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Influence of Violation of Regeneration in People over 35-40 Years Old on Decrease in Production of Sexual Hormones

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Abstract

With age the quantity of pluripotent stem cells gradually decreases. It leads to violation of renewal of tissues, including tissues of endocrine organs, at people over 35-40 years old. Artificial formation of the chimeral individual may be used for recovery of the pool of pluripotent stem cells in patients older than 40-50 years old. Transfusions of mononuclear fraction of peripheral blood procured from young donors 18-23 years old with the same blood groups and sex as the recipient (patent of the Russian Federation № 2350340), allows people over 40 years old to reestablish the pool of pluripotent stem cells, the process of tissue renewal and the production of sex hormones, and can also be seen as a promising way to reduce biological age, while providing a significant prolongation of life and while maintaining a high quality of life. Transfusions of mononuclear fraction of peripheral blood are required to be carried out multiple times before the restoration of the numerical strength of the recipients' pool of pluripotent stem cells is complete. The effectiveness of regeneration reconstitution in individuals older than 40 years old upon receiving transfusions of allogeneic pluripotent stem cells depends on the difference in age between the recipient and the young donor. When there is a major difference in age between young donors and recipients over 40 years old, the proliferative potential of pluripotent stem cells of donors (their ability to maintain the number of own pool) is higher than the proliferative potential of the recipient. In response to the formation of colony-stimulating growth factors, this leads to the dominance of transfused and formed colonies in the bone marrow of the donor's pluripotent stem cells over the analogous cells of recipients when renewing all of their tissues. The latter occurs mainly due to the donors' stem cells.

Keywords: stem cells, regeneration, immune system, testosterone, estradiol, progesterone, andropause, menopause

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Introduction

Cell death takes place over the course of all of ontogenesis as a manifestation of normal vital functions of the organism. Death and the breakdown of old cells with following regeneration are constantly taking place in the organism, which thereby ensures the organism's normal vital functions [1, 2, 3].

After 35-40 years of age, the numbers of the pool of pluripotent stem cells and poorly differentiated progenitor epithelial cells and progenitor cells of other tissues that they replenish, are reduced [3, 4], which makes it impossible to replace old dead cells with an adequate number of progenitor cells or directly migrated stem cells [3]. With increasing age, production of cell growth factors (fibroblast growth factor basic – bFGF and another) aimed at stimulation of cell proliferation for increase in the number of progenitor cells of an epithelium and other tissues increases. Excess, increased mitogenic stimulation, which increases proportionally to age, is observed in all tissues in people older than 35-40 years of age, leading to activation of fibroblasts and their formation of fibrous tissue [3]. The numbers of the pool of pluripotent stem cells after 35 years of age go down by 1% per year [3]. For this reason, among people over 35-40 years of age, sclerosis in most tissues and organs develops with an intensity equal to the rate of the decrease of the pool of pluripotent stem cells – by 1% per year [3, 5]. The changes taking place in people over 35 years old is proven by the development of atrophy and fibrous changes in other tissues and organs, including tissues of endocrinal organs.

The Influence of Disorders of Renewal of Tissues on Gonadal Function in People over 35 Years Old

In particular, among men one can note atrophy of the testicles, which shows itself through the development of fibrosis of the basal membrane of tubular testicles, the reduction in the quantity of Leidig cells, and other changes. The rate at which the general testosterone level decreases among men over 35 years old: 1% per year accord to these values of the intensity of sclerosis of tissues [3, 6]. The reduction in testosterone production leads to the appearance of partial androgen deficiency of aging men (PADAM) with compensatory increase in levels of 5α-dihydrotestosterone and 17β-estradiol [7, 8], and initiates the development of metabolic syndrome (X-syndrome) [9, 10].

The age-related decrease in testosterone production has a significant influence on factors of the extracell environment which regulate cell aging [3]. Apoptosis develops in cells of normal mouse embryos (which are capable of division in the according conditions without signs of aging) when adding the blood serum of an older individual [11]. Some of the leading inductors of apoptosis are glucocorticoids and tumor necrosis factor α (TNFα) [2]; their levels increase under PADAM [9, 10].

Stem cells of peripheral blood among men have androgen receptors and, likewise, are androgen-dependent. The age-related reduction in production of sex hormones has a negative impact on the development and proliferation of pluripotent stem cells which are dependent on the levels of these hormones, which is an additional negative factor which is capable of reducing their quantity. The subsequent breakdown in the regeneration of tissues of the gonads (the testicles in men with a reduction in Leidig cells, which increte testosterone) testifies to the formation of a vicious circle with the phenomenon of mutual dependency [3].

Similarly, the onset of menopausal period in women is due to infringement of tissues renewal of ovaries, leading to primary ovarian deficiency [3]. In menopausal period women, there is an increase in the number of atresial (degenerative) follicles in the ovaries and relative progression of ovarian failure. The flow of the menopausal period depends on the rate of the decline in estrogen production. The average age for the onset of menopause is 50 years old. The main sign of primary ovarian insufficiency is an increase in follicle-stimulating hormone (FSH). Primary ovarian failure is characterized by a decrease in the level of estradiol in the blood serum <5 ng% and increased FSH > 40 ME/l [12].

The follicles are located in the cortex of the ovary, and they are surrounded by connective tissue and are at different stages of development. Each menstrual cycle there is a selection of several

primordial follicles, one of which later gives rise to the ovum, while the rest undergo atresia. The pimordial follicle (Figure 1) consists of a single layer of granulose cells and a 1st order oocyte, stopped at the prophase of the first meiotic division [13, 14].

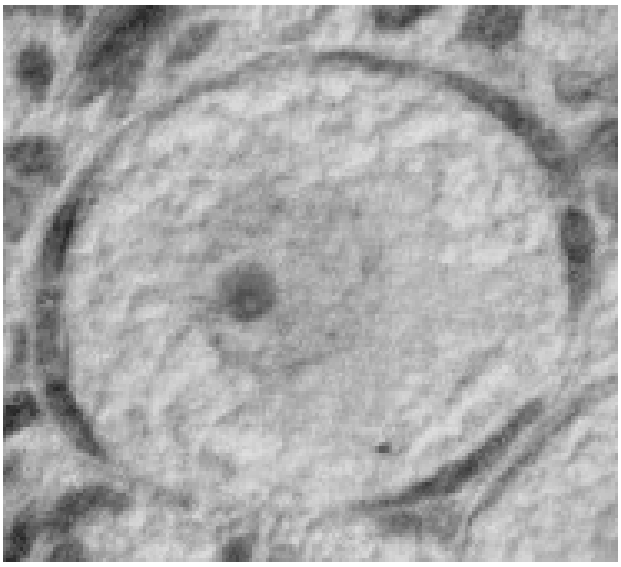


Figure 1. Primordial ovarian follicle (single follicle): 1st order oocyte surrounded by a single layer of flattened follicular cells.

At this stage, the chromosomes condense and synthesize RNA, initiating synthesis of proteoglycans bearing tissue-specific antigens. The crossover, taking place during the first meiotic division [11], changes the antigenicity of the 1st order oocyte. In order to prevent autoimmune reactions of rejection before crossing over, a blood-ovary barrier appears in the prophase of the 1st order oocyte, the functions of which are conducted by the layer of granulose cells.

During the differentiation process in granulose cells of certain primordial follicles there appear FSH receptors [15]. With increasing FSH in the beginning of the follicular phase, these cells, taking a cubic and cylindrical shape, begin to increte estradiol [13, 14]. Estradiol is required for the differentiation and division of the granulose cells themselves, having estrogen receptors.

Following the conversion of the primordial follicle to a primary follicle (Figure 2), a basal membrane appears on the latter, upon which granulose epithelial cells are arranged in one row, and take a cubic and cylindrical shape [13, 14].

At the stage of the secondary follicle (Figure 3), the 1st order oocyte increases in size [13, 14]. Replenishing the composition of granulose epithelial cells by using migrating committed stem cells (tissue-specific stem cells), as well as mitotic division of granulose cells, lead to the formation of a multilayer epithelium follicle.

There is formation of a shell of the follicle – the theca, represented by a multilane layer of fibroblasts and other components of the extracellular matrix. Around the follicle there is formed a vasculature of arterioles and capillaries that do not penetrate through the basal membrane [13, 14], which are necessary for entry of migratory committed (tissue-specific) stem cells into the epithelial cell layer of granulose cells, and into the connective-tissue layer of the follicle shell – the theca. Granulose cells increte a follicular fluid, which forms a space required for cell migration, and is a medium containing factors regulating migration and cell division. According to Yen SSC (1986), the follicular fluid includes proteoglycans, steroid and peptide hormones, hyaluronic acid, and other components [16].

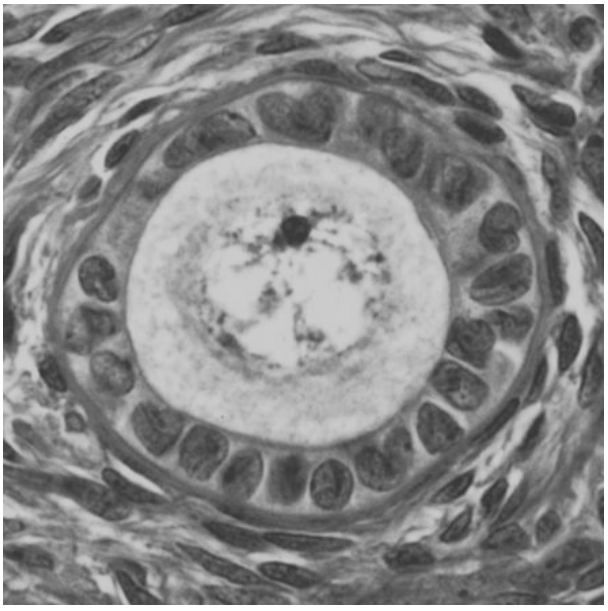


Figure 2. Primary ovarian follicle: 1st order oocyte surrounded by a single layer of cubic and cylindrical follicular granulose cells, separated from the ovarian stroma by the basal membrane. The acquisition by flattened follicular cells of cubic and cylindrical form is connected to the emergence among them in the process of differentiation of receptors to gonad-stimulating hormone and the beginning of their formation of estrogen.

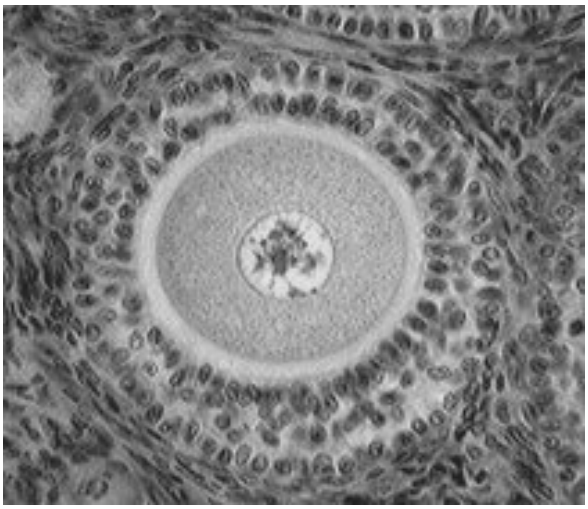


Figure 3. Secondary ovarian follicle: 1st order oocyte surrounded by a multilayered follicular epithelium (by granulose cells). A transparent zone (zona pellucida) is formed between the oocyte and the granulose cells. The basal membrane is located between the granulose epithelial cells and the stromal ovarian cells, which are beginning to form the connective tissue sheath of the follicle (theca), which represents, together with the basal membrane, the extracellular matrix.

The basal membrane and the connective tissue sheath (theca) are the extracellular matrix, providing growth and renewal of the granulose follicular epithelium [11]. The proteoglycans formed by granulose cells contain tissue-specific antigens that are captured and present themselves as macrophages to T-helper cells. After this, the T-helper cells, through the mediation of antigen-presenting cells, formed in pluripotent stem cells the appropriate tissue-specific receptors that are required for their subsequent directional migration [3]. Proteoglycans of the follicle (supporting tissue-specific antigens) become signaling molecules that attract migrating committed (tissue-specific) stem cells.

Committed (tissue-specific) stem cells migrate to tissue specific antigens of proteoglycans of fibronectin (part of the basal membrane, converts to the extracellular matrix) that are complementary to their receptors. The basal membrane does not prevent the migration of a macrophages and committed (tissue-specific) stem cells which support regeneration. After passing through the basal membrane, committed (tissue-specific) stem cells bind their tissue-specific receptors bind to their complementary antigens of proteoglycans of laminin (part of the basic membrane, converted to the epithelial layer), and are retained in the epithelial layer. Committed (tissue-specific) stem cells keep their connection with the basal membrane also keep contact with the underlying connective tissue, which executes control over differentiation of epithelial cells. The direction of differentiation of migrated committed (tissue-specific) stem cells is determined by the specific set (corresponding to the morphogenetic code) of cell growth factors on fibronectin and laminin of the basal membrane, as well as on collagen fibrils of the extracellular matrix [3, 11].

The tertiary follicle stage (Figure 4) is characterized by an increase in the follicle. Under the influence of FSH by granulose cells, follicular fluid continues to be produced. The oocyte, surrounded by granulose cells, shifts to one of the poles of the follicle, forming an oviparous tubercle [13, 14]. The concentration of estradiol increted by the granulose cells is higher in the follicular fluid than in plasma, creating the necessary conditions for the growth of the follicle. In particular, the high level of estradiol in the follicular fluid is required to complete the first meiotic division and differentiation and division of granulose cells having estrogen receptors.

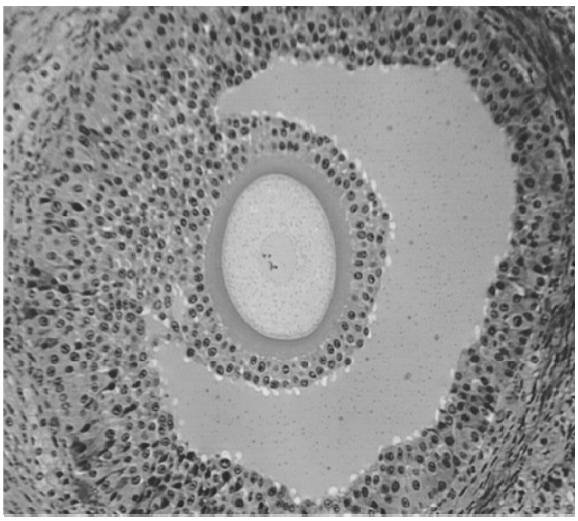


Figure 4. Tertiary ovarian follicle (mature follicle, Graaf follicle): 1st order oocyte is located on the local eccentric thickening of the layer of granulose cells – the cumulus oophorus. One or more layers of cylindrical granulose cells – the corona radiate, is located around the oocyte and accompanies it after ovulation. The follicle has a follicular cave filled with follicular fluid.

Of the group of follicles selected at the beginning of the menstrual cycle, one becomes dominant, and grows, and then its ovulation begins. In response to the rise in estrogen incretion at the end of the follicular phase of the menstrual cycle, there is an increase in luteinizign hormone (LH) level, needed for ovulation. In order for the LH level to rise to the ovulatory level, it is necessary for the concentration of estradiol in the blood to exceed 200 pg/ml and that it will be maintained at that level for at least 48-50 hours [17]. Exceeding this threshold value by estradiol is reached by the achievement of the number of granulose cells of the follicle of a necessary value (mainly by the replenishment of the granulose epithelium by migrating committed (tissue-specific) stem cells, as well as by the mitotic division of the granulose cells of the follicular epithelium themselves). The time needed to reach the critical level of increted estradiol required for increase peak LH and ovulation, that is, the length of the follicular phase, depends on the intensity of replenishment of granulose epithelium of the follicle by committed (tissue-specific) stem cells.

After determining the dominant follicle, the remaining follicles (necessary in the early follicular phase to quickly create the necessary level of estradiol) go through regression (atresia) [13]. The cause of atresia of these follicles is probably competed by the dominant follicle cell for molecules of growth factors and hormones (including FSH). Those follicles that lose the competition for the regulatory factors undergo atresia. Atresia of these follicles, the granulose epithelial cells of which have estrogen receptors, is contributed to be the lack of formation of estradiol by these follicles. During the differentiation process, by the beginning of the next follicular phase, new follicles with granulose cells appear, capable of expressing FSH receptors. The menstrual cycle is repeated.

The luteal phase begins after ovulation [13]. The exit of the ovum outside the follicle makes the blood-ovary barrier unnecessary: the basal membrane collapses, and capillaries grow onto the granulose follicle cells, to replenish their numbers by migrating committed (tissue-specific) stem cells (differentiated into granulose cells). A corpus luteum is formed in place of the mature follicle. The maximum blood flow to the corpus luteum, and thus the formation of the maximum number of granulose cells in its composition (due to migration of tissue-specific stem cells, differentiating into granulose luteocytes) is seen 7-8 days after ovulation, which corresponds to the peak of progesterone incretion.

By analogy with the end of the follicular phase (when the level of estradiol exceeds the threshold, initiating a peak increase of incretion of LH and ovulation), at the end of the luteal phase the increased incretion of FSH and the subsequent increase in the incretion of estradiol are probably also initiated by the progesterone level exceeding the threshold level [16]. The exceeding of the threshold level by progesterone is caused by a progressive increase up to a maximum number of granulose luteocytes of corpus luteum by the end of the luteal phase.

Under action of estradiol the quantity of LH receptors in granulose cells of a mature follicle increases. After ovulation the quantity of LH receptors in cells of a corpus luteum becomes increased, and receptors FSH - reduced [18]. Thus hormones and their corresponding receptors are the indivisible regulatory system [19].

After that (if pregnancy does not occur) from the 8th day after ovulation the reverse development (involution) of the corpus luteum begins. By the 8th day after ovulation, thecaluteocytes, forming testosterone, reach their maximum size [13], which is accompanied by their highest formation of testosterone (further contributing to the involution of the corpus luteum). The increase in the formation of estradiol also promotes atresia of the corpus luteum.

Thus, the leading factor in the change of the phase of the menstrual cycle is a progressive increase in the numbers of the epithelial layer of granulose cells of follicles, and then in the numbers of granulose luteocytes of corpus luteum due to migratory committed (tissue-specific) stem cells and their mitotic division.

Exceeding the threshold level by estradiol at the end of the follicular phase leads to an increase of incretion of LH and to a subsequent beginning of incretion by granulose cells of progesterone. Similarly, achieving the threshold level of progesterone in the late luteal phase leads to increased formation of FSH and to stimulation of incretion of estradiol by granulose cells of some primordial follicles (which, in the process of differentiation,

developed receptors for gonadotropin-releasing hormone) [16, 18].

A reduction in the pool of pluripotent stem cells in people after 35 years of age leads to insufficient replenishment of their numbers by tissue-specific stem cells, including both granulose cells of the epithelial layer of follicles, and granulose leteocytes of corpus luteum. As a result, by age 50, for most women the total production of estradiol by granulose cells of the epithelial layer of the mature follicle cannot reach the threshold required for initiation of the peak of incretion of LH and ovulation. This leads to the fact that in the menopausal period, menstrual cycles become anovular, and their duration increases. If it is impossible to begin ovulation, then the continuing incretion of follicular fluid leads to the formation of cysts. Factors negatively affecting the migration of committed (tissue-specific) stem cells and macrophages through the extracellular matrix, including basal membranes, as well as on the formation of tissue-specific receptors in pluripotent stem cells and on the participation in this process of antigen-presenting cells and T-helper cells, likely, will lead to the same result.

The epithelium of all tissues is located on the basal membrane, which is part of the extracellular matrix. Renewal of old epithelial cells takes place through the division of poorly differentiated progenitor cells of the epithelial layer and replacing them. Progenitor cells are replenished by committed stem cells (tissue-specific stem cells) that migrate through the extracellular matrix and basal membrane [3, 11]. After age 35, humans develop insufficient arrival of migratory committed stem cells (tissue-specific stem cells) to the epithelial layer due to a lower pool of pluripotent stem cells, which is compensated for by excessive stimulation of division of poorly differentiated progenitor epithelial cells by cell growth factors (formed paracrinally and autocrinally). Continuous excessive stimulation of mitogenic activity of epithelial cells leads to their malignant transformation – to cancer. Similarly insufficient arrival of committed stem cells (tissue-specific stem cells) for replenishment of progenitory cells in other tissues can lead to development of malignant process [3, 20].

Under insufficient replenishment by committed (tissue-specific) stem cells of the composition of the granulose cells of the epithelial layer of growing follicles, as well as the lack of replacement by them of dead old cells of the germinal epithelium covering the ovaries, there is compensatory autocrine and paracrine production of cell growth factors [20] for stimulation of mitotic division of the germinal and granulose epithelial cells themselves (of their low-grade forms). In the extracellular matrix of the germinal epithelium and the epithelium of follicles, the levels of cell growth factors of the ovaries become constantly high. The increased constant mitogenic stimulation leads to increased risk of benign, transitional, and malignant ovarian tumors originating from different cells (with their predominant formation from epithelial germ cells). For this reason, the formation of ovarian tumors is naturally associated with age.

Ovarian tumors form more often in nulliparous women compared with multiparous women (because multiparous women have a history of nine-month breaks in mitogenic stimulation of ovary cells). For the same reason, children of women who waited a long time (the follicles of which were under the influence of cell mitogenic growth factors and a number of hormones for a long time) have an increased risk of genetic abnormalities.

Atresia of ovarian follicles leads to menopause [13]. In the menopausal period, insufficient formation of estradiol contributes to atresia of primordial follicles since their granulose cells have estrogen receptors.

In addition, reduced formation of estradiol and atresia of primordial follicles contributes to the violation in menopausal period ultradian rhythm of formation of gonadotropin-releasing hormone, FSH and LH, required for estradiol incretion [13].

The physiological rhythm of incretion of hormones helps the neuroendocrine system to complete its main function, which is integration of various biological processes into a single organism. These processes take place on the molecular, cell, tissue, organ, and system levels. The success of the transfer of the biological signal depends not only on the level of the hormone but also on the frequency of the incretion of the hormone [21]. The pulse of the rhythm of the incretion of hormones from the point of view of cybernetics is related to “discrete messages”, which are capable of sending a significantly large volume of information, unlike “continuous

messages," which have a constantly changing size [22]. Information from the central nervous system, which is transferred in the form of nerve impulses which follow one after another at regular intervals, is transformed into an impulse rhythm of formation of hormones. An age-related decrease in the production of sex hormones leads to a loss of impulse ultradian rhythm of formation of LH and FSH. These changes lead to limitation and distortion of the information being transferred, which regulates a whole series of physiological processes including proliferation activity [21].

The lack of sex hormones that develops during andropause and menopausal period / postmenopause leads to compensatory excessive formation of their precursors – cholesterol and low density lipoprotein for support of synthesis of the end products (sex hormones) [9, 10]. An increase in the levels of cholesterol and low density lipoproteins contributes to atherosclerosis.

Three types of cells – thecocytes, granulose cells of follicles, and leutocytes of corpus luteum together form a complete set of enzymes required for the synthesis of sex hormones. Progesterone and 17-hydroxyprogesterone are formed in corpus luteum cells, androgens are formed in thecocytes and in ovarian stroma, and estrogens are formed in granulose cells of follicles [13]. The decrease in the menopausal period of the number of granulose cells of the epithelium of follicles, and their total enzyme activity, leads to excessive formation of androgens – progenitors of estrone and estradiol synthesized by granulose cells. The lack of granulose cells is compensated for by extragonadal aromatization of androgens (adipose and other tissues), but this is not always enough for the fermentation of androgens being produced. A consequence may be the appearance of virilization.

The involvement of adipose tissue in the process of compensation leads to an increase in its mass [10].

The lack of estrogen in the menopausal period and in the postmenopause is accompanied by insulin resistance, dyslipidemia, and obesity [13], and the risk of tumor diseases also increases for estrogen-dependent tissues, the differentiation and division of cells of which require estrogen. For men with partial androgen deficiency, there is a mirror image increase in the formation of estrogen, the appearance of dyslipidemia, obesity and increased risk of tumor diseases of the androgen-dependent prostate tissues. These changes occurring in men and women should be considered to be compensatory reactions, developing in response to the decrease in the production of sex hormones [7, 8, 9, 10, 19, 20, 21].

Perspective Methods of Correction of Reduced Production of Sex Hormones in People Older than 45-50 Years Old

Sex hormones are necessary for the division and differentiation of cells having receptors complementary to them. Under an age-related decrease in production of sex hormones, atrophy of tissues consisting of such cells develops, with depletion of the progenitor cells and violation of replacement of old dying cells in them. Therefore, in patients over 40 years old, it is expedient to prescribe supplemental hormone replacement therapy (androgen replacement in men, and pregnenolone, tibolone replacement in women), as well as to prescribe therapies aimed at restoring the numbers of the pluripotent stem cell pool [3, 6, 7, 8, 9, 19, 20, 23].

Replenishment of the pool of pluripotent stem cells in people over 40-50 years old will help promote adequate renewal the progenitor cells of an epithelium and other tissues with the subsequent replacement of old dying cells (necrosis or apoptosis) with an adequate quantity of new cells. There must be support of a normal quantity of cells in the pool of pluripotent stem cells in order to ensure that the constant process of tissue renewal in people over 40-50 years old can go on unharmed [3, 6, 20].

Renewal of epithelial-reticular cells of the thymus (conducting the teaching of T-lymphocytes) through transfusions of allogenic stem cells with their subsequent perception of tissue-specific antigens of the immune system of the recipient as "their own" makes it possible to consider transfusions of allogenic pluripotent stem cells as a part of mononuclear fraction of peripheral blood from young donors 18 to 23 years with the same blood groups and sex as the recipient as the most promising way of supporting a normal quantity of the pool of pluripotent stem cells among people over 40-50 years old (RF patent

number 2350340). After transfusing pluripotent stem cells form their own pool in bone marrow, which takes part in the renewal of the majority of the quantity of tissues of the organism. The individual becomes a chimera [3, 6, 20].

Considering the widespreadness of chimerism in natural conditions, artificial formation of the chimeral individual may be used for recovery of the pool of pluripotent stem cells, reconstitution renewal of tissue (reconstitution of regeneration) and production of sex hormones in patients older than 40-50 years old. The effectiveness of regeneration reconstitution in individuals older than 40 years old upon receiving transfusions of allogeneic pluripotent stem cells depends on the difference in age between the recipient and the young donor. When there is a major difference in age between young donors and recipients over 40 years old, the proliferative potential of pluripotent stem cells of donors (their ability to maintain the number of own pool) is higher than the proliferative potential of the recipient. In response to the formation of colony-stimulating growth factors, this leads to the dominance of transfused and formed colonies in the bone marrow of the donor's pluripotent stem cells over the analogous cells of recipients when renewing all of their tissues. The latter occurs mainly due to the donors' stem cells. Transfusions of mononuclear fraction of peripheral blood are required to be carried out multiple times before the restoration of the numerical strength of the recipients' pool of pluripotent stem cells is complete [3, 6, 20].

The effectiveness of therapy aimed at recovery of tissues renewal (recovery of regeneration) is demonstrated by the clinical example of a 60 year-old patient. In order to restore the tissue renewal (restoration of regeneration), the recipient was given 7 transfusions of mononuclear fraction of peripheral blood at intervals of two to three months between each transfusion. Harvesting of mononuclear fraction of peripheral blood done made from a 20-year-old donor with the same gender (male) as the recipient and the same antigenic systems: AB0, Rh-factor, phenotype Rh-factor, Kell. Three months after the seventh transfusion of mononuclear fraction of peripheral blood, the levels of hematopoietic progenitor cells CD34+ of peripheral blood increased by 5 times (from 1 to 5 cells per 1 mcl). A comparison the results received with data about 117 healthy people of different age groups, with a maximum value of CD34+ of peripheral blood in young people of 6.5 cells/mcl, and a minimum value in older people of 0.5 cells/mcl, showed that following completion of the course of transfusions of mononuclear fraction of peripheral blood from a 20-year-old donor, the patient's level of CD34+ of peripheral blood approached the maximum value characteristic for young people. Thus, the 60-year-old patient was able to restore the pool of stem cells and the regeneration process to a level common in young people. Furthermore, the observed patient's ESR following the seventh transfusion decreased from the initial 6 mm/hr to 3 mm/hr (Table 1, 2; Figures 5, 6) [6].

Table 1. Increase in the level of hematopoietic progenitor cells CD34+ in peripheral blood following seven transfusions to a 60-year-old patient of mononuclear fraction of peripheral blood from a 20-year-old donor [6]

Indicators	Transfusions of mononuclear fraction of peripheral blood	
	Before transfusions	Three months after the seventh transfusion
Hematopoietic progenitor cells $CD34^+$, cells/mcl	1	5
ESR, mm/hour	6	3

Table 2. Reference values of hematopoietic progenitor cells $CD34^+$ in peripheral blood of healthy individuals of various ages

	$CD34^+$ in peripheral blood of healthy individuals (cells/mcl)			
Manual method, n = 117	Minimal contents	Maximum contents	Average contents	Standard deviation
	0.50	6.50	2.36	1.14

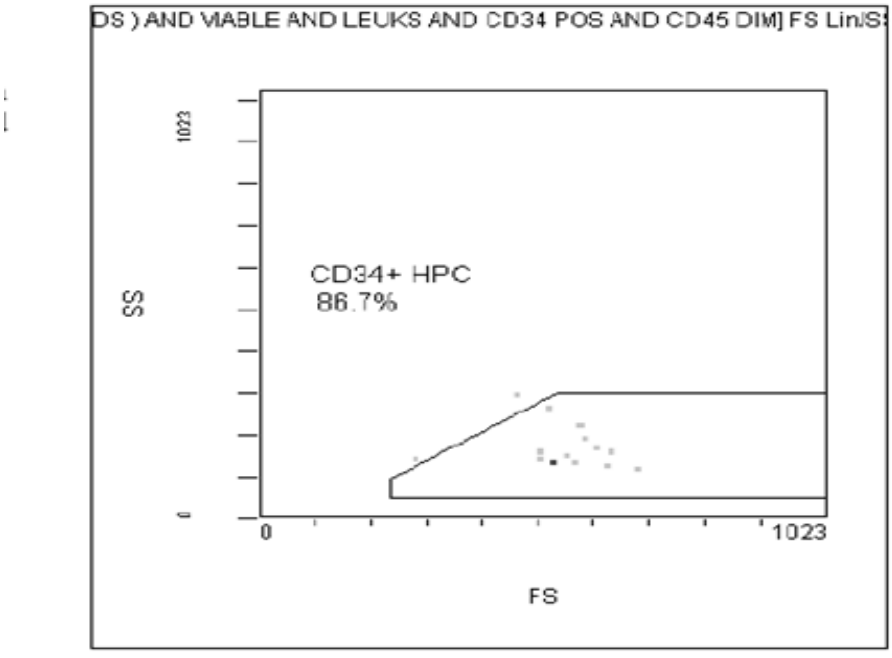
Note: these data are given from the instructions of Stem-Kit Reagents, Beckman Coulter Company, France, section 13.1. The range of normal values was determined in blood samples from healthy subjects of various age groups (n = 117; 58 men and 59 women).

Before and during the course of regenerative therapy, the patient was given androgen replacement therapy in an individually selected dose of testosterone drug, 1.6 ml per day of Androgel 1%, to treat partial age-related androgen deficiency. The patient showed an increase in recovery of total testosterone thanks to tissue regeneration following the 4th transfusion of mononuclear fraction of peripheral blood. This made it possible to change the ongoing androgen replacement therapy by reducing the dose of Androgel 1% from 1.6 ml to 0.5 ml per day. After the reduction in the dose of Androgel 1%, total testosterone levels returned to its previous sufficient level (Table 3). The patient's total PSA level remained normal [6].

After completing the course of transfusions of mononuclear fraction of peripheral blood, taking into account normalization of testosterone production thanks to restoration of the number of the patient's own Leidig cells, androgen replacement therapy can be stopped. If there continues to be testosterone deficiency due to residual irreversible changes in the testes, androgen replacement therapy should be continued, while reducing the dose of testosterone drug to the lowest sufficient level [23].

Table 3. Change in the level of total testosterone in a 60-year-old patient following transfusions of mononuclear fraction of peripheral blood from a 20-year-old donor [6]

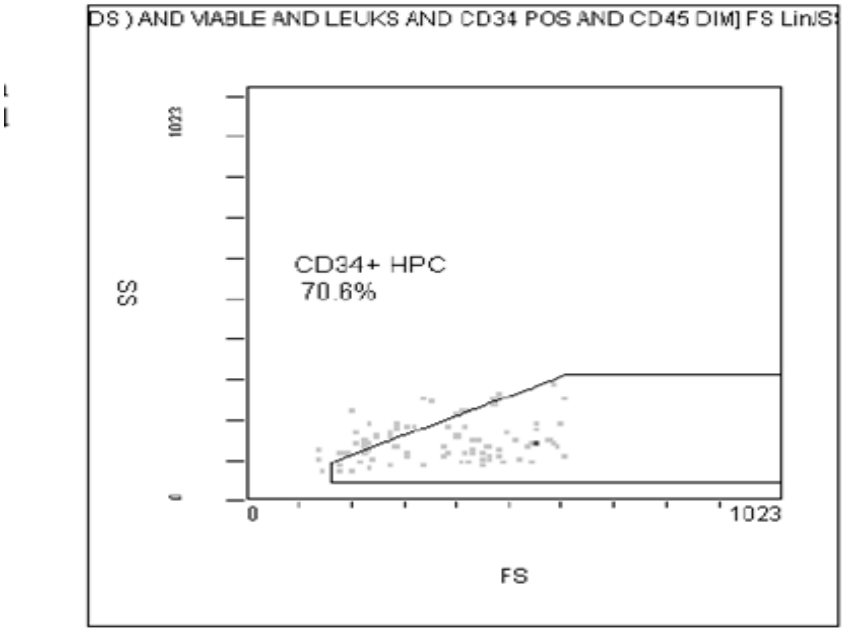
Indicators	Transfusions of mononuclear fraction of peripheral blood		
	Before transfusions	After 4 transfusions	After 5 transfusions
Total testosterone, nmol/l	13.5	26.6	17.6
Total PSA, ng/ml	1.27	1.60	1.16
Dose of Androgel 1%, ml	1.6	1.6	0.5



[NOT (BEADS) AND VIABLE AND LEUKS AND CD34

Region	Cells/µL	Number	%Total	%Gated
ALL	1	15	0.01	100.00
CD34+ HPC	1	13	0.01	86.67

Figure 5. Protocol for determining hematopoietic progenitor cells CD34+ in peripheral blood in a 60-year-old patient (before transfusions of mononuclear fraction of peripheral blood) [6].



[NOT (BEADS) AND VIABLE AND LEUKS AND CD34

Region	Cells/µL	Number	%Total	%Gated
ALL	7	85	0.09	100.00
CD34+ HPC	5	60	0.06	70.59

Figure 6. Protocol for determining hematopoietic progenitor cells CD34+ in peripheral blood in a 60-year-old patient (three months after the seventh transfusion of mononuclear fraction of peripheral blood) [6].

Since atresia of primordial follicles during menopausal period makes it impossible to restore female physiological regulation, carried out by sex hormones with infradian, circadian and ultradian rhythms of their formation, thus the prevention of follicular atresia and the preservation of their functions is one of the most important tasks of medicine. Recognition of the reduction in the pool of stem cells, being the main cause of ovarian follicular atresia in menopausal period, suggests that the recovery of the stem cell pool in women 45-50 years old by transfusions of mononuclear fraction of peripheral blood (harvested from a young donor with the same blood group and gender as the recipient) can restore regeneration of tissues and maintain the physiological regime of endocrine regulation carried out by sex hormones. This should be done in the menopausal period before the onset of menopause.

Given the development in men of irreversible fibrotic changes in the testes after 35-40 years of age, it is advisable to carry out restoration of the pool of stem cells in men at the age of 40-50 years old as well.

Conclusion

Artificial formation of the chimeral individual may be used for recovery of the pool of pluripotent stem cells in patients older than 40-50 years old. Transfusions of mononuclear fraction of peripheral blood procured from young donors 18-23 years old with the same blood groups and sex as the recipient (patent of the Russian Federation № 2350340), allows people over 40 years old to reestablish the pool of pluripotent stem cells, the process of tissue renewal (restoration of regeneration) and the production of sex hormones, and can also be seen as a promising way to reduce biological age, while providing a significant prolongation of life and the ability to work (while maintaining a high quality of life). Transfusions of mononuclear fraction of peripheral blood are required to be carried out multiple times before the restoration

of the numerical strength of the recipients' pool of pluripotent stem cells is complete.

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