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REVIEWS

Human Chorionic Gonadotropin: Unknown about Known

M. A. Borisova, D. Yu. Moiseenko, and O. V. Smirnova*

Moscow State University, Moscow, 119991 Russia

**e-mail: smirnova_ov@mail.ru*

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Abstract—The last two decade discoveries shift the accent from the consideration of human chorionic gonadotropin (hCG) as a hormone that controls progesterone production by corpus luteum cells to a powerful paracrine regulator that, in the tandem with its hyperglycosylated analog (h-hCG), induces successful implantation and coordinated dialog between blastocyst and uterine tissues. The ability of hCG and h-hCG to interact with TSH receptor and TGF-beta-RII, respectively, significantly extend the spectrum of processes controlled by these molecules. Differences between intracellular pathways of signal transduction between hCG and LH mediated by the same receptor (LH/hCG-R) impugn the unity of their effector mechanisms, which was previously considered as obvious. The paracrine properties of hCG include the control of fusion of trophoblasts into syncytiotrophoblasts, angiogenesis, immunity regulation, and endometrium predisposition to implantation. Angiogenesis is associated with LH/hCG-R expressed on mural cells of uterine spiral arteries as well as induced secretion of soluble VEGF form by endometrial cells. hCG regulates the ratio between different types of T-helper cells in maternal organism at the initial gestation stage determining a high level of Th2 cells. hCG supports local immunotolerance, functioning as a chemoattractant for T-suppressors (T-Treg) and an apoptotic factor for T-lymphocytes. Endometrial susceptibility arises from the activation of osteopantin secretion and the decline of mucin secretion by epithelial cells. h-hCG affects the same tissues as hCG, functioning as a paracrine agent regulating multiple cascades of cytokines. h-hCG plays the key role in the trophoblast invasion into the uterine decidua as a result of gelatinase secretion by these cells. The degree of the angiogenic effect of h-hCG is compatible with that of hCG, but its signal transduction is mediated by the TGF-beta signal transduction pathway that stimulates mural cell proliferation. h-hCG acts as a mitogen on NK-cells and is able to activate them and direct to angiogenesis maintenance. In this article, we attempted to elucidate the most important discoveries about the role of hCG and its hyperglycosylated analog, both accomplished and still upcoming.

Keywords: human chorionic gonadotropin, hyperglycosylated human chorionic gonadotropin, trophoblast, implantation, immunotolerance

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Human chorionic gonadotropin (hCG) is a hormone belonging to the class of gonadotropins that is secreted primarily by the placenta and serves to maintain pregnancy. hCG is a placental analogue of LH and interacts with its receptors. For a long time it was believed that the main function of the hormone is the maintenance of the corpus luteum during pregnancy and the stimulation of the synthesis of progesterone. In recent years, new major functions of this hormone are being discovered. These functions arise primarily due to the existence of several structural analogues interacting with a number of cells in a manner other than LH, whose concentration significantly exceeds the normal concentration of LH during pregnancy. In particular, hCG stimulates the development of the placenta by stimulating angiogenesis and the growth and invasion of trophoblasts, thereby enhancing the fetus trophism, is involved in the preparation of the endometrium for implantation, reduces the endometrial immune response, affects mother brain cells, etc. Due to its specific secretion by placental syncytiotrophoblasts, hCG serves as a molecular indicator of pregnancy, being found in the mother's blood as early as on the second day after implantation.

Since hCG has a similar but significantly enhanced effect in comparison to LH, it is used to control ovulation in humans and other mammals and to treat infertility in both sexes and may serve as a marker of development of hormone-producing tumors. β-Subunit of this protein is synthesized in gonadal neoplastic cells and in non-gonocyte tumors at the last stages. Due to the early production by trophoblasts at high quantities, hCG is a hormone marker of fetal chromosomal abnormalities.

STRUCTURE

Human chorionic gonadotropin (hCG) is a glycoprotein with a molecular weight of approximately

36.0 kDa, which is composed of 237 amino acid residues and comprises two non-covalently bound subunits. α-Subunit is homologous to α-subunits of anterior pituitary hormones: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH). It consists of 92 amino acids and comprises two *N*-glycosylated (at Asn52 and Asn78) regions. β-Subunit is unique for hCG and is specific for the receptor. The pharmaceutical pregnancy test is based on the analysis of its content in the urine. β-Subunit consists of 144 amino acids and is homologous to the β-subunit of LH by approximately 85%. It is characterized by the presence of an additional 24-aa region and has two *N*-glycosylated and four *O*-glycosylated (at Ser121, Ser127, Ser132, and Ser138) regions [1–4]. Each subunit contains a cysteine- knot motif—two antiparallel β-sheets bound through two disulfide bonds, with a third disulfide bond passing through the loop formed by the first and second bonds. The dominant antigenic sites are located on the loops forming the knot structure [2]. An additional hydrophobic loop on the β-subunit between Cys38 and Cys57 often serves as a site of the hormone inactivation by proteases [2, 5].

Modification with saccharides. Saccharides account for approximately 25–41% of the hCG molecule (by weight). It is believed that a normally glycosylated hormone contains 25–30% of saccharides [1, 2, 6, 7]. Carbohydrate structural elements greatly affect the biological activity of the hormone. In particular, the affinity of the hormone for the LH/hCG receptor (LH/hCG-R) depends on the content of sialic acid residues. It was shown that a more basic hCG devoid of part of sulfate or sialic residues, has a lower affinity for the LH/hCG receptor but a higher affinity for the TSH receptor [1, 7]. The duration of free presence of the hormone in mother's circulation system and the half-life of the hormone in blood also depend on the degree of glycosylation. The lifetime increases with increasing acidity of the isoelectric point of the glycoprotein [3, 5].

hCG forms. Currently, four compounds with a specialized physiological effect are distinguished, which are structurally related by the common β-subunit. These are placental hCG and hyperglycosylated hCG (h-hCG), pituitary hCG (p-hCG), and the hyperglycosylated β-subunit secreted primarily by cancer cells. Hormonal function (i.e., distant effects on target cells) is characteristic for typical hCG and p-hCG, whereas its hyperglycosylated analogues exhibit a more local effect, being autocrine/paracrine factors [1, 8].

hCG contains primarily *N*-linked side chains consisting of 8 (linear structure) and 11 (dichotomic structure) saccharide residues and *O*-linked chains formed by three residues. h-hCG is characterized by a large number of three-ray chains consisting of 15 saccharides modified with fucose and six-mer *O*-linked chains [1, 3]. Due to this fact, the molecular weight of h-hCG is 40–41 kDa versus 36.0–37.0 kDa (according to different data) kDa for hCG. A large number of carbohydrate residues hampers normal folding of α-β dimer and, due to the presence of the characteristic cysteine loop, opens another binding site, structurally similar to the binding sites of the transforming growth factor β (TGF-β), Nerve growth factor (NGF), and platelet-derived growth factor (PDGF) [1].

Homodimers of hyperglycosylated β subunits, which can be formed in blood, are structurally similar to the glycoproteins of the TGF-β family [19]. h-hCG is an agonist of TGF-β receptors, which exhibit serine/threonine kinase activity. Possibly, there are yet unstudied types of h-hCG interaction with tyrosine kinase receptors for PDGF and NGF [18].

The pituitary hCG (p-hCG) differs from the placental form of the hormone by the presence of sulfated oligosaccharides. Sulfate groups are located on the *N*-acetylgalactosamine residues, which replace the sialic and galactose residues on both *N*- and *O*-linked oligosaccharide chains [10].

BIOSYNTHESIS

Placental hCG is synthesized by trophoblasts, chorionic villus cells. Trophoblasts are cells with the fetal karyotype; depending on the degree of differentiation, they are classified into the cytotrophoblasts, syncytiotrophoblasts, and intermediate trophoblasts. The main contribution to the synthesis of hCG is made by syncytiotrophoblasts, which form syncytium on the outer layer of chorionic villi. Cytotrophoblasts synthesize primarily h-hCG. Small amounts of hCG are also synthesized in cells of gonadal and nongonadal neoplasm [10].

α-Subunit is encoded by a single *CGA* gene on chromosome 6 (6q21.1-23) and is common for hCG, LH, FSH, and TSH. β-Subunit is encoded by a cluster of *CBG* genes, formed in primates as a result of duplication of the *LHB* cluster, encoding LH β-subunit. The latter led to the emergence of genes encoding the new hCG β-subunit, which is specifically expressed in the placenta but retains the ability to expression by pituitary gonadotrophs. The *CBG* cluster is located on chromosome 19 (19q.13.3) adjacent to the *LHB* and consists of six nonallelic genes. The *CGB5* gene is most actively transcribed in trophoblasts and accounts for approximately 65% of all β-subunit molecules. The *CGB7* gene encodes a β-subunit isoform differing by three amino acid residues [8].

The expression of the *CGA* gene and six genes of the *CGB* cluster is regulated by transcription factors AP2, SP1 (for the *CBG5* promoter), and some others. The amount of hCG produced by syncytiotrophoblasts depends on the concentrations of h-hCG, progesterone, 17-β-estradiol, corticosteroids, growth factors, cytokines (e.g., TNF-α and EGF), oxygen, and

gonadotropin-releasing hormone (GnRH), as well as nuclear receptor ligands PPAR-γ and RXR-α [8, 11, 12]. Translation is performed both on rough endoplasmic reticulum (ER), in the lumens of the cisterns of which mannose and galactose residues are attached to nitrogen atoms, and on free polyribosomes [12]. Glycosylation at the oxygen atom and the attachment of fucose and sialic residues is performed in the Golgi apparatus.

Expression dynamics and blood content. Human chorionic gonadotropin is secreted by trophoblasts as early as at the stage of blastocyst formation, before implantation. In mother's blood, it is already detected on the second day after implantation. The concentration of hCG reaches a peak in the first trimester of pregnancy [8]. In the early days of pregnancy, the biosynthetic activity of individual trophoblasts, related to the synthesis of hCG and its derivatives, is maximal. In the period between the second and sixth weeks of gestation, h-hCG, which is actively synthesized by invasive cytotrophoblasts, prevails in the blood of pregnant women [10, 14]. After implantation, the hormone concentration increases exponentially, peaking at approximately 10–12 weeks of gestation and then decreasing to a level of approximately 1/5 of the maximum value (table) [15].

Since the lifetime of hCG in the bloodstream and its affinity for LH/hCG-R is greater than that of LH, its biological activity is also greater, which makes it a promising drug for the stimulation of LH activity in the body [3, 10].

The average content of hCG in the blood of nonpregnant women is less than 5 mIU/mL, and in women after menopause it is below 9.5 mIU/mL (mIU/mL is the international milliunit per milliliter; for hCG it is currently 2.35×10^{-12} moles or 6×10^{-6} grams (per milliliter)). Serum test is usually performed using fluorescence immunoassay with a hCG β-subunit detection sensitivity below 5 mIU/mL [13].

Pituitary hCG is normally synthesized in the anterior pituitary gland during the peak of LH synthesis before ovulation and in postmenopausal women. Gonadotropin-releasing hormone (GnRH) directly increases the blood content of p-hCG in both men and women. The hormone level in male urine (0.03–1.7 mIU/mL) is significantly correlated with the blood level of GnRH [1]. The production of p-hCG is parallel to the production of LH. The fact of hCG production can be explained by the adjacent location of genes encoding β-subunits of LH and p-hCG on the chromosome.

METABOLISM

The half-life of LH in the bloodstream is approximately 0.5 h, whereas for hCG it is 37 h, which is due to the much lower pI value of the latter (approximately 3.5 versus 8.0 for LH). Nevertheless, hCG undergoes numerous catabolic transformations [3, 10]. At the initial stage, the heterodimer of the glycoprotein can either dissociate into two free subunits or be cleaved by proteases at the protruding loops. The half-life of dissociating hCG dimers in blood at 37°C is approximately 700 h. h-hCG dissociates much faster, within only 140 h [10]. Macrophage or leukocyte elastase cleaves hCG β-subunit at the amino acid residues Val44 or Gly47. After this modification, hCG, in turn, dissociates much faster. After cleavage by elastase, the structural integrity of hCG can be retained for a long time due to the presence of five disulfide bonds; however, the biological activity in this case is lost. The cleaved hCG is much more rapidly degraded by kidney glycosidases and exoproteases. Glycosidases cleave terminal residues of sialic acid, galactose, and *N*-acetylglucosamine, so that the glycoside chains after the treatment with glycosidases are ended with mannose residues. The relative content of degraded hCG forms in urine increases many times from the first weeks of gestation to the end of pregnancy [6, 10].

Blood levels of hCG in women at different stages of pregnancy [15]

Week of gestation	Overall level of hCG β-subunit, mIU/mL
4–7	0–233200
8–11	11440–455300
12–15	14300–510400
16–19	6490–33770
20–23	550–72930
24–27	8240–107470
28–31	12780–152900
32–35	12980–128480
35–39	5390–114730

RECEPTION

hCG reception. The main hCG receptor is the G-protein–coupled LH/hCG-R. The biological activity of hCG is associated primarily with its interaction with LH/hCG-R. The affinity of hCG for LH/hCG-R is greater than that of LH [3]. In addition, hCG can interact with a low affinity with the TSH receptor [5].

LH/hCG-R consists of 674 amino acid residues and contains glycosylation sites. It comprises a large *N*-terminal domain encoded by 10 exons, C-domen associated with the membrane, which is composed of seven transmembrane helices and a cytoplasmic *C*-terminus [16, 17]. The *N*-terminal domain contains numerous leucine-rich repeats and consists of 340 amino acid residues, which is not typical for the

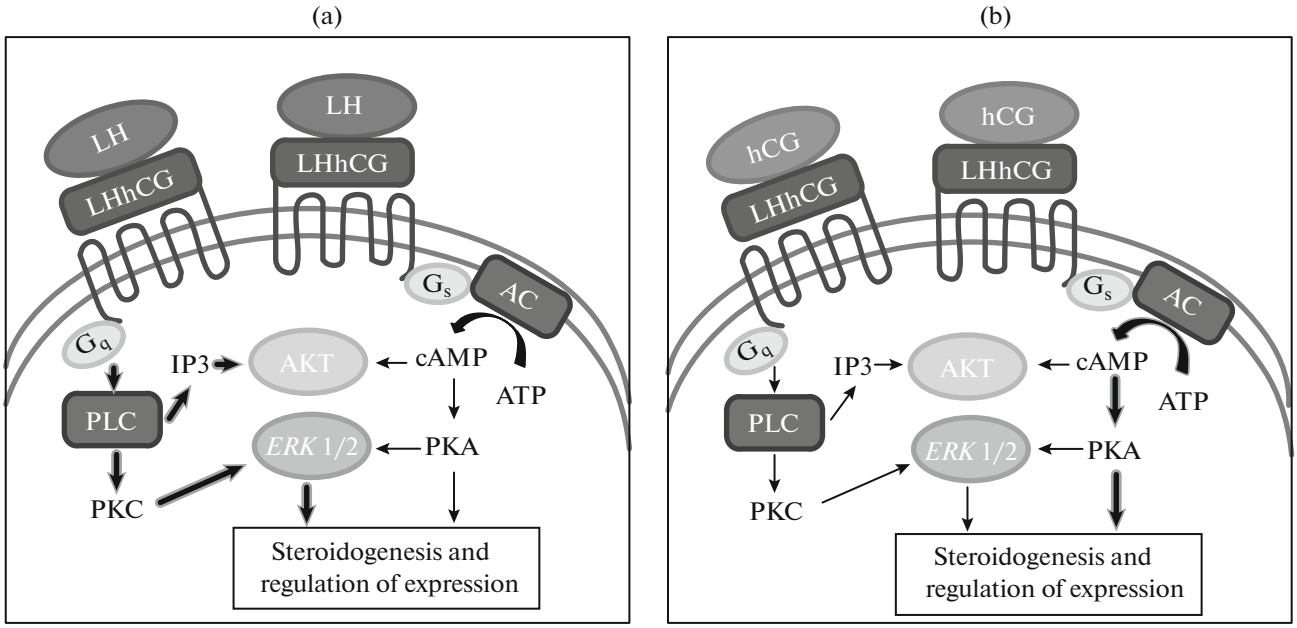

Fig. 1. Pathways of interaction of LH and hCG with the LH/hCG receptor. Designations: PKA—protein kinase A, PKC—protein kinase C, AKT—protein kinase B, ERK1/2—serine/threonine MAP kinases. The thick arrows indicate the most characteristic signal transmission pathway for this hormone [19].

G-protein–coupled receptors of this type, which usually bind to small ligands [11].

The hormone binding sites of the receptor and the key amino acid residues in these sites are known [7]. The large range of hCG concentrations in the first weeks of gestation is paradoxical. Even taking into account the corrections for the cycle phase in which conception occurred and the refined time of implantation, the hCG normal levels in blood of pregnant women may vary by almost a thousand times [1, 15]. To explain the normal course of pregnancy at such enormous range of concentrations of a regulatory hormone, the spare receptor concept has been proposed, according to which the activation of even a small number of receptors in the cell causes a complete physiological response. It should be noted that high concentrations of hCG cause a decrease in the number of its receptors, as a result of which, for example, the progesterone production by the corpus luteum cells at low hCG concentrations may be greater than at very high concentrations [1].

h-hCG reception. The facts of analysis of the structural (spatial) homology as well as the known data on the increased h-hCG content at the early stages of gestation, when this signaling compound promotes cytotrophoblast invasion by activating metalloproteinases, led to assumption on its ability to interact with TGF-β receptors. This assumption (for TGF-β-RII) was confirmed in experiments with antagonists of these receptors in angiogenesis models by coimmunoprecipitation and with the use of the competitive ligand ^{125}I-TGF-β [18].

The positive results of the h-hCG test on the activation of progesterone production by Leydig cell testify to its weak ability to interact with LGCH-R. hCG and h-hCG can also interact with the mannose receptor CD206 (C-type lectin receptor) of NK cells [18].

SIGNAL TRANSDUCTION

LH/hCG-R belongs to the superfamily of G-protein–coupled receptors. In [19] it was shown that LH/hCG-R can transmit signals via two signaling pathways. In the case of LH, signals are transduced primarily via the Gq proteins and activation of phospholipase C and protein kinase C (Fig. 1a). For hCG, the main pathway is through the Gs proteins and activation of adenylate cyclase (Fig. 1b). For example, increased progesterone production is achieved by enhancing the expression of the cholesterol side chain cleavage gene (P450scc). Both pathways ultimately result in the activation of serine/threonine kinase B (AKT) and mitogen-activated kinases 1 and 3 (ERK 1/2); however, a more characteristic pathway for hCG is the effector response directly via protein kinase A.

It was demonstrated that hCG can affect cells (particularly, the immune system cells) through phosphoinositol pathway and protein kinase C [1, 20]. Other studies showed the effect of hCG on calcium-dependent potassium channels, in particular, on BK-potas-

sium channels, which is important for the relaxation of uterine muscles [1].

Signal transduction through TGF-β receptors. Transmembrane receptor TGF-β-RII transducts signals through the intracellular serine/threonine kinase domain, activating Smad proteins. Smad proteins, joining together to form trimers, function as transcription factors. In particular, it was demonstrated that the appearance of h-hCG accelerates phosphorylation of regulatory Smad2 in endothelial and mural cells and leads to genomic activation of Smad-dependent cells [18].

PHYSIOLOGICAL EFFECT

hCG possesses diverse hormonal functions, and its hyperglycosylated homologues exhibit a wide range of paracrine properties.

LH activity (*doubling of LH functions*). As an agonist of LH receptor, hCG exhibits its activity. The receptor common for LH and hCG is located on the theca and granulosa cells in ovaries. Similarly to LH, hCG can stimulate the growth of the primary follicle immediately after the appearance of the LH/hCG receptor. hCG can stimulate the meiosis of diploid cells (the transition of oocytes to the stage of second-order oocytes), stimulate the production of collagenase (which fractures the follicular vesicle, thus leading to ovulation), and act as a differentiation factor on the remaining follicular cells, leading to the corpus luteum formation. Although hGH shares all these functions with LH, due to the greater lifetime in the bloodstream and higher affinity for LH/hCG-R, it has a great physiological effect at equal concentrations [21].

Over 80 years it was considered that the primary function of hCG is the stimulation of progesterone production in the corpus luteum in the ovary. The addition of placental hCG to corpus luteum sections stimulates progesterone production. If the blastocyst is not implanted into the human uterine endometrium, the corpus luteum undergoes regression 12–14 days after ovulation. Thus, it is hCG that delays the menstrual phase onset. hCG increases the functional activity of the corpus luteum during the luteal phase of the menstrual cycle. The synthesis of steroid hormones is enhanced due to increased transcription of the gene encoding *P450scc*, the enzyme that cleaves the side chain of cholesterol. Additionally, LH and hCG activate transcription of 3-β-hydroxysteroid dehydrogenase (3β-HSD), which directly converts pregnenolone into progesterone [3]. The initial stages of steroidogenesis are the same in all cells and include the cholesterol ester hydrolysis, the side chain cleavage, and the synthesis of pregnenolone. These reactions are the rate-limiting stages of the biosynthesis of steroid hormones and the main targets of trophic hormones [3, 22].

By enhancing *CYP17* or *P450c17* transcription, hCG enhances the synthesis of androgens (testosterone and androstenedione) in the theca cells of follicles and the extrafollicular compartment of ovaries in women and in Leydig cells of the testes in men. Androgens, in turn, serve as intermediates in the synthesis of estrogens. CYP19 (P450-aromatase) acts in the granulosa cells and Sertoli cells on the lumenal side of the seminiferous tubules. Follicle-stimulating hormone (FSH) accelerates the conversion of androgens to estrogens. By acting together with FSH, LH/hCG accelerates the maturation of follicles and spermatogenesis [3, 22, 23]. hCG also stimulates the synthesis of relaxin by corpus luteum cells [7].

LH and hCG are responsible for the regulation of expression of many genes. In granulosa cells, LH through ERK 1/2 triggers the final stage of differentiation into the progesterone-producing corpus luteum cells. Apparently, hCG can activate the signaling pathway via the epidermal growth factor (EGF), which is characteristic of LH (shown in mice using equine CG), leading to the maturation of granulosa cells; however, the degree of coincidence of the regulated gene clusters for LH and hCG is not known exactly [24]. LH suppresses the expression of many genes of the actin cytoskeleton, which may contribute to clustering organelles for steroidogenesis. In addition, a decrease in the expression of adhesive and apoptotic proteins is detected in the granulosa cells exposed to LH. Desensitization of the target cells by hCG is associated with the decrease in the adenylyl cyclase activity, whereas the content of LH/hCG-R itself usually increases [22].

In recent years it became clear that the overview of hCG only as a hormone maintaining the corpus luteum in pregnancy cannot explain the changes in the blood content of hCG during pregnancy, because the high basal level of hCG is retained throughout pregnancy, even when progesterone begins to be produced by the placenta itself, reaching a level of 150–200 mg [5].

Specific Types of hCG Activity

The development of the placenta and fetus trophism. hCG is involved in angiogenesis by affecting LH/hCG-R located on uterine myometrial cells and spiral arteries. LH/hCG-R are found on endothelial cells and smooth muscles of spiral arteries and other vessels around the placenta. LH/hCG-R is expressed on endothelial and mural cells (pericytes and smooth muscle cells). These receptors mediate the mitogenic effect of hCG on vascular cells and the activation of the VEGF synthesis by the endometrium and ovarian granulosa cells [25, 26]. Endometrial cells secrete the soluble form of VEGF, which affects the endothelial spiral arteries in a paracrine manner [18]. After reaching the region of chorionic invasion into the uterine myometrium, the spiral arteries are involved in the

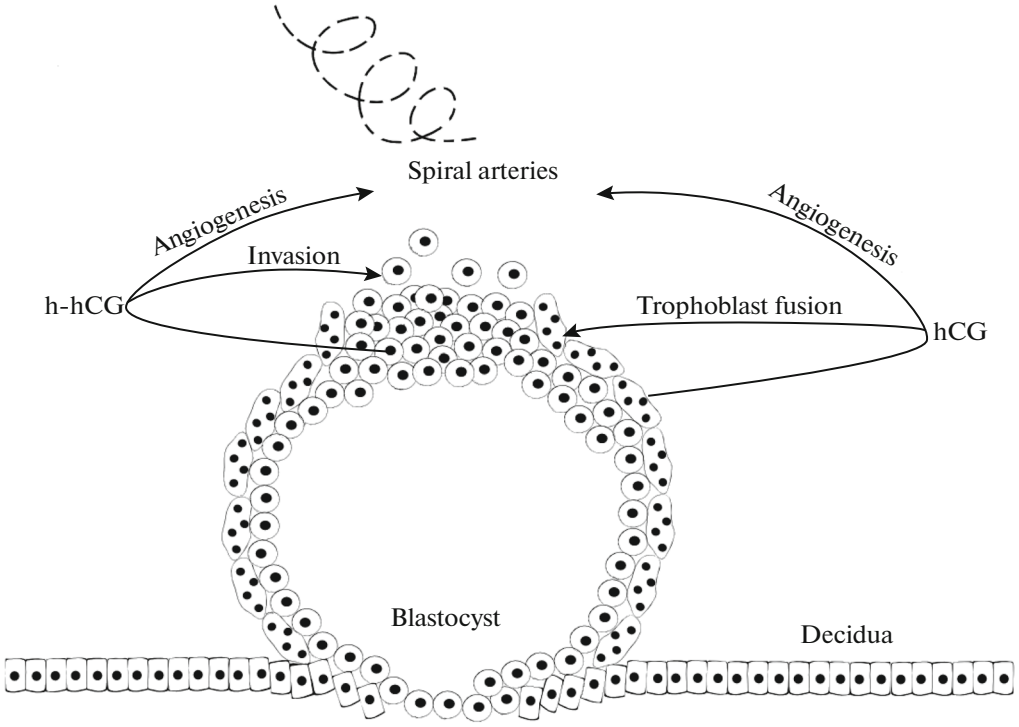

Fig. 2. Blastocyst implantation and trophoblast invasion on week 3–5 of gestation. Spiral arteries are depicted with dashed lines, because they appear only at later stages. Multinuclear and mononuclear cells are syncytiotrophoblasts and trophoblasts, respectively. Arrows indicate the functions of h-hCG and hCG [10].

formation of the blood-placental barrier. Large multinucleated syncytiotrophoblasts are located along the edges of the placenta and can perform a barrier function [1, 10]. hCG triggers the fusion of cytotrophoblasts into syncytiotrophoblasts. These two functions are performed throughout pregnancy and, probably, explain the stably high basal level of the hormone concentration in blood.

Differentiation of trophoblasts. The role of hCG in the initiation of fusion of cytotrophoblasts into syncytiotrophoblasts was shown in vitro in trophoblast cell cultures, where hCG via cAMP-dependent kinases increases the mRNA content of the fusogenic (causing syncytium formation) protein syncytin-1 [8, 10, 11] (Fig. 2).

Possibly, hCG also affects the further development of the placenta and the umbilical cord formation. This is evidenced by the retained sensitivity of umbilical cord cells to the effects of hCG [1].

Effect on the uterus. hCG simulates the growth of uterine tissues. LH/hCG-R is presented in large amounts on myometrium and endometrium cells. It is assumed that hCG is involved in the growth coordination between the fetus and the uterine tissues [1, 10, 18]. hCG inhibits any type of contractile activity of the myometrium throughout pregnancy by influencing the calcium-dependent BK potassium channels, activating them and increasing K^+ release from the myometrial cells (repolarization), which leads to the relaxation of uterine muscles. There is evidence that contractions in the last weeks of pregnancy are associated with a decrease in the hCG levels [1].

Regulation of implantation process. Before the contact with the endometrium, the blastocyst releases hCG into the uterine space, where it interacts with endometrial and transmits information about the impending implantation [14, 27]. Experiments in mixed cultures of trophoblast and epithelial cells showed an increase in the number of invaded trophoblasts after the addition of hCG. The hormone increases the secretion of proimplantation factors (the leukemia-inhibiting factor (LIF) and trophinin) by the epithelium, whose concentration correlates with the implantation frequency [27]. In the presence of IL B1 (IL-B1), hCG causes a decrease in the mucin mRNA expression (particularly, *MUC16*). The blockade of their synthesis with interfering RNAs in vitro demonstrated the negative impact of mucins on the process of implantation, because mucins inhibit the binding of osteopantin to integrins and other adhesion proteins. hCG significantly enhances the expression of the gene encoding osteopantin, the primary adhesive protein that promotes the blastocyst attachment (shown in ECC-1 carcinoma cells and SW1 cells of the first-trimester trophoblasts) [27].

Regulation of immunity. hCG nonselectively blocks any type of immune response or the activity of macro-

phages of the mother's body directed to proliferating cells of the placenta, ensures the secretion of a factor preventing the macrophage migration (possibly, MIF) towards the chorionic proliferation region, preventing the destruction of genetically foreign placental tissue. In addition, it regulates the activity of antigen-presenting dendritic cells and natural killer cells [1, 8].

hCG reduces the mitogenic activity of cytotoxic T-and B-cells by activating T-suppressors (Th3). These cells of the CD4+CD25+Foxp3+ lineage, which play an important role in the regulation of autoimmune processes by inhibiting the proliferative response of autologous CD4+CD25–T-cells and allogeneic dendritic cells as well as by releasing a number of immunomodulating signaling compounds [28]. The analysis of decidual tissues of the placenta, obtained from patients with spontaneous miscarriages, showed reduced levels of mRNA of TGF-β, IL-10, neurolipin-1, and Foxp3. All these compounds are secreted by T-suppressors. In women with spontaneous miscarriages and some types of infertility, the content of T-suppressors in the placental tissue is significantly below normal. In vitro analysis of the migration of Th3 cells showed that trophoblasts or JEG-3 carcinoma cells producing hCG exhibit attractant properties. Interestingly, the attractant activity, which was demonstrated in experiments with recombinant HCT116 cells with inserted *CGB3* and *CGB7* genes, is apparently characteristic of both normal hCG, and homodimers of its both subunits. Since interleukin IL-2 is missing in the blastocyst attachment area, T-suppressors probably migrate from the peripheral tissues immediately after division [28]. On gestation week 30, at least 25% of Th3 cells carry LH/hCG-R. It should be noted that this receptor may be expressed on Th3 cells of nonpregnant women after their treatment with hCG. At very low doses hCG can attract neutrophils, monocytes, and lymphocytes. The migration occurs in accordance with the positive concentration gradient [29].

There is also evidence that the amount of apoptotic T-cells increases under exposure to hCG. Experiments with cell cultures demonstrated the presence of antiproliferative and apoptotic activity in endometrial cells by activating *FasL* mRNA expression, which also stimulates apoptosis of T-cells [30]. It was shown that cultivation of a mixed culture of syncytiotrophoblasts and allogeneic lymphocytes causes apoptosis of T-cells, whose intensity depends on the concentration of hCG [29]. hCG mobilizes the dendritic cells that cause immune tolerance, by regulating the secretion of IL-10 and the production of the MHC II complex. Other dendritic cells, conversely, may undergo apoptosis [29].

hCG increases the production of indoleamine 2,3-dioxygenase, a tryptophan-degrading enzyme, in syncytiotrophoblasts and dendritic cells. The tryptophan degradation leads to a decrease in the activity of effector immune cells in the placental tissue. hCG induces expansion of regulatory B-cells, which, in turn, increases the level of IL-10 and decreases the level of TNF-α produced by Th1 cells [29].

Thus, on the system level, hCG stimulates the transition of immunity from the cytotoxic Th1-dependent type to the humoral type, which is based on the production of large amounts of antibodies by B-cells and is regulated by T-suppressors [27–29].

Thyroid-stimulating effect. hCG exhibits thyrotrophic activity. The addition of hCG (25–42 IU/mL) to rat thyroid cell cultures caused the uptake of radioactive iodine (iodides) due to increased expression of the gene encoding sodium/iodide symporter [5]. Direct stimulation of maternal thyroid gland is observed primarily at the end of the third trimester of pregnancy. At this time, the enhancement of the thyroid-stimulating effect of hCG can lead to a decrease in the TSH concentration. The concentration of hCG during pregnancy is positively correlated with the concentration of thyroxine and negatively correlated with the level of unbound TSH receptor [5, 31–33]. Normally, only the peak hCG concentrations, exceeding the plateau level of the late stages of pregnancy, can lead to temporary hyperthyroidism [5].

hCG cleaved by elastase often retains its thyrotropic activity and even may increase it. For example, the stimulation of recombinant TSH receptor with a hCG preparation obtained from patients with trophoblastoma and containing a large amount of cleaved molecules gave a twice stronger physiological response compared to the stimulation with normal hCG. The affinity for the TSH receptor is higher in the hCG forms that are devoid of sialic groups, even though such forms have a shorter lifetime in blood [5, 32].

Placental transport of iodides can be regulated by sodium/iodide transporter (NIS) and pendrin, a sodium-independent chloride/iodide transporter. Genes of both proteins are actively expressed in placental cells, although to a lesser extent than in the thyroid gland. NIS is synthesized throughout the cytotrophoblast layer, whereas pendrin is synthesized primarily in the peripheral areas on syncytiotrophoblast membranes. Apparently, hCG affects the synthesis of both transporters [32, 33].

Anti-HIV activity of hCG. The anti-HIV activity of hCG (more precisely, its free β-subunit) has been discussed since 1995, when its therapeutic effect on Kaposi's sarcoma, associated with HIV type 1, was detected. It was assumed that hCG is responsible for the extremely low rates of infection of embryos with HIV-1 in the first trimester of pregnancy, when its concentration is maximum. To date, the apoptotic effect of hCG on Kaposi's sarcoma cells is explained by the interaction with the TGF-β receptor [34].

In one study, the components of the hCG preparation affecting HIV in vitro were revealed. These were compounds similar to the urinary forms of lysozyme

C, RNase U, and RNase A. These enzymes exhibit a strong antiviral activity and are able to destroy HIV structure. Nevertheless, the possibility of dependence of the synthesis of these compounds on hCG itself cannot be ruled out [34, 35].

hCG function in men. hCG promotes embryonic development by the male type, stimulating the Leydig cells, already differentiated in the embryo, to synthesize testosterone. Embryonic Leydig cells appear already on week 8 of gestation. Under the influence of LH or hCG, the activity of cholesterol desmolase (P450scc) increases, as a result of which Leydig cells synthesize a number of androgens: testosterone, dehydroepiandrosterone, and androstenedione. They can also synthesize small amounts of estrogens. hCG stimulates testicular Leydig cell function, enhances the synthesis and production of testosterone, as well as promotes spermatogenesis, the development of secondary sexual characteristics, and the lowering of the testicles into the scrotum [7, 22]. LH/hCG-R were also found in spermatozoa, where their role remains unclear [1].

Other functions of hCG. hCG influences the mother's brain, possibly causing hyperemesis gravidarum ("vomiting in pregnancy") or just lingering persistent feeling of nausea usually in the first trimester of pregnancy [1, 5].

There is evidence of fetal tissue growth stimulation. LH/hCG-R is found in kidney and liver tissues as well as, according to other data, in lungs, spleen, and intestine of the embryo, whereas none of these organs in adults expresses this receptor in their cells [1, 19, 36].

Functions of Hyperglycosylated hCG (h-hCG)

h-hCG exhibits paracrine and autocrine effects, having a binding site on TGF-β receptors other than the LH-like site on cysteine knot loops and similar to that of some growth factors. h-hCG is secreted by cytotrophoblasts (median layers of chorionic villi) and regulates their invasion into the decidua. This signaling compound has an antiapoptotic effect and activates metalloproteases in cytotrophoblasts, thus stimulating the invasion of trophoblasts. h-hCG plays a particularly important role in the early implantation, when its concentration may exceed the concentration of hCG [14, 18].

Experiments with Leydig cells showed a small yield of progesterone under the influence of h-hCG. This fact suggests that h-hCG, to some extent, is able to interact with LH/hCG-R, causing a weak physiological response [18]. h-hCG enhances the production of gelatinases (matrix metalloproteinases MMP2 and MMP9). The effect of h-hCG on leukocytes was predicted, preventing their adhesion by means of E-selectin. In addition, h-hCG has a mitogenic effect on NK cells, at high concentrations it can affect NK cells via the mannose receptor. NK cells are the dominant type of endothelial lymphocytes in the first trimester of pregnancy, showing a strong angiomodulating effect and increasing the diameter of vessels [1, 18].

In addition, h-hCG has a strong angiogenic effect. Experiments in the "aortic annulus" model in mice knockout for LH/hCG-R showed a 4.73-fold increase in vascular anastomoses compared to h-hCG-devoid control. In this case, h-hCG acts via the TGF-β-RII receptor. It was shown in vitro that h-hCG enhances proliferation of endothelial and mural cells via the TGF-β signaling pathway, revealed by the blockade of these biological effects after the treatment with antibodies to the TGF-β receptor (type 1 and 2). In this case, the competition for the receptor between TGF-β and h-hCG is observed [18]. TGF-β has a mitogenic effect on vascular smooth muscle cells, causing their further differentiation, and regulates the interplay with endothelial cells. The interplay between trophoblasts and vascular cells through the TGF-β receptor plays the key role in placental development and tumorigenesis [18].

h-hCG is the key invasion factor, which determines its autocrine and paracrine functions. The treatment of JEG-3 carcinoma cells with the B152 antibody to h-hCG showed a decrease in invasion but not in migration. This fact suggests that the effect of h-hCG is determined by its positive impact on the production of matrix metalloproteinases MMP2 and MMP9 [18].

hCG LEVEL TESTING METHODS

The first pregnancy test, also known as Aschheim–Zondek test, was based on the fact that hCG, which exhibits LH activity, stimulated the production of steroid hormones by the ovaries and testicular Leydig cells.

Today, the immunological methods of pregnancy diagnosis are used. Usually, the glycoprotein is determined by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies to the β-subunit. Currently, there are many antibodies to hCG and at least one effective antibody to h-hCG (B152, binds in the *O*-glycosylated site) [10]. In the blood and, particularly, in the urine of pregnant women, there are various forms of hCG, many of which lose their primary biological activity, being cleaved by proteases or glycosidases. For this reason, the precise determination of the biological activity of hCG by immunological methods is hampered, and the search for new more specific antibodies, whose epitope is a vulnerable functional site or glycosylation site, is constantly performed. As a result, the international unit of biological activity per hormone weight also changes. Today it is 10.300–15.400 IU/mg [2].

Pathophysiological Effect in hCG Deficiency and Excess

When speaking about hCG, it should always be borne in mind whether the effect of the hormone with the normal structure, it hyperglycosylated analogue, or free hyperglycosylated β-subunit is considered.

hCG deficiency. Since the activation of even a small amount of LH-hCG-R in the cell may lead to a full physiological effect, the content of hCG in mother's blood varies significantly. Very low levels of the hormone in the first trimester of pregnancy are associated with an increased risk of miscarriage due to poor fetus trophicity and slowly developing placenta (poor angiogenesis). Modern hCG preparations are obtained primarily using recombinant DNA technology in yeast cells for proper glycosylation. Recombinant hCG (hCG-rec) causes a smaller immune response and, therefore, can be administered by patients themselves in the form of intramuscular injection [3].

hCG excess. In some women, there is a complication of pregnancy known as "vomiting in pregnancy," which is regarded as early toxemia and is accompanied by weight loss, dehydration, and ketoacidosis, which is caused with increased blood levels of hCG (Fig. 3). In this case, transient hyperthyroidism also may develop. Hyperemesis gravidarum may symptomatically resemble Graves disease and can be distinguished from the latter by the presence of specific antibodies [1, 5, 10, 31]. At hCG concentrations greater than 200 IU/mL, hyperthyroidism may develop for several weeks [5].

h-hCG deficiency. Low levels of hyperglycosylated hCG are associated with preeclampsia, which is characterized by hypertension in the mother and inefficient supply of the fetus with oxygen and nutrients due to inefficient angiogenesis in the uterine myometrium. Preeclampsia is one of the most dangerous complications of pregnancy, which often entails damaging the brain tissue of the embryo. Its development is attributed to inefficient trophoblast invasion and slow angiogenesis in the first trimester of pregnancy [10]. Inefficient implantation is one of the major causes of miscarriage in humans. In the majority of cases (13 out of 20 examined), sudden miscarriages were associated with h-hCG concentrations below the norm [6, 10].

h-hCG excess. Hyperglycosylated hCG functions as an antiapoptotic factor affecting trophoblasts and, probably, uterine cell. Its excess causes malignization of tissues, cell invasion, and uncontrolled proliferative activity of cytotrophoblasts. h-hCG interacts with the TGF-β receptor, inhibiting its cellular activity [10, 38]. Choriocarcinoma and other trophoblast tumors are accompanied by increased levels of hCG and h-hCG. h-hCG can trigger the development of malignant tumors through the TGF-β signaling pathway. Vascularization of gestational tumors, such as choriocarcinoma, may be associated with the influence of h-hCG. It was shown that nearly all

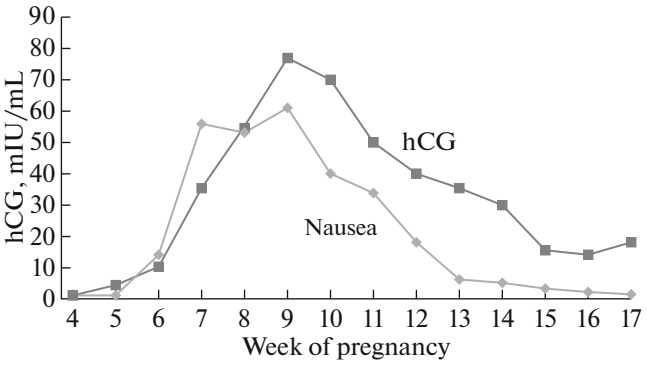

Fig. 3. Correlation between the development of symptoms of nausea in the first trimester of pregnancy and the increase in the hCG concentration in urine. The severity of symptoms was estimated on a scoring scale by self-diagnosis performed by pregnant women [37].

hCG produced by choriocarcinoma is hyperglycosylated. Choriocarcinoma cells are commonly used to produce h-hCG for research purposes [18].

Ectopic expression of β-subunit. Ectopic expression of free β-subunit was shown for carcinomas of the endometrium and neck, as well as for many types of cancer of the lungs, liver, kidneys, ovaries, breast, prostate, pancreas, and colon. In bladder cancer, ectopic expression is observed in 35% of cases [34]. Ectopic expression of β-subunit is sometimes explained by dedifferentiation, when cells return to the pluripotent state. Nevertheless, intact hCG is very rarely found at advanced stages of carcinoma development and is almost never found in epithelial types of cancer. The appearance of β-subunit in epithelial cancers is usually indicative of the advanced stages of the disease and active metastasizing [34].

The use of hCG as a marker of certain pathophysiological conditions. Since hCG is synthesized in trophoblasts (cells that form the chorionic villi and have the fetal karyotype), its biochemical analysis can give reason to assume chromosomal aberrations of the embryo at the earliest stages of pregnancy. In particular, disorders affecting the expression of glycosylation enzymes can be detected [1, 5, 8]. For example, it was shown that trophoblasts of embryos with trisomy for chromosome 21 (Down syndrome) contain substantially greater amounts of sialyltransferase 1, which attaches sialic acid to the terminal region of the polysaccharide chain, and fucosyltransferase 1, which attaches 6-deoxygalactose to one of *N*-acetylglucosamines. Blood analysis by immunoelectrophoretic focusing and Western blot hybridization showed that the band corresponding to hCG is shifted relative to the normal isoelectric point to more neutral values (from pI 4.5 in normal conditions to pI 7.3 in trisomy for chromosome 21) [6]. hCG and especially h-hCG, together with estriol and α-fetoprotein, are used in the triple test for Down syndrome identification. Down syn-

drome manifests itself in a strong decrease in the level of hCG at the early stages of pregnancy and an excessive increase in its level due to prolonged immaturity of the placenta in the middle of pregnancy [10].

h-hCG is a marker of choriocarcinoma. Choriocarcinoma cells produce h-hCG in large quantities. The blockade of the function of this molecule by specific antibodies results in a nearly complete inhibition of choriocarcinoma growth [1, 5, 11]. Hyperglycosylated β-subunit is used as a tumor marker in trophoblastomes, both in women and men, and appears at advanced stages of many types of cancer. The efficacy of chemotherapy is estimated by the curve showing the decline in the blood concentration of this marker [11]. Trophoblastomas, choriocarcinoma, and molar pregnancy lead to the appearance in blood of large amounts of hCG and h-hCG as well as free β-subunit. This makes these molecules good candidates for markers of diseases of this type [10, 39].

In combination with α-fetoprotein, hCG β-subunit is an excellent marker of germ cell tumors [5, 39]. For nontrophoblast tumors, significant amounts of hCG are usually released only at the advanced stages of disease. Elevated hCG levels in bladder, brain, neck, and breast cancers are usually a reason for a poor prognosis [2]. The study of hepatoblastoma showed that the amplitude and rate of decrease in the concentration of α-fetoprotein and hCG β-subunit are positively correlated with the survival prognosis [5, 39].

Free β-subunit modified with saccharides by the h-hCG β-subunit type is produced in large amounts by choriocarcinoma cells and other malignant tumors. It is detected in various cancer cells, especially at the late stages of malignization. Its presence in blood is significantly correlated with a negative prognosis. To date, attempts were made to use the free glycosylated β-subunit as a vaccine or as material for generating differentiated forms of killer T-cells in the treatment of non-gestational types of cancers [1, 10].

Antiserum to hCG derivatives (in particular, to the hCG β-subunit) reduces the number of bladder cancer cells. Currently, it is believed that β-subunit influences these cell lines as an antiapoptotic factor rather than as a proliferation activating agent [34]. Therapy with anti-h-hCG antibodies may also be useful for the treatment of gestational trophoblast disease, when trophoblasts remaining after childbirth become aggressively metastatic and invasive [18].

The blockade of the signaling pathway associated with the TGF-β receptor by hCG derivatives can also explain its therapeutic effect on Kaposi's sarcoma cells, which is combined with the antiapoptotic (malignizing) effects on some bladder sarcoma cell lines [34].

Prospects for the Therapeutic Use of hCG

Treatment of autoimmune diseases. hCG exhibits immunosuppressive and antiinflammatory properties. The therapeutic effect of recombinant hCG in the treatment of rheumatoid arthritis and Sjogren's syndrome was shown clinically [29]. In laboratory studies, rheumatoid arthritis is often simulated by injecting streptococcal cell-wall material to rats. The characteristic symptoms, which are manifested several days after injection, include the inflammation of joints, bones, and ligaments; cartilage destruction; and an increase in the levels of tumor necrosis factor TNF-α, interleukins IL-6 and IL-B1, and inducible nitric oxide synthase iNOS. In this case, the injection of recombinant hCG 2 days before injecting the inflammatory agent resulted in a dose-dependent reduction in symptoms. However, only the mature hCG has the immunosuppressive effect, whereas individual isolated subunits have only a minimal effect [29].

Sjogren's syndrome (autoimmune inflammation and destruction of lacrimal and salivary glands associated or not associated with other autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus) is simulated using NOD (non-obesity) mice with diabetes mellitus type 1. On week 12 of postnatal development, these mice have increased amounts of CD4+ T- and B-cells in the salivary glands, and saliva production in them is markedly reduced. The injection of hCG for 6–12 weeks significantly weakens both symptoms. The number of invasive lymphocytes and the concentrations of interferon-γ, TNF-α, IL-1B, IL-10, iNOS, and metalloproteinase-9 decrease, which leads to an increase in saliva production and reduces damage of salivary glands [29].

In NOD mice, diabetes develops as a result of destruction of β-cells of pancreatic islets by immune cells. The injection of hCG leads to a significant increase in the number of suppressor T-cells in the spleen and lymph nodes of the pancreas, which is accompanied by an increase in the concentration of IL-10 and TGF-β. A possible mechanism of action of hCG is the recruitment of suppressor T-cells to the inflammation site [29].

The therapeutic properties of hCG are associated primarily with its effect on the immune cells. In addition, the symptoms of multiple sclerosis, ankylosing spondylitis, thyroiditis, and diabetes mellitus type 1 during gestation are alleviated [29].

Treatment of ectopic pregnancy. High-affinity antibodies selective to h-hCG may be useful for the medical treatment of ectopic pregnancy developing in the fallopian tubes, because surgical intervention today usually leads to their removal [18].

Attempts to use hCG in dietetics and therapy of nonsexual disturbances. The so-called—a very low-calorie diet that have become very popular in Western Europe for the treatment of obesity—is known since 1954.

This diet is based on the data obtained by Simeons (1954) during the treatment of Frohlich syndrome (depletion of adipose tissue in the gonads), who showed that hCG promotes proper fat distribution between tissues and reduces the feeling of hunger. According to the diet, 125 mIU/mL hCG daily help patients to reduce hunger and enhance lipid metabolism. According to the results of meta-analysis conducted in 1995 [40], none of over 25 studies in which observations were performed sufficiently accurately described the effect of hCG on fat redistribution, appetite reduction, or direct weight reduction.

Repeated attempts were made to use hCG for the treatment of various diseases of the digestive system. In the Russian literature, the possibility of a positive effect of hCG on liver diseases is discussed. For example, a significant reduction in the pathological manifestations of CCl_4-induced cirrhosis as a result of antifibroblastic (decrease in disturbances in the beam arrangement of hepatocytes), lipolytic, choleretic, and hyperbilirubinemic effects of hCG was reported in [41].

CONCLUSIONS

Thus, when studying the physiological effect of human chorionic gonadotropin, it is necessary to differentially consider the effects of its normally glycosylated and hyperglycosylated forms, which act through receptors of different superfamilies and trigger different signal transduction pathways. Another important point is focusing on the less comprehensively studied paracrine effects of hCG variants, which determine the implantation success by at least several mechanisms: (1) involvement in trophoblast cell differentiation, (2) stimulation of their invasion, (3) induction of endometrial decidualization, (4) stimulation of angiogenesis and involvement in the blood–placental barrier formation, and some others. Noteworthy are also the immunomodulatory functions of hCG variants not only in relation to the mother immunotolerance with respect to the fetus but also in terms of its use as an immunotherapeutic agent.

Nevertheless, despite the century elapsed after the discovery of hCG, it has not been studied completely, neither structurally nor in terms of reception and signal transduction and diversity of physiological effects. Currently, it is obscure whether p-hCG, which is stably detected in blood during the peak LH concentration before ovulation, is of any significance. The effect of hCG on the development of fetal tissues and differentiation by the male type is also unclear. It is necessary to elucidate its therapeutic properties, at least to assess the range of its immunomodulatory properties and confirm its effect on the pathologically changed liver. Despite the fact that h-hCG plays the key role in the invasion of chorionic cells and placental development at the earliest stages, the role of h-hCG in malignant transformations of the placenta and the development of neoplasms is unclear. It is necessary to perfect the cancer therapy technique based on the antibodies to the hCG β-subunit. All of these issues require further study.

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