

SOME REASONS WHY PROSTAGLANDIN E2 CAN BE ASSOCIATED WITH NAUSEA AND VOMITING IN PREGNANCY

Introduction

P. 1-2

We have presented the case that nausea and vomiting of pregnancy (NVP) has an organic cause by applying information from our own work and the work of many other researchers in this field. We divided the subject into twelve headings.

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A temporal relationship between maternal serum hCG and NVP during weeks 7-14 from first day of last menstrual period (LMP) for a group of patients was a starting point, while other investigators found raised maternal serum HCG and increased NVP in women who had hyperemesis gravidarum, twin pregnancies, or with hydatidiform moles. However, the very high levels of serum hCG in women with choriocarcinoma, which were not associated with nausea and vomiting, mean that hCG itself cannot be the cause of these symptoms. Using the medical literature, we pointed 14 hormones, hormone-releasing factors, cytokines and prostaglandins which stimulate the synthesis and release of hCG in syncytiotrophoblast cells in early pregnancy. Only Prostaglandin E2 (PGE2) and Prostaglandin F2 alpha (PGF2 α) of these substances are known to cause NVP which was shown when these PG's were given to procure a legal termination in early pregnancy. PGE2 has been demonstrated to be produced by the syncytiotrophoblast cells of the human

trophoblast at 7-11 weeks from LMP. These cells are in direct contact with maternal blood at the materno-fetal interface. The production of PGE2 in those cells is stimulated by several hormones and cytokines, notably Interleukin-1 and HCG itself.

In order for the synthesis of PGE2 in early pregnancy to be properly controlled, the activity of the enzyme Prostaglandin dehydrogenase (PGDH) is seen in early trophoblast cells. PGDH oxidises PGE2 into its inactive metabolite 15 Ketoprostaglandin E2. The syncytiotrophoblast is devoid of PGDH and therefore contains high levels of PGE2. The syncytiotrophoblast cells of the chorionic villi are in direct contact with maternal blood. PGDH is under progesterone control. As progesterone levels fall during weeks 5-9 from LMP, so will PGDH activity in decidual and chorionic villi levels fall, while PGE2 in trophoblast and maternal serum levels will rise. A rise in PGE2 could also be associated with the increased NVP when anti-progesterones are given to obtain a therapeutic abortion in modern times. We have shown that maternal serum PGE2 was higher when women had NVP than when they had no NVP on the same day. However, it would not be advisable to reduce maternal PGE2 due to its functions of immuno-suppression and glycogenolytic effects in early pregnancy.

Section 1. **HUMAN CHORIONIC GONADOTROPHIN (hCG)** **AND PREGNANCY SICKNESS**

Clinical relationship between maternal serum hCG and pregnancy sickness.

Several studies comparing emetic pregnancy to serum maternal hCG have been written spanning more than half a century. Schoeneck et al (1) presented evidence that there was an increased concentration of gonadotrophin in the urine of pregnant women who have the symptoms of nausea and vomiting, compared to pregnant women who are free of these symptoms. They also stated that the greatest concentration of maternal urinary hCG was present from the 6th to the 14th week of pregnancy when nausea and vomiting were mostly encountered. However, Soules et al (2) observed there was no obvious correlation between the concentration of maternal hCG and the severity of nausea and vomiting of pregnancy. These symptoms were graded 0-4 in severity but the mean levels of serum hCG did not differ significantly among any of the individual grades.

Masson et al (3) agreed with the findings of Schoeneck for a group of women rather than individual women, finding higher serum hCG levels in pregnant women with nausea and vomiting than in asymptomatic women. Jarnfelt Samsioe et al (4) formed a similar opinion, finding that serum levels of hCG in emetic women were significantly higher in early pregnancy, than the serum hCG levels of non-emetic women. Kaupilla et al (5) confirmed this view, observing that women suffering from hyperemesis gravidarum had serum hCG concentrations significantly higher than normal between weeks 7 and 14 of gestation.

Barnie-Adshead et al (unpublished work 1979) found that in a series of 13 women the correlations between maternal serum hCG levels and pregnancy sickness symptoms in individual patients were so limited that they were clearly insignificant, agreeing with the findings of Soules. However, when serum hCG levels of those women with severe symptoms were compared with those with mild symptoms, raised hCG and β hCG levels were

significantly associated with severe symptoms during weeks 7 and 9-12 from first day of last menstrual period (LMP), agreeing with the findings of Kaupilla, Masson and Jarnfelt Samsioe. In addition, when the overall maternal serum hCG levels were compared with overall sickness symptoms for several weeks throughout early pregnancy, there was a correlation for maternal serum hCG ($P < 0.02$) and β hCG ($P < 0.01$) levels with sickness symptoms from weeks 6-11 from LMP. This association became highly significant when β hCG and hCG serum levels and sickness symptoms were compared for weeks 6-17 from LMP ($P < 0.001$).

A series of seven authors have shown that hyperemesis gravidarum is more common in hydatidiform moles than in normal singleton pregnancies. Hydatidiform moles are associated with raised serum hCG's, and similarly a sample of seven authors have demonstrated an increased incidence of hyperemesis gravidarum or NVP in twin pregnancies compared to singletons. Again, twin pregnancies are associated with increased maternal serum hCG's.

There are then some pointers to a relationship between maternal serum hCG and pregnancy sickness when viewed together over a number of weeks in early pregnancy, namely weeks 7-14 from LMP, and yet, when individual women's symptoms of pregnancy sickness are related to their maternal serum hCG, no significant association is formed. Added to that, we have been given the important information by Professor K D Bagshawe, "very raised serum and cerebrospinal fluid levels of human chorionic gonadotrophin occur with choriocarcinoma in the absence of nausea and vomiting provided there are no gastrointestinal or cerebral metastases". Therefore, maternal hCG itself cannot be causing nausea and vomiting, but a certain relationship, particularly over a period of 6 or 7 weeks, has been established between maternal serum hCG and pregnancy sickness symptoms. Therefore, in seeking the aetiology of this condition, we may look at substances which stimulate the synthesis of hCG in syncytiotrophoblast cells (6) being particularly interested in any such substance which is known to cause nausea and vomiting.

HUMAN CHORIONIC GONADOTROPHIN AND PREGNANCY SICKNESS

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Section 2.

THE SOURCE OF HUMAN CHORIONIC GONADOTROPHIN FROM WITHIN HUMAN TROPHOBLAST CELLS

The chorionic villi of the human trophoblast contain two very significant cell types, namely cytotrophoblast cells which secrete very little hormone but fuse together to form syncytiotrophoblast cells in which hormones are synthesised in very large quantities, and secreted into the maternal circulation. One of these hormones is human chorionic gonadotrophin. (1)

hCG synthesis can be increased by stimulating this fusion process (1) or by direct stimulation within the syncytiotrophoblast cells via their cell receptors. (2)

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Section 3.

ISOFORMS (VARIANTS) OF HUMAN CHORIONIC GONADOTROPHIN

Human Chorionic Gonadotrophin consists of two units, alpha hCG and beta hCG which combine to form the biologically active hormone hCG.

Several factors have to be considered in the relationship of hCG to pregnancy sickness.

hCG exhibit's a considerable heterogeneity in maternal blood during pregnancy. (1) Typical isoelectric focusing (IEF) pattern of immuno-reactive (IR) hCG in sera of normal pregnant women at 7 weeks of pregnancy (these components were designated for convenience). A PI (isoelectric point) 3.9, B PI 4.1, C PI 4.4, D PI 4.7, E PI 5.0, F PI 5.8, G approx PI 6.7. In general the reactive amounts of B and C were large while A, D, E, F and G were small. (2)

The isoforms of hCG produced in early and late pregnancy are different. (1) The isoforms of hCG in sera from early pregnancy were more acidic (have a lower isoelectric point) than those obtained from later pregnancy. (1) The hCG molecules with the highest in vivo biological activity were produced during the first trimester. (1) hCG from early pregnancy has a longer half-life (when tested in mice) than hCG from late pregnancy which explains the higher activity in the in vivo bioassay of hCG in early pregnancy. (1)

In the alpha unit of hCG, carbohydrate constitutes 30% of the total weight of which 27.4% is sialic acid. Similarly, the beta subunit contains 36% carbohydrate of which 28% is sialic acid. The sialic acid residues as well as the entire carbohydrate moiety of hCG have been shown to be essential for the full expression of hCG's in vitro and in vivo gonadotrophic activities. (3)

Intact hCG from first trimester pregnancy serum showed multiple peaks on Sephadex G 100. The dominant peak eluted with apparent molecular weight (72,000) higher than that of hCG from third trimester serum (63,000). First trimester hCG is more glycosylated than other forms of hCG. hCG's isolated from first trimester pregnancy showed considerable heterogeneity. The major peaks are apparent at molecular weight 72,000. The data suggests that differences in carbohydrate content account for most of the observed heterogeneity of hCG. (4) The electrophoretic technique measures the overall charge of the isoforms of hCG and the change in the median charge of the isoforms of hCG reflects a change in the carbohydrate of the polypeptide structure. (1)

This change of iso-electric charge during pregnancy is not a continuous process but occurred at a limited period of time, namely 11-15 weeks of gestation. All the values of median mobility were higher at weeks 6-10 than at weeks 16-43 of gestation. The mean values of the degree of charge of heterogeneity of hCG was significantly ($P < 0.05$ and $P < 0.01$ respectively) higher at 11-15 weeks and at 16-43 weeks than at 6-10 weeks. (1) The concentration of serum hCG decreased at the same period of gestation as the median charge of hCG changed. There was a significant ($r = 0.394$; $P < 0.01$; $n = 39$) positive correlation between the median mobility and the concentration of hCG in serum during the weeks 6-10 or the weeks 16-43 of gestation. (1)

The usual time for nausea and vomiting of pregnancy (N.V.P) to cease is similar at 12-14 weeks pregnancy. (5)

Binding of hCG variants to Liver Receptors

The biological activities of desialyted forms of hCG are greatly reduced in vivo due to the

high affinity of asialo-glycoproteins for hepatic receptors and their consequent rapid clearance from the circulation. Intact purified hCG on the other hand has little affinity for hepatic receptors and has a relatively long life in vivo. (6)

The beta-subunit sialic acid seems to be more critical than the alpha-sialic acid in preventing hepatic binding and prolonging plasma half-life. (6) Intact hCG exhibited the slowest metabolism as, very little, if any, of it was removed from the circulation during the thirty minute observation period (in mice). Asialo hCG by contrast has rapidly downgraded and only 2% of the initial concentration was detectable after the first two minutes and less than 0.4% remaining after 30 minutes. Intact alpha-asialo beta was also rapidly removed as about 1% of its residual concentration was detectable even after 30 minutes. Asialo-alpha intact beta was cleared much more slowly as more than 30% of the initial concentration was present at two minutes, whereas, at 30 minutes nearly 10% was still detectable. (6)

Yoshimura et al state other mutations of hCG cause it to be unable to dimerise i.e. for alpha and beta subunits to join which they must for normal activity, such mutations may manifest as elevated free B-hCG. This is seen in molar gestations. They found that compared to 23 gestational age-matched controls, 39 molar patients with hyperemesis had elevated free B-hCG 101 ± 70 ng/ml, 31 ± 31 ng/ml $p < 0.001$. There was also a significant difference between groups for total HCG $9,327 \pm 3,613$ ng/ml, $5,543 \pm 2,290$ ng/ml $p < 0.01$ but not for free alpha hCG 399 ± 231 , $377 \pm$ ng/ml. (7)

Whereas the human alpha-gene is present as a simple copy gene on chromosome 6 there are six genes which encode hCG beta-like products on chromosome 19. Each of these hCG-beta genes are transcribed in vivo but with highly variable levels of expression. The genes hCG beta 5 and hCG beta 3 are expressed at about a 7:1 ratio. Furthermore, analysis of first trimester RNA expression using gene-specific oligonucleotide indicated a 20:1 ratio for the two hCG-beta genes. Could it be that the increased beta hCG unit found in molar gestations, or the change in charge of hCG isoforms around 13 weeks of gestation are due to a change in the relative expression between the genes encoding different hCG proteins? (1)

What was commonly referred to as immuno-reactive hCG is a mixture of free-beta hCG, free-alpha hCG, deglycosylated hCG, desialyted (asialo) hCG and hCG completely lacking the four sites of glycosylation (BCTP). Molar tissue in which only 3% of the measured hCG is fully glycosylated and a majority of hCG is not intact. Large amounts of the basic fractions of HCG were much more potent than intact HCG in stimulating the release of cyclic '5' adenosine monophosphate (CAM) when combined with human TSH or LH Receptors. These basic fractions correspond to the deglycosylated hCG lacking the B-CTP. Thus, part of the puzzle of the relationship between hCG and NVP may lie in the fact that measurements of intact hCG do not reflect various fractions of different potency. (7)

In patients with hyperemesis gravidarum (HG) the hCG profiles differed significantly from those without HG ($p < 0.001$). The HG subjects had higher concentrations in the acidic third when compared with control subjects. Peak 6 (PH 3.3) was observed only in hCG profiles of women suffering from HG. Peak 5 (PH 3.6) occurred significantly more frequently in hCG

profiles of HG women, than with control subjects ($p < 0.05$). Therefore, the acidic forms might be responsible for pregnancy related nausea either by direct effect on the brain stem centres or in intestinal mobility or by indirect effects through secretion of hormones normally related to other glycoproteins, such as thyroid hormones. (8)

“Nicking of hCG”

A proportion of hCG molecules in pregnancy serum and urine samples have nicks or a missing peptide linkage between either beta subunit residues 44 and 45 or beta subunit residues 47 and 48. The nick causes a rapid dissociation of hCG into free alpha and free beta subunits, with consequent ablation of the steroidogenic activity of hCG. (9)

Once nicked, hCG rapidly dissociates into free alpha and beta subunits. Standard hCG (batch CR 127, 20% nicked) and hCG preparation C5 (100% nicked) were incubated for varying times in whole blood. C5 hCG dissociated rapidly into free alpha and beta subunits (dissociation half-life 22 ± 5.2 hours) over 30 times faster than standard hCG (dissociation half-life 700 hours). It was inferred that nicked hCG rapidly dissociates and that the relative amount of nicked molecules produced by trophoblast considering circulation and dissociation half-life be 3.4-3.6 times higher than measured in serum samples. (9)

Levels of total hCG (nicked and non-nicked) and intact hCG (non-nicked) were determined in 233 serum and 168 urine samples from 4-40 weeks of pregnancy. A linear relationship was indicated between advancing weeks of gestation and increasing extent of nicking. Minimum ‘nicking’ was observed in serum from the first two months of pregnancy (mean = 9% of HCG molecules) and increased ‘nicking’ in the months thereafter, with maximum ‘nicking’ in samples from the last months of pregnancy (mean = 21% of hCG molecules $P < 0.00005$). It was concluded that nicking is more prevalent after hCG peak (after two months of pregnancy). (9)

The increased degree of ‘nicking’ of the hCG molecules was reported to be a gradual process throughout gestation, whereas, the median charge changed at a restricted period and then remained constant throughout the second and third trimesters. It, therefore, seems unlikely that the two processes are related. (1)

The Source of “Nicking”

Human leukocyte elastase secreted by neutrophils can ‘nick’ hCG. Type IV collagenases secreted by macrophages are also elastases with the same specificity as the leukocyte enzyme. This enzyme may also ‘nick’ hCG. We postulate that an elastase or type IV collagenase like enzyme, associated with or present in trophoblast tissue, specifically ‘nicks’ and thus deactivates hCG. The progressive increase in proportions of ‘nicked’ hCG may simply reflect the increase of placental mass that occurs throughout pregnancy. We infer that ‘nicking’ occurs before or immediately upon secretion of hCG by trophoblast tissue. (9)

Summary

hCG exhibits considerable heterogeneity in maternal blood during pregnancy. Isoforms of hCG in sera from early pregnancy were more acidic than those obtained from later pregnancy. This acidic hCG is also the most glycosylated hCG and has a longer half-life than basic hCG. A significant change in the charge of hCG and its glycosylation takes place between 11-15 weeks gestation, the hCG becoming more basic with increased deglycosylation. The usual time for nausea and vomiting of pregnancy (NVP) to cease is similar at 12-14 weeks gestation. There is also evidence that fully acidic and highly glycosylated hCG is associated with hyperemesis gravidarum.

Deglycosylated hCG has a high affinity for specific liver receptors and is, consequently, rapidly removed from the circulation, while intact hCG has little affinity for these liver receptors and, therefore, has a relatively long circulation half-life. hCG can also be 'nicked' when a peptide linkage becomes missing at the beta subunit residues 44 and 45 or 47 and 48. The nick causes a rapid dissociation of hCG into free alpha and full beta subunits with consequent ablation of its steroidogenic activity. This 'nicking' is minimal until 8 weeks of gestation and gradually increases throughout pregnancy.

If either of these processes (a) deglycosylation of hCG with increased binding to liver receptors or (b) 'nicking' of hCG does not act efficiently, the resultant fully glycosylated hCG may continue later into pregnancy, possibly causing nausea and vomiting of pregnancy to persist longer than usual until these changes occur.

In order to relate maternal serum hCG to the symptoms of NVP it would be necessary to identify the maternal serum level intact (non-nicked) hCG, the iso-electric focussing pattern of the HCG or its degree of deglycosylation at a specific stage of gestation and determine the severity of the women's NVP at the same stage of gestation.

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Section 4.

HORMONES, CYTOKINES AND PROSTAGLANDINS WHICH INCREASE hCG SYNTHESIS OR RELEASE FROM SYNCYTIOTROPHOBLAST CELLS

Many substances, hormones, cytokines and prostaglandins are known to stimulate the production or release of hCG from maternal syncytiotrophoblast cells in the first trimester of pregnancy.

1. **Gonadotrophin Releasing Hormone (GNRH)**
 - 1a. Pulsatile release of hCG into the medium. Pulse amplitude and frequency were increased in response to GNRH in higher dosage. (1)
 - 1b. GNRH stimulated hCG secretory response by 80%. IL1 beta stimulated a rapid and transient hCG stimulatory response increase approximately 150%, but lower

concentrations were ineffective. Combined treatment stimulated response by 150%. GNRH and IL-1 beta produce an increased stimulation but by different pathways. 8-12 weeks placental trophoblast used. (2)

2. **Epidermal Growth Factor (EGF)**

2a. EGF 7-10 week (gestational weeks placenta). In superficial explants, short (1-4 minutes) pulses of EGF increased both rate and amplitude of spontaneous pulsatility of hCG. The frequency increased from 3 to 5 hours. This effect was dose-dependent and the concentration of 50 ng/ml was the lowest tested and the most effective. In explants cultured for 24 hours EGF caused a two-fold increase in hCG secretion compared to controls. EGF added daily for the first week caused 180% increase in hCG secretion.

2b. EGF stimulated proliferate potential of cytotrophoblast in early (4-5 week) placental explants. The EGF stimulation of trophoblast proliferation was apparent at a 12 hour EGF treated period. By contrast, 6-12 week placental explants did not respond to EGF increase in trophoblast proliferation. Instead, in early placental explant culture, EGF stimulated hCG and Human Placental Lactogen (HPL) secretion with a lag period of 72 hours, whereas, very early placental explants did not respond to EGF with increase in hCG and HPL secretion. Therefore, EGF stimulated trophoblast proliferation in 4-5 week placenta and stimulates differentiated trophoblast function in 6-12 week placenta. (4)

2c. EGF binding sites and EGFR production increase in human placenta throughout the gestation period. (5)

3. **Parathyroid Hormone (1-34PTH)**

Gestational age-dependent effects of parathyroid hormone (1-34 PTH) were noted. In static cultures 1-34 PTH stimulated hCG secretion in 7-9 week placenta in a biphasic fashion, the maximal effect being noted at 10-25ng/ml concentration (250-270%) while at 1 and 100ng/ml the effect was mild. Effects of 1-34 PTH at 11-14 weeks were inhibitory. In static cultures at 7-9 weeks the stimulatory effects of 25ng 1-34 PTH was increased by 70% when EGF 100 ng/ml was added. (6)

4. **Oxytocin (OT) Arginine-Vasopressin (AVP) and Prolactin (PRL) Effects on hCG**

In static cultures OT and AVP significantly increase hCG secretion, whereas PRL had no effect. In superperfusion, one minute pulses of OT induce a significant 2 to 10 fold rise in hCG pulse amplitude. PRL pulses caused a progressive inhibition of spontaneous hCG pulsatility. (7)

5. **Calcium**

Secretion of hCG by first trimester human placental minces. Depletion of calcium (Ca) in the medium by addition of EGTA resulted in a dramatic decrease in the levels of immunoreactive hCG in the medium with consequent accumulation of hCG in the tissue. Calcium is essential for normal secretion of hCG by human placentae. (8)

6. **C'AMP**

Any factor which increases C'AMP in trophoblast tissue will increase maternal hCG secretion. (9, 10)

Factors known to increase C'AMP in trophoblast cells:

- (i) Catecholamines
- (ii) GNRH
- (iii) hCG itself, hCG variants,
- (iv) Prostaglandin E2

7. **Catecholamines stimulate C'AMP in early Trophoblast Cells**

- 7a. The catecholamine Epinephrine stimulated adenylate cyclase activity from 10 weeks of gestation to term. (11)
- 7b. High density of B adrenergic receptors in human placentae composed of B1 and B2 receptors. It is likely that cell size or membrane surface area changes contribute greatly to the decrease in beta adrenergic receptor densities observed with increasing gestational age. (12)
3H-DNA binding to early human placentae, the binding capacity of early crude placental membrane is about three times higher than described in term placentae. (13)

8. **hCG and its Variants Stimulate C'AMP in early Trophoblast Cells**

The acute in vitro effects of human chorionic gonadotrophin (hCG) on human placental glycogen metabolism have been studied in immature placental villi (8-20 weeks) in short term culture. hCG elicited within 15 minutes of culture an acute glycogenolytic response in placental tissue which included a decrease in placental glycogen, an activation of the glycogen phosphorylase enzyme system and a pronounced elevation in the cyclic AMP concentration of the placenta. (14)

9. **hCG Variants**

The principal difference between hCG and other glycoprotein hormones (leuteinizing hormone LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) is the large 31 amino acid tail, the β -carboxyterminal portion (β -CTP). LH is ten times more potent than intact HCG at stimulating the TSH receptor; mutant hCG's lacking the β -CTP were much more potent than intact hCG in stimulating the release of C'AMP when they combined with human TSH and LH

receptors. (15)

Normal human placenta express hCG/LH receptor gene. (16)

10. **Prostaglandin E2 Stimulates C'AMP in early Trophoblast Cells**

The acute in vitro effects of Prostaglandin E2 on human placental glycogen metabolism have been studied in immature placental villi in short term culture. 10 ng/ml Prostaglandin E2 induced a similar glycogenolytic effect including a larger decrease in placental glycogen than 50 iu/ml of hCG and an increase in tissue cyclic AMP concentration. (14)

11. **Interleukin-1 β Stimulates hCG Secretion**

IL1 β (10⁻⁹m) increased basal hCG secretion in placental trophoblast. The response peaked within 25 minutes after IL1 β perfusion was initiated, and hCG secretion returned to basal concentration 10 minutes later. IL1 β (10⁻⁹m) stimulated a rapid and transient hCG secretory response. hCG release increased by approximately 150% in response to the cytokine, but lower concentrations were ineffective. IL1 β (10⁻⁹m) is within the physiological range. 8-12 week placental trophoblast. (17)

12. **Tumour Necrosis Factor Alpha (TNF ALPHA) Stimulates hCG Secretion**

Trophoblast stimulated with rTNF alpha released hCG in a dose-dependent fashion. Simultaneous stimulation of trophoblasts (placentas at 7-9 weeks gestation) with rTNF alpha and IL-1 alpha resulted in synergistic enhancement of IL-6 release, subsequently leading to enhanced hCG release. Although TNF alpha and IL-1 share the intracellular signalling pathway, a comparative study of their potency to stimulate IL-6 production demonstrated that the level induced by rTNF alpha is much lower than the level induced by rIL-1. Similar results were obtained with regard to the capacity of these cytokines to induce hCG release. rTNF alpha induced IL-6 release at doses greater than 200 ng/ml while IL-1 alpha induced IL-6 releases at doses 2.0 ng/ml. (18)

13. **Human Macrophage Colony-Stimulating Factor (M-CSF)**

When human cytotrophoblast cells in the early stage of pregnancy (6-11 week human villous tissue used) were cultured in a serum-free medium in the presence of M-CSF, the cytotrophoblast cells fused and formed a typical syncytiotrophoblast. On the other hand, cytotrophoblasts incubated with anti- M-CSF antibody showed hardly any syncytiotrophoblast formation.

When cytotrophoblasts were incubated in the presence of M-CSF the supernatant of the culture showed an increase in human chorionic gonadotrophin and human placental lactogen (HPL) levels in proportion to the concentration of M-CSF added. When cytotrophoblasts were incubated in the presence of anti-M-CSF antibody or anti-FMS antibody, hCG and HPL secretion were suppressed. Thus, M-

CSF was morphologically and endocrinologically found to induce the differentiation of chorionic cells. (19)

14. **Transforming Growth Factor B1 (TGF B-1) Suppresses hCG Release**

Trophoblast-derived TGF β -1 suppresses cytokine but not GNRH induced release of hCG by normal human 7-9 week trophoblasts. Trophoblast produced predominantly a latent rather than an active form of TGF β -1. (19) rTGF β markedly suppressed rIL-1 alpha and rTNF alpha and IL-6 induced hCG release. In contrast to the TGF β -mediated regulatory activity on IL-6 and HCG release, TGF β exerted no inhibitory or augmenting effect on IL-6 or hCG production. These findings, together with TGF β 's effect on GNRH-induced hCG release exclude the possibility that rTGF β is toxic to trophoblasts and thereby reduces IL-6 and hCG release. This indicates that TGF β , produced by trophoblasts, platelets and monocytes in the placenta might act as a physiological regulator of cytokine-dependent hCG release mechanism in an autocrine or paracrine fashion. (20)

These are some of the organic substances which increase hCG synthesis from syncytiotrophoblast cells in early pregnancy: gonadotrophin releasing hormone, epidermal growth factor, parathyroid hormone, oxytocin arginine-vasopressin hormones, cyclic AMP, catecholamines, hCG itself and variants of hCG, Prostaglandin E₂, Interleukin-1 β , tumour necrosis factor alpha and macrophage colony-stimulating factor. Prostaglandin E₂ is the only substance in this group that is known to cause nausea and vomiting.

HORMONES, CYTOKINES AND PROSTAGLANDINS WHICH INCREASE hCG SYNTHESIS OR RELEASE FROM SYNCYTIOTROPHOBLAST CELLS

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Section 5.

PROSTAGLANDIN E2 IS KNOWN TO CAUSE NAUSEA AND VOMITING IN EARLY PREGNANCY WHEN USED FOR TREATMENT

Nausea and vomiting were the most troublesome side effects when PGE₂ was first used to procure a termination of pregnancy in the early 1970's. These side effects of nausea and vomiting associated with giving Prostaglandin E₂ and F₂ α (PGs) have been clearly and persistently described when given by intravenous infusion (1) (2) by single intra-amniotic injection (3) by intrauterine extra-amniotic infusion (4 and 5). The oral route of PG administration is quite unsuitable because of the severity of side effects (6). A more modern paper describing the use of Prostaglandin E₂ pessaries 10mg given pre-operatively before termination of pregnancy states, the use of these pessaries was found to be associated with an unacceptably high incidence of nausea and vomiting. The incidence of nausea and vomiting is only too apparent to those who provide an anaesthetic service to patients who have received prostaglandins (7). Wiqvist (2) found the continuous intravenous infusion of PGF₂ α caused nausea and vomiting, these symptoms disappeared on reducing the infusion rate, and the dose which caused the side effects varied considerably from one case to another. The side effects of nausea and vomiting caused by intravenous infusion of Prostaglandin E₂ were found by Karim (1) to be worse in patients suffering from hyperemesis gravidarum. Gillett (8) showed raised plasma levels of prostaglandins were associated with an increased incidence of nausea and vomiting, and that those side effects regressed rapidly when the infusion was terminated. These authors have clearly described the relationship between Prostaglandins E₂ and F₂ α with the side effects of nausea and vomiting in pregnancy when used for treatment.

PROSTAGLANDIN E2 IS KNOWN TO CAUSE NAUSEA AND VOMITING IN EARLY PREGNANCY

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Section 6.
THE PRESENCE OF PROSTAGLANDIN E2 IN THE EARLY
SYNCYTIOTROPHOBLAST

As we have found a relationship between hCG and pregnancy sickness between weeks 7 and 14 from LMP and a relationship between hCG and PGE2 synthesis in early trophoblast cells, it seems reasonable to make further investigation of the possible association between Prostaglandin E2 levels and pregnancy sickness.

Kelly et al wrote, a mechanism by which the trophoblast could control its immediate environment in the decidua and maternal blood may be through the release of prostaglandins and cytokines. This possibility has been investigated here by measuring PG and several cytokines released from first trimester trophoblastic villi cultured for 24 hours. PGE is the main primary prostaglandin with a release of 240 ± 95 pg/mg/24 hours (1). Because PGE can influence the function of many leukocytes (particularly macrophages) by raising intracellular c'AMP concentrations, the release of hormonal factors from the villous trophoblast might be important in pregnancy maintenance. Trophoblastic villi were obtained from women undergoing a first trimester 7-11 week termination of pregnancy (1). Johnson et al found the PGE2 was produced by a syncytial (differentiated) trophoblast. Cells grown beyond 24 hours in fibrin showed sustained expression of cyclo-oxygenase and this enzyme protein expression correlated with increased PGE2 production in differentiated (syncytial) trophoblast. Term placentas were used (2). Cheng et al found there was clear distinction in the distribution of immunoreactive PGE2 among the different cell types of trophoblastic villi at 56 days of amenorrhoea or less. The cytoplasm of syncytiotrophoblast cells stained positively for PGE2, whereas, there was very little, if any, staining in the cytotrophoblastic layer (3). The syncytiotrophoblast is devoid of Prostaglandin dehydrogenase (PGDH) and, therefore,

contains high levels of PGE₂ (4). This distribution of catabolising enzyme leaving unmetabolised PGE₂ only on the maternal aspect of syncytiotrophoblast cells which might be important where these villi, and particularly the syncytiotrophoblast cells are in direct contact with maternal blood (1).

THE PRESENCE OF PROSTAGLANDIN E₂ IN THE EARLY TROPHOBLAST

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Section 7.

HORMONES AND CYTOKINES WHICH INCREASE THE SYNTHESIS OF PROSTAGLANDIN E₂ IN SYNCYTIOTROPHOBLAST CELLS

Activators which stimulate cells by means of cell surface receptors also cause release of prostaglandins from these cell membranes. This mechanism of prostaglandin stimulation is illustrated in Figure 1 from Dinarello's paper Biology of Interleukin-1 (1). Examples of this mechanism include Interleukin-1 which induces a five fold increase in Prostaglandin E₂ production, a response that was density, time and dose dependent. 8-10 week human placentas used (2). HCG itself has also been shown to stimulate PGE₂ synthesis in 9-12 week placentas at physiological conditions. The rate of PGE₂ synthesis increased with a longer

incubation period, particularly in placentas of younger gestation. Significant stimulation of PGE₂ synthesis occurred at 10⁴ iu/1 HCG and continued to increase in a dose-dependent manner up to 5 x 10⁶ iu/1 as seen in 9-10 week placental organ cultures. There was considerable variation of PG production between placentas of the same gestation (3).

Prostaglandin E₂ is also synthesised in early pregnancy by macrophages and monocytes present in the decidua (4) and at the materno-fetal interface (5) and by decidual stroma cells (6). There is therefore, clearly evidence of the presence of prostaglandin synthesis and stimulation of Prostaglandin E₂ production in the various cells of the materno-fetal interface in early pregnancy.

This study investigated the regulation of cyclo-oxygenase-2 (COX-2) gene by human CG (hCG) in mucosal cells from fallopian tubes. Culturing mucosal cells with increasing concentrations of hCG also resulted in a dose-dependent increase in media PGE₂ levels suggesting that the COX-2 protein increased by hCG is catalytically active. Although hCG treatment had no effect on the transcription rate of COX-2 gene, it significantly increased the stability of COX-2 transcripts from 3.7_h in the control to 7.3_h after treatment. (7)

HORMONES AND CYTOKINES KNOWN TO INCREASE THE SYNTHESIS OF PGE₂ IN SYNCYTIOTROPHOBLAST CELLS

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Section 8.

PROSTAGLANDIN DEHYDROGENASE ACTIVITY IN TROPHOBLAST CELLS OF THE EARLY HUMAN PLACENTA

Prostaglandin dehydrogenase is an enzyme required for the first step of prostaglandin metabolism. In human uterine and intrauterine tissues, oxidation of the 15-hydroxyl group of prostaglandins is catalysed almost entirely by a NAD-linked 15-hydroxyprostaglandin dehydrogenase (PGDH) (1). PGDH activity was measured in homogenates of 25 human placentae between 7 and 10 weeks of gestation. Between 7 and 8 weeks of gestation and 15 and 16 weeks, mean values for PGDH increased 10 fold. The data indicate that the development of terminal villi and the migration of trophoblast into the maternal spiral arteries is associated with a substantial increase in the placental capacity for prostaglandin metabolism (1). There was a large variation in PGDH activity between individual placentae of each of the early gestational stages. (1)

Prostaglandin E2 is a better substrate for this enzyme than Prostaglandin E1. The first step in the metabolism of Prostaglandin E2 involves oxidation of the 15-hydroxyl group to its inactive metabolite 15 Ketoprostaglandin E2 (2). There is a wide variation in the PGDH content in individual placentas (2). The syncytiotrophoblast has the ability to synthesise Prostaglandin E2 and is devoid of PGDH but PGDH is present in cytotrophoblast cells. (4)

In early human placentae 15-OH PGDH activity decreases between 5 and 9 weeks and then gradually increases in week 9. The activity was significantly lower than at other times during gestation. The progesterone content of the placenta changes in a similar way to PGDH activity and is lowest in week 9. On regression analysis there is a fairly close positive correlation ($r^2 = 0.69$) between PGDH activity and the concentration of progesterone in the placenta during the period studied (3). Prostaglandin dehydrogenase is under progesterone control in reproductive tissues. That is, if progesterone is raised, PGDH will be raised and PGE2 will be lowered. After treatment with anti-progesterone RU 486, in vivo levels of PGDH fall in uterine tissues and PGE2 will be raised. (4) (5)

It is relevant to relate anti-progesterone treatment for induction of early termination of pregnancy to nausea and vomiting of pregnancy. Epostane, the competitive inhibitor of the

enzyme 3 β -Hydroxysteroid dehydrogenase, therefore a specific anti-progesterone, when given in a dose of 200mg orally every 6 hours for 7 days in 50 women in the 5th through the 8th week of pregnancy, nausea occurred in 86% of women (6). Thirty-four early pregnant women (duration of amenorrhoea for up to 49 days) admitted to hospital for termination of their pregnancy received 25mg RU 486 (anti-progesterone, a progesterone receptor antagonist) twice or four times daily for 4 days. Seven patients (20%) had increased nausea during RU 486 treatment (7). It is reasonable to suggest that the cause of increased nausea in both instances is due to lowered progesterone in decidual and chorionic villus tissues, with lower prostaglandin dehydrogenase and raised PGE₂ tissue and maternal serum levels.

PROSTAGLANDIN DEHYDROGENASE ACTIVITY IN TROPHOBLAST CELLS OF THE EARLY HUMAN PLACENTA

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Section 9.

WHY IS RAISED MATERNAL SERUM PROSTAGLANDIN E2 NOT ASSOCIATED WITH AN INCREASED INCIDENCE OF MISCARRIAGE?

Both anti-progesterones such as Mifepristone (RU 486) and Prostaglandins have achieved considerable success in their ability to induce abortion. The most effective treatment has been the administration of a primary dose of anti-progesterone and the subsequent treatment with a small dose of synthetic prostaglandin, eg. Misoprostol (1). Only a small dose of PGE2 needs to be used, a considerable advantage due to the side effects of NV (nausea and vomiting) it causes.

If a raised maternal serum PGE2 is associated with increased NVP (nausea and vomiting of pregnancy) as we suggest (2), why is raised maternal serum PGE2 not associated with an increased incidence of miscarriage?

Prostaglandin dehydrogenase (PGDH) is the main inactivating enzyme for prostaglandins and, therefore, controls local levels of prostaglandins. PGDH is under the control of progesterone, as we have described in this paper. As progesterone synthesis increases in pregnancy, so PGDH will also increase and inactivate PGE2. Cheng et al (3) wrote, in early pregnancy at 55 days of gestation PGDH staining in the cells of the decidual mucosa taken from a patient who had undergone surgical termination of pregnancy with no prior treatment, showed intense staining for PGDH in decidual stroma cells, in decidual glands and in the endothelial lining of cells of small blood vessels, both veins and arteries, of the decidua. In the small arteries the cells of the muscle layer were also PGDH positive, and intensely positive decidual stroma cells were arranged around the circumference of the vessel. In contrast in RU 486 treated tissue, the endothelial cell lining of small blood vessels was commonly immunonegative for PGDH or with only weak and focal reactivity, and with reduced immunoassaying for PGDH of the surrounding muscle and decidualised stroma cells. Thus, PGDH in decidual tissues would normally protect the uterine myometrium from the side effects of maternal PGE2 (3). Indeed, Bygdeman et al (4) have found that within 24 hours of the administration of Mifepristone (RU 486 anti-progesterone which lowers PGDH activity in cells) to women in early pregnancy, there is an increase in the contractility of the uterus and the uterus becomes very sensitive to exogenous prostaglandins.

In contrast, the chorionic villi from a patient after surgical termination of pregnancy stained intensely for PGDH in the cytotrophoblast cells, but with much less staining for PGDH in the syncytiotrophoblast cells (3) suggesting the Prostaglandin E2 would still be synthesised by these syncytiotrophoblast cells which bathe in maternal blood at the materno-fetal interface. This would enable the maternal serum PGE2 to be raised at this stage of pregnancy. In the natural state it is PGDH in decidual cells and small blood vessels which prevents the PGE2 from causing an abortion, although the maternal PGE2 produced by the syncytiotrophoblast

cells of the chorionic villi can still be the cause of NVP.

Cheng et al (5) investigating the effect of Mifepristone (RU 486) on the immunohistochemical distribution of Prostaglandin E and its metabolite in decidual and chorionic tissue in early pregnancy wrote, there was a clear distinction in the distribution of immunoreactive PGE₂ among the different cell types. The cytoplasm of syncytiotrophoblast cells stained positively for PGE₂, whereas, there was very little, if any, staining in the cytotrophoblast layer. There was no obvious difference in staining between control villi from women who had a surgical termination of pregnancy and villi from women treated with RU 486. The intensity of PGE₂ immunoassaying was greater in decidual tissue after RU 486 treatment compared to that in controls. These changes were most obvious in blood vessels (P<0.001) after only 12 hours of RU 486 treatment. Overall, PGE₂ staining of stroma cells increased compared to that in controls, although changes were less marked than the changes in corresponding blood vessels. Cheng continues, the early appearance after RU 486 administration of a change in metabolism as indicated by the striking increase in PGE₂ in blood vessels, suggests that one of the main mechanisms by which progesterone ensures a continuing successful pregnancy is the maintenance of low PG levels in the vasculature.

In summary, the reason that raised maternal serum PGE₂ which is associated with increased NVP is not associated with an increased incidence of miscarriage, may be that the presence of PGDH in decidual cells and decidual small arteries and veins results in a low level of PGE₂ in the decidua and small vessels, thus protecting the uterine myometrium from PGE₂. On the other hand, PGE₂ continues to be synthesised in syncytiotrophoblast cells, where there is very little PGDH, and which bathe in maternal blood. Released from these syncytiotrophoblast cells, PGE₂ can pass from the materno-fetal interface into the uterine veins, as Human Chorionic Gonadotrophin must also do, with little effect, on the uterine decidua or myometrium.

WHY IS RAISED MATERNAL SERUM PROSTAGLANDIN E2 NOT ASSOCIATED WITH AN INCREASED INCIDENCE OF MISCARRIAGE?

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Section 10.

POSSIBLE REASONS NAUSEA AND VOMITING DOES NOT OCCUR IN CHORIOCARCINOMA

PGE₂ is not or is only minimally synthesised by choriocarcinoma cells.

The authors were unable to demonstrate the presence or synthesis of prostaglandins in human malignant choriocarcinoma cells (four lines BEWO, JAR, 2 and Omega 2) growing in continuous culture. (1)

To determine the production of PGE₂ and PGF₂ α by JEG-3 choriocarcinoma cells, the cells were incubated in either (a) control media or (b) media containing TNF- α (1-20ng/ml). After 15 mins - 23 hours media were collected and analysed directly for PGF₂ α and derivatised for the determination of PGE₂ as the methyloxinate derivative. TNF α (1-20ng/ml) appeared to have no effect on the viability of JEG-3 cells. The effect of TNF α on the formation of PGE₂ and PGF₂ α was studied at 15 mins and 24 hours. Levels of PGE₂ and PGF₂ α were below the limit of assay sensitivity in all cultures (1.25 and 3.1pg respectively). We were unable to demonstrate any effect of TNF- α on the production of either PGE₂ or PGF₂ α . (2)

Decrease in Nicotinamide Adenine Dinucleotide-dependent 15-Hydroxyprostaglandin Dehydrogenase Activity (PGDH) may be important in the accumulation of PGs in neoplastic tissues. The activity of PGDH in neoplastic trophoblast tissue, namely hydatidiform mole tissues and in choriocarcinoma cells grown in monolayer culture and the activity of PGDH in term placenta which is known to be high, was compared at the same time. The specific activity of PGDH in hydatidiform mole tissue (0 to 1.2nmol 15 ketoprostaglandin E₂ formed x min⁻¹ x mg⁻¹ cytosolic protein) and in choriocarcinoma cells (BEWOline) (1.0nmol 15-ketoprostaglandin E₂ x min⁻¹ x mg⁻¹ protein) was strikingly less than that found in normal term placental tissue (11.4 \pm 2.3 (SE) nmol ketoprostaglandin x min⁻¹ x mg⁻¹ protein). PGDH activity in neoplastic tissues was found to be one tenth or less of that in normal term human placentae. (3) If choriocarcinoma cells were able to synthesise prostaglandin E₂ or PGF₂ α then the very limited amounts of PGDH in these cells would allow considerable quantities of these prostaglandins to be produced. The lack of PGDH in these cells suggests that very little

or no PGE₂ is synthesised in choriocarcinoma cells.

POSSIBLE REASONS WHY NAUSEA AND VOMITING DOES NOT OCCUR IN CHORIOCARCINOMA

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Section 11.

CLINICAL FEATURES OF NVP WHICH CAN BE RELATED TO MATERNAL SERUM PROSTAGLANDIN E₂

There are some features present in the symptom complex of NVP which can be associated with maternal serum PGE₂.

Firstly, the variation in NVP from pregnancy to pregnancy, indeed no two pregnancies have exactly similar symptoms, and the variation in NVP from one pregnancy to the next in the same mother, which occurs in between 33-50% of pregnant women. These variations can be explained by the many different maternal serum concentrations of placental specific hormones and cytokines in each individual pregnancy. They stimulate the synthesis of hCG which, consequently, varies in each pregnancy. hCG itself is one of the factors which will stimulate PGE₂ production, which consequently differs in each pregnancy. There is also a large variation in the amount of Prostaglandin dehydrogenase (PGDH) activity between individual placentae at the same stage of gestation.

Secondly, the finding that whether NVP begins early or late, severely or mildly, it ceases at approximately day 84 from LMP (1) suggests that another substance, possibly PGDH activity, is required to reduce NVP.

Thirdly, the median week of peak NVP is week 9 from LMP (ration 8-10 weeks) (1). This week corresponds with the week of peak maternal hCG serum levels, and the nadir of maternal serum progesterone. High serum hCG gives maximum stimulation of maternal PGE₂ synthesis. Low maternal progesterone leads to reduced placental PGDH. Both raised PGE₂ and low PGDH will be related to increased NVP at that time of gestation.

Fourthly, the positive correlation with NVP and non-smoking status (2), agreeing with the finding of 10 other authors, can be due to the damage cigarette smoking in pregnancy causes to the placental cells, with resultant marked decrease in maternal hCG (3) and PGE₂.

Fifthly, we have published the paper Nausea and Vomiting of Pregnancy: An Association Between Symptoms and Maternal Prostaglandin E₂ (4), which demonstrates a positive relationship between NVP and maternal serum PGE₂ levels. For each of 18 women the maternal serum PGE₂ was higher when she had NVP than when she had no NVP on the same day. This remained true whether the sample taken at the time she had NVP was before or after midday. However, we would not recommend any attempt to reduce maternal PGE₂ during early pregnancy, because of the vital functions PGE₂ has at that time of gestation. We are, however, of the opinion that treatment of NVP can and should be given safely and effectively as it is now in Canada.

CLINICAL FEATURES OF NVP WHICH CAN BE RELATED TO MATERNAL SERUM PROSTAGLANDINS E₂

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Section 12.

SOME FUNCTIONS OF PROSTAGLANDIN E2 IN EARLY PREGNANCY

Although we attempt to make the case for maternal serum Prostaglandin E2 being a cause of pregnancy sickness, we realise that no attempt should be made to reduce the synthesis of Prostaglandin E2 in early pregnancy because of the vital functions of Prostaglandin E2 at that time. These functions include:

A. **Immunosuppression of Decidual CD-16 CD56 Bright NK Cells (LGL White Cells)**

Uterine large granulated lymphocytes (LGL), constitute 70% of the decidual white cells, together with macrophages 20% and CD₃ T Cells 10%. Peripheral LGLs respond to Interleukin-2 (IL-2) by proliferating and becoming potent lymphokine activated killer cells capable of greatly increased killing of K 562 cells (1) and human trophoblast cells (2). These decidual CD-16 CD56 Bright NK cells possess a high affinity receptor for IL-2. These NK cells have both Interleukin-2 receptors alpha and beta (3). Their NK (killer) activity is markedly elevated even by treatment with small amounts of IL-2 (3). Therefore, there needs to be suppression of IL-2 at the fetomaternal interface.

Prostaglandin E2 inhibited T lymphocyte proliferation by 80-90% of control values. This was associated with a similar degree of inhibition of IL-2 production while the expression of IL-2 receptors was not affected. This was in marked contrast to the expression of the transferrin receptor, which was inhibited 65% after 72 hours of in vitro action (4). These studies demonstrate that Prostaglandin E2 exerts its inhibitory effect on T cell activation and proliferation via two distinct pathways; inhibition of IL-2 production and inhibition of transferrin receptor expression. The transferrin receptor expression is mediated via the C'AMP pathway and is IL-2 dependent. (4)

B. **Stimulation of Cyclic AMP Levels in Immature Placental Villi**

The presence of Prostaglandin E2 in the culture medium evoked a glycogenolytic effect in immature human placental villi, 8-20 weeks placentae used, including the increase in tissue cyclic AMP levels. The effects of 10mg/ml Prostaglandin E2 were more marked than those of hCG on activation of phosphorylase. Prostaglandin E2 induced a larger decrease in placental glycogen and a 149% increase in cyclic AMP concentration in immature placental tissue. (5)

The fact that Prostaglandin E2 stimulates C'AMP and glycogenolysis has significant effects on trophoblast cells as C'AMP mediates the action of a wide variety of hormones on target tissues. (6)

Prostaglandin E2 has other functions in early pregnancy, for example, the synthesis of matrix metalloproteinases which assist cytotrophoblast invasion of the decidua is partly dependent upon PGE2 production (7). However, the two significant functions mentioned above alone serve to show the importance of Prostaglandin E2 in early pregnancy. Therefore, any attempt to improve pregnancy sickness by reducing Prostaglandin E2 synthesis in early pregnancy could adversely effect that pregnancy.

SOME FUNCTIONS OF PROSTAGLANDIN E2 IN EARLY PREGNANCY

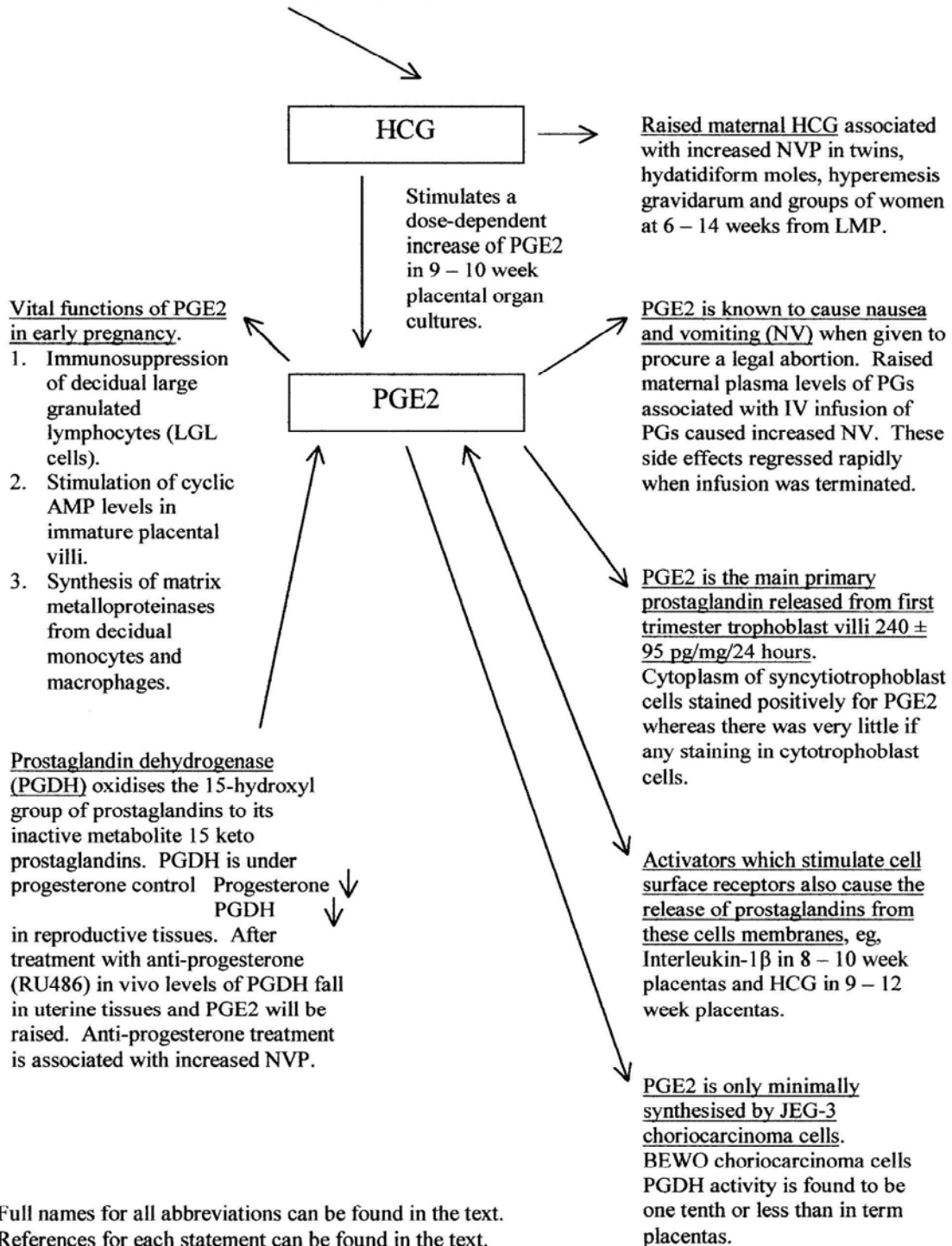
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HORMONES AND CYTOKINES WHICH STIMULATE AND RELEASE HUMAN CHORIONIC GONADOTROPHIN (HCG) FROM SYNCYTIOTROPHOBLAST CELLS

GNRH, EGF, 1-34 PTH, OT, AVP, Ca, C'AMP, factors which stimulate C'AMP, ie, catecholamines, HCG itself, HCG variants, PGE2 and separately IL-1 β , TNF α and MCSF



Full names for all abbreviations can be found in the text.
References for each statement can be found in the text.