

Research Article

Food Supplement 20070721-GX May Increase CD34⁺ Stem Cells and Telomerase Activity

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Few rejuvenation and antiaging markers are used to evaluate food supplements. We measured three markers in peripheral blood to evaluate the antiaging effects of a food supplement containing placental extract. Samples were evaluated for CD34⁺ cells, insulin-like growth factor 1 (IGF1), and telomerase activity, which are all markers related to aging. To control the quality of this food supplement, five active components were monitored. In total, we examined 44 individuals who took the food supplement from 1.2 months to 23 months; the average number of CD34⁺ cells was almost 6-fold higher in the experimental group compared with the control group. Food supplement intake did not change serum IGF1 levels significantly. Finally, the average telomerase activity was 30% higher in the subjects taking this food supplement. In summary, our results suggest that the placental extract in the food supplement might contribute to rejuvenation and antiaging.

1. Introduction

Human placenta, also called *zihche* in Chinese, has been used as a medicine in Korea and China for over 1,400 years due to its anti-aging and cosmetic properties [1]. In traditional oriental medicine, human placental extract has been used for wound healing. More recently, human placental extract was approved for treatment of abnormal liver function and menopausal symptoms and has no known toxic effects [2–4]. In both rodent and human cells in vitro, placental extract induces significant nitric oxide production, which is an important cellular mediator of tissue repair [5]. Placental extract contains several enzyme inhibitors, anti-coagulant proteins, and antioxidants [6–8]. Furthermore,

placental extract is a rich source of bioactive molecules such as hormones, proteins, lipids, nucleic acids, glycosaminoglycan, amino acids, vitamins, and minerals [2, 9]. It also contains many uncharacterized compounds believed to have various bioactivities involved in inhibition or delay of aging, inflammation, sunburn, mutagenicity, anaphylaxis, and oxidation [1].

Similar to human placenta, pig placenta has been used as a source of biomedical material. In fact, freeze-dried pig placenta contains equal or, in certain circumstances, higher nutritive properties than human placenta [10].

Hematopoietic stem cells (HSCs) represent a long-lived cell population that provides blood cells through hematopoiesis throughout the human lifespan. These cells are

identified by the surface marker CD34⁺. Hematopoiesis is regulated by a balance between self-renewal, proliferation, and differentiation. The capacity of HSCs to maintain their population through self-renewal replications throughout the aging process may imply that the HSC population itself does not age. However, diminishing proliferative capacity after serial transplantation [11], repeated exposure to irradiation [12], or age-related loss of telomeric DNA [13, 14] indicates that the life of HSCs may be limited. In humans, CD34⁺ stem cells represent about 0.01% to 0.03% all cells in peripheral blood [15]. Regulation of hematopoiesis is altered during aging, impairing the ability of older people to respond appropriately to the physiological demand for blood cell replacement triggered by stimuli such as blood loss or cytoreductive chemotherapy [16]. In the peripheral blood and bone marrow, both the quantity and the clonogenic capacity of HSCs decline with age [15, 17, 18]. Furthermore, there are age-dependent differences in lineage maturation and cell-cycle phases [19], as well as an accumulation of genomic mutations and an imbalance in cytokine production [16]. Thus, HSCs are a key determinant in aging.

It is well established that a reduced concentration of insulin-like growth factor 1 (IGF1) plays a role in extending lifespan in invertebrates [20]. In mammalian evolution, IGF1 pathways have diverged from a single receptor to multiple receptors and even more complicated pathways and regulatory networks [21]. Insulin, IGF1, and growth hormone regulate metabolic pathways to control growth and differentiation [22]. This process depends mainly on the concentration of circulating IGF1. In mice, knocking out the insulin receptor (IR) pathway reduces lifespan and increases age-related diseases [23]. In contrast, both spontaneous and targeted genetic disruptions of the GH/IGF pathway are associated with small size (dwarfism), numerous indices of delayed aging, enhanced stress resistance, and a major increase in lifespan in mice [24]. These results suggest a role for the GH/IGF pathway in murine longevity. Furthermore, caloric restriction, well known to extend lifespan and slow down age-related pathology, is also associated with a reduced concentration of circulating IGF1 in nematodes, fruit flies, and mice [25]. On the other hand, reducing the concentration of IGF1 in humans, although protective against cancer, decreases the risk of cardiovascular disease and diabetes [26]. Moreover, human aging is associated with a decline in GH and IGF1 levels. It has been proposed that GH therapy may reverse some of the physiological features of aging [27].

Telomerase is pertinent to a cell's potential for self-renewal and is required for long-term cellular proliferation and survival [28, 29]. Because human cells progressively lose telomerase activity, telomeric DNA is lost during successive rounds of DNA replication. Shortening of telomeres has been reported to correlate with cellular senescence [30, 31]. This indicates that replicating cells that lose the telomerase needed to maintain telomeres will eventually senesce [32].

The aim of the present study was to evaluate the rejuvenation and anti-aging effects of placental extract on healthy individuals by determining CD34⁺ cell counts, serum IGF1 levels, and monocyte telomerase activity in peripheral blood.

2. Materials and Methods

2.1. Source of 20070721-GX. The ingredients of 20070721-GX are pig placental extract, royal jelly, avocado oil, and wheat germ oil. 20070721-GX is a proprietary formulation available from Gwo Xi Stem Cell Applied Technology, Inc. To control the quality of each batch of the food supplement of 20070721-GX, the placental extract component is measured by ELISA. The evaluation includes concentrations of progesterone (Pgt), estriol (E3), estradiol (E4), human chorionic gonadotropin (HCG), human placental lactogen (hPL), and total protein (tPrt). For each batch, the concentrations are 10 ± 5 mg/mL tPrt, 30 ± 5 ng/mL Pgt, 10 ± 5 ng/mL E3, 10 ± 5 ng/mL E4, 25 ± 5 mIU/mL HCG, and 0.15 ± 0.1 mg/L hPL. Batches that fit these six indicators are chosen into chemistry, manufacturing, and controls (CMCs) manufacturing for standard operation and SGS examination.

2.2. Volunteers. This is a double-blind placebocontrolled trial. This research project was approved by the Institutional Review Board of China Medical University (DMR99-IRB-32). Forty-four subjects were recruited from China Medical University Hospital; 22 people have the product treatment (20070721-GX group), and the other 22 people were placebo treatment (Control group). The control group was 54.5% male, and the 20070721-GX group was 50% male. The mean age was 43 ± 16 years in the control group and 46 ± 9 years in the 20070721-GX group. Basic characteristics of individual subjects are listed in Tables 1 and 2.

2.3. Sample Collection. Individuals in the experimental group took three capsules (20070721-GX or placebo) daily for months. Peripheral blood samples from control and 20070721-GX subjects were drawn into BD Vacutainer CPT Tubes (Becton, Dickinson and Co., Franklin Lakes, NJ). Within 2 h of blood collection, blood samples were centrifuged at room temperature (18–25°C) in a horizontal rotor for 20 min at $1500 \times g$. After centrifugation, approximately half of the plasma was aspirated without disturbing the whitish cell layer consisting of mononuclear cells and platelets. The cell layer was collected with a Pasteur pipette and transferred to a microcentrifuge tube.

2.4. Flow Cytometry of CD34⁺ Hematopoietic Stem and Progenitor Cells. Hematological Cell Counts. The total number of CD34⁺ cells from peripheral blood samples was measured by flow cytometry (Trucount, BD). For the two-platform method, results were expressed as % CD34⁺ cells, and the absolute number of CD34⁺ cells per microliter was calculated as % CD34⁺ cells \times white blood cells $\times 10^3 \mu\text{L}$. For the one-platform method, the number of CD34⁺ cells per microliter was calculated as $\text{CD34}^+ \text{ cells}/\mu\text{L} = (\text{number of CD34}^+ \text{ cells} \times \text{bead count per test} \times \text{dilution factor})/\text{number of beads collected}$.

2.5. Sample Acquisition. Cells were acquired on a three-color FACSCalibur flow cytometer (BD) equipped with a 488-nm argon laser and analyzed with CellQuest 3.1 software. The

TABLE 1: Demographics, CD34⁺ Cells, telomerase activity, and IGF1 concentration in control subjects.

Sample No.	Age (y)	Gender	Duration (m)	CD34 (%)	Telomerase (U)	IGF-1 (ng/mL)
<i>Control</i>						
1	19	m	0	0.10	0.14	138.04
2	20	f	0	0.10	0.08	150.61
3	20	f	0	0.20	0.08	128.54
4	21	m	0	0.20	0.10	78.95
5	21	f	0	0.20	0.08	145.85
6	22	m	0	0.30	0.08	71.82
7	32	f	0	0.70	0.15	133.39
8	39	f	0	0.50	0.09	129.83
9	41	m	0	0.10	0.13	165.89
10	46	m	0	0.20	0.13	77.93
11	47	f	0	0.30	0.15	114.37
12	48	m	0	0.10	0.10	60.95
13	53	m	0	0.20	0.08	141.97
14	53	m	0	0.30	0.08	72.16
15	54	f	0	0.20	0.11	95.00
16	56	f	0	0.30	0.10	104.71
17	56	m	0	0.05	0.11	141.96
18	57	f	0	0.10	0.09	153.90
19	58	m	0	0.50	0.12	109.15
20	60	m	0	0.50	0.08	109.91
21	64	m	0	0.15	0.09	110.00
22	64	f	0	0.05	0.08	108.00
Average	43	54.5%[#]		0.24	0.10	115.59
SD	15.8			0.17	0.02	29.37

[#]The ratio of male.

instrument was aligned and calibrated daily using a three-color mixture of CaliBRITE beads (BD) with FACSComp software (BD). To comply with statistical requirements, 50,000 events were acquired for each sample in the Milan/Mulhouse protocol [33]. For international society of hematology and graft engineering (ISHAGE) (single and two platform), a total of 100,000 leukocytes (G1) were collected; for single-platform ISHAGE, 2,000 bead events were collected.

2.6. Enzyme-Linked Immunosorbent Assay (ELISA) for IGF1. Human IGF1 was determined by ELISA kit (Quantikine, Minneapolis, MN, USA). Briefly, the assay utilized mouse anti-human IGF1 for immobilization on the microtiter wells and goat anti-human IGF1 along with streptavidin conjugated to horseradish peroxidase for detection. The human serum sample was allowed to react simultaneously with the two antibodies, thus sandwiching the IGF1 molecules between the solid-phase and enzyme-linked antibodies. After incubation, the wells were washed to remove unbound, labeled antibodies. The horseradish peroxidase substrate 3,3',5,5'-tetramethylbenzidine (TMB) was added for development of a blue color. This was stopped with the addition of Stop Solution, changing the color to yellow. Absorbance was measured spectrophotometrically at 450 nm (Bio-Tek Instruments, Winooski, VT). The concentration of IGF1 was directly proportional to the color intensity of the test sample.

2.7. Analysis of Telomerase Activity (TRAP Assay). Analysis of telomerase activity was assessed using the telomeric repeat amplification protocol (TRAP) assay [34] according to the manufacturer's protocol (Roche, Pleasanton, CA, USA). The TRAP assay was performed on 30 μ g protein per sample extract. Thirty cycles of PCR were performed at 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s. Sample absorbance was measured at 450 nm using a PowerWave \times Microplate ELISA Reader (Bio-Tek Instruments).

2.8. Statistical Analysis. Univariable, multivariable, and stepwise regression analyses with two-sided *t* tests were used to evaluate the effect of 20070721-GX, gender, and age on changes in number of CD34⁺ progenitor cells, IGF1 concentration, and telomerase activity; *P* < 0.05 was considered statistically significant.

3. Results

3.1. 20070721-GX-Induced Increase in Progenitor CD34⁺ Cells. Circulating cells were analyzed according to cell-surface markers, with progenitor cells defined as CD34⁺. The number of CD34⁺ progenitor cells differed significantly between the control and 20070721-GX groups (Figure 1(a), Table 3, *P* < 0.001). The average level of CD34⁺ progenitor cells was about 6-fold higher in the 20070721-GX group compared with the control group (Tables 1 and 2). More than

TABLE 2: Demographics, CD34⁺ Cells, telomerase activity, and IGF1 concentration in 20070721-GX subjects.

Sample No.	Age (y)	Gender	Duration (m)	CD34 (%)	Telomerase (U)	IGF-1 (ng/mL)
<i>20070721-GX</i>						
23	27	m	2	4.60	0.27	68.08
24	27	f	2	3.90	0.12	172.53
25	35	f	2	1.35	0.14	113.52
26	39	f	8	1.70	0.14	74.43
27	40	m	12	1.70	0.13	117.07
28	41	m	3	1.00	0.10	133.14
29	43	m	12	0.60	0.08	79.82
30	44	f	5	0.70	0.09	99.85
31	44	f	6	0.30	0.09	142.48
32	45	f	1.7	5.95	0.10	113.79
33	46	f	2	0.20	0.12	97.65
34	46	f	8	0.40	0.23	129.58
35	46	m	23	1.70	0.22	118.71
36	48	m	1.2	0.30	0.13	75.49
37	49	m	14	0.35	0.15	88.34
38	51	m	12	1.25	0.13	79.32
39	54	f	12	0.30	0.12	99.85
40	57	m	12	1.10	0.10	126.29
41	58	f	8	1.10	0.08	155.78
42	58	f	11	0.60	0.11	74.34
43	59	m	2	0.65	0.10	93.82
44	61	m	3	1.50	0.15	123.91
Average	46	50%[#]		1.42	0.13	108.08
SD	9.2			1.47	0.05	27.75

[#]The ratio of male.

half of 20070721-GX subjects (12/22, 54.5%) had more than 1% CD34⁺ progenitor cells in peripheral blood. Regression analysis showed that 20070721-GX significantly increased the number of CD34⁺ progenitor cells in peripheral blood. However, age, gender, and the duration of 20070721-GX intake did not affect the amount of CD34⁺ progenitor cells (Table 3).

3.2. 20070721-GX-Induced Increase in Telomerase Activity.

With sufficient telomerase enzyme, it is possible to prevent cells from dying. The clinical trials showed that telomerase activity differed significantly between the control and 20070721-GX groups (Figure 1(b), Table 4, $P = 0.016$). The average telomerase activity increased about 30% in the 20070721-GX group compared with the control group (Tables 1 and 2). Regression analysis showed that 20070721-GX significantly increased telomerase activity in subjects. The duration of 20070721-GX intake had a small but significant effect on the increase in telomerase activity ($\beta = 0.003$, $P = 0.022$) using univariable regression analysis; however, this was not significant after adjusting other variables, and duration was consequently excluded from stepwise regression analysis. Age and gender did not affect telomerase activity (Table 4).

3.3. *Effect of 20070721-GX on Serum IGF1 Concentration.* To evaluate IGF1 concentrations in this cohort, we used ELISA. Serum IGF1 ranged from 60 to 165 ng/mL (mean, 115 ± 30 ng/mL) among control subjects and from 68 to 172 ng/mL (mean, 108 ± 28 ng/mL) among the 20070721-GX subjects (Tables 1 and 2). Although the average IGF1 concentration in 20070721-GX subjects decreased slightly, it did not reach statistical significance (Figure 1(c), $P = 0.40$).

4. Discussion

To control the quality of each batch of the food supplement of 20070721-GX, the placental extract component is measured by ELISA. The evaluation includes concentrations of progesterone (Pgt), estriol (E3), estradiol (E4), human chorionic gonadotropin (HCG), human placental lactogen (hPL), and total protein (tPrt). For each batch, the concentrations are 10 ± 5 mg/mL tPrt, 30 ± 5 ng/mL Pgt, 10 ± 5 ng/mL E3, 10 ± 5 ng/mL E4, 25 ± 5 mIU/mL HCG, and 0.15 ± 0.1 mg/L hPL. Batches that fit these six indicators are chosen into chemistry, manufacturing, and controls (CMCs) manufacturing for standard operation and SGS examination. Because this placental extract is derived from pig at 8 weeks pregnancy (full term is 20 weeks), it is full of nutrition and

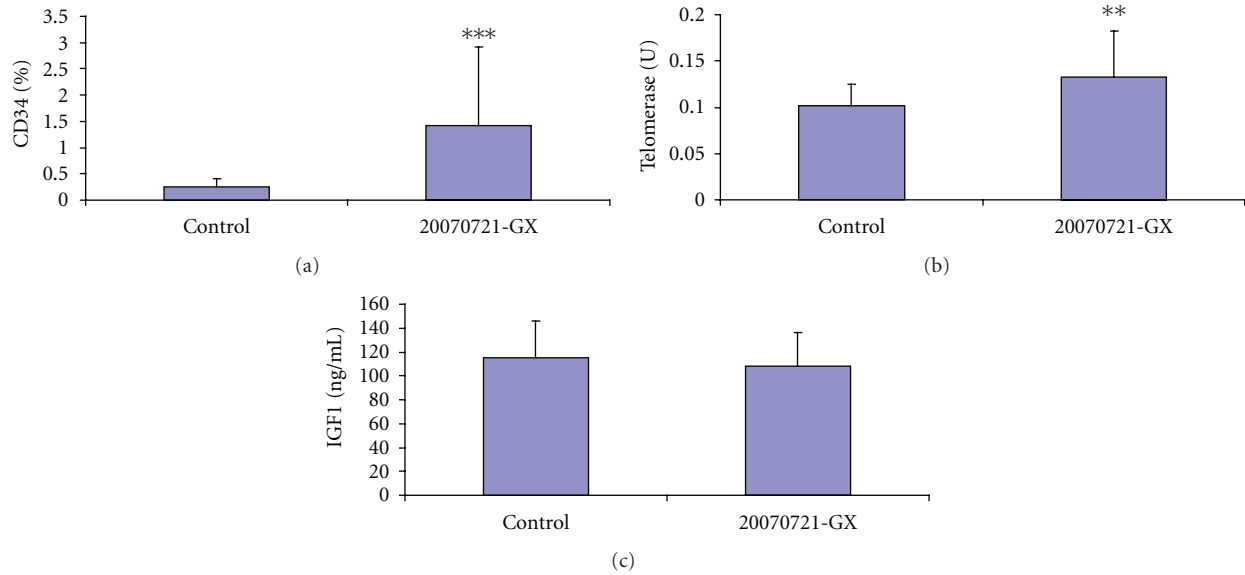


FIGURE 1: Effect of 20070721-GX on human rejuvenation. (a) Flow cytometric enumeration of CD34⁺ hematopoietic stem cells in control and 20070721-GX-treated subjects. (b) Telomerase activity (TRAP assay). (c) Enzyme-linked immunosorbent assay for IGF1. Data represent means \pm SEM. Differences between groups were examined by *t* test. ***P* < 0.05; ****P* < 0.001.

TABLE 3: Regression analysis for CD34⁺ cells.

Variable	Univariable		Multivariable		Stepwise	
	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
20070721-GX	1.177 (0.527 ~ 1.827)	0.001*	1.732 (0.851 ~ 2.613)	<0.001*	1.177 (0.527 ~ 1.827)	0.001*
Duration (months)	0.036 (-0.033 ~ 0.105)	0.296	-0.068 (-0.151 ~ 0.015)	0.106	Excluded	
Male	-0.153 (-0.899 ~ 0.592)	0.680	0.052 (-0.597 ~ 0.700)	0.873	Excluded	
Age (years)	-0.015 (-0.043 ~ 0.014)	0.306	-0.018 (-0.043 ~ 0.007)	0.157	Excluded	

**P* < 0.05 considered statistically significant.

the peptide concentrations are relatively high compared with other placental extracts collected at full term.

In our study, 95% (21/22) of individuals had an increase of more than 20% of CD34⁺ stem cells in peripheral blood compared with the control group (Tables 1 and 2). The enhancing effect was being observed in two elderly people (Tables 1 and 2). One subject was 59 years old (no. 43) and showed a 2.7-fold increase over control, and another was 61 years old (no. 44) with a 6.25-fold increase. In human, CD34⁺ cells can increase by 15% in young people but not in elderly people after taking exercise [15]. However, the food supplement 20070721-GX can increase health not only in the young but also in middle-aged or older people. In the present study, the CD34⁺ stem cells in peripheral blood of subjects that took 20070721-GX ($1.42 \pm 1.50\%$) were significantly higher than in control subjects ($0.24 \pm 0.17\%$, *P* = 0.001) (Tables 1 and 2).

In addition, the CD34⁺ status of two young individuals increased 7 times and 24 times after taking the supplement for only 2 months in comparison to the mean of the control group (Tables 1 and 2). This result indicates that duration of

food supplement intake was not closely related to the increase in CD34⁺ cells (Table 3).

Other issues arise from this result. Is the increase in CD34⁺ cells enough to repair injured cells or cause rejuvenation? According to a study by Khosrotehrani et al. [35], a total of 701 male (XY+) microchimeric cells were identified (mean [SD], 227 (128) XY+ cells per million maternal cells [0.02%]). Interestingly, in maternal epithelial tissues (thyroid, cervix, intestine, and gallbladder), 14% to 60% of XY+ cells expressed cytokeratin. This result implied that only a small amount of stem cells from the son is needed to repair injured cells derived from maternal tissues. Stem cells can not only transdifferentiate different organs, but they are also involved in renewal and proliferation. Because this food supplement increased CD34⁺ stem cells by 20% in 95% of subjects, we suggest that it could play a role in repairing injured tissue. Direct evidence, however, requires further study.

Using the TRAP assay, we found that 2.2 to 2.3-fold (to 0.22–0.23 U) increases in telomerase activity after two 46-year-old subjects (nos. 34 and 35) took 20070721-GX for 8 or

TABLE 4: Regression analysis for telomerase activity.

Variable	Univariable		Multivariable		Stepwise	
	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
20070721-GX	0.030 (0.006 ~ 0.053)	0.016*	0.022 (−0.011 ~ 0.055)	0.186	0.030 (0.006 ~ 0.053)	0.016*
Duration (months)	0.003 (0.000 ~ 0.005)	0.022*	0.001 (−0.002 ~ 0.004)	0.372	Excluded	
Male	0.010 (−0.015 ~ 0.035)	0.436	0.011 (−0.013 ~ 0.035)	0.349	Excluded	
Age (years)	0.000 (−0.001 ~ 0.001)	0.422	−0.001 (−0.002 ~ 0.000)	0.172	Excluded	

* $P < 0.05$ considered statistically significant.

23 months (Table 2). Although an increase in telomerase activity has an antisenesescence effect, increasing telomerase activity may also cause tumorigenesis [36, 37]. Therefore, we measured the telomerase activity in two malignant brain tumor cell lines: DBTRG and 8401. Telomerase activity in these cells was 1.8 U and 2.7 U, respectively, which is much higher than the average activity of 0.13 U in the experimental subjects. Furthermore, according to Fu and Chen [38], telomerase activity in normal bone marrow is 0.3 U, which is close to the increased level of activity in our experimental subjects. These results suggest that this food supplement does not cause tumorigenesis.

Telomerase activity is inversely related to senescence, IGF1 is associated with rejuvenation, and CD34⁺ cells are related to injured organ repair and anti-inflammatory activity. Thus, it is important to monitor the phenotypic features of people taking food supplements. Skin became more fine and tight, and wrinkles decreased in some people. In addition, more energy, increased sexual impulses, and improvement of menstrual disorder symptoms were described by others. These features are related to rejuvenation and antisenesescence. Furthermore, decreased serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase liver enzymes and improved nasal allergies, arthritis, and lower back pain were also observed. These features could be related to anti-inflammatory activity and injured cell repair. These phenotypic features are subjective and only represent a preliminary finding but are worth noting when designing future studies.

In our study, taking the food supplement increased the number of CD34⁺ cells and increased telomerase activity, but decreased IGF1 in the same subjects (nos. 23, 25, 26, 33, 36, 37, 38, 39, and 42; 9/22 = 40%). Thus, an important issue is whether the placental extract affects the same cellular target in each case. Because there are so many compounds in placental extract, it is possible that different targets of different compounds may lead to three different effects. However, because CD34⁺ stem cells have a relatively high telomerase activity [39], these effects are likely to be linked in placental extract that likely affects both. However, because IGF1 is an independent biomarker for antisenesescence, none of the subjects that took the food supplement had only a decrease in IGF1. The placental extract should be fractionated to determine which components are responsible for which effects.

Abbreviations

IGF1:	Insulin-like growth factor 1
HSC:	Hematopoietic stem cells
IR:	Insulin receptor
Pgt:	Progesterone
E3:	Estriol
E4:	Estradiol
HCG:	Human chorionic gonadotropin
hPL:	Human placental lactogen
tPrt:	Total protein
CMC:	Chemistry manufacturing, and controls
ISHAGE:	International society of hematotherapy and graft engineering
TMB:	3,3',5,5'-Tetramethylbenzidine
TRAP:	Telomeric repeat amplification protocol.

Authors' Contribution

The first two authors contributed equally to this work.

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