Myeloproliferative syndromes, Hyperplastic disorders of lymphatic system, Phagocytic disorders, Disorders caused by the absence or malfunctioning of enzymes, Histiocytic disorders, Inherited red blood cell abnormalities, Inherited immune system disorders, Hereditary thrombotic

---

<sup>1</sup><http://stemcells.nih.gov/info/basics/pages/basics1.aspx>

<sup>2</sup>[http://www.stemcellresearchnews.net/Diseases\\_Treated.aspx](http://www.stemcellresearchnews.net/Diseases_Treated.aspx)

disorders, Plasma cell disorders.

In 1988, the first successful and documented transplantation of the family cord blood was done in Paris by the American doctors Hal Broxmeyer and Joanne Kurtzberg headed by Professor E. Gluckman<sup>3</sup>. Newborn sister's cord blood was transplanted to a young American male US diagnosed with an inherited Fanconi anemia .

Cord blood stem cells transplantation is a natural result of bone marrow transplantation. Because of numerous advantages of cord blood cells compared to bone marrow, the former type of cells are more frequently used for life saving treatments. Cord blood, like in the case of bone marrow transplantation, can be used for both autologous (cells from one's own cord blood) or allogeneic transplantations (to different individual such as siblings). Among siblings cord blood transplantations are done much more frequently compared to autologous transplants. However, approximately 60% of bone marrow transplants are autologous transplantations. Considering the importance of stem cells, it is critical to conserve them in stable conditions during the time until the stem cells reach the laboratory. That leads to the research question; "How does the time affect the number of useful stem cell colonies in 1 ml of the cord blood which have been taken from the females aged between 20 and 35 years?" As the time required for the cord blood taken at birth to reach the laboratory increases, a reduction in the number of stem cells occurs. That's why more stem cell colonies are formed in the cord blood that are delivered to the laboratory in a short period of time and we will have less colonies in blood that are delivered in a longer period of time. So the independent variable will be both the number of the useful stem cell colonies in cord blood and the duration of time until the cord blood has reached to the laboratory. Because number of the stem cell colonies depends on the arrival time as stated.

## **METHOD**

Since my aim is to utilize the number of the useful stem cells, at the beginning of the experiment, cord blood was collected from 40 females with the age intervals of 20-35 during birth (Picture I). The collection of stem cells made in Ankalife Kadın Sağlığı ve Tüp Bebek Merkezi after getting permission from the laboratory. Procedure consists of the insertion of a needle into the umbilical vein (following the umbilical severance) and blood collection from umbilical cord and placenta. Procedure was same both vaginal or a cesarean section births. Cord blood was taken with the cord blood collection bag (Picture II). Procedure of cord blood collection generally performed by a midwife or gynecologist after umbilical cord separation during a birth (Picture I). It poses no risk either to mother or the baby. The blood sample was transported into a special collecting kit (picture III-IV).

Under 36 weeks premature births or patients with chronic diseases such as hypertension, heart diseases and diabetes were excluded from the study because these kinds of illnesses can affect the number of the stem cells in the cord blood. However, the aim of the experiment was to determine how time affects the stem cells. Therefore except time, every single variable kept constant.

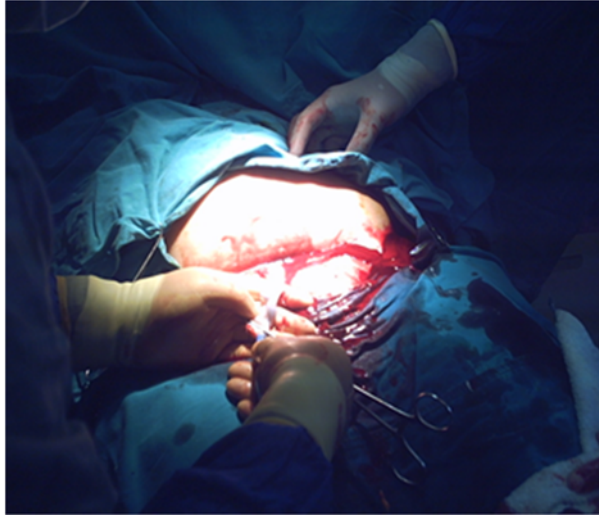
In transportation of cord bloods, they kept in unique boxes which maintain the temperature at 20°C-25°C. Transporting blood samples isn't necessary to kept constant with cooler ice molds or cooling batteries. It is enough to protect the kit from the sun and hot places. (Picture III-IV) Blood samples taken from delivery room, were reached to the laboratory.

---

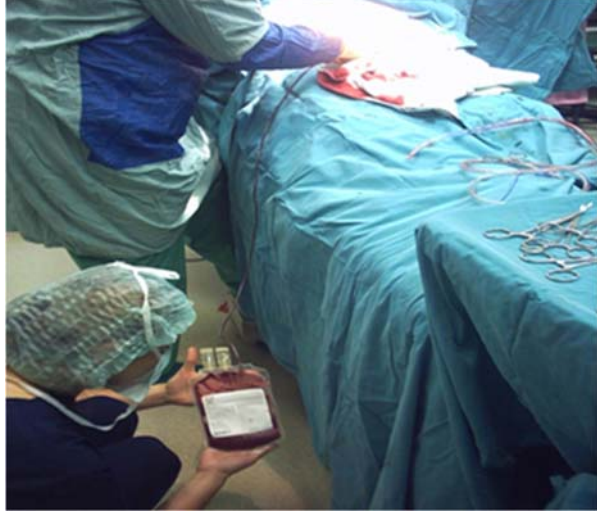
<sup>3</sup><http://unidirectory.auckland.ac.nz/profile/pd-gluckman>

Each blood examined with the same person, same procedure and the same laboratory. The laboratory was at the standard conditions which is called “Standard Temperature and Pressure” or STP. Pressure was 1 bar and temperature was 25°C (298.15K). Temperature kept constant with a thermometer and air pressure kept constant with a barometer.

Picture I



Picture II



Picture III



Picture IV

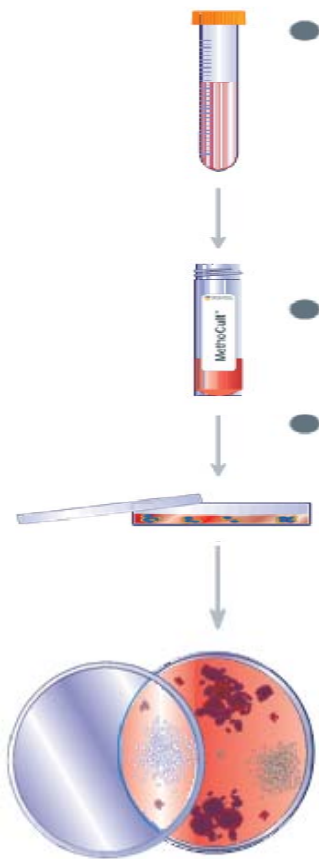
The exact birth time and the delivery time was recorded. The difference between those times was taken as variable for our experiment. The efficiency of the blood defined as the maximum number of the cells which can form colonies in a 50 mm petri dishes (Falcon™, BD Biosciences, USA). A culture medium could be liquid or gel designed to support the growth of microorganisms or cells. MethoCult™ (STEMCELL Technologies, Vancouver, CA) was used as a medium in the study. This was the best method for collection of stem cells. Another method for the collection of stem cells was the Peripheral Blood Stem Cell Harvest but it has some side effects during the collection of stem cells like there would be a decrease in the platelet count.

On the other hand, also cell number and cell viability could be determined as a functionality of blood samples but, because of the erythrocytes, thrombocytes, white blood

cells within the blood, they would not give detailed information about stem cells productivity.

For the colony culturing, 1 ml blood added in a sterile 5 ml tube (Falcon™), with 3 ml of MethoCult™ medium and stirred, then the mixture spreaded to a petri dish (Falcon™). (Picture V) Petri dishes were incubated for 14 – 16 days, in humidified incubator (Elektro-mag, Turkey) at 37°C and 5% CO<sub>2</sub> (picture VI). After the growth of the Granulocyte-Machrophage (CFU-GM) colonies, their numbers calculated under a microscope (Olympus, Japan) with a 10x40 zoom (Picture VII, VIII, IX). Erythropoid colonies were not be on the count.

Picture V



Picture VI



Picture VII



Picture VIII



CFU-GM

Picture IX



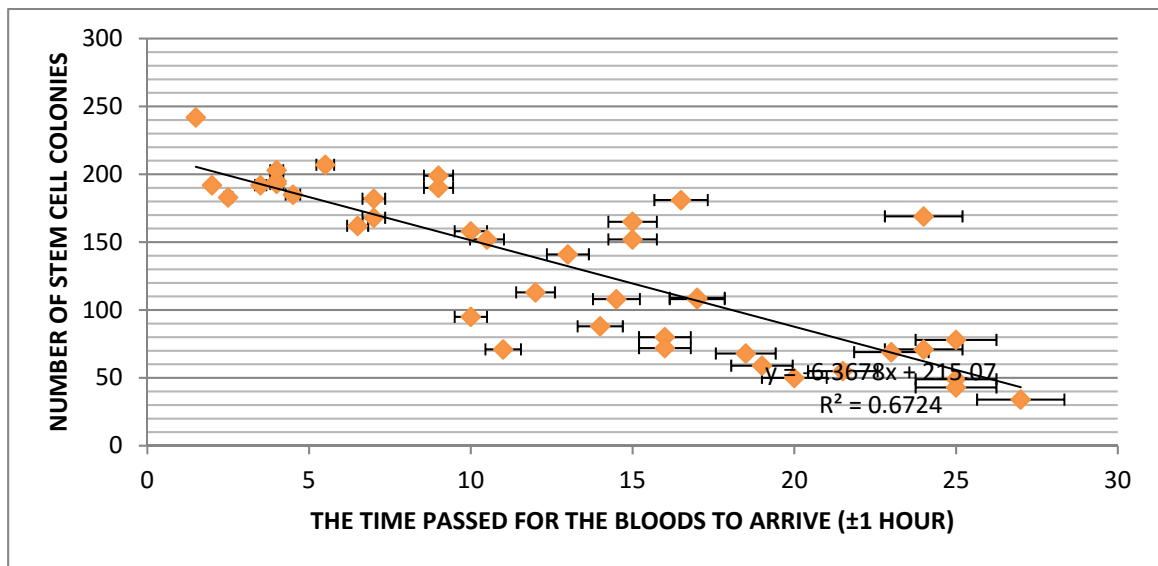
CFU-GM

## DATA PROCESSING

	THE TIME PASSED FOR ARRIVAL ( $\pm 1$ HOUR)	NUMBER OF STEM CELL COLONIES
PATIENT 1	2	183
PATIENT 2	7	168
PATIENT 3	5	207
PATIENT 4	10	95
PATIENT 5	2	242
PATIENT 6	27	34
PATIENT 7	12	113
PATIENT 8	14	88
PATIENT 9	6	162
PATIENT 10	10	152
PATIENT 11	9	190
PATIENT 12	7	182
PATIENT 13	10	158
PATIENT 14	15	152
PATIENT 15	3	192
PATIENT 16	2	192
PATIENT 17	11	71
PATIENT 18	25	49
PATIENT 19	4	193
PATIENT 20	9	199
PATIENT 21	17	109
PATIENT 22	18	68
PATIENT 23	16	80
PATIENT 24	25	43
PATIENT 25	4	185
PATIENT 26	13	141
PATIENT 27	16	181
PATIENT 28	20	50
PATIENT 29	23	69
PATIENT 30	14	108
PATIENT 31	4	195
PATIENT 32	16	72
PATIENT 33	19	59
PATIENT 34	15	165
PATIENT 35	25	78
PATIENT 36	4	203
PATIENT 37	17	108
PATIENT 38	24	169
PATIENT 39	21	55
PATIENT 40	24	71



**TABLE 1:** Raw data table of the number of stem cell colonies and the passing time until they reach to the laboratory. All these 40 female patients were conducted with the same age intervals of 20-35.



**GRAPH 1:** The correlation graph of the number of the stem cells versus the time passed for the bloods to arrive with respect to the raw data table in each patient (Table 1) with the tangent,  $y = -6,3678x + 215,07$ .

### Sample Calculations

**Mean:** For 1 ml of the cord blood, all values both time and the number of stem cell colonies will be summed up and then will be divided into the total number of patients. The mean estimated as 13 hour and 130 stem cell colonies in each patient.

**Standard Deviation:** For 1 ml of the cord blood, calculating Standard Deviation of the time and the number of stem cell colonies, the average value is calculated, variance (the average value of the squared differences from the mean) is worked out and finally, square root of variance is calculated which is the Standard Deviation.

Formula of Standard Deviation is: 
$$s = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x - \bar{x})^2}$$

Since the average time is 13, their differences from the average will be calculated in each patient.

Sum of squared values of differences from the mean will give us the variance which is;

$$\text{Variance} = s^2 = 57.3553 = 57.3$$

Standard Deviation =  $s = 7.573333 = 7.5$  for the duration of time until bloods arrived to the laboratory.

	THE TIME PASSED FOR ARRIVAL ( $\pm 1$ HOUR)	NUMBER OF STEM CELL COLONIES
Mean	13	130
Mod	13.5	146.5
Standard Deviation	7.5	58.7
Maximum	27	242
Minimum	2	43

**TABLE 2:** The data table of the average, mod, standard deviations, maximum and minimum values for the time passed for arrival and number of stem cell colonies with respect to Table 1.

For other statistical values, the one-way analysis of variance (ANOVA) is used to determine whether there are any significant differences between the means of two or more independent groups. ANOVA is beneficial to understand whether number of stem cell colonies differed based on the time passed for arrival time amongst patients, dividing patients into five independent groups. Also, it is important to realize that the one-way ANOVA is an omnibus test statistic and cannot tell you which specific groups were significantly different from each other; it only tells that at least two groups were different. Since there are three, four, five or more groups in study design, determining which of these groups differ from each other is important.

To determine a precise and accurate pattern between the arrival time of the blood and the stem cell colonies, it was beneficial to create 5 different time intervals to make ANOVA test between groups which are the bloods that arrived between less than 5 hours, between 5-10 hours, 10-15 hours, 15-20 hours and more than 20 hours.

		NUMBER OF THE STEM CELL COLONIES							
THE TIME PASSED FOR ARRIVAL ( $\pm 1$ hour)	$\leq 5$	183	242	192	192	193	185	195	203
	5-10	168	207	95	162	190	182	158	199
	10-15	113	88	152	152	141	108	165	71
	15-20	109	68	181	50	72	59	108	80
	$\geq 20$	37	49	43	69	78	169	55	71

**Table 3:** Data table with 5 different groups containing 8 patients which are the bloods of the patients that has arrived between less than 5 hours, between 5-10 hours, 10-15 hours, 15-20 hours and more than 20 hours.

NUMBER OF STEM CELL COLONIES	THE TIME PASSED FOR BLOODS TO ARRIVE ( $\pm 1$ HOUR)														
	$\leq 5$			5-10			10-15			15-20			$\geq 20$		
		x-mean	(x-mean) <sup>2</sup>		x-mean	(x-mean) <sup>2</sup>		x-mean	(x-mean) <sup>2</sup>		x-mean	(x-mean) <sup>2</sup>		x-mean	(x-mean) <sup>2</sup>
183	-15	225	168	-2	4	113	-10	100	109	19	361	37	-34	1156	
242	44	1936	207	37	1369	88	-35	1225	68	-22	484	49	-22	484	
192	-6	36	95	-75	5625	152	29	841	181	91	8281	43	-28	784	
192	-6	36	162	-8	64	152	29	841	50	-40	1600	69	-2	4	
193	-5	25	190	20	400	141	17	289	72	-18	324	78	7	49	
185	-13	169	182	12	144	108	-15	225	59	-31	961	169	98	9604	
195	-3	9	158	-12	144	165	42	1764	108	18	324	55	-16	256	
203	5	25	199	29	841	71	-52	2704	80	-10	100	71	0	0	
SUM	1585	2461	1361		8591	990		7989	727		12435	571		12337	
MEAN	198		170			123			90			71			

**TABLE 4:** The data table for the time passed for the bloods to arrive and number of stem cell colonies between 5 groups with their difference from means and squares of the difference from means to calculate sum of squares within groups (SSW) for the one way ANOVA test.

For Anova test, squares of the difference between mean and number of stem cells will be added which is known as squares within groups (SSW).

$$2,461 + 8,591 + 7,989 + 12,435 + 12,337 = 43,813 \text{ (SSW)}$$

NUMBER OF STEM CELL COLONIES	(x-mean)	(x-mean) <sup>2</sup>
183	53	2809
242	112	12544
192	62	3844
192	62	3844
193	63	3969
185	55	3025
195	65	4225
203	73	5329
168	38	1444
207	77	5929
95	-35	1225
162	32	1024
190	60	3600
182	52	2704
158	28	784
199	69	4761
113	-17	289
88	-42	1764
152	22	484
152	22	484
141	11	121
108	-22	484
165	35	1225
71	-59	3481
109	-21	441
68	-62	3844
181	51	2601
50	-80	6400
72	-58	3364
59	-71	5041
108	-22	484
80	-50	2500
37	-93	8649
49	-81	6561
43	-87	7569
69	-61	3721
78	-52	2704
169	39	1521
55	-75	5625
71	-59	3481

**TABLE 5:** The data table of the difference between mean and number of stem cell colonies and their squares for calculation of the SST known as total sum of squares for Anova test.

As the difference between number of stem cell colonies and mean value which is 130 in each patient was found, the square values summed up to estimate the total sum of squares (SST) .

**Total sum of squares (SST): 133,898**

For the analysis of variance, sum of squares between groups was determined with the difference between sum of squares (SST) and sum of squares within groups (SSW)

$$\text{Sum of squares (SST)} = \text{Sum of squares between groups} - \text{Sum of squares within groups (SSW)}$$

$$133,898 = \text{Sum of squares between groups} - 43,813$$

**So the sum of squares between groups is 90,085 (SS)**

$$\frac{\text{Sum of squares between groups}}{\text{Number of groups} - 1} = \frac{90,085}{4} = 22,521.25 \text{ (MS without error)}$$

SOURCE	SS	df	MS	F	P
TREATMENT BETWEEN GROUPS	90,085 (SS)	4	22,521.25	17.97	<.0.001
ERROR	43,813 (SSW)	35	1,252.45	10.8 (F <sub>crit</sub> )	
TOTAL	133,898 (SST)	39	23,773.7	17.97	<.0.001

**TABLE 6:** The ANOVA test results with their treatment between groups, errors and total values.

## CONCLUSION

Until now, more than 70 different diseases have been treated by the use of treatment cord blood. The most frequent and important of these disease categories has been Leukemia. Inherited diseases of red blood cells, the immune system and certain metabolic disorders are the next largest group. Cord blood transplantation has been successfully performed for the treatment of lymphoma, myelodysplasia and severe aplastic anemia. This procedure has numerous advantages to donors and transplant recipients. It is easy to obtain and generally more likely to provide a suitable match. Moreover cord blood can be stored frozen and it is ready to use. Thus we preferred to use cord blood stem cells for this investigation.<sup>\*4</sup> Another advantage of using cord blood stem cells is that cord blood is also less likely to transmit viruses, including Epstein-Barr virus (EBV) and cytomegalovirus (CMV). These viral infections are potentially lethal for transplant patients. Only less than 1 percent of babies are born with CMV while nearly 50 percent of the adults in USA carry it as a latent virus .

For these reasons, it easy to understand the importance of the stem cells. Because of that, the aim of the experiment is to utilize the number of the useful stem cell colonies. So the research question was “How does the time affect the number of useful stem cell colonies in 1 ml of the cord blood which have been taken from the age intervals of 20-35 females?” This experiment estimated the number of the stem cell colonies affected by different arrival times to laboratory. A total of 40 females were conducted with the same age intervals of 20-35. Their cord blood cells have taken with the Cord Blood Collection Procedure and the number of functional stem cells calculated with the Colony-Forming Unit Assay Procedure. The hypothesis supported that as the time increases until the cord blood reaches a laboratory, the number of the stem cells could decrease. The hypothesis stated that due to the natural selection of the stem cells. It is the survival of the fittest and as the metabolic wastes increase, there will be more competition between stem cells and that leads a reduction of stem cells.

<sup>4</sup><http://www.cordblood.com/benefits-cord-blood>

On the Graph 1, it's possible to determine that as the time increase, the number of the stem cell colonies decrease. They have a strongly negative correlation with each other. Since there are two independent variables such as mean, standard deviation, minimum and maximum values determined separately (Table 2). For the duration of time it is estimated that the mean is 13, standard deviation is 7.5 and the minimum and maximum values are 2 and 27 respectively. On the other hand, for the number of stem cell colonies, mean calculated as 130, standard deviation as 58.7 and the minimum and maximum values for the colonies as 43 and 242 respectively.

To estimate a statistical value, it was the best way to use the ANOVA (the one-way analysis of variance) test to demonstrate a pattern between the number of the stem cell colonies and the time taken for blood to reach to the laboratory. ANOVA is used to estimate a mathematical value whether two independent groups are different from each other or not. By separating patients into different time intervals, it was possible to make a comment on whether which time interval is the most efficient one for the formation of the colonies. All patients divided into five groups which are bloods arrived to the laboratory less than 5 hours, between 5-10, 10-15, 15-20 and more than 20 hours (Table 3). It was the most logical way to demonstrate five different groups because the range for the arrival time was 25. Each group consists of 8 patients.

At the end of the ANOVA test, F ratio and F critical value will be obtained. For the calculation of the F ratio, sum and mean of the groups should be found. For example, for the first group, which is the bloods that have arrived less than 5 hours, the mean of the colonies calculated as 198 and the sum of the colonies calculated as 1,585 (Table 4). After calculating all the means and the sums of the five groups, each x value were subtracted from the mean of the belonging group, then the final values were squared. All squared values were summed up to obtain the squares within groups known as (SSW). The squares within groups calculated as 43,813. Then for the total sum of squares, the mean of all x values, which is 130, subtracted from each patient's number of the stem cells colonies. After that, all obtained values squared and summed. The final data will be the total sum of squares (SST), which is 133,898. Sum of squares between groups was determined with the difference between sum of squares (SST) and sum of squares within groups (SSW) for the analysis of variance. So the sum of squares between groups is 90,085. After obtaining the sum of squares between groups, it was divided by one less than the total number of groups which is 4 and at the end of the calculation, with the error propagation, 23,773.7 was obtained which is known as MS. F and P value\*<sup>5</sup> calculated with respect to Table 6 as 17.97 and 0.001. The error propagation was made with the ANOVA test in the Table 6.

The maintained p-value, helps to determine the significance of results\*<sup>6</sup>. A small p-value ( $\leq 0.05$ ) indicates strong evidence and a large p-value ( $> 0.05$ ) indicates weak evidence. Since the p-value is 0.001 in our experiment, it means the experiment is statistically and strongly significant. The p-value is determined from the F ratio and the two values for degrees of freedom shown in ANOVA table (Table 3). The ratio of two mean square values is called the F ratio\*<sup>7</sup>. The critical value is the number that the test statistics must exceed to reject the test. In this

---

<sup>5</sup> <http://vassarstats.net/anova1u.html>

<sup>6</sup> <http://blog.minitab.com/blog/adventures-in-statistics/how-to-correctly-interpret-p-values>

<sup>7</sup> [http://graphpad.com/guides/prism/6/statistics/index.htm?f\\_ratio\\_and\\_anova\\_table\(one-way\\_anova\).htm](http://graphpad.com/guides/prism/6/statistics/index.htm?f_ratio_and_anova_table(one-way_anova).htm)

experiment,  $F_{crit}$  is 10.8 and less than the F ratio which is 17.97, the results are significant at the %5 significance level. There is strong evidence that the 5 groups differ. As a result, each group differs from each other statistically and that means the hypothesis is considerable.

It has been made some tests for understanding the importance and the efficiency of stem cells for medicine. The most common test for estimating the number of stem cells is Viability Test and Colony Forming Unit Test (CFU). In standard temperature and pressure, those stem cells can reproduce themselves when they needed and later they form structures called colonies. That's why in a sample that has taken from cord blood, if there are more stem cells that means more colony formation can be observed. Stem cell colonies are easy to count under a microscope so there won't be any errors while counting those colonies. However, for the duration of time, it is estimated that there is only one hour uncertainty because of the delays in traffic while bloods are arriving to the laboratory.

Standard plastic blood bag contains Acyte Cytrate Dextrose (ACD)<sup>8\*</sup>. This liquid prevents coagulation and feeds cells. It is a solution of citric acid, sodium citrate and dextrose in water. It is mainly used as an anticoagulant to preserve blood specimens required for tissue typing, it is also used during procedures such as plasmapheresis instead of heparin. As a hypothesis, as the duration of time increases while transporting cord blood in a standard plastic blood bag, the nutrients in the bag decrease. On the other hand, metabolic wastes increase and because of those reasons, the rate of cell death increases. In our experiment Acyte Cytrate Dextrose (ACD) was used in the standard plastic blood bags to keep the stem cells alive. For other experiments, other nutrients and preventions can be used in blood bags. After using different liquid preventions, the best medium can be found for bloods to be more efficient. Also the experiment only evaluated 40 patients. It wasn't enough to make an accurate statistical value. However, since there is less data, it's a fact that as the duration of time increases, number of the stem cells decrease. Another limitation for this experiment was to work with different patients. Since every individual is different, it was impossible to maintain every single thing that they have done in the past. For example, each patient has gone through different psychological events, has different anxiety and stress levels. Those factors may effect the production of the stem cells. On the other hand, this experiment can be made with the patients that are between the ages of 18 and 20. Because, especially in Turkey, births in early ages are more common and fertile. By decreasing the range of age might have different outcomes.

As a result of this investigation, cord blood cells should delivered in a short period of time (It should be less than the average which is 13). The result of this experiment was expected because of the natural selection. As the time passes, the chance for stem cells in the blood bags to live decreases constantly and this refers to every cell type. Only by that, there is more chance for stem cells to live and reproduce themselves. The Parent's Guide to Cord Blood Foundation<sup>\*9</sup> recommends shipping with a courier that has a division specializing in transportation of the cord bloods. This insures the bloods and helps critical shipment that is not misplaced, arrives promptly, and is maintained within the acceptable temperature range during transport from the hospital to the lab. The first priority for parents is to consider the cord blood shipping time: Once the cord blood is harvested, the blood cells and stem cells gradually begin to die. Public cord blood banks set a limit of 48 hours on the time between


---

<sup>8</sup> <http://www.usp.org/>

<sup>9</sup> <http://parentsguidecordblood.org/en/faqs/why-is-it-important-to-ship-cord-blood-with-a-special-courier>

birth and processing the blood for cryogenic storage. So this experiment showed that it would be a "best practice" if family banks also followed the 48 hour window.

## APPENDIX



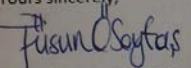
YAŞAM BANKASI  
Kordon Kanı Bankası  
FamiCord Group

To whom it may concern,

Yaşam Bankası is the first Family Cord Blood Bank in Turkey. It has been established in 2002 and in September 2013 acquired by the second biggest Cord Blood Bank in Europe – FamiCord Group. It is specialized in collection, processing, testing, cryopreservation and storage of Cord Blood Units (CBUs). CBUs are stored long-term to be used, if needed, as a stem cell source for transplantation in the treatment of specific diseases.

Talya Polat has done an internship study at Yaşam Bankası Cord Blood Bank from 15<sup>th</sup> September till 15<sup>th</sup> October of 2015. She has mainly worked on Colony Forming Unit (CFU) Assay that detects and enumerates hematopoietic progenitor cells in a CBU. After cord blood is processed to separate total nucleated cells including stem cells, it is important to analyze for cell number, viability and functionality. CFU assay proves us that the existing cells are alive and functional.

Her results from the assays were fruitful for her internship and for our laboratory quality control assurance as well.

Yours sincerely,  
  
Füsün ÖNER SOYTAŞ, M.Sc., MBA  
CEO and General Manager



