Human chorionic gonadotropin (hCG) and hyperglycosylated hCG, seven semi-independent critical molecules: A review.

Laurence A. Cole*

USA hCG Reference Service, 34 Broadmoor Way, P.O. Box 950 Angel Fire, New Mexico

Abstract

hCG exists in two analogous structures, as typified by the hormone hCG which binds an LH/hCG hormone receptor and the TGFß receptor binding autocrine hyperglycosylated hCG. Both share a common α-subunit and ß-subunit amino acid sequence. From the common hormone hCG and autocrine hyperglycosylated hCG structures there are seven common hCG variants with wildly varying hormone hCG or autocrine hyperglycosylated hCG functions. There is placental hormone hCG and placental autocrine hyperglycosylated hCG which together control pregnancy and start hemochorial placentation during pregnancy. There is pituitary sulfated hCG, part of the hormone hCG library which works with LH in driving ovarian steroidogenesis, ovulation and luteogenesis. Fetal hCG a fetal hormone that promotes fetal organ growth during pregnancy, and ovarian hyperglycosylated hCG which drive the final proteolytic enzyme step of ovulation. Cancer hyperglycosylated hCG and cancer hyperglycosylated hCG free ß-subunit drive malignancy in most cancers. The seven variants of hCG drive critical functions during pregnancy, cancer and other bodily functions.

Keywords: Hyperglycosylated hCG, Steroidogenesis, Ovulation, Luteogenesis.

Accepted on 09 July, 2017

Introduction

The hormone hCG was founded in the early twentieth century by Aschner, Fellner, and Ascheim and Zondek [1]. In 1912, Aschner promoted the genital tract of guinea pigs with injections of water-soluble extracts of human placenta [1-4]. In 1913, Fellner promoted ovulation in immature rabbits with a saline extracts of human placenta [2]. In 1919, Hirose induced ovulation and normal luteal function in immature rabbits by repeated injections of human placental tissue [3]. All of these studies show that there was a clear link between the placenta and the female gonads [1-3]. In 1927, Ascheim and Zondek demonstrated that pregnant women produce a gonad-stimulating molecule [4]. They showed that injecting this hormone into intact immature female mice let to follicular maturation, ovulation, and hemorrhaging into the ovarian stroma.

The realization around this time that the placenta was producing a molecule that promoted ovarian progesterone production led researchers to coin the name human chorionic gonadotropin (hCG): Chorion comes from the Latin chordata meaning afterbirth; gonadotropin because the hormone was considered a gonad tropic molecule, or a stimulator of gonad (or ovary) steroid production.

I am far from sure that the name is correct today. Firstly, because the hormone and autocrine dealt with today are produced by placenta cells and cells other than placenta cells. Secondly, because the hormone and autocrine do not act primarily on gonad cells. Considering that it is both a hormone (hCG) and a autocrine (hyperglycosylated hCG), a general name like human pregnancy glycoprotein or human acidic glycoprotein might have been more appropriate.

The hormone hCG and the autocrine hyperglycosylated hCG evolved in primates from a deletion mutation in luteinizing hormone (LH) ß-subunit [5]. The original molecules, anthapoid CG (aCG), was not very acidic, isoelectric point (pI) 6.3. Over 55 million years it evolved through baboon hCG, orangutan hCG and hominid hCG to form acidic human hCG (pI=3.5) [6].

hCG characteristically had a 92 amino acid α-subunit and a 145 amino acid ß-subunit. The α-subunit has two N-linked oligosaccharides and the ß-subunit has two N-linked and four O-linked sugar side structures [7,8]. Through evolution, hCG subunits contains a cystine knot structure inherited from transforming growth factor-ß (TGF-ß). aCG evolved from primate LH, fish LH evolved from fish gonadotropin ancestral hormone (GAH), which directly evolved from fish TGF-ß [9].

Hyperglycosylated hCG was discovered in 1997 by Laurence A. Cole PhD, the author of this review [7]. While looking at the carbohydrate structure of hCG it was very apparent that two separate molecules of different molecular weights that were present in pregnancy urine, molecular weights 36,100 and 39,000 [6,7]. Hyperglycosylated hCG had an identical α and β-subunit amino acid sequence to the hormone hCG, identical N-linked sugar structures [7,8-10], but at least 3 of 4 very different O-linked carbohydrate side chains [7,8-10]. This is the carbohydrate side chains on the 145 amino acid β-subunit C-terminal peptide at β121, β127, β132 and β138. It is difference in these carbohydrate side chains that causes hyperglycosylated hCG to fold very differently to hCG, so that hCG is a hormone, and hyperglycosylated hCG an autocrine or cytokine binding a transforming growth factor-β-II (TGF-β-II) receptor. hCG has four type 1 O-linked oligosaccharides, all of which are trisaccharides and tetrasaccharides. Hyperglycosylated hCG

has 3 or 4 type 2 O-linked oligosaccharides, all of which are pentasaccharides and hexasaccharides [7,8-10].

While the hormone hCG has essential functions in forming the hemochorial placentation fetal feeding apparatus, it also promotes progesterone production by the ovarian corpus luteum, suppresses fetal contractions during pregnancy, and suppresses maternal macrophage attack on fetal and placental tissues, immunologically foreign tissues. The hormone hCG is also found as pituitary placental hCG, and as the fetal hormone hCG. In the pituitary it function to control ovarian steroidogenesis, ovarian follicular growth, oocyte ovulation and luteogenesis [11]. In the fetus during pregnancy it controls fetal organ growth and organ development [12,13].

Pregnancy Hyperglycosylated hCG has critical functions in pregnancy driving blastocyst implantation [14,15], and in deep implanting hemochorial placentation [16], and in promoting growth of the placenta as pregnancy advances [17]. It also has critical functions as cancer hyperglycosylated hCG and cancer hyperglycosylated hCG free ß-subnit in human cancers, where it uses a mechanisms parallel to blastocyst implantation to drive malignancy or viciousness of cancer [18,19]. As described in this review, ovarian hyperglycosylated hCG also has a function in ovarian ovulation, driving a hole in the follicle and in the ovary to permit ovulation to occur [20].

Hyperglycosylated hCG is secreted by trophoblast cells in vesicles by endocytosis. Secreted molecules are autocrines acting on a TGF-β-II receptor on the same cell surface [21-23]. In simple autocrine pathway, all of the minuscule amount secreted acts on the receptor, in complex autocrine pathways, the higher concentrations of hyperglycosylated hCG circulate before acting on the autocrine receptor [24-26].

Figure 1 shows the three dimensional structure of the hormone hCG and of the TGF-ß autocrine or autocrine hyperglycosylated hCG. These are unpublished models created by Butler SA from X-ray crystallography data on hCG and thermodynamic computer models of the remaining structure [27-30]. The principal difference between the two different forms of hCG are the ß-subunit C-terminal peptides (ß110-145) (Figure 1). On the hormone hCG the Type 1 O-linked sugar structures cause the C-terminal peptide to fold into loop ß40-58. This blocks nicking or cleavage of this loop. On hyperglycosylated hCG, the Type 2 O-linked O-lined oligosaccharides cause the C-terminal peptide to avoid this ß40-58 loop and to not block nicking or cleavage of this loop.

The result of this tiny structural difference between the hormone hCG and the autocrine hyperglycosylated hCG, is that hyperglycosylated hCG, but note the hormone hCG is nicked or cleaved at β47-48 by leukocyte elastase immediately upon secretion. The nicked autocrine is rapidly dissociated because of the cleavage to release a nicked hyperglycosylated hCG separated β-subunit. This is the molecule which acts on the TGF-β-II receptor (Figure 2) [21-23]. Nicked hyperglycosylated hCG ß-subunit binds the TGF-ß-II receptor through the fingers on the dissociated ß-subunit not present on α-ß subunit dimers (Figure 1) [9].

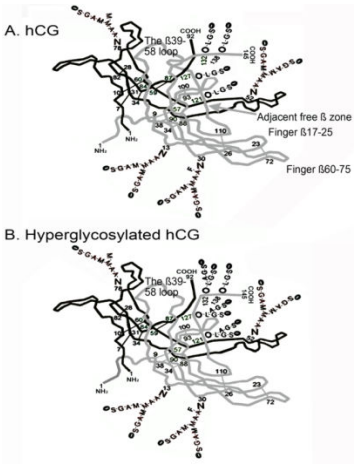

Figure 1: The three dimensional structure of the hormone hCG and the autocrine hyperglycosylated hCG, as shown by Butler SA.

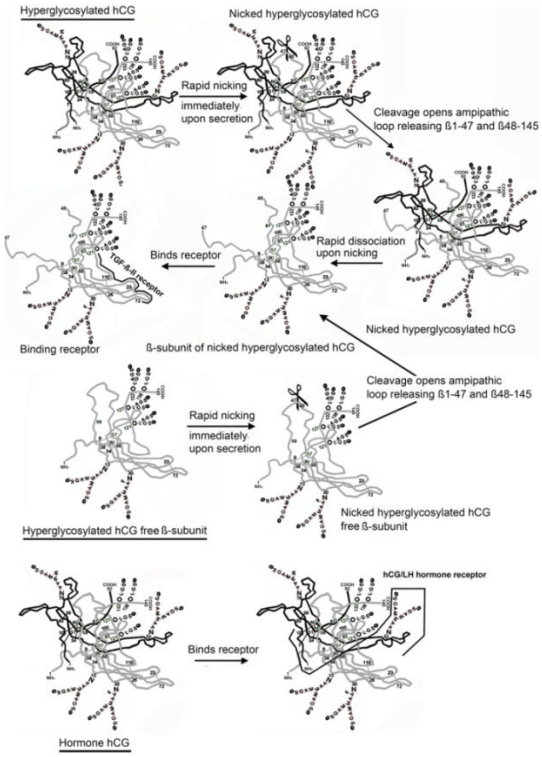

Figure 2: The processing of hyperglycosylated hCG before binding the TGF-β-II receptor.

The result is that we have two very separate independent molecules, the hormone hCG, which is an intact αß dimer, and the completely different structure nicked hyperglycosylated hCG ß-subunit. The hormone hCG acts on an hCG/LH joint hormone receptor, while the autocrine nicked hyperglycosylated hCG ß-subunit act on an ancestral TGF-ß-II receptor (Figure 2) [21-23].

All told, there appears to be seven semi-independent variants of the two major forms of hCG molecules, produced by different tissues and acting at different sites in the body (Table 1). Each taking on a hormone hCG or an autocrine hyperglycosylated hCG function. There is the placental hormone hCG, and the placental autocrine hyperglycosylated

hCG. These are the primary molecules. There is the pituitary hormone hCG, known structurally as placental sulfated hCG, and the fetal form of hCG known as the fetal hormone hCG. There is the ovarian hyperglycosylated hCG molecule and the cancer hyperglycosylated hCG molecules. Finally there is the hyperglycosylated hCG free ß-subunit produced by cancer cells (Table 1). Here I examine the literature today regarding all these variants of hCG to complete the hCG story.

I then examine the hCG assay, the pregnancy test, as used today commercially and what it detects and does not detect, and then examine false positive pregnancy tests or false-positive hCG tests. I then review the role that the two major pregnancy forms of hCG played in the development and evolution of humans, a very critical function. I also review what this can tell us about our development and the future of humans. Finally, I review the synthesis of hCG molecules, and the degradation of hCG molecules to make this review complete.

Table 1: The 7 semi-independent variants of the hormone hyperglycosylated hCG and the autocrine hyper-glycosylated hCG . MW is molecular weight, SO4 is sulfated sugars.

Parameter	Placental Hormone hCG	Placental Hyper-glycosylated hCG	Pituitary Sulfated	Cancer hyper-glycosylated hCG	Cancer hyperglycosylated free ß-subunit	Ovarian hyper-glycosylated hCG	Fetal hCG
Source cells	Syncytio-trophoblast cells	Cytotrophoblast cells	Pituitary Gonadotrope cells	Trophoblastic malignancy cells	Non-trophoblastic cancer malignancy cells	Ovarian Theca cell	Fetal kidney & liver cells
Mode of action	Endocrine	Autocrine	Endocrine	Autocrine	Autocrine	Autocrine	Endocrine
Total MW	36525	39149	35943	40461	26271	Not determined	Not determined
Site of action	LH/hCG receptor	TGFß antagonism	LH/hCG receptor	TGFß antagonism	TGFß antagonism	Ovarian follicle	Fetal organ
Amino acids α-subunit	92	92	92	92	92	92	92
Amino acids ß-subunit	145	145	145	145	145	145	145
Peptide MW	25813	25813	25813	25813	15543	Not determined	Not determined
O-linked sugar units	4	4	4	4	4	4	4
Type O-linked sugars sugars	Type 1	Type 2	Type 1 + SO	Type 2	Type 2	Not determined	Not determined
N-linked sugar units	4	4	4	4	2	4	4
Type N-linked sugars	Biantennary	Biantennary	Biantennary + SO	Triantennary ß	Triantennary	Not determined	Not determined
Sugar side chain MW	10712	13336	10130	14648	10728	Not determined	Not determined
Percentage sugars	0.29	0.34	0.28	0.36	0.41	Not determined	Not determined

Placental Hormone hCG

The placental hormone hCG is the original form of hCG as discovered by Aschner, Fellner, and Ascheim and Zondek at the turn of the twentieth century [1-4]. It is a hormone produced by placental syncytiotrophoblast cells, acting on placental cytotrophoblast cells, maternal macrophages, maternal muscular cells and maternal gonad luteal cells. The size of the hormone hCG including carbohydrate side structures is molecular weight 36,525, carbohydrate comprising 29% of the composition (Table 1).

The hormone acts on a joint LH/hCG hormone receptor that functions through promotion of cAMP. The principal function of the hormone hCG is to drive growth and maintenance of hemochorial placentation, the fetal feeding system during pregnancy. (Figure 3) illustrates the human hemochorial placentation system. Human placenta contain 4-6 hemochorial placentation chambers.

In synthesis of hemochorial placentation chambers, placental hyperglycosylated hCG first drives growth of a tree-like structure of cytotrophoblast cells [17]. These are then fused by the action of placental hormone hCG to form a single cell thick skin of syncytiotrophoblast cells surrounding the cytotrophoblast cells. These are the villus trophoblast cells (Figure 3). Placental hormone hCG then promotes growth of maternal uterine spiral arteries to reach the hemochorial placentation chambers [31-33]. It also promotes growth of the umbilical circulation to link the villus structures with the fetal

circulation (Figure 3) [34-36]. Hemochorial placentation chambers are complete around the 10th week of gestation, and then are deep implanted in the uterus.

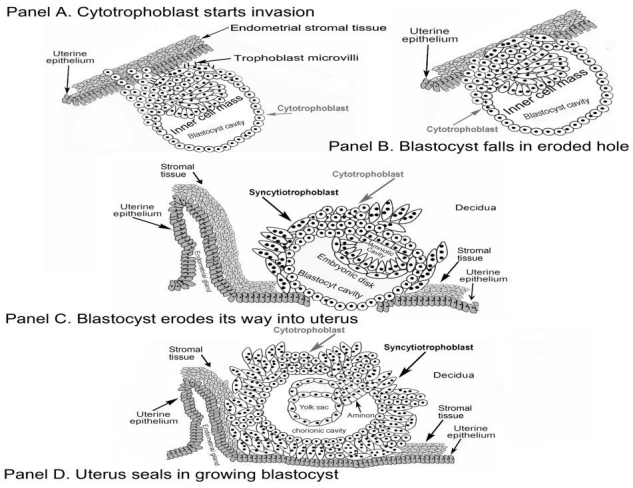

Figure 3: Human hemochorial placentation during pregnancy.

In functioning, hemochorial placentation chambers fill with maternal blood. Oxygen, glucose and essential nutrients passage from the maternal blood across the single layer of syncytiotrophoblast skin and into the villus chamber (Figure 3). They passage from here into the umbilical circulation and into the fetus.

Other functions of the placental hormone hCG include tempering of blastocyst implantation, the first function in pregnancy [14,15]. Promoting ovarian luteal cells to produce progesterone at 4 to 7 weeks gestation, when menstrual cycle pituitary LH is depleted [37]. The placental hormone hCG also suppresses myometrial muscular contractions during pregnancy [38,39]. It also It promotes formation of macrophage migration inhibitory factor to protect fetal and placental tissues from maternal macrophages during pregnancy [40,41].

Table 2: Concentration of total hCG and hyperglycosylated hCG (hCG-H) in 496 serum samples from 310 women with term pregnancies measured using the Siemens Immulite 1000 total hCG assay. Data from 50 pregnancies that failed due to miscarriage were excluded from this table.

Gestation age (weeks since start of menstrual period)	N	Median Total hCG	Range Total hCG	Median hCG-H	Range hCG-H	hCG-H %
3-weeks	n=42	0.26	0.04 – 5.5	0.2	0.01–6.45 (645X)	87%
4 weeks	n=42	3.4	0.21–173 (824X)	2.5	0.18–160 (888X)	51%
5 weeks	n=67	65	1.86–1308 (704X)	8.6	0.96–698 (731X)	43%
6-weeks	n=29	252	3.80–855 (225X)	86	0.76–629 (827X)	36%
7 weeks	n=30	3278	203–7,766 (38X)	359	27–931 (34X)	16%
8 weeks	n=33	4331	1,064–10,057 (9.4X)	386	67–1050 (15.6X)	7%
9 weeks	n = 24	5832	1,031 – 11,586 (11.2X)	430	102–1158 (11.3X)	5%
10 weeks	n=20	10352	1,952–19,958 (10.2X)	521	188–1855 (9.9X)	4%
11 - 13 weeks	n=41	5953	1,440–15,318 (10.6X)	137	24–330 (13.7X)	2%
14 - 17 weeks	n=57	2934	311–4,757 (15.2X)	26	6.7–129 (19.3X)	1%
18 - 26-weeks	n=62	1931	210–6,223 (30.3X)	15.8	5.3–95 (17.9X)	1%
27 - 40 weeks	n=49	1911	184–8,530 (46.4X)	2.95	0.3–12.2 (40.6X)	0%

The placental hormone hCG marks a person as being pregnant, the hCG test being the pregnancy test. Many physicians call hCG "the pregnancy hormone", placental hormone hCG founding some of the basic commodities of pregnancy (Table 2) shows the concentration of total hCG (placental hormone hCG + placental hyperglycosylated hCG) and placental hyperglycosylated hCG (there is no specific immunoassay for placental hormone hCG) in serum samples during the course of pregnancy.

Placental Autocrine Hyperglycosylated hCG

The placental autocrine hyperglycosylated hCG is seemingly the first hCG molecule functioning during pregnancy, driving implantation of the blastocyst of pregnancy. The placental autocrine hyperglycosylated hCG can be detected in serum and urine as early as two days before pregnancy implantation [14,15]. In driving implantation of the blastocyst (Figure 4) cytotrophoblast cells of the blastocyst produce hyperglycosylated hCG, which acts through a TGF-ß-II receptor to promote metalloproteinase and collagenase secretion by the blastocyst cells. This erodes uterine cells

permitting implantation to occur (Figure 4). Implantation is tempered by the placental hormone hCG, diminishing implantation [14,15]. Placental hyperglycosylated hCG also promotes growth of the implanting blastocyst (Figure 4). The size of placental hyperglycosylated hCG is molecular weight 39,149, with sugars accounting for 34% of the composition.

Under the promotion of placental hyperglycosylated hCG the growing implanted blastocyst cytotrophoblast cells develop arms. The arms grow out to form the hemochorial placentation tree-shaped villus tissue (Figure 5). These form over multiple weeks the root villus structure of the hemochorial placentation chambers (Figure 3).

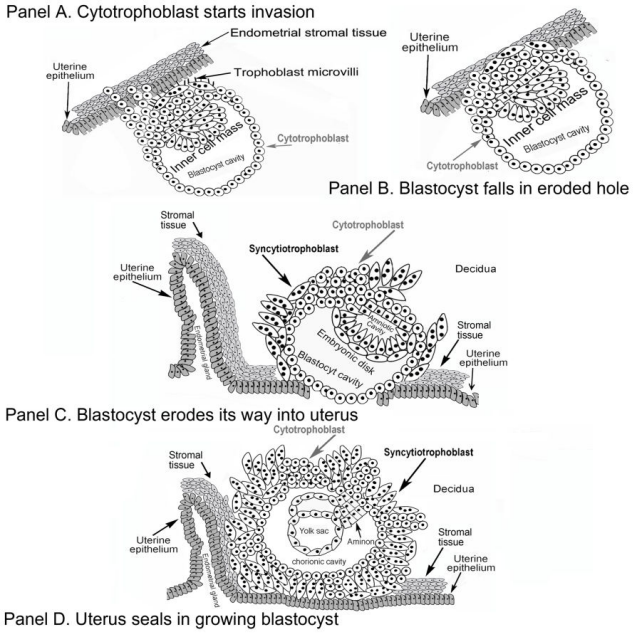

Figure 4: Implantation of the blastocyst.

Placental hyperglycosylated hCG also deep implants the hemochorial placentation structure, once again using hyperglycosylated hCG driven metalloproteinases and collagenases acion [16]. Placental hyperglycosylated hCG also has the overall responsibility of promoting placental growth as the fetus expands in pregnancy [17].

Deficiency of placental hyperglycosylated hCG is a common problem with the autocrine synthesis of placental hyperglycosylated hCG. In the U.S.A. 42% of pregnancies are failures, 25% of pregnancies become biochemical pregnancies, and 17% of pregnancies are spontaneous abortions or miscarriage [42-44]. This is due to failure of pregnancies to implant the blastocyst completely [42-44]. Further examination of this field shows that it actually is due to incomplete action of placental hyperglycosylated hCG (Figure 6) [14,15,45,46].

As shown in Figure 6, term childbirth outcome pregnancies produce a significant proportion hyperglycosylated hCG on the day of implantation, 111 of 111 produced >40% hyperglycosylated hCG. In contrast, 19 of 27 spontaneous abortion or miscarried pregnancies produced <40% hyperglycosylated hCG on the day of implantation, and 38 of 46 biochemical pregnancies produce <40% hyperglycosylated hCG on the day of implantation (Figure 6). The 8+8 pregnancies that did produce >40% hyperglycosylated hCG were later shown to be gross genetic abnormalities. Clearly, a deficiency of hyperglycosylated hCG causes those 42% of pregnancy failures.

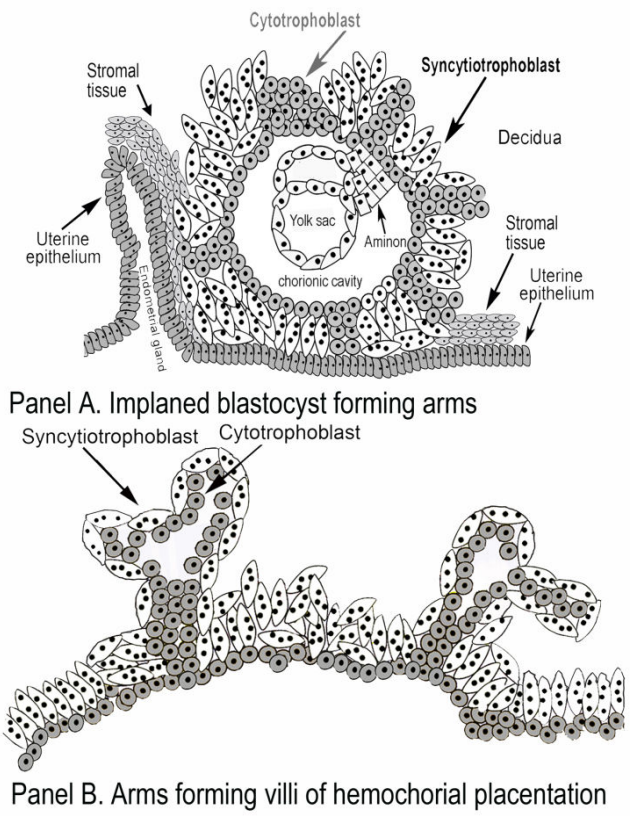

Figure 5: Blastocyst forming arms of cytotrophoblast tissue.

As shown, (Figure 7), a deficiency of hyperglycosylated hCG also cause failure of deep implantation at 10-18 weeks gestation. During evolution hyperglycosylated CG correspond drove deep implantation [47-49]. With increase in the acidity of the hormone hCG and the autocrine hyperglycosylated hCG, hemochorial placentation deep evolution implanted at 1% uterine thickness in anthropoid primates, and deeper at 10% in orangutan and 30% in humans [47-50]. Hyperglycosylated hCG drove invasive implantation, as such deep evolution must be driven by placental hyperglycosylated hCG. (Figure 7) shows the hyperglycosylated hCG production at the time of deep implantation, 10-18 weeks of gestation [16-50].

As shown, (Figure 7), the majority of preeclampsia and gestational hypertension cases produce exceptionally low concentrations of pregnancy hyperglycosylated hCG, less than the 3rd percentile or <3%. It is inferred that placental hyperglycosylated hCG deficiency at this time leads to incomplete deep implantation. This leads to failure of fetal feeding and preeclampsia and gestational hypertension [16].

All told, placental hyperglycosylated hCG appears to be the tissue growth and tissue invasion promoter of pregnancy. Hyperglycosylated hCG works alongside the hormone hCG in setting up and regulating pregnancy. (Table 2) shows the concentration of total hCG (placental hormone hCG +

placental hyperglycosylated hCG) and placental hyperglycosylated hCG (there is no specific immunoassay for placental hormone hCG) in serum samples during the course of pregnancy.

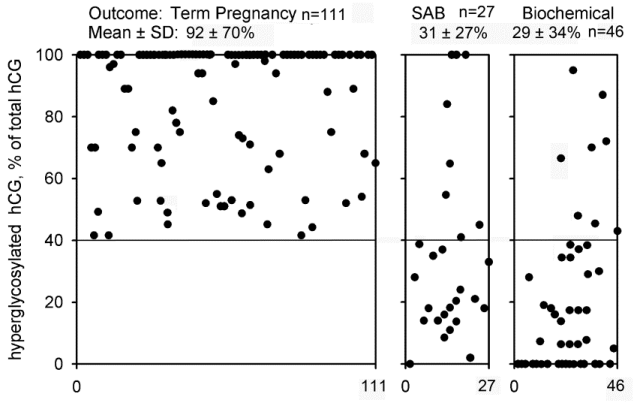

Figure 6: Placental hyperglycosylated hCG and pregnancy failure.

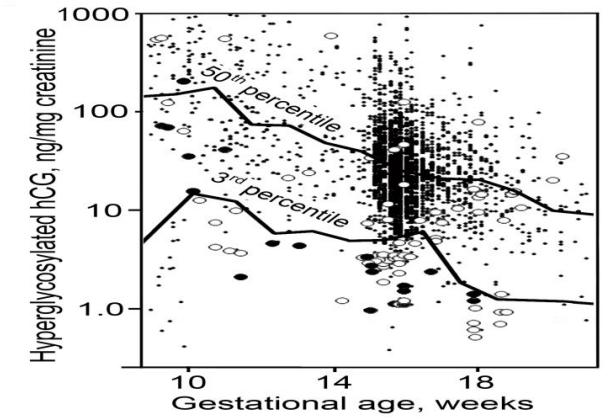

Figure 7: Placental hyperglycosylated hCG and deep implantation failure. Black circle mark gestation hypertension cases, open circles mark preeclampsia cases and small black mark show controls.

Pituitary sulfated hCG

hCG evolved from a deletion mutation in the ß-subunit of LH. LH is a pituitary gonadotrope cell hormone. hCG should also therefore be primarily a pituitary gonadotrope hormone. hCG was turned on in pituitary gonadotrope cells by hypothalamic gonadotropin releasing hormone (GnRH), the signal which originally turned on LH. The problem was that placental hormone hCG was also turned on by a placenta form of GnRH [51].

hCG is produced in low concentrations (0.1–10 mIU/ml) by pituitary gonadotrope cells during the menstrual cycle [11,52-55]. It is called pituitary sulfated hCG because the sulfated side chains terminate in a sulfated N-acetylgalactosamine residues [11]. These reduce the acidity of hCG and 50% reduce the biological potency [11].

It is believed that pituitary sulfated hCG supplement and guarantees LH biological activity. Pituitary sulfated hCG taking over enhancing ovulation and enhancing steroidogenesis, ovulation and luteogenesis whenever LH production is insufficient. Placental hCG is approximately 108-fold more potent than LH because of its long circulating half-life. Both hCG and LH act on the same joint LH/hCG receptor [20].

Studies in my laboratory investigated pituitary sulfate hCG function [20]. Table 3 marks 111 women achieving pregnancy. The table shows the concentrations of LH and hormone hCG on the day of LH peak. The urine LH peak concentration and the hormone hCG concentration (total hCG minus hyperglycosylated hCG) were determined at the time of the LH peak in the menstrual cycle immediately preceding a pregnancy. It is inferred that ovulation had to have occurred in this cycle or pregnancy could not occur [20].

If one considers a low urine LH peak being <23 mIU/ml as deficient LH in urine (serum equivalence of 4.6 mIU/ml LH), too low to have driven ovulation, could pituitary sulfated hCG have substituted, and driven ovulation [20]. Looking at (Table 3) case 6, LH peak=6.8 mIU/ml, pituitary sulfated hCG=0.15 ng/ml (equivalent considering 108 fold x50% difference to 89 mIU/ml LH), could pituitary sulfated hCG have substituted for deficient LH in this case? Looking at case 9, LH peak=22 mIU/ml, pituitary sulfated hCG=0.10 ng/ml (equivalent to 59 mIU/ml LH), could pituitary sulfated hCG have substituted for deficient LH in this case? Looking at case 29, LH 15 mIU/ml, pituitary sulfated hCG=0.22 ng/ml (equivalent to 130 mIU/ml LH), could pituitary sulfated hCG have substituted for deficient LH in this case? Looking at case 43, LH peak=19 mIU/ml, pituitary sulfated hCG=0.10 ng/ml (equivalent to 59 mIU/ml LH) indicating once again that pituitary sulfated substituted for deficient LH.

Looking at case 47, LH peak=7.1 mIU/ml, pituitary sulfated hCG=0.11 ng/ml (equivalent to 65 mIU/ml LH), could pituitary sulfated hCG have substituted for deficient LH in this case? Looking at case looking at case 49, LH peak=4.7 mIU/ml, pituitary sulfated hCG=0.15 ng/ml (equivalent to 85 mIU/ml LH), could pituitary sulfated hCG have substituted for deficient LH in this case; looking at case 53, LH peak=5.2 mIU/ml, pituitary sulfated hCG=0.40 ng/ml (equivalent to 238 mIU/ml LH), could pituitary sulfated hCG have substituted for deficient LH in this case; looking at case 60, LH peak 22 mIU/ml, pituitary sulfated hCG 0.75 ng/ml (equivalent to 446 mIU/ml LH); looking at case 78, LH peak=3.9 mIU/ml, pituitary sulfated hCG=0.07 ng/ml (equivalent to 41 mIU/ml LH) could pituitary sulfated hCG have substituted for deficient LH in this case; looking at case 79, LH peak=22 mIU/ml, pituitary sulfated hCG=0.03 ng/ml (equivalent to18 mIU/ml LH), could pituitary sulfated hCG has substituted for deficient LH in this case; looking at case 62, LH peak=2.9 mIU/ml, pituitary sulfated hCG=0.05 ng/ml (equivalent to 30 mIU/ml LH), could pituitary sulfated hCG have substituted for deficient LH in this case; looking at case 66, LH peak=17 mIU/ml, pituitary sulfated hCG=0.07 ng/ml (equivalent to 41 mIU/ml LH); looking at case 111, LH peak=19 mIU/ml, pituitary sulfated hCG=0.07 ng/ml (equivalent to 41 mIU/ml LH) [20].

Again results show that in a least 13 of 111 (12%) cases that pituitary sulfated hCG substituted for deficient LH, and was seemingly responsible for ovulation [20]. This shows that

pituitary sulfated hCG was important, at least as an LH stand-by (Table 3). It is assumed that in the other 98 cases that pituitary sulfated hCG supplemented LH in achieving ovulation (except in cases 44, 65, 90 and 94 where clearly pituitary sulfated hCG is deficient, <1.0 mIU/ml). It is concluded, that in approximately 12% of cases, pituitary sulfated hCG substitutes for LH in ovulation.

Table 3: One hundred and eleven women achieving pregnancy, peak LH, and hormone hCG at LH peak in cycle immediately preceding pregnancy

Case	Day of LH peak	Conc. LH mIU/ml	Conc. HCG ng/ml	Case	Day of LH peak	Conc. LH mIU/ml	Conc. HCG ng/ml	Case	Day of LH	Conc. LH mIU/ml	Conc.
1	13	197	0.02	38	17	119	0.05	75	17	74	0.07
2	14	29	0.05	39	14	81	0.06	76	14	136	0.01
3	12	78	0.03	40	14	272	0.03	77	13	164	0.02
4	12	93	0.07	41	15	144	0.01	78	14	3.9	0.07
5	12	110	0.02	42	17	275	0	79	16	22	0.03
6	17	6.8	0.06	43	14	19	0.02	80	13	124	0.03
7	14	143	0.02	44	17	109	0.09	81	17	99	0.06
8	17	56	0.06	45	12	180	0.03	82	15	122	0.07
9	17	22	0.02	46	14	169	0.01	83	12	42	0.05
10	14	187	0.02	47	14	7.1	0.07	84	14	82	0.06
11	14	111	0.04	48	16	134	0.06	85	14	82	0.06
12	13	197	0.03	49	14	4.7	0.07	86	15	109	0.05
13	13	101	0.03	50	14	174	<	87	15	195	0.03
14	16	336	0.04	51	14	117	0.02	88	14	112	0.02
15	13	32	0.06	52	13	517	0.23	89	15	160	0
16	17	43	0.06	53	14	5.2	0.12	90	14	185	0.02
17	13	112	0.15	54	16	242	0.01	91	14	187	0.02
18	13	270	0.02	55	17	126	0.02	92	12	380	0.01
19	14	156	0	56	14	490	0.07	93	14	84	0.02
20	13	152	0.42	57	14	109	0.03	94	14	138	0.04
21	17	99	0.01	58	14	122	0.06	95	16	137	0.01
22	13	238	0.07	59	12	127	0.04	96	12	110	0.01
23	12	110	0.01	60	15	22	0.75	97	14	174	0,02
24	15	64	0.06	61	14	121	0.05	98	14	459	0.01
25	13	47	0.01	62	14	2.9	0.05	99	14	183	0.03
26	17	76	0.06	63	14	258	0.08	100	14	61	0.08
27	17	134	0.01	64	14	333	0.02	101	14	165	0.01
28	14	200	0.02	65	13	123	<	102	17	376	0.03
29	14	15	0.03	66	17	17	0.07	103	14	84	0.02
30	16	108	0.07	67	14	104	0.01	104	17	52	0.02
31	14	111	0.05	68	14	43	0.04	105	14	160	0.03
32	14	138	0.04	69	17	75	0	106	14	90	0.06
33	14	160	0.03	70	14	297	0.02	107	14	99	0.06
34	14	88	0.01	71	14	116	0.02	108	14	179	0.01

35	15	120	0.04	72	14	84	0.04	109	16	331	0.01
36	14	110	0.05	73	12	197	0.03	110	14	209	0.05
37	13	56	0.02	74	15	223	0.05	111	14	19	0.07

Fetal Hormone hCG

Four independent research groups [12,13,55,56] have demonstrated that a variant of hCG is independently produced in the pregnancy fetus, by fetal liver and fetal kidney cells. It was demonstrated that this variant of hCG controls fetal organ growth and development. It was also demonstrated that production of fetal hormone hCG is halted at parturition [12,13,55,56].

Fetal research is today highly controlled and limited by ethics boards. Today there is no information on the structure and physical properties of fetal hCG, nor whether it is a hCG hormone or an autocrine like hyperglycosylated hCG. The USA hCG reference service discovered recently an inherited disorder of hCG production, Familial hCG Syndrome [57,58]. In this syndrome people produce a mutant forms of hCG free ß-subunit possible produced by the liver or kidney. It is thought that this syndrome may derive from fetal hCG or be the continued production of fetal hCG during a life time.

Ovarian Autocrine Hyperglycosylated hCG

Just my laboratory has discovered ovarian autocrine hyperglycosylated hCG, we await other laboratories to test for it and confirm our findings [20]. Basically a tiny peak of hyperglycosylated hCG was detected (Figure 8), about 1.5 ng/ml at the time of the LH peak in every menstrual cycle. This low concentration may limit discovery by others. The final step of ovulation is that metalloproteinases and collagenase make holes in the ovary and the follicle to permit oocyte ovulation. It is inferred since hyperglycosylated hCG drives such proteolytic actions in implantation and deep implantation, that this is driven by a theca cells hyperglycosylated hCG (Figure 8). This has to be confirmed.

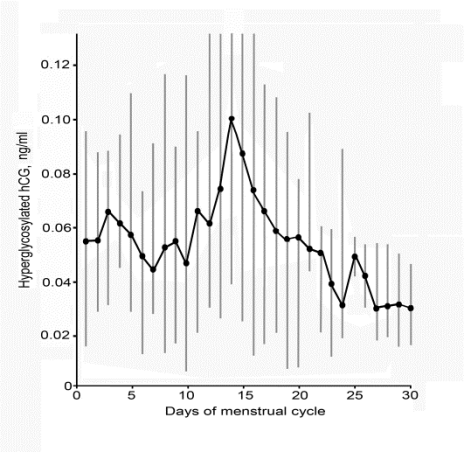

Figure 8: The mid-menstrual cycle peak of hyperglycosylated hCG, mean of 42 cycles. Bars shows range of concentrations.

Cancer Autocrine Hyperglycosylated hCG and Cancer Hyperglycosylated hCG free ß-subunit

My original discovery of hyperglycosylated hCG in 1997 was a discovery of cancer hyperglycosylated hCG produced by choriocarcinoma cells [7]. Choriocarcinoma cells produced a form of hCG which promoted cancer cell growth, blocked choriocarcinoma apoptosis and promoted cancer cell invasion of other cells, malignancy processes, that was clearly independent of the hormone hCG [7,18].

As found, trophoblastic cancers (choriocarcinoma, ovarian germ cell malignancy, testicular germ cell malignancy) produced hyperglycosylated hCG αß subunit dimer, while all other cancers make a hyperglycosylated hCG free ß-subunit composed of only the ß-subunit of hyperglycosylated hCG. As shown by Beebe et al. [59], a simple explanation describes why non-trophoblastic cancer only produces a free ß-subunit. A trophoblast cell and pituitary cell disulfide isomerase adds the final two disulfide bonds, ß93-ß100 and ß26-ß110. In the absence of this enzyme and the absence of these disulfide bridges ß-subunit is not recognized by α-subunit for combination and only a free ß-subunit is made [59]. This happens with ectopic production of hCG by non-trophoblastic cancers. The TGF-ß-II receptor is antagonized by either hyperglycosylated hCG or the cleaved hyperglycosylated hCG free ß-subunit [18,60].

Hyperglycosylated hCG and its free ß-subunit are useful tumor markers for cancer. They are best measured as their urinary degradation product, ß-core fragment [61-65]. Table 4 shows a ten year tumor marker study completed by the author at Yale University [61-65]. As shown, the tumor marker urine ß-core fragment detected 44% of a wide range of 2167 very different

cancer cases. I asked why did it only detect 44% of cases, did the other 56% of cases not express the hCG ß-subunit gene?

To investigate the issue a super-sensitive ß-core fragment assay was developed (sensitivity ≥ 0.1 fmol/ml). ß-core fragment is the terminal degradation product of hyperglycosylated hCG and its ß-subunit. New cancer cases were identified, n=56. As shown, all cancer cases, no exceptions, actually produced ß-core fragment. In 17 cases they just produced miniscule quantities of tumor marker ß-core fragment (<3 fmol/ml).

Table 4: Urine degradation products hyperglycosylated hCG ß-subunit + ß-core fragment (B204 assay) as tumor markers.

Source	**Cut-off >3 fmol/ml**		
	#Cases	#Positive	Sensitivity
A. Trophoblastic malignancies			
Choriocarcinoma	63	63	100%
Ovarian germ cell cancer	11	11	100%
Testicular germ cell cancer	17	17	100%
Total	110	110	100%
B. Non-trophoblastic malignancy			
Bladder cancer	140	62	44%
Breast cancer	456	156	34%
Cervical cancer	410	197	48%
Colorectal cancer	80	29	36%
Endometrial cancer	233	103	44%
Gastric cancer	205	90	44%
Hepatic cancer	46	21	44%
Lung cancer	154	38	25%
Intestinal cancer	17	8	47%
Lymphoma	41	13	32%
Ovarian cancer	207	145	70%
Pancreatic cancer	29	16	55%
Prostate cancer	12	9	75%
Renal cancer	66	32	48%
Uterine cancer	63	26	41%
Vulvar cancer	8	4	50%
Total	2167	949	44%
C. Healthy			
NED, post cancer chemotherapy	33	2	6%
NED, post cancer surgery	21	1	5%
Healthy female, no cancer history	72	2	3%
Healthy male, no cancer history	28	1	4%
Total	154	6	4%
D. Benign Disease			
Benign gynecological lesion, tumor	28	0	0%
Benign lung lesion	4	0	0%

Follicular ovarian cyst, benign	67	1	1%
Benign ovarian cyst, non-functional	26	0	0%
Benign prostate hyperplasia	8	0	0%
Cervical carcinoma in-situ	12	0	0%
Cervical dyskaryosis	66	2	3%
Condyloma	30	0	0%
Endometriosis	16	1	6%
Myoma	27	3	11%
Total	284	7	3%

This showed that in reality 100% of cancer expressed hyperglycosylated hCG or hyperglycosylated hCG free ß-subunit. The studies of Acevedo et al. [66] and Regelson [67] used a double antibody method with flow cytometry techniques to examine the expression of hCG ß-subunit by cancer cells. They detected that 100% of cancer cells express the ß-subunit gene. This very much confirms our combined conclusion.

Seemingly, some cancers produce miniscule amounts (e.g. 0.1-3.0 fmol/ml) of ß-subunit as simple autocrines (TGF-ß autocrines secreted and act directly on the TGF-ß-II receptor), while other cancer produce higher concentrations (3.0–54 fmol/ml) that act as complex autocrines (TGF-ß autocrines that are secreted, circulate and then acts back on cellular TGF-ß-II receptor, according to TGF-ß simple an complex autocrine models [24-26].

In the last 10 years nine independent groups have each shown that hyperglycosylated hCG and hyperglycosylated hCG free ß-subunit feeds back to the cancer cell and directly promotes cancer cell growth, blocks cancer cell apoptosis and promotes cancer cell invasion, the properties of malignancy [19,21-23,65,68-74]. Shown in Figure 9 is my experience using hyperglycosylated hCG and hyperglycosylated hCG ß-subunit to enhance the growth of cancers. As shown hyperglycosylated hCG and hyperglycosylated hCG ß-subunit significantly enhanced the growth of 7 of 7 cancers, or of all cancer tested.

It is our own belief, studying all this research, that hyperglycosylated hCG and its free ß-subunit are the actual signals in all cancer cells that promotes malignancy in all human cancers [65]. As shown in (Table 4), ß-core fragment is virtually not detected in benign diseases (detection=3.0%), but is probably present in 100% of active cancers. The presence of hyperglycosylated hCG and its free ß-subunit in benign and normal tissues seemingly institutes a malignancy. The exception being pregnancy, where hyperglycosylated hCG has clear normal functions in tissue growth and implantation (Table 5).

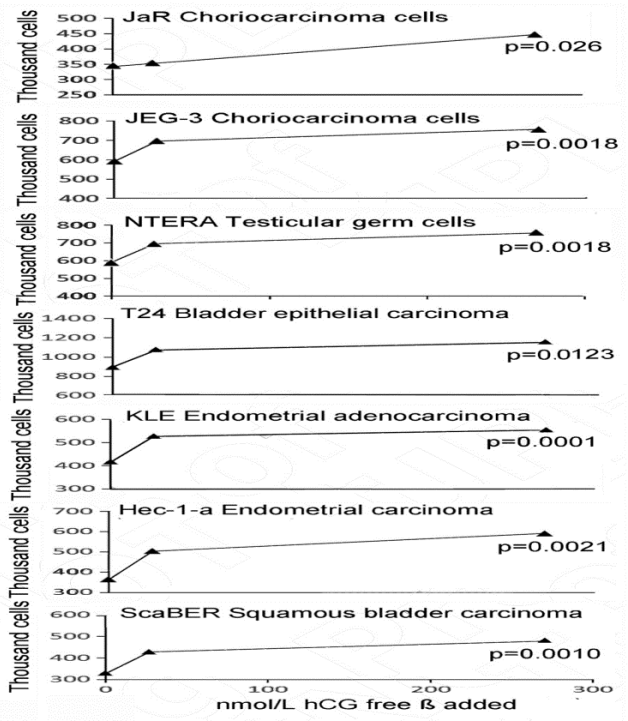

Figure 9: The use of hyperglycosylated hCG (JaR, JEG-3 and NTERA) and hyperglycosylated hCG ß-subunit to enhance the growth of cancers.

Table 5: Examination of new ovarian cancer cases, (n=56) using extra-sensitive urine ß-core fragment (B204 assay) assay (sensitivity >0.1 fmol/ml).

Pathologic Diagnosis	Stage	Status	ß-core fragment >0.1 fmol/ml
Ovarian Endometrioid carcinoma	IV	Persistent	0.12
Ovarian Endometrioid carcinoma	IV	New, not treated	0.15
Ovarian Serous cystadenocarcinoma	IIIc	New, not treated	0.2

Ovarian Serous cystadenocarcinoma	IIIc	Persistent	0.2
Ovarian Serous cystadenocarcinoma	IIIc	Recurrent	0.25
Ovarian Serous cystadenocarcinoma	IV	Recurrent	0.4
Ovarian Serous cystadenocarcinoma	IV	Recurrent	0.4
Ovarian Brenner tumor	Ia	New, not treated	0.58
Ovarian Serous cystadeno carcinoma	IIIc	New, not treated	0.6
Ovarian Serous cystadenocarcinoma	IIIc	New	0.75
Ovarian Brenner tumor	Ia	New, not treated	0.88
Ovarian Serous cystadenocarcinoma	IIIc	Recurrent	1.2
Serous cystadeno carcinoma	II	Recurrent	1.6
Ovarian Serous cystadenocarcinoma	IIIc	Recurrent	2.1
Ovarian Endometrioid carcinoma	III	Recurrent	2,3
Ovarian Serous cystadenocarcinoma	IIIc	Persistent	2.5
Ovarian Mucinous carcinoma	Ia	New, not treated	2.9
Ovarian Endometrioid carcinoma	I	New, not treated	3.1
Ovarian Granulosa-theca cell malignancy	IIIc	Recurrent	3.1
Ovarian Serous cystadenocarcinoma	II	New, not treated	3.5
Ovarian Granulosa-theca cell malignancy	IIIc	Persisent	3.7
Ovarian Mucinous cystadenocarcinoma	III	Recurrent	4.2
Ovarian Serous cystadenocarcinoma	III	New	4.5
Ovarian Serous cystadenocarcinoma	III	New, not treated	4.3
Ovarian Clear cell carcinoma	IIIc	New, not treated	4.8
Ovarian Clear cell carcinoma	IIIc	Recurrent	5.5
Ovarian Serous cystadenocarcinoma	IIIc	New	6.3
Ovarian Serous cystadenocarcinoma	IIIc	New, not treated	6.6
Ovarian Mixed epithelial tumor	IIIc	New, not treated	7.1
Ovarian Mixed epithelial tumor	III	New	7.9
Ovarian Serous cystadenocarcinoma	IIIc	New, not treated	8.1
Ovarian Serous cystadenocarcinoma	IIIc	Persistent	8.8
Ovarian Serous cystadenocarcinoma	IIIc	New, not treated	9
Ovarian Serous cystadenocarcinoma	III	Persistent	9.5
Ovarian Mixed epithelial tumor	IIc	New, not treated	10
Ovarian Mixed epithelial tumour	IIb	New, not treated	11.3
Ovarian Serous cystadenocarcinoma	III	New	11.4
Ovarian Serous cystadenocarcinoma	IV	Recurrent	11.4
Ovarian Serous cystadenocarcinoma	IV	Recurrent	12
Ovarian Serous cystadenocarcinoma	IIIc	Recurrent	12
Ovarian Serous cystadenocarcinoma	IIIc	Recurrent	12
Ovarian Endometrioid carcinoma	IIc	New, not treated	12.2

Ovarian Endometrioid carcinoma	III	Persistent	13.1
Ovarian Mixed mesodermal carcinoma	III	Recurrent	14.4
Ovarian Mixed mesodermal carcinoma	III	Recurrent	16.3
Ovarian Serous cystadenocarcinoma	IV	Recurrent	16.9
Ovarian Serous cystadenocarcinoma	IV	Recurrent	17.5
Ovarian Serous cystadenocarcinoma	IIIc	New, not treated	18.8
Ovarian Serous cystadenocarcinoma	IIIc	Persistent	20
Ovarian Mixed epithelial tumor	IIIc	Recurrent	20
Ovarian Mixed epithelial tumor	III	Recurrent	21
Ovarian Serous cystadenocarcinoma	IV	New, not treated	28
Ovarian Serous cystadenocarcinoma	IV	New, not treated	29
Ovarian Serous cystadenocarcinoma	III	New	32
Ovarian Serous cystadenocarcinoma	IIb	New, not treated	41
Ovarian Malignant dermoid cyst/teratoma	IIb	New	54

hCG assay

hCG today marks all pregnancies, the total hCG assay is the pregnancy test. It is measured today in clinical laboratories by any of 12 completely automated immunometric assays. Literally, the blood samples is injected into one region of the automated machine, and 15-20 minutes later, the final results prints out a another region of the automated machine. These are the tests run by all hospital and clinical laboratories all over the world.

These automated assay range from the Abbott AxSym test, to the Abbott Architect test, to the Beckman Access, Beckman Dxi, Ortho Vitros, Roche Elecsys, Siemens Centaur, Siemens Dimension, Siemens Immulite, Siemens ACS180, Siemens Stratus and the Tosoh A1A test. Each company makes kits for using the machines to run a large range of assays. There is one other total hCG assay that is not tested here, the Perkin-Elmer Delfia test, this is currently not used in the U.S.A.

I have carefully evaluated the sensitivity and specificity of each commercial assay [75,76]. All use a generic antibody to the ß-subunit core structure, however, 11 of the 12 use an antibody to the ß-subunit C-terminal peptide as a second antibody or capture antibody. This is all tests except the Siemens Immulite, which use two separate antibodies to the ß-subunit core structure [75,76].

There are major problems with the ß-subunit C-terminal peptide as an hCG antigen. The ß-subunit C-terminal peptide is mostly carbohydrate, the four O-linked oligosaccharides by molecular weight, so using it as antigen forces carbohydrate specificity on the test. The 11 assays poorly detect carbohydrate variants of hCG, like hyperglycosylated hCG, cancer hyperglycosylated hCG, and pituitary sulfated hCG [75,76]. Because the ß-subunit C-terminal peptide can fold into the region nicked or cleaved on hCG, loop ß40-58 (Figure 1), nicking or cleavage of hCG can interfere with the assays detection. (Table 6) shows the complete specificity of these 12 hCG assays used in the U.S.A.

As shown in the complete specificity table (Table 6), the 11 assays using ß-subunit C-terminal peptide antibodies, poorly detected, 8%-81% detection, nicked hCG, hyperglycosylated hCG, nicked hyperglycosylated hCG and asialo hCG. They also do no detect at all nicked hCG missing the ß-subunit C-terminal peptide. When hCG, hyperglycosylated hCG and hyperglycosylated hCG free ß-subunit are secreted into serum, they are all normally nicked, dissociated into a free ß-subunit, and loose the ß-subunit C-terminal peptide. These are the normal intermediates present in blood. The result is that in the standard pregnant patient serum the assay may only detect 50% or less of the total hCG. This is completely unacceptable and inappropriate data for a physician to receive.

As the director of the USA hCG Reference Service I have written to the manufacturers of all 11 test devices that use a ß-subunit C-terminal peptide antibody, and complained bitterly about the inappropriateness of their assay and the problem created by the specificity of their assay. All 11 test manufacturers took no notice of my letter. Saying that the assay sells very well, that they receive no complaints about the assay from physicians or laboratories, so why should they change them. I have written back saying that 50% detection of pregnancy hCG is not acceptable. They all responded again saying that nobody is complaining. When a physician or a laboratory get a patient hCG result back of 10,000 mIU/ml, they have nothing that tells them that he assay is only detecting 50% of the hCG. The only method that could tell them that a

patient is producing much more hCG is to run the Siemens Immulite assay, which is almost never done.

Table 6: The specificity of today's automated immunometric total hCG assays .CTP is ß-subunit C-terminal peptide. Percentage values are the result in ng/ml expressed as a percentage of the actual concentration of the standard (determined by amino acid analysis). A poor detection, <75% or >125% of standard serum concentrations is shown by bold underlined numbers.

Calibrated standards	Abbott Architect	Abbott AxSYM	Beckman Access 2	Beckman DxI	Ortho Vitros	Roche Elecsys	Siemens ACS180	Siemens Centaur	Siemens Dimension	Siemens Immulite	Siemens Stratus	Tosoh A1A
hCG	96%	103%	103%	100%	112%	109%	105%	104%	96%	96%	92%	95%
Hyperglycosylated hCG	86%	86%	127%	98%	68%	78%	102%	81%	59%	103%	66%	97%
Nicked hCG	70%	99%	84%	71%	73%	69%	85%	66%	65%	108%	8%	ND
Nicked hCG missing CTP	0%	0%	0%	0%	0%	12%	0%	0%	10%	109%	28%	16%
Nicked hyperglycosylated hCG	40%	46%	46%	51%	80%	100%	70%	40%	80%	103%	88%	70%
Asialo hCG	35%	69%	48%	46%	85%	46%	81%	39%	65%	114%	73%	59%
hCGß	86%	90%	142%	136%	47%	102%	126%	47%	47%	111%	73%	66%
Nicked hCGß	33%	51%	56%	63%	19%	53%	72%	19%	41%	107%	70%	60%

The only assays that remains acceptable to the USA hCG Reference Service, that quantitatively and correctly measures hCG is the Siemens Immulite assay. This is today, unfortunately, one of the poorest selling assays in the USA today, almost nobody, or no commercial laboratory is using it. What a mess we have with poor hCG assays today.

There are specific assays for just measuring hyperglycosylated hCG (the B152 assay), for measuring intact hCG only (the Roche Intact hCG assay), nicked hCG only (the B151 assay), free ß-subunit only (FBT11 assay), and ß-core fragment only (B210 assay). There is no assay and no antibody that detects the hormone hCG only, pituitary sulfate hCG only, or cancer hyperglycosylated hCG or cancer hyperglycosylated hCG free ß-subunit only.

Quest Diagnostics owns the patent on the B152 assay, an assay that detects hyperglycosylated hCG and hyperglycosylated hCG free ß-subunit only. They offer a specific test for measurement of these molecules available at Quest Diagnostics Inc. Multiple manufacturers offer specific antibodies for detecting intact hCG only and free ß-subunit only, but that is it. I would be useful to have a commercial assay for pituitary sulfated hCG, but none is available.

Table 7 illustrates the problem with the 11 automated total hCG assays that have problems detecting nicked molecules, dissociated molecules, carbohydrate variant molecules and molecules missing the ß-subunit C-terminal peptide. Shown are 31 serum samples, primarily pregnancy serum samples, detected by the USA hCG Reference Service. As shown, overall, immunoassay results can vary by as much as 50%, with two different assays giving very widely variable results (Table 7). This is not acceptable. A patient may be tested for hCG in one physician's office, and then passed on to a second physician. Each physician may use a separate clinical laboratory that uses a different total hCG immunoassay. The patients serum hCG may go down instead of up, or up instead of down, because of the use of differing commercial assays. This can wrongly very much confuse or alarm physicians.

Table 7: USA hCG Reference Service samples, 31 cases, tested by multiple different commercial laboratories.

441.624	100%	Siemens Immulite	3100%	100%	Siemens Immulite
262.556	59%	Simens Centaur	2800%	90%	Siemens Dimension
292.136	66%	Siemens Dimension			
			1900%	100%	Simens Immulite
9.358	100%	Siemens Immulite	1400%	74%	Siemens Dimension
6.451	69%	Siemens Dimension	1200%	63%	Beckman DxI
16.621	177%	Beckman Access 2			
8.875	94%	Tosoh A1A	1800%	100%	Siemens Immulite
			4.5	25%	Roche Elecsys hCG +ß
7,370	100%	Siemens Immulite			
5,743	78%	Siemens Dimension	17	100%	Siemens Immulite
			<2.0	<12%	Siemens Centaur
6,464	100%	Siemens Immulite	14	82%	Roche Elecsys hCG +ß ß
6,717	104%	Siemens Dimension			
			16	100%	Siemens Immulite

444	100%	Siemens Immulite	8	50%	Siemens Centaur
103	23%	Siemens Centaur			
			15	100%	Siemens Immulite
275	100%	Siemens Immulite	9	60%	Siemens Dimension
263	96%	Siemens Dimension	7	47%	Abbott Architect
48	20%	Abbott Architect			
132	48%	Siemens Dimension	13	100%	Siemens Immulite
			9	69%	Siemens Centaur
206	100%	Siemens Immulite			
87	42%	Siemens Dimension	12	100%	Siemens Immulite
			10	83%	Beckman DxI
168	100%	Siemens Immulite	8	67%	Ortho Vitros Eci
50	30%	Siemens Dimension	7	58%	Siemens Centaur
156	100%	Siemens Immulite	9.2	100%	Siemens Immulite
103	66%	Siemens Centaur	7.1	77%	Roche Elecsys hCG +ß
			6.1	66%	Siemens Centaur
148	100%	Siemens Immulite			
38	26%	Siemens Centaur	8	100%	Siemens Immulite
38	26%	Siemens Dimension	8	100%	Siemens Centaur
140	100%	Siemens Immulite	5.9	100%	Siemens Immulite
66	47%	Ortho Vitros Eci	4.6	78%	Siemens ACS180
66	47%	Siemens Centaur			
			4	100%	Siemens Immulite
137	100%	Siemens Immulite	3	75%	Siemens Dimension
120	87%	Siemens Dimension	<2.0	<50%	Siemens Centaur
93	68%	Tosoh A1A			
			3	100%	Siemens Immulite
118	100%	Siemens Immulite	<1.0	<33%	Siemens Dimension
70	59%	Abbott AxSym			
66	56%	Beckman Access 2	2.4	100%	Siemens Immulite
60	51%	Siemens Centaur	<2.0	<83%	Beckman Access 2
110	100%	Siemens Immulite	47	100%	Siemens Immulite
74	67%	Siemens Centaur	38	81%	Siemens Dimension
			44	93%	Abbott Architect
88	100%	Siemens Immulite			
61	69%	Siemens Centaur	32	100%	Siemens Immulite
58	66%	Siemens Dimension	14	44%	Siemens Dimension
78	89%	Roche Elecsys hCG+ß ß ß ß ß `ß`	17	53%	Abbott Axsym
75	85%	Tosoh A1A	7	22%	Siemens Centaur

False Positive hCG

Obtaining false positive pregnancy test results is a common problem with hCG testing today. It seeming occurs in approximately 2% of patients tested for pregnancy today. A woman is tested for pregnancy today using a total hCG test. If the test result is positive indicating pregnancy, in about 2% of cases at 6 weeks of pregnancy, when the women is tested by ultrasound, no fetal sac may be found indicating definitively that the woman is not pregnant. This is a false positive pregnancy test.

The problem is that a total hCG test is not solely a pregnancy test. An hCG test will detect pituitary sulfated hCG and not solely placental hCG. An hCG test will also detect the molecule produced by quiescent gestational hCG, if the it is coming month later following a complication of a pregnancy or a gestational trophoblastic disease. An hCG test will detect familial hCG syndrome, if a person is genetically producing low concentrations of hCG for life. An hCG test will detect a gestation trophoblastic disease malignancy or benign disease, or can detect hyperglycosylated hCG or hyperglycosylated hCG free ß-subunit if the person has active cancer. An hCG test can also be false and detect heterophilic antibodies in the blood. All of these are cause of false positive pregnancy tests that physicians need to be aware of [77-79].

Table 8 shows the USA hCG Reference Service experience with 440 cases of false positive pregnancy test. As shown, 7 reasons have been identified by the USA hCG Reference Service to explain 440 or 440 cases of false positive pregnancy test. The USA hCG Reference Service uniquely investigates each case. The finding of the USA hCG Reference Service are explained here.

The most common cause of false positive pregnancy test is Quiescent Disease [77-79]. Classically, a woman has a pregnancy with fails ending in a spontaneous abortion. The fetus and most of the placenta clears at the spontaneous abortion. A small amount of hemochorial placentation tissue remains deeply imbedded in the uterus after the spontaneous abortion. The hormone hCG and the autocrine hyperglycosylated hCG in the circulation clears quite rapidly. The small amount of remaining tissue deeply implanted in the uterus produces a tiny amount of the hormone hCG and hyperglycosylated hCG. Among the tissue remaining, the cytotrophoblast cells can die off and months after spontaneous abortion just the hormone hCG is produced, 2.0-256 mIU/ml, by syncytiotrophoblast cells. These residual syncytiotrophoblast cells may linger for 6 months producing a constant small amount of the hormone hCG, 2.0-256 mIU/ml, causing the false positive hCG result. This is quiescent disease, a low concentration of hormone hCG 2.0-256 mIU/ml produced two months to eight months after a spontaneous

abortion pregnancy [77-79], that may wrongly lead to a false positive pregnancy test (Table 8).

Quiescent gestational trophoblastic diseases may also follow the evacuation of a complete or partial hydatidiform mole, or chemotherapy for hydatidiform mole or choriocarcinoma. In approximately 10% of cases, persistent trophoblastic disease needing chemotherapy can follow a quiescent pregnancy and a quiescent hydatidiform mole, and in about 25% of cases flow quiescent choriocarcinoma.

Quiescent disease is best detected by the finding of low persistent production of the hormone hCG, 2.0 – 256 mIU/ml, in the months following a failed pregnancy, a terminated hydatidiform mole, or chemotherapy treated choriocarcinoma case. Quiescent disease can be demonstrated by the absence, or near absence (<10% of total hCG) of hyperglycosylated hCG, showing the death of cytotrophoblast cells. Hyperglycosylated hCG concentration can be measured using the Quest Diagnostics ultra-sensitive hyperglycosylated hCG test. These results are measured in pictogram/ml. This can be easily converted to nanogram/ml. It is known that 1 nanogram/ml is equivalent to 11 milli-international units/ml (mIU/ml) of hCG.

The second most common cause of a false positive pregnancy test is pituitary hCG. In the incidence pituitary sulfated hCG is produced by the pituitary gland. In menopause and perimenopause when ovarian estradiol production halts, inhibition and control of hypothalamic GnRH pulses is stopped. This leads to extreme GnRH pulses sent to pituitary gonadotrope cells. Pituitary gonadotrope cells then can produce extreme pituitary sulfated hCG and follicle stimulating hormone (FSH) production. Serum hCG can range from 1.4 to 39 mIU/ml [78,79]. This is a common cause of false-positive total hCG results (Table 8). Pituitary hCG is best detected by demonstrating an unduly high (>30 mIU/ml) FSH concentration. It is not unusual to find women with FSH concentration as high as 150-200 mIU/ml.

Table 8: False-positive hCG cases, 440 cases total, examined by the USA hCG Reference Service. H-hCG is hyperglycosylated hCG.

Diagnosis	Number Cases	Median hCG mIU/ml	Range hCG mIU/ml	Median H-hCG ng/ml	Median FSH mIU/ml
1. Pituitary hCG	134				
Perimenopause, age 38-51	60	8.4	1.4 - 28	<0.05	69.9
Postmenopause, age 52-70	47	11.4	2.5 – 33	<0.05	68
Bilateral oophorectomy cases	14	10.1	1.8 - 39	<0.05	59
Amenorrhea cases	1	6.1		<0.05	55.7
hCG peak	10	2.5	<1.0 – 11.8	<0.05	
		Median hCG mIU/ml	Range hCG mIU/ml	Proportion H-hCG ng/ml	
2. Quiescent disease	184				
Quiescent pregnancy	73	16	6.0 - 256	<5% total hCG	
Quiescent hydatidiform mole	83	19	1.7 - 207	<5% total hCG	
Quiescent choriocarcinoma	28	8	2.0 - 117	<5% total hCG	
		False Positive hCG, mIU/ml	False Positive Range, mIU/ml	hCG Immulite mIU/ml	hCG range mIU/ml
3. Heterophilic antibodies	86				
Abbott Axsym, 1998-2005	45	81	6.0 - 1010	12.5	0 – 179
All methods, 2006-2016	38	24	7.1 - 404	4	0 – 74
		Median hCG mIU/ml	Range hCG mIU/ml	Median free ß ng/ml	Mean free ß % of total hCG
4. Familial hCG syndrome	20	29.5	<1.0 - 287	0.45	0.68
		Median hCG mIU/ml	Range hCG mIU/ml	Median H-hCG ng/ml	Range H-hCG ng/ml
5. Gestational trophoblastic disease	6	239	60 - 2362	9.8	0.36 - 283
		Median hCG mIU/ml	Range hCG mIU/ml	Median free ß ng/ml	Mean free ß % of total hCG
6. Cancer	7	8	<1.0 - 274	1.3	0.88

		Median hCG mIU/ml	Range hCG mIU/ml
7. Munchausen's syndrome	3	44150	7900 – 80400

Heterophilic antibodies are also a cause of false positive serum pregnancy tests. This is interference by heterophilic antibodies, human cross-reactive antibodies generating an anti-animal antibody like response in blood. In these cases, the human anti-animal like antibodies can link together the capture and tracer antibodies used in the immunometric assay causing false-positive pregnancy hCG test results.

Normally, these interfering antibodies are very large immunoglobulin molecules that do not get into urine. Classically, heterophilic antibody interference can easily be demonstrated by a positive hCG result in serum, 7.1–1010 mIU/ml, and a negative hCG result in urine. Urine pregnancy test use dip stick devices and are general insensitive, 50 mIU/ml. Measure urine hCG using a sensitive serum total hCG test. Dilute patient urine 1:1 using normal male serum or control serum and measure it in a serum test.

Familial hCG syndrome is an inherited or genetic condition in which some organ in the body produced low concentration of hCG or its free ß-subunit (<1.0–287 mIU/ml) [57,58]. Classically, this is a mutated form of hCG with distorted amino acid sequence causing invariable results in different hCG assays. It is mostly a free form of hCG free ß-subunit since it is being produced by cells other than trophoblastic or pituitary hCG cells [59]. The amazing thing about familial hCG syndrome is that it is the only symptom may be low level hCG production [57,58].

The USA hCG Reference Service has identified familial hCG syndrome cases all over the world. They have found athletes, football stars, native peoples and city people with familial hCG syndrome all over the world. Familial hCG syndrome is best identified by the finding of widely varying hCG results in different assays, due to the mutation of hCG, and by the finding of a significant proportion of total hCG being free ß-subunit (>40%). Familial hCG syndrome is then confirmed by showing that at least one parent has the same inherited syndrome [57,58].

Gestational trophoblastic disease as a cause of pregnancy test false positive results is very rare. Thee USA hCG Reference Service has observed 6 of 440 cases (Table 8). Gestational trophoblastic disease, or a chemorefractory, minimally aggressive hidden form of choriocarcinoma can present as a case with 60–2362 mIU/ml total hCG. The choriocarcinoma can be identified by the demonstration of the presence of 10%-40% of total hCG being hyperglycosylated hCG. It only shows as active choriocarcinoma with higher hCG concentrations and higher proportions hyperglycosylated hCG months later when the concentrations start or rise.

The USA hCG Reference Service has also rarely seen cancer cases causing false-positive total hCG results. Cancer is generally demonstrated by all the immunoreactivity being hCG free ß-subunit. No information is provided as to the source of cancer. This generally has to be confirmed by finding tumors using MRI scans.

The final cause of false-positive total hCG test as found by the USA hCG Reference Service is Munchausen's Syndrome. Three cases have been observed. In these cases, physicians and nurses administered to themselves hCG to make themselves look sick (Munchausen's syndrome is a psychiatric condition). In these cases the physicians and nurses administered to themselves Ovidrel, an ultra-pure recombinant form of the hormone hCG to avoid side effects. In these case intact hCG and not degradation products was detected in blood, with levels rising and falling with each shot. Munchausen's syndrome was identified by the purity of the intact hCG, and lack of normal serum degradation products and hyperglycosylated hCG, indicating that they were administering shots of recombinant ultra-pure Ovidrel.

hCG and Evolution of Humans

Seemingly, by far the greatest and most significant roles that the hormones hCG and the autocrine hyperglycosylated hCG have played is in the evolution of humans [47-49,80]. Somehow, they governed the specific evolution of human beings, driving human being evolution.

It was like they were generated as a plan by someone, possibly God?, specifically to make or to generate humans. Fifty million years ago, with the lemur, simian primates evolved, with a tiny brain size, 0.07% of total body weight. The only way to expand the brain size of simian primates was to change fetal placentation from inefficient epitheliochorial placentation to a more efficient fetal feeding system. This would allow brain growth genes to function and to expand and develop the brain. The evolution of the hormone hCG, which followed simian primates, introduced hemochorial placentation a more efficient fetal feeding system. Amazingly, both hCG the hormone driving hemochorial placentation and hyperglycosylated hCG, an independent and separate TGF-ß invasion molecule, one that would drive deep implantation of the hemochorial placentation apparatus, evolved.

As the hormone hCG and the autocrine hyperglycosylated hCG were made more and more acidic by advancing evolution they drove more and more efficient hemochorial placentation and implantation, driving brain growth, super brain growth and ultra-brain growth. At the level of humans a super acidic form of the hormone hCG (pI 3.5) and the autocrine hyperglycosylated hCG (pI 3.2) were made, needed to derive the super-large human brain [47-49,80].

The hormone hCG and the autocrine hyperglycosylated hCG are the most acidic proteins made in primates, at the limit of protein acidity or the limit that evolution could drive these molecules. Making humans the very end of this specific hCG

evolution pathway, or the end result of this hCG-driven evolution scheme [47-49,80].

This evolution story started 55 million years ago with the evolution of the lemur, early prosimian primates [47-49,80]. These early primates produced an LH but not an hCG. They used inefficient epitheliochorial placentation or an unproductive fetal feeding system which limited the action of the seven brain growth genes, or limited brain growth.

The seven brain growth genes were the microcephalin gene [81], WDR62 gene [82], CDK5RAP2 gene [83], CEP152 gene [84], ASPM gene [85], CENPJ gene [83] and STIL gene [86]. The development of the brain started with prodecessors, with Aotus and Callicebus, early Anthropoid primates that expressed the hCG genes, 37 million years ago [6]. With these species, the LH ß-subunit gene duplicated and underwent a deletion mutation generating a new gene coding for a longer amino acid C-terminal peptide, 145 verses 121 amino acids. The ß-subunit combined with the root glycoprotein α-subunit to make hCG. Both a hormone hCG and an autocrine hyperglycosylated hCG were produced. Figure 10 illustrates the deletion mutation on LH ß-subunit that happened with the evolution of Aotus and Callicebus.

The initial Aotus and Callicebus hormone hCG and autocrine hyperglycosylated hCG were very neutral (pI 6.3) having evolved from the neutral molecule LH (pI 8.0). As a result of their neutrality they were only minimally biologically active molecules, installing a minimal hemochorial placentation system with just deep implantation of 1% of uterine thickness [47-49,80]. The hemochorial placentation was still were more efficient than what they replaced, epitheliochorial placentation. This allowed some brain growth, the brain developing from 0.07% of body weight, found in their predecessors, Tarsier and Lemur, to 0.17% of body weight found in Aotus and Callicebus (Table 9). This is a 2.4 fold expansion.

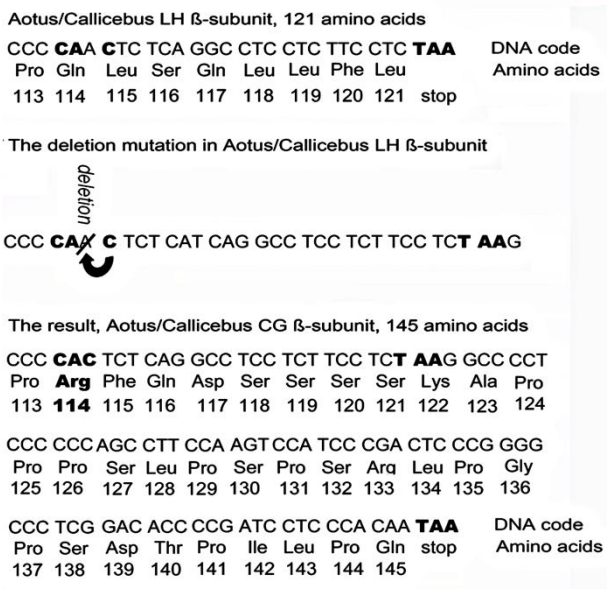

Figure 10: The deletion mutation in LH ß-subunit gene that created hCG ß-subunit [6].

Table 9: The evolution of CG. Shown in bold is species in which all information is available on CG. Shown in regular text are established evolutionary between links between species for which information is available. Some species like chimpanzee, orangutan, bonobo and lemur are divergences and not direct lines of human evolution. The abbreviation ya is years ago, ND is not determined, As is assumed. Human evolution as indicated by species, pI and brain sizes are published data.

Species	Family Evolved ya	Sugar side chains on CG ß-subunit	pI	Clearance rate	Depth of implantation	Brain mass % body weight (vs. lemur)
Homo sapiens	**200,000 ya**	**6 sugar chains**	**pI=3.5**	**36 h**	**30%**	**2.4% (34X)**
Homo heidelbergensis, Hominini	400,000 ya		Extinct	Extinct	Extinct	2.1% (30X)
Homo erectus, Hominini	1,000,000 ya		Extinct	Extinct	Extinct	1.6% (23X)
Homo ergaster, Hominini	1,800,000 ya		Extinct	Extinct	Extinct	1.1% (16X)
Homo habilis, **Hominin**i	2,000,000 ya	5 sugar chains (As)	Extinct	Extinct	Extinct	1.2% (16X)
Australopithecus garni, Hominini	2,500,000 ya		Extinct	Extinct	Extinct	0.86% (12X)
Australopithecus africus, Hominini	2,700,000 ya		Extinct	Extinct	Extinct	0.83% (12X
Australopithecus afarensis, Hominini	3,500,000 ya		Extinct	Extinct	Extinct	
Australopithecus anamensis, Hominini	4,000,000 ya		Extinct	Extinct	Extinct	
Ardipithecus ramidus, Hominini	4,300,000 ya		Extinct	Extinct	Extinct	

Orrorin tugensis, Hominini	6,000,000 ya		Extinct	Extinct	Extinct	
Sahelanthropus tchadensis, Hominini	7,000,000 ya		Extinct	Extinct	Extinct	
Chimpanzee, Hominidae	6,000,000 ya		Extinct	Extinct	Extinct	0.74% (11X)
Orangutan, Hominidae	**12,000,000 ya**	**4 sugar chains**	**pI=4.9**	**6.0 h**	**10%**	**0.74%(11X)**
Hominidae great apes	4-12,000,000 ya					
Bonobo, Hominidae	4-12,000,000 ya					
Hominidae great apes	4-22,000,000 ya					
Hominoidea	12-22,000,000 ya					
Colobus, Hominoidea	20,000,000 ya					
Homoinoidea	12-22,000,000 ya					
Baboon, Catarrhine	20,000,000 ya	4 sugar chains	ND	ND	ND	0.47% (6.7X)
Macaque	24,000,000 ya					
Callicebus, Anthropoid	**37,000,000 ya**	**3 sugar chains**	**pI = 6.3**	**2.4 h**	**1%**	**0.17% (2.4X)**
Aotus, Anthropoid	37,000,000 ya	3 sugar chains	pI = 6.3	2.4 h	1%	0.17% (2.4X)
Tarsier, Tarsiidae family	45,000,000 ya					
Catarrhine monkeys	45,000,000 ya					
Lemur, Strepsirrhine	**55,000,000 ya**	**LH ß-subunit: 1 sugar chain**	**pI=8.0**	**0.33 h**	**No implantation**	**0.07% (1.0X)**

What happened next, and how the hormone hCG and the autocrine hyperglycosylated hCG controlled advanced primate, hominid and human evolution involved increasing the acidity of hCG. The acidity of hCG was enhanced, Pi 6.3 to pI 3.5 by increasing the number of acidic amino acids, and by increasing the number of acidic carbohydrate side chains (Table 9). The Aotus and Callicebus ß-subunit has just 3 sugar side chains (pI 6.3).

Over 17 million years with the evolution of Baboon hormone chorionic gonadotropin (CG) and autocrine hyperglycosylated CG a molecule evolved with four acidic sugar side chains. With this more acidic CG and more efficient hemochorial placentation a brain developed of 0.47% of body weight, or of 6.7 fold expansion (Table 9)

Over a further 8 million years with the evolution of Orangutan hormone CG and autocrine hyperglycosylated CG a molecule evolved that had four sugar side chains with further acidic amino acids, the pI of the molecule was reduced to Pi=4.9. With this more acidic hCG and more efficient hemochorial placentation a brain developed that increased to 0.74% of body weight, or of 11 fold expansion (Table 9).

Over a further 8 million years with the evolution of hominids or Homo habilis hormone CG and autocrine hyperglycosylated CG a molecule evolved that had 5 acidic sugar side chains. With this more acidic hCG and even greater efficiency hemochorial placentation a brain developed that was increased to 1.2% of body weight, or of 16 fold expansion (Table 9). Homo habilis is an extinct species, brain size told from excavated skull capacity.

Then over just two million years with the evolution of humans, hCG and the autocrine hyper glycosylated hCG evolved with 6 acidic sugar side chains. With this more acidic hCG, pI 3.5 and 3.2, and ridiculously efficient hemochorial placentation a brain developed that was increased to 2.4% of body weight, or of a 34 fold expansion (Table 9). This marks the end of how hCG drove brain expansion. With expanding brain size species became more and more bipedal and upright, developed tools and hand and finger control, or human developed.

It is estimated that hCG ß-subunit had as part of the acidity and carbohydrate transfer Aotus and Callicebus hCG to human hCG underwent 45 amino acid changes or mutations. This indicates that almost every species in between Aotus, Callicebus and humans underwent specific mutations in hCG ß-subunit aimed at the development of humans [6]. Seemingly, as many as 45 species in a row, Aotus/Callicebus to humans, underwent mutations in the same hCG ß-subunit gene, each leading to brain expansion.

Under evolution rules/laws the same gene does not undergo 45 mutations in a row. This has never been observed in evolution previously. This once again suggests possibly God's involvement interfering with evolution. This is how CG controlled human evolution and development [47-49,80].

hCG and the Future

In many respects predicting what will happen in the future is very subjective and pure guess work. Here we try and predict how advancement in the hCG field could change the world.

I am at this time working with others writing a National Institutes of Health (NIH) grant to carry out clinical trial with injections of placental hyperglycosylated hCG to try and totally prevent pregnancy failures [14,15,45,46]. Hopefully we will be successful and pregnancy failures will come to a complete end.

Research with B152 antibody to hyperglycosylated hCG is showing promise in the treatment of cancer [19]. B152 completely block cancer malignancy in trial cases, generating oncostasis or non-growing, non-invasive cancers. There appears to be promise in the future for B152 as a cancer treatment [19].

In many respects the biggest discovery with the hormone hCG and the autocrine hyperglycosylated hCG is the discovery that they drove human evolution. Furthermore, that without the evolution of these molecules that humans never would have come about [47-49].

The evolution of hCG from LH first occurred in Aotus and Callicebus (pI=6.3), in early anthropoid primates, hCG became more acidic and advanced in potency in Baboon, advanced further with Orangutan, advanced in potency even further with hominids and finally advanced extremely with humans (pI=3.5). With its stepwise advance in potency, hemochorial placentation and implantation advanced in potency in parallel. With the stepwise advance in hemochorial placentation function, brain growth promoting genes were able to promote the growth and development of a larger and larger brain in parallel. Thus the advanced primate and human brain came about, and advanced primate and humans evolved. It could correctly be stated based on research that the hCG ß-subunit gene is the one gene that drives human evolution, in that this gene along with the inherrant brain promotion genes drove the final creation of the human brain [47-49].

I now bring up the question of whether the evolution of humans is the end of the evolutionary road or will more advanced species, super-humans, evolve from humans? hCG is by far the most acidic molecule in the human genome or the human library of proteins, or in fact in the entire library of primate genomes (hormone hCG, pI=3.5). This is an extreme. It appears that hCG has reached the extreme that a molecule can go to in acidity, and it appears unlikely that a molecule can become more acidic or more glycosylated and be stable. If hCG became more acidic it would be unstable in the body's circulation and likely destroy itself.

Human hCG came about by increasing its acidity, increasing its acidity and increasing its acidity further. It very much appears that this advancing hCG theory, the following hemochorial placentation theory, and the large brain theory are all at the very end of the evolutionary road and can go no further [47-49]. It is therefore concluded that humans are a final product and the end of their evolutionary road.

The observation that "the hCG ß-subunit gene is seemingly the one gene that signifies humans" is a little scary. A little scary because of all the non-ethical hCG ß-subunit gene experiments that scientists could so quickly imagine. How about transplanting the hCG ß-subunit gene into a dog zygote or a dog blastocyst. This is very doable. In theory the hCG ß-subunit gene could make a human hCG ß-subunit which can combine with dog hCG α-subunit to make the hormone hCG and the autocrine hyperglycosylated hCG. This process could be promoted by giving the developing dog GnRH.

The hCG should theoretically promote the dog to develop hemochorial placentation and implantation. While the dog does not have primate brain growth genes it does have mammalian brain growth genes, and should develop a larger more advanced brain. You can easily imagine rich people paying a lot of money for an advanced canine species with a larger brain. For a dog that thinks better, this is what we would be creating with this scientific craziness.

Other future studies, might include transplanting hCG ß-subunit gene into orangutans and making much more intelligent or human-like orangutans. Looking at possible experiments non-ethical scientists might consider making a more acidic variant of hCG in the laboratory. Additional O-linked oligosaccharides might be added to the hCG α-subunit or ß-subunit structure, or additional glutamic acid amino acid residues added to hCG α-subunit or ß-subunit NH2 or COOH terminal. DNA could then be synthesizing to make a matching hCG α-subunit or ß-subunit gene. This gene could transplant into a human zygote or blastocyst to make a super-human, with a larger more advanced brain coming from the super-active variant of hCG. Is this craziness in our future?

Cancer cells steal hCG or activate hCG genes and use them to drive cancer malignancy. If we develop or evolve more advanced forms of hCG, it will expand our brain size and intelligence, but it will also generate cancers more potent and make it harder to treat malignancy. Is this what we want, a more advanced human that can experience much worse cancers?

hCG has taken us to where we are as human todays. Who knows where it could take us in the future?

Synthesis of Hormone hCG and Autocrine Hyperglycosylated hCG

The synthesis of the hormone hCG and the autocrine hyperglycosylated hCG, for instance in placental cytotrophoblast cells and syncytiotrophoblast cells follow similar synthetic pathways.

The α-subunit and ß-subunit undergo separate amino acid synthesis on ribosomes in the rough endoplasmic reticulum. It is generally thought that excess α-subunit is made and that newly synthesized ß-subunit combines with the excessα α-subunit [87-89]. Immediately after peptides are synthesized disulfide bridges are formed to tie polypeptides together [88,89]. Disulfide-linked polypeptides then each receive in the endoplasmic reticulum two high mannose/glucose N-linked oligosaccharides from dolichol phosphate. This is a Man 9 Glucose 3 N-linked oligosaccharide structure [90,91]. The high mannose/glucose structures are processed by mannosidases and glucosidase to a basic Man 5 structure in the endoplasmic reticulum.

Combination of the α-subunit and the ß-subunit to form hCG dimer is not limited by the rate of glycosylation, but rather is limited by the rate of disulfide formation [59]. As discussed previously, incompletion of ß-subunit disulfide bonds leads to non-combination of hCG subunits and the formation of free subunit molecules [59]. Combination starts in the endoplasmic reticulum [59]. Combined hCG molecules at this stage are transferred to the Golgi apparatus [87-89].

In the Golgi apparatus the N-glycosylation of hCG is completed, α-mannosidases are used to degrade the Man 5 structure further, and α-mannosyl-transferases, ß-N-acetyl-glucosaminyl-transferases, ß-galactosyltransferases and α-N-aceylneraminyl-transferases used to complete the N-linked oligosaccharide structures. O-glycosylation of the ß-subunit and N-acetyl-galactosaminyl-transferase activity occurs in the Golgi apparatus. It is ß-N-acetylglucosaminyl-transferase activity at the O-glycosylation stage that differentiates the hormone hCG and the autocrine hyperglycosylated hCG. The Type 1 O-linked oligosaccharide does not have ßN-acetyglucosamine sugars, while the Type 2 O-linked oligosaccharides on hyperglycosylated hCG has these sugars.

In the Golgi apparatus the hormone hCG and the autocrine hyperglycosylated hCG are packaged into seminal vesicles for shipment across the cell membrane and delivery outside of the cell [92-94].

Degradation of Hormone hCG and Autocrine Hyperglycosylated hCG

The dissociation and degradation of the hormone hCG and the autocrine hyperglycosylated hCG occur in parallel, mostly in the blood stream. Because they occur in the blood, intact molecules and degradation products are detected in blood or serum hCG assays or pregnancy tests.

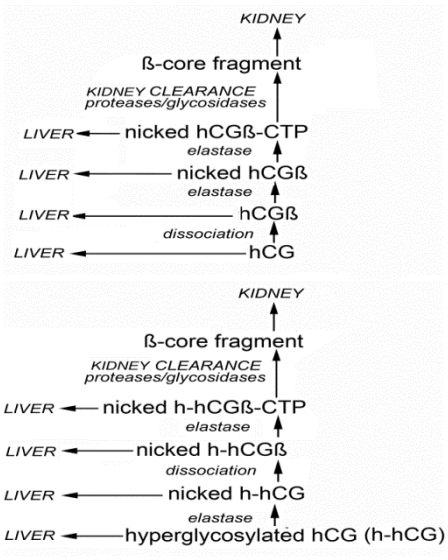

Figure 11: The degradation of the hormone hCG and the autocrine hyperglycosylated hCG in serum and the kidney.

Nine specific articles describe the degradation of hCG in serum and urine. All show that the dissociation of hCG into subunits, the nicking or cleavage of hCG or its ß-subunit, and the cleavage of the ß-subunit C-terminal peptide are all degradation inevitabilities, and that they all happen in no set order [94-102]. All conclude that ß-core fragment formation occurs in the kidney and is detected only in urine. ß-core fragment is the degradation end product of the hormone hCG and the autocrine hyperglycosylated hCG.

From these nine articles a figure is drawn illustrating the degradation of the hormone hCG and the autocrine hyperglycosylated hCG (Figure 11).

A difference is shown in the degradation of the two form of hCG. Because the hormone hCG structurally is blocked from cleavage or nicking [60], I place dissociation as a first degradation step and nicking as a likely later degradation step. Similarly, because the autocrine hyperglycosylated hCG is not structurally blocked from nicking, I place nicking or cleavage as the first structural step (Figure 11). Realistically, nicking, dissociation, and cleave of the ß-subunit C-terminal peptide are all degradation inevitabilities [94-102].

References

1. Aschner B. Ueber die function der hypophyse. Pflug Arch Gest Physiol. 1912;146:1-147.
2. Fellner OO. Experimentelle untersuchungen uber die wirkung von gewebsextrakten aus der plazenta und den weiblichen sexualorganen auf das genital. Arch Gynakol. 1913;100:641.
3. Hirose T. Experimentalle histologische studie zur genese corpus luteum. Mitt Med Fakultd Univ ZU. 1919;23:63-70.
4. Aschheim S, Zondek B. Das Hormon des hypophysenvorderlappens: testobjekt zum Nachweis des hormons. Klin Wochenschr. 1927;6:248-52.
5. Fiddes JC, Goodman HM. The cDNA for the ß-subunit of human chorionic gonadotropin suggests evolution of a gene by readthrough into the 3-untranslated region. Nature J. 1980;286:684-87.
6. Maston GA, Ruvolo M. Chorionic gonadotropin has a recent origin within primates and an evolutionary history of selection. Mol Biol Evol. 2002;19:320-34.
7. Elliott MM, Kardana A, Lustbader JW, et al. Carbohydrate and peptide structure of the alpha- and beta-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. Endocrine J. 1997;7:15-32.
8. Valmu L, Alfthan H, Hotakainen K, et al. Site-specific glycan analysis of human chorionic gonadotropin beta-subunit from malignancies and pregnancy by liquid chromatography - electrospray mass spectrometry. Glycobiol. 2006;16:1207-18.
9. Isaacs NW. Cystine knots. Curr Opin Struct Biol. 1995;5:391-95.
10. Cole LA. hCG and Hyperglycosylated hCG Carbohydrate Structures Corrected. J Glycobiol. 2014;3:1000114.

11. Birken S, Maydelman Y, Gawinowicz MA, et al. Isolation and characterization of human pituitary chorionic gonadotropin. Endocrinol. 1996;137:1402-11.
12. Goldsmith PC, McGregor WG, Raymoure WJ, et al. Cellular localization of chorionic gonadotropin in human fetal kidney and liver. J Clin Endocrinol Metab. 1983;57:54-61.
13. Abdallah MA, Lei ZM, Li X, et al. Human Fetal nongonadal tissues contain human chorionic gonadotropin/ luteinizing hormone receptors. J Clin Endocrinol Metab. 2004;89:952-56.
14. Sasaki Y, Ladner DG, Cole LA. Hyperglycosylated hCG the source of pregnancy failures, Fertil Steril. 2008;89:1871-1786.
15. Cole LA, Brennan MC, Hsu CD, et al. Hyperglycosylated hCG, A Sensitive Screening Test For Preeclampsia / Gestational Hypertension. Preg Hypertension. 2017.
16. Brennan MC, Wolfe MD, Murray-Krezan C, et al. First Trimester Hyperglycosylated Human Chorionic Gonadotropin and Development of Hypertension. Prenat Diagn. 2013;33:1075-9.
17. Cole LA, Butler SA. Hyperglycosylated hCG, hCGß and Hyperglycosylated hCGß: Interchangeable Cancer Promoters. Molec Cellul Endocrinol. 2012;349:232-8.
18. Cole LA, Butler SA, et al. B152 anti-hyperglycosylated human chorionic gonadotropin free ß-Subunit in a new possible treatment for cancer. J Reprod Med. 2015;60:13-20.
19. Cole LA, Naftolin F. Hyperglycosylated hCG Controls the Proteolytic Step of Ovulation. Am J Obstet Gynecol. 2017.
20. Butler SA, Ikram MS, Mathieu S, et al. The increase in bladder carcinoma cell population induced by the free beta subunit of hCG is a result of an anti-apoptosis effect and not cell proliferation. Brit J Cancer. 2000;82:1553-56.
21. Berndt S, Blacher S, Munuat C, et al. Hyperglycosylated human chorionic gonadotropin stimulates angiogenesis through TGF-ß receptor activation. FASEB J. 2013;213686.
22. Ahmad F, Ghosh S, Sinha S, et al. TGF-ß-induced hCG-ß regulates redox homeostasis in glioma cells. Molec Cellul Biochem. 2015;399:105-112.
23. Weigand M, Hantel P, Kreienberg R, et al. Autocrine vascular endothelial growth factor signalling in breast cancer Evidence from cell lines and primary breast cancer cultures in vitro. Angiogenesis. 2005;8:197–204.
24. Grivennikov S, Karin Michael. Autocrine IL-6 Signaling: A Key Event in Tumorigenesis?. Cancer Cell. 2008;13:7–9.
25. Namachivayam K, Blanco CL, Kumar KM, et al. Smad7 inhibits autocrine expression of TGF-ß2 in intestinal epithelial cells in baboon necrotizing enterocolitis. Am J Physiol. 2013;304:G167-G180.
26. Wikipedia. TGFß signaling pathway. https:// en.wikipedia.org/wiki/TGF_betasignaling_pathway.
27. Lustbader JW, Yarmush DL, Birken S, et al. The application of chemical studies of human chorionic gonadotropin to visualize is three-dimensional structure. Endocr Rev. 2013;14:291-310.
28. Wu H, Lusbader JW, Yee L, et al. Structure of human chorionic gonadotropin at 2.6Å resolution from MAD analysis of the selenmethionyl protein. Structure. 1994;2:545-58.
29. Lapthorn AJ, Harris DC, Littlejohn A, et al. Crystal structure of human chorionic gonadotropin. Nature J. 1994;369:455-62.
30. Toth P, Li X, Rao CV, et al. Expression of functional human chorionic gonadotropin/human luteinizing hormone receptor gene in human uterine arteries. J Clin Endocrinol Metab. 1994;79:307-15.
31. Zygmunt M, Herr F, Keller-Schoenwetter S, et al. Characterization of human chorionic gonadotropin as a novel angiogenic factor. J Clin Endocrinol Metab. 2002;87:5290-96.
32. Zygmunt M, Herr F, Münstedt K, et al. Angiogenesis and vasculogenesis in pregnancy. Eur J Obstet Gynecol Reprod Biol. 2010;1:S10-18.
33. Rao CV, Li X, Toth P, et al. Expression of epidermal growth factor, transforming growth factor-alpha and their common receptor genes in human umbilical cords. J Clin Endocrinol Metab. 1995;80:1012-20.
34. Rao CV, Li X, Toth P, et al. Novel expression of functional human chorionic gonadotropin/luteinizing hormone receptor in human umbilical cords. J Clin Endocrinol Metab. 1993;77:1706-14.
35. Wasowicz, Derecka K, Stepien A, et al. Evidence for the presence of luteinizing hormone–chorionic gonadotrophin receptors in the pig umbilical cord. J Reprod Fertil. 1999;117:1-9.
36. Strott CA, Yoshimi T, Ross GT, et al. Ovarian physiology: relationship between plasma LH and steroidogenesis by the follicle and corpus luteum; effect of hCG. J Clin Endocrinol Metab. 1969;29: 1157-67.
37. Eta E, Ambrus G, Rao V. Direct regulation of human myometrial contractions by human chorionic gonadotropin. J Clin Endocrinol Metab. 1994;79:1582-86.
38. Doheny HC, Houlihan DD, Ravikumar N, Human chorionic gonadotrophin relaxation of human pregnant myometrium and activation of the BKCa channel. J Clin Endocrinol Metab. 2003;88:4310-15.
39. Akoum A, Metz CN, Morin M. Marked increase in macrophage migration inhibitory factor synthesis and secretion in human endometrial cells in response to human chorionic gonadotropin hormone. J Clin Endocrinol Metab. 2005;90:2904-10.
40. Matsuura T, Sugimura M, Iwaki T, et al. Anti-macrophage inhibitory factor antibody inhibits PMSG-hCG-induced follicular growth and ovulation in mice. J Assist Reprod Genet. 2002;19:591-595.
41. Norwitz E, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. N Engl J Med. 2001;345:1400-08.
42. Semprini AE, Simoni G. Not so inefficient reproduction. Lancet. 2000;356:257-58.

43. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. N Engl J Med 1999;340:1796-99.
44. Cole LA. Pregnancy implantation. Hum Res.
45. Cole LA. Hyperglycosylated hCG and pregnancy failures. J Reprod Immunol. 2012;93:119-22.
46. Cole LA, Khanlian SA, Kohorn EI, Evolution of the Human Brain, Chorionic Gonadotropin and Hemochorial Implantation of the Placenta: Origins of Pregnancy Failures, Preeclampsia and Choriocarcinoma. J Reprod Med 2008;53:449-557.
47. Cole LA. hCG and hyperglycosylated hCG in the establishment and evolution of hemochorial placentation. J Reprod Immunol. 2010;82:112-18.
48. Cole LA. The evolution of the primate, hominid and human brain. Primatology. 2015;4:100124.
49. Bahado-Singh RO, Oz AU, Kingston JM, et al. The role of hyperglycosylated hCG in trophoblast invasion and the prediction of subsequent pre-eclampsia. Prenat Diagn. 2002;22:478-81.
50. Siler-Khodr TM, Khodr GS, Valenzuela G, et al. Gonadotropin-releasing hormone effects on placental hormones during gestation: I Alpha-human chorionic gonadotropin, human chorionic gonadotropin and human chorionic somatomammotropin. Biol Reprod. 1986;34:245-54.
51. Odell WD, Griffin SG, Carrell DC. Human chorionic gonadotropin-like proteins: Secretion in non-pregnant humans and production by bacteria. Trans Am Clin Climatol Assoc. 1992;103:238-54.
52. Stenman UH, Alfthan H, Rant T, et al. Serum levels of human chorionic gonadotropin in nonpregnant women and men are modulated by gonadotropin-releasing hormone and sex steroids. J Clin Endocrinol Metab. 1987;64:730-36.
53. Odell WD, Griffin J. Pulsatile secretion of human chorionic gonadotropin in normal adults. N Engl J Med. 1987; 317:1688-91.
54. Cole LA, Gutierrez JM. Production of hCG during the menstrual cycle. J Reprod Med. 2009; 54:245-50.
55. Huhtaniem IT, Korenbrot CC, Jaffe RD. Content of chorionic gonadotropin in human fetal tissues. J Clin Endocrinol Metab. 1976;46:994-97.
56. Cole LA. Familial HCG syndrome. J Reprod Immunol. 2001;93:52-7.
57. Cole LA, Butler SA. Familial hCG syndrome: production of variable, degraded or mutant form of hCG. J Reprod Med. 2014;59:435-42.
58. Beebe JS, Huth JR, Ruddon RW. Combination of the chorionic gonadotropin free beta-subunit with alpha. Endocrinology. 1990;126:384-91.
59. Cole LA. Human chorionic gonadotropin two separate very independent molecules sharing the same common amino acid sequence. Glycobiol. 2007;in press.
60. Nam JH, Cole LA, Chambers JT, et al. Urinary gonadotropin fragment, a new tumor marker: I. Assay development and cancer-specificity. Gynecol Oncol. 1990;36:383-90.
61. D'Agostino RS, Cole LA, Ponn RB, et al. Urinary gonadotropin fragment in patients with lung and esophageal disease. J Surg Oncol. 1992;49:147-50.
62. Kardana A, Braunstein GD, Cole LA. Gonadotropin subunit nicking enzyme (GBNE), a potential test for detecting early malignancies. Oncology Res. 1996;8:13-6.
63. Cole LA, Tanaka A, Kim GS, et al. Beta core fragment (?-core / UGF / UGP), a tumor marker: Seven year report. Gynecol Oncol. 1996;60:264-70.
64. Cole LA, Cancer malignancy. Gynecol Oncol. 2007.
65. Acevedo HF, Krichevsky A, Campbell-Acevedo EA, et al. Flow cytometry method for the analysis of membrane-associated human chorionic gonadotropin, its subunits, and fragments on human cancer cells. Cancer J. 1992;69:1818-28.
66. Regelson W. Have we found the "definitive cancer biomarker"? The diagnostic and therapeutic implications of human chorionic gonadotropin-beta statement as a key to malignancy. Cancer J. 1995;76:1299-01.
67. Iles R. Ectopic hCGß expression by epithelial cancer: Malignant behavior metastasis and inhibition of tumor cell apoptosis. Mol Cell Endocrinol. 2006;260:264-70.
68. Jankowska A, Gunderson SI, Warchol JB. Reduction of hCGß subunit expression by modified U1 snRNA caused apoptosis in cervical cancer cells. Mol Cancer. 2008;7:26-9.
69. Li D, Wen X, Ghali L, et al. hCG ß expression by cervical squamous carcinoma-in vivo histological association with tumour invasion and apoptosis. Histopathol. 2008;53:147-55.
70. Gillott DJ, Iles RK, Chard T. The effects of beta-human chorionic gonadotrophin on the in vitro growth of bladder cancer cell lines. Br J Cancer. 1996;73:323-26.
71. Guo X, Liu G, Schauer IG, et al. Overexpression of the ß Subunit of Human Chorionic Gonadotropin Promotes the Transformation of Human Ovarian Epithelial Cells and Ovarian Tumorigenesis. Am J Pathol. 2011;179:1385–93.
72. Hamade AL, Nakabayashi K, Sato A, et al. Transfection of antisense chorionic gonadotropin ß gene into choriocarcinoma cells suppresses the cell proliferation and induces apoptosis. J Clin Endocrinol Metab. 90:4873-79.
73. Isklander YL, Byrant JL, Zeman RA, et al. Tumorigenesis and metastasis of neoplastic Karposis's Sarcoma cell line in immunodeficient mice blocked by a human pregnancy hormone. Nature. 1995; 375:64-68.
74. Cole LA, Sutton JM, Higgins TN, et al. Between-method variation in human chorionic gonadotropin test results. Clin Chem. 2004;50:874-82.
75. Cole LA, DuToit S, Higgins TN. Total hCG tests. Clin Chim Acta. 2011;412:2216-22.
76. Cole LA, Muller CY. Hyperglycosylated hCG in the management of quiescent and chemorefractory gestational trophoblastic diseases. Gynecol Oncol. 2010;116:3-9.

77. Cole LA. USA hCG Reference Service. http://hcglab.com/about/understanding-gestational-trophoblastic-diseases/quiescent-gestational-trophoblastic-disease/.
78. Cole LA, Laidler LL, Muller CY. USA hCG reference service, 10-year report. Clin Biochem 2010;43: 1013-22.
79. Cole LA. Human Evolution,The Complete Story. Biosci Reports. 2017.
80. Jackson AP, Eastwood H, Bell SM, et al. Identification of microcephalin, a protein implicated in determining the size of the human brain. Am J Hum Genet. 2002;71:136-42.
81. Nicholas AK, Khurshid M, Désir J, et al. WDR62 is associated with the spindle pole and is mutated in human microcephaly. Nat Genet. 2010;42:1010-14.
82. Bond J, Roberts E, Springell K, et al. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. Nat Genet. 2005;37:353-55.
83. Guernsey DL, Jiang H, Hussin J, et al. Mutations in centrosomal protein CEP152 in primary microcephaly families linked to MCPH4. Am J Hum Genet. 2010;87:40-51.
84. Kouprina N, Pavlicek A, Mochida GH, et al. Accelerated evolution of the ASPM gene controlling brain size begins prior tohuman brain expansion. PLoS Biol. 2004;2:E126.
85. Kumar A, Girimaji SC, Duvvari MR, et al. Mutations in STIL, encoding a pericentriolar and centrosomal protein, cause primary microcephaly. Am J Hum Genet. 2009;84:286-90.
86. Ozturk M, Bellet D, Manil L, et al. Physiological studies of human chorionic gonadotropin (hCG), alpha hCG, and beta hCG as measured by specific monoclonal immunoradiometric assays. Endocrinol. 1987;120:549-58.
87. Cole LA. Sructures of hCG free ?-subunit and free ß-subunit. In: Human chorionic gonadotropin, Elsiever. 2015;51-7.
88. Wu H, Lustbader JW, Liu Y. Structure of human chorionic gonadotropin at 2.6 A resolution from MAD analysis of the selenomethionyl protein. Structure. 1994;2:545-58.
89. Chojnacki T, Dallner G. The biological role of dolichol. Biochem J. 1988;251:1-9.
90. Burda P, Aebi, M. The dolichol pathway of N-linked glycosylation structure of human chorionic gonadotropin at 2.6A resolution from MAD analysis of the selenomethionyl protein. Biochim Biophys Acta. 1999;1426:2239–57.
91. Fujikura T, Mukai M. Syncytiotrophoblastic vesicles in placental intervillous space. Am J Obstet Gynecol. 2006;195:304–07.
92. Tao YX, Lei ZM, Rao CV. Seminal vesicles are novel sites of luteinizing hormone/human chorionic gonadotropin-receptor gene expression. J Androl. 1998;19:343-47.
93. Birken S, Gawinowicz MA, Kardana A, et al. The Heterogeneity of hCG: II. Characteristics and origins of nicks in hCG reference standards. Endocrinol. 1991;129: 1551-58.
94. Cole LA, Kardana A. The biological and clinical significance of nicks in human chorionic gonadotropin and its free ß-subunit. Yale J Bio Med. 1991;64:627
95. Cole LA, Kardana A, Park SY, et al. The deactivation of hCG by nicking and dissociation. J Clin Endocrinol Metab. 1993;76:704-10.
96. Cole LA. Degradation products of hCG, hyperglycosylated hCG, and free ß-subunit. In: Human chorionic gonadotropin (hCG). Elsevier. 2010;113-22.
97. Okamoto T, Matsuo K, Niu R, et al. Human chorionic gonadotropin (hCG) ß-core fragment is produced by degradation of hCG or free hCGß in gestational trophoblastic tumors: a possible marker for early detection of persistent postmolar gestational trophoblastic disease. J Endocrinol. 2001;171: 435-43.
98. Bidart JM, Puisieux A, Troalen F, et al. Characterization of a cleavage product in the human choriogonadotropin beta-subunit. Biochem Biophys Res Commun. 1988;154:626-32.
99. Nishimura R, Ide K, Utsunomiya T, et al. Fragmentation of the ß-subunit of human chorionic gonadotropin produced by choriocarcinoma. Endocrinol. 1988;123:420-25.
100. Nisula BC, Blithe DL, Akar A, et al. Metabolic fate of human choriogonadotropin. J Steroid Biochem. 1989;33:733-37.
101. Cole LA. The hCG assay or Pregnancy Test. Clin Chem Lab Med. 2012;49:617-30.

*Correspondence to

USA hCG Reference Service, 34 Broadmoor Way,

P.O. Box 950Angel Fire, New Mexico

Tel: 575-377-1330

E-mail: larry@hcglab.com