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Hyperglycoslated hCG and Pregnancy Failure

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Abstract

The purpose of this report is to combine the data from different resources and investigate the impact of rising hCG-H level during early pregnancy in woman. Giving an in-depth review of hCG molecules and the possible consequences of insufficient hyperglycosylated hCG in pregnancy failure. Three articles have been selected containing corresponding information, which could be combined and used as a strong evidence to explore the correlation between hCG-H and pregnancy failure plus the roles of hCG-H in embryo implantation. The data collected from the articles have suggested a very strong correlation between hCG-H level and pregnancy failure. All studies managed to achieve a mean success rate of 74% indicating three-quarter of the pregnancy failure are detected due to insufficient level of hCG-H during early implantation. The results collected do comply with the theory suggested for pregnancy failure. it is known two-third of pregnancy failures are caused by insufficient hCG-H during implantation and the other one-third is caused by chromosome abnormalities. The result is a good indication hCG-H does play a major role in successful pregnancy. It is also a preferred method over hCG due to its consistency throughout different gestation stages. hCG-H managed to yield better results over hCG, which means there could be a possibility in the future hCG is replaced by hCG-H as the main molecules to monitor pregnancy outcome. It is also worth to note serum samples can be a superior alternative for urine samples. Both samples are deemed to share similar sensitivity but it is claimed that urine samples have a 5 folds higher chances of returning false positive results.

Introduction

Extensive researches have been done on early pregnancy detection to understand the mechanisms behind early spontaneous abortion, biochemical pregnancy and the reason why it happens. Biochemical pregnancy is a common form of pregnancy failure accounting for 25% of cases. The pregnancy will terminate immediately after a short rise in hCG level. This occurs at a very early stage, even ultra sound detection wont be able to diagnosis it. Another 17% of female pregnancy will fall into the spontaneous abortion category. It normally occurs during the first and second trimester of pregnancy (Norwitz et al., 2001).

Pregnancy failure can be caused by many different factors. Environmental exposure, damages induced by physical impact to the embryo or the uterus causing unsuccessful implantation or triggering spontaneous abortion. Surgical operation and tumours growth can disfigure the uterus, inducing scaring, damaging the ability of successful embryo implantation. It is widely accepted conceptus chromosome abnormalities contribute to at least one-third of the pregnancy failure (Wright et al., 2004). Other factors that can contribute to pregnancy loss can be common diseases like diabetes, t cell immune defects, or hormonal disorder etc. (Barnhart, 2012). However the effect of these disorders is normally ignore due to its minimal impact on pregnancy failure. Researches have found evidence suggesting the other two-third of pregnancy failure is

caused by improper implantation during pregnancy. It is known that hCG and hCG-H are vital for successful embryo implantation. Both hCG molecules can also used to determine the chance of early pregnancy failure (Jauniaux et al., 2006).

Human Chorionic Gonadotropin widely known as hCG is a hormone promoting the production of progesterone in woman by corpus luteal cell or trophoblastic cells, which later forms the major part of the placenta. During pregnancy the level of hCG can rise substantially due to the extra production of hCG by syncytiotrophoblasts cells in the placenta, in preparation for the fetal development. The hormone plays a very important role for successful pregnancy (Urbancsek el al., 2002). The main beneficial features of hCG are the promotion of progesterone production, angiogenesis in uterine vasculature, growth of the uterus and fetal organs development and many other important factors, which contribute to body changes in the female dictating pregnancy success rate (Cole, 2010).

It has been a question why 5 different molecules with different biological functions are given the same name. Other forms of hCG were initially assigned a different name. When hyperglycaostlated hCH was first discovered in 1997 it was named Invasive trophoblasts antigen (ITA) (Cole, 2012).4 Years later World Health Organization have denied the name ITA and stated all molecules with the same set of amino acid sequence must be given the same name. Therefore part of the name must contain the word “hCG”. Hyperglycostlated hCG were later given, because its resemble the oversized sugar structure form of hCG (Cole, 2012).

In the early discovery of hCG, the hCG hormone has always been thought of as a single molecules. Recent advancement in technology has totally changed the hCG concept established years ago. The hCG molecule compromises 3 independent molecule, the major component defining hCG from others is the beta-subunit peptide structure and it is the common side chain exist in all other type of hCG. The beta-subunit peptide structure contains 145 amino acid Beta subunit. All hCG share the common beta-subunit side chain, hence the name given, but its functions and working mechanism can be totally different to others (Cole, 2007).

hCG in the science world is the only known example in biochemistry of one amino acid that could describe 5 independent molecules (Cole, 2012). Researches showed hCG molecules are composed of mainly glycoprotein, 28-39% of the molecular weight is due to the sugar side chain. Apart from this similarity, each of the individual hCG behave very differently. hCG is produced by the placental syncytiotrophoblast cells Sulfated hCG is produced by pituitary gonadotrope cells. hCG-H autocrine is produced by placental cytotrophoblast cells and the two other type of hCG, cancer promoter hyperglycoslated hCG, hCGβ, are produced by cancerous tumour. Hyperglyocasted hCG and sulphated hCG share the same subunit combination but vary in carbohydrate structure, and the other 3 independent hCG molecules have different carbohydrate structure and subunit combinations (Cole, 2012).

The reason other forms of hCG variant existed is believed due to the hCG’s evolution origination (Cole, 2012). However there are no detailed researches

have been found to support the current theory of how hCG variant molecules originated. The only known fact, limited by current technology, is hCG has molecular evolutionary origins with growth factor TGFβ. They are sharing similar DNA and amino acid sequences suggesting the possibility of hCG origination. hCG share 4 unique peptide cystine knot structure, which resemble 3 other cytokine. The hyperglycoslated version of hCG, and hCGβ contain a larger carbohydrate subunit which limit subunit folding resulting the exposure of similar structures found on TGFβ. Therefore hCG-H, hyperglycoslated hCG-b and hCGβ all compete with TGFβ growth factor to bind to TGFβ receptor. Regular hCG doesn't have the circulate reproduction capability like hCG-H and it doesn't bind to TFGβ receptor, it only target hCG/ LH receptor (Ticonni et al., 2007).
β
Gestational diseases can also trigger the rising level of hCG indicating possible cancer development. The cancer of trophoblastic cells or choriocarcinoma and all other type of cancer cells often involve TGFβ growth factors. The natural job of TGFβ is to block apoptosis, promote cell growth and most important of all allow invasive enzyme synthesis. It is a vital component in during implantation stage of pregnancy. However the property of this growth factor are also used by tumours cell to perform invasive growth and indefinitely replicate (Cole, 2012). Researches have found the accumulation of hCG-H during pregnancy could result in cancerous growth after given birth (Zygmount et al., 2002).

hCG molecules can be separated into endocrine and autocrine according to their functions. hCG and sulphated hCG stimulate the production of progesterone by binding to hCG/LH receptors, which in term will trigger the growth of myometrium in preparation for baby development inside the womb. Unlike hCG and sulphated hCG, the other 3 hCG molecules play an autocrine role within the body. The functions of hyperglycostlated hCG, hCGβ and hyperglycostlated hCGb play more of an autocrine role within the body rather than endocrine. All 3 hCG molecules target transforming Growth factor TGFβ receptor acting as a cytokine and antagonise a TGFβ receptor. Those 3 autocrine hCG can modulate the apoptosis of trophoblasts cells, indicate cancers and act as a tumour markers. This method of molecule and receptor interaction is defined as autocrine (Cole, 2012).

Hyperglycoslated human chorionic Gonadotopin, acronym hCG-H, contain a similar beta-subunit component but a different obligosaccirde, which gives it a different biological function. hCG-H is produced by the cytotrophoblast cell promote invasion during the implantation stage of the embryo inside the uterus lining. Apart from aiding the implantation stage of pregnancy, hCG-H also drives the growth of placental tissue (Cole et al., 2006). Unfortunately there is also a chance of it triggering the malignancy of choriocarcinoma and gem cell malignancies on cytotrophoblast tissue. In summary the concept of hCG-H is very straightforward, it is produced by the cytotrophoblast cell and circulate around the body before acting back on antagonizing TGFb receptor to promote growth and cells invasion (Guibounche et al., 2010).

For successful implantation it is vital to establish fetal blood supply to deliver fundamental nutrients and excrete toxic waste during different stages of

pregnancy. To achieve this there are two steps involved requiring both hCG and hCG-H. During the first 3 weeks of early pregnancy, hCG promotes angiogenesis in the uterine vasculature to ensure maximum amount of blood can reach to the invading placenta (Cole, 2009). On the other hand hCG-H promote the fusion of cytotrophoblast and differentiate it from synctiotrophoblast resulting villous trophoblast tissue formation. The combination of villous trophoblast tissue formation and angiogenesis will form the maternal fetal blood supply interface, also known as hemochorial placentation (Guibounche et al., 2010).

In 2006 hyperglycoslated human chorionic gonadotropin beta have been proven containing a similar structure to hCG-H. Both hCG molecules share similar amino sequence apart from hyperglycoslated hCG-b contain a larger sugar structure. Hyperglyoslated hCG-b and hCG-b both are produced by all advanced maglicancy except the choriocarcinoma and gem cell malignancies, which produces hCG-H. hCG-H, hyperglycoslated hCGb and hCGb. They are cancer growth promoters well known to the scientists (Cole and Butler, 2012). All 3 hCG molecules are involved in advanced cancer growth but the proportion of hCG molecules can vary according to the type of cancers. This kind of cancers aid their own growth by further producing hCG molecules to repeatedly stimulate malignant growth. Therefore an elevated hCG autocrine molecules doesn't necessarily mean pregnancy have taken place (Cole et al., 2006).

Pregnancy failure commonly occur during 5 -20 weeks of gestation. The rate of successful implantation is mainly dependant on the age and ethnic group, the chances of implantation failure could be inbetween 30-60% (Cole, 2012). Recent case studies have shown the main cause of early pregnancy failure could be due to ineffective embryo implantation or rejection of embryo due to immune interaction (Cole et al., 2003). The repeatedly antagnosation of TGFb receptors inducing higher hCG-H production seems to be the primary reason for pregnancy failure. The deficiency of hCG-H causes incomplete blastocyst implantation, chemical pregnancy and miscarriage. (Kovalevaskaya et al., 2002)

In the past hCG was the main molecule used for pregnancy failure detection. It measures the doubling rate of hCG over 2 days and conclude the chance of a pregnancy failure. However this method is deemed unfavourable due to its high rate of false positive results. It can only detect 28% of miscarriage with a 36% false positive rate, which could results in unnecessary stress to the mother. The predominant form of hCG presence in serum and urine samples seems to be hCG-H. It can be detected at the very early stage of pregnancy, especially during the implantation stage and the month that follow (Sutton-Riley et al., 2006).

The more predominant hCG-H seems to provide much accurate results in pregnancy failure detection. Multiple investigations have established hCG-H is primarily produced during and after implantation. (Kovalevaskaya et al., 2002). The proportion of hCG-H vs hCG found in women suffering from spontaneous abortion pregnancies is significantly lower. Its has been determined only hCG-H can absolutely differentiate pregnancy that will progress to term or pregnancy that will fail. It is suspected the concentration of hCG-H can provide an indication of pregnancy failure (Barnhart et al., 2004).

Even though as stated above hCG and hCG-H both work together to establish fetal blood supply interface and promote the embryo implantation process. However more researches have established extra information on the reason why hCG isn't very suitable for pregnancy failure detection other than the fact its test results have a low reliability. hCG was originally used to determine the chance of pregnancy failure to occur, but its inconsistence level in serum and urine making selection of the right moment much harder to achieve (Sasaki et al., 2007).Unlike hCG, the production of hyperglycoslated hCG remain extremely consistent in early stage of pregnancy with limited differential status of trophoblast cell. hCG is not favourable for early pregnancy detection because its abundance in samples can vary significantly between different individual (Cole, 2009). Also there is one major flaw resulting unsuitability of hCG for pregnancy failure detection. The early level of hCG present in serum and urine sample are produced by only matured and differentiated syncytiotrophoblast cells resulting inconsistent results according to the rate of maturation and how well cells differentiated. hCG-H is totally opposite and it is produced by stem cytotrophoblast cell, which giving a very stable level of hCG-H production during early stage of pregnancy (Sutton-Riley et al., 2006).

The major aim of this report is to explore the correlation between hCG-H and pregnany failure using existing data from different studies, combining them to give a bigger picture of the current knowlodge on role of hCG-H in pregnancy failure. Although hCG-H is currently not as widely employed as hCG but its significance can't be ignored.

Method

The literature materials used in this report were found using several methods and resources. The process of gathering information were mainly dependant on the usage of scientific journals search engines, google and Middlesex university library. The keywords that were used in search engines include: hCG, hCG-H, hyperglycoslated hCG, pregnancy failure, abortion and spontaneous abortion.

The process of gathering information were done using search engine Science direct and google with the keyword stated above. The 2 separate search criteria were set so only subject related results are shown. The results must contain either hCG-H, hCG, human chorionic gonadotropin, hyperglycoslated hCG, or hyperglycoslated human chorionic gonadotropin plus pregnancy failure, abortion, or spontaneous abortion. The search results have returned 1,125 articles, which is further filtered by the date published and its usefulness toward this report. Only articles with a relevant title and focus will be investigated, if the information contained in abstract is deemed viable, the articles will be fully analysed and useful information are used to write different section of this report.

Data Analysis

Some of the data are already available collected by studies, as hCG-H is raising the awareness of its advantage over the current pregnancy detection method. Scientific Studies that contain research data that deemed useful will be further analysed statistically and make a possible conclusion to answer the question raised by this report regarding the correlation of hyperglycoslated hCG and pregnancy failure. There are 3 studies found containing statistical and graphical data closely related to the research topic.

Apart from general statical analysis minitab will also be employed to further analysed the data to give an overall picture of the results. Studies carried out by Saski Y et al., and Cole, L.A, both used urine sample with the same cut off point. Other factors such as stage of gestation measured are the same. Therefore the data collected by both studies can be combined to give a larger set of data. The P-value will be analysed to conclude the findings of both studies. However the other two studies done by Sutton Riley et al., will not be used due to different time of gestation was measure and it only measure the concentration of hCG-H rather than hCG-H to hCG ratio.

Results

The following results were collected from 3 articles, which were deemed to fulfil the requirement to answer the main question addressed by this report. Part of the results are converted from graphical data into statistical data to achieve a more standardise results for easy understanding and interpretation. These data do share very similar characteristic and their method used to collecting the data will be further analysed in the discussion section.

Table-1 hCG-H Studies for Data Analysis

The following data were collected from 3 articles and any significant data collected are included in the table below. The number of participant in the studies and might not equal to term pregnancy, spontaneous pregnancy plus biochemical pregnancy due to some participant unable to achieve pregnancy during the study. The cut off point is the point/line separate results, where any results failing below 13ug/l hCG-H or 50% hCG-H level of total hCG are detected. Results are the number of pregnancy failure cases that were detected, it is the number of cases that fall below the cut off point and suffer pregnancy failure.

Author	Year published	Number of participant (Term/fail)	Sample used	Detection Period	Cut off point	Number of pregnancy failure below cut off point.	Results

Sutton-Riley et al.	2006	120 (87/33)	Serum	3-8 week	>13ug/l	24/33	73%,
Sutton-Riley et al.	2006	167 (139/28)	Urine	3-7week	>13ug/l	21/28	75%,
Sasaki Y et al.	2008	110 (42/20)	Urine	First day after implantation	>50% hCG-H of total hCG	13/20	65%
Cole, L.A.	2012	127 (81/46)	Urine	First day after implantation	>50% hCG-H of total hCG	38/46	83%

Table 2 – The significant findings from each author

Author	Significant finding
Sutton-Riley et al. 2006	• It is statistically proven hCG-H is a more effective method in assessing pregnancy outcome. • Serum and Urine samples detection method give similar sensitivity but urine samples gives 5 folds higher false positive results • Serum hCG-H samples can detect 31% more pregnancy cases over hCG samples (73%-42%) • There is a correlation between hCG and hCG-H in both urine and serum sample. Both reflect on gestation progress.
Sasaki et al. 2007	• 28/42 successful pregnancy produced 95% or above hCG-H, • All 42 cases of termed pregnancy produced over 50% or hCG-H, • 2/3 of the 20 fail pregnancy have a hCG-H level below 50%, consistent with the theory of 2/3 improper implantation
Cole, L.A. 2012	• In the 81 successful term pregnancy cases all of them have 51% or over hCG-H of total hcg, • It also suggested hCG results are insignificant. • Only 4 out of 18 Spontaneous abortion cases have over 51% hCG-H of total hCG • Only 4 out of 28 biochemical pregnancies have over 51% hCG-H of total hCG • Biochemical pregnancies and spontaneous abortion do show a significant difference between the level of hCG-H mean concentration.

Table 3 - The Analysis of research data from Sasaki Y et al., and Cole, L.A using statistic analysing software Minitab.

The pregnancy failure cases from both articles are separated into two different category using the cut off point. Man-Whitley test is employed to compare the median of the two separated cases. The results obtained from the test is the difference between the separated data.

Total cases of pregnancy failure	The median % hCG-H of total hCG for cases above cut off point	The median % hCG-H of total hCG for cases below cut off point	Results
66	97%	18%	70%±10% (p-value 0.0000)

Discussion

hCG molecules is a very important that play an important part in successful pregnancy. The pregnancy-promoting processes involved, pregnancy recognition by the mother, survival of the corpus luteum, stimulation of progesterone production, enhancement of embryo implantation, control of trophoblast differentiation and function, and regulation of maternal/fetal immune relationships (Cole, 2009). hCG-H has been found to link to improper implantation leading to errors in embryo invasive process. However it has also been noted apart from hCG molecules other factors like genetic, diseases can contribute to pregnancy failure. The inconsistency of hCG has also been a problem in detecting pregnancy outcome. hCG can fluctuate making it difficult to predict the exact day implantation occurred. Apart from inconsistency, hCG only have half of the efficiency of hCG-H, resulting an unfavourable molecules for pregnancy outcome detection (Sasaki et al., 2006).

Three articles researched online are analysed and critiqued to explored their significance in pregnancy failure. The data from each of the experiment are studied to help conclude the role of hCG-H in detecting pregnancy failure.

The main objective of Sutton Riley et al. (2006) is to explore the difference between serum and urine samples. Up until today Serum wasn't a popular method in detecting hCG-H due to insufficient researches and hCG urine doubling test is still currently the common method used to monitor pregnancy progress. This method requires two separate visits date to the clinic and it have a relatively high false positive rate (26-40%). More importantly it can only yield results with limited sensitivity varying from 62-78% of the cases. Sutton Riley et al. (2006) wanted to find an alternative method replacing the current maternal hCG doubling method due to its inconvenience. Also it is not a viable method when it comes to emergency situation (Cervinski et al., 2009).

Similar to the other two studies (Sasaki Y et al. 2007 and Cole, L.A. 2012), Sutton riley have focused on researching on hCG-H rather than hCG because researches have gradually reviewed the significance of the molecules in early pregnancy and its job as a pregnancy outcome detector (Cole et al., 2003). It was mentioned in the introduction section, hCG-H is the vital autocrine factor determining the success of

implantation and suppresses body invasive immunity (Behery et a., 2013). Apart from investigating the significance of hCG-H, he also studied the impact of using different type of samples on the results. According to the data collected it seems the concentration of hCG-H is almost equal in serum and urine samples. During the 4^{th}-7^{th} week of gestation, a linear correlation was found between serum and urine hCG-H level with a r2 value equal to 0.97. This means serum and urine samples are very closely correlated (Sasaki el al., 2007).

Using the 13ug/l as a cut off point the data Sutton Riley et al. 2006 collected suggest urine and serum samples do share a very similar sensitivity with both tests giving 73% and 75% pregnancy failure detection rate. This is supported by the other two studies Sasaki Y et al. 2007 and Cole, L.A. 2012 , they both have obtain 65% and 77% for hCG-H detection sensitivity. Although Sutton Riley et al. have chosen to use concentration of hCG-H as a cut off point rather than using the hCG-H to total hCG ratio like the two other studies. All 4 sets of data have managed to detect 65-77% of the pregnancy cases with a false positive percentage ranging from 0-15%. The cut off point set by the other two authors are 50% of hCG-H to total hCG. It is very important to note the cut off point used in the three articles stated above is derived from using data collected during their research to construct an ROC curve for optimum cut off point (Sasaki et al., 2007).

Sasaki Y et al. 2007 and Cole, L.A. 2012, both have used a similar method to collect their data immulite automated hCG test and with the same cut off point both set of data can be categorised to form into one big set of data. Both set of data contain 123 cases of term pregnancy and 66 cases of pregnancy failure this data trends do fit the theory mentioned in the introduction. One-third of the pregnancy normally doesn't proceed to terms due to conceptus chromosome abnormalities (Nash and Fisher. 2004). The combined data from table 2 is also showing 51 out of 66 cases of pregnancy failure have hCG-H to hCG ratio below 50%, which gives the percentage of 72%. As stated in the introduction scientists have agreed roughly two-third of the pregnancy failure is caused by insufficient hCG-H during implantation. (Cole, 2009). The data is suggesting only 15 pregnancy failures cases managed to achieve hCG-H level over the cut off point. Lacking of hCG-H can resulting inappropriate implantation procedure, eventually leading to pregnancy failure. (Grenache et al., 2009). The remaining 15 cases it very likely caused by non hCG-H related matter.

The 3 articles selected for further analysis do show some interesting differences Sasaki Y et al. and Cole, L.A. have chosen to collect the data after the first day of implantation and filter out any participant who have failed to conceive. Over 4 years Cole, L.A. have collected 215 urine sample from female volunteer, who wanted to achieve pregnancy. Urine samples were tested for hCG, hCG-H and LH daily for up to 6 menstrual cycles. Data collected during this time will be used as a reference in detecting elevated level of hCG production to determine the day of implantation. After implantation has taken place Cole, L.A. using antibody B152 and antibody 5008 as a chaser and detector in specific microtiter plate assay.

It is worth noting all 3 articles have managed to achieve similar data even thought data were collecting during different period of gestation. According to the Table 1 in Sutton Riley et al. The level of hCG-H and % hCG-H of total hCG-H will decrease and increase as the pregnancy goes on. By following the trend in Sutton Riley et al. regarding the hCG molecules changes. It is best to perform hCG-H concentration test for studies carried out after third week of gestation and % hCG-H of total hCG test as soon as implantation have taken place. Hence the reason why Cole, L.A and Sasaki et al., have input so much effort prior in LH, hCH and hCG-H data collection, this is to ensure the precise implantation moment can be detected. This is because once the peak of LH occurred the % hCG-H of total hCG will fall gradually as gestation goes on (Sutton Riley et al. 2006).

The results from Sasaki Y et al. 2007 and Cole, L.A 2012 are done in almost the same manner. Both author have chosen to use urine samples obtained from the same measuring period. Therefore I have created Table 3 by combining the data from both articles to create a single larger group of data. The data was later separated using the cut off point established by both studies's ROC curve (Sasaki et al., 2007). The data was later analysed using statistical software Minitab to explore the differences or similarity of the data. It has been found the differences of %hCG-H of total hCG between pregnancy failure can be as great as 70% (p-value 0.0000). This means the data anyalsed is showing an extremely strong correlation between the level of hCG-H and pregnancy failure with 100% confidence. It is also suggested by all 3 articles (table 2.) the level of % hCG-H of total hCG all exceed 50% or above, two-third of successful term pregnancy even have 95% hCG-H to hCG ratio or above. This suggests hCG-H plays an extremely important role in successful implantation.

The data from all 3 studies have also shown hCG is a considerable less useful molecules in pregnancy outcome detection comparing to hCG-H. Sutton Riley et al. have suggested hCG failed to detect 31% of pregnancy failure cases compared to hCG-H. hCG also provide a relative low detection rate for biochemical pregnancy. hCG can barely detect half of the biochemical pregnancy , whereas hCG-H can offer as high as 100% detection rate. Cole, L.A. and Sasaki et al. both have similar results suggesting hCG is insignificant for pregnancy outcome detection.

False positive results have always been an unavoidable problem in science. Due to the nature of the research, false positive results will always occur. This is because hCG-H isn't the only factor dictating pregnancy outcome. Some cases of pregnancy with insufficient level of hCG-H still manage to proceed without experiencing any problems. (Sutton-Riley et al., 2006). Although False positive results doesn't affect the studies, because all data are collected after pregnancy, meaning it wouldn't affect the studies. Sutton Riley et al., 2006 did suggest the sensitivity or urine and serum samples tests do give very similar sensitivity. However the down side is the 5 folds higher false positive results. This could have a huge impact realistically. This is because false positive results can always create anxiety and unnecessary distress. However serum testing is very difficult to be carried out at home, which means urine testing would always be the more convenient testing method.

Even though table 3 have suggested hCG-H molecule do have a strong impact on pregnancy success rate. But there are a few limitations, which scientists have no control of when studying hCG-H. It is never possible to achieve 100% positive results, because hCG-H alone doesn't cause all of the pregnancy failure. Diseases, chromosome abnormalities or even damages to the womb can induce pregnancy failure. Therefore there are always cases that don't fit the trends stated by table 2 and table 3 in the results section. (Cole, 2009).

It is also worth mentioning the genetic variation between different races and environmental impact can have an effect on the level of hCG-H molecule production. Although there are currently no statistical data available, but it is always something that should be taken into consideration when conducting any data collection researches. However is it difficult to target one specific race, as the data will only reflect on that specific ethnic group. It is also unfeasible to do a worldwide study as it requires too tremendous amount of resources. (cole, 2012).

Another problems of the data are different detection methods were used. Each author has chosen to use their own method in detecting hCG-H. The non-standardised detection method can introduce errors due to different mechanism of detection.

Another possible errors that should be considered is every single study a different cut off point is used, The data of hCG-H , total hCG for all samples were first inputted into Microsoft excel to obtain a True positive and false positive value. The predictive value were worked out based on other studies assuming 16% clinically recognised pregnancy will end in spontaneous abortion and 2% of the cases will end in biochemical pregnancy. Afterward the predictive value will be used to create a Receiver operating characteristics (ROC) curve by using software AccuROC. It works by fitting the data into an existing trends established by other scientists to create an ROC curve. The ROC curve will then determined the optimum cut off point. This means every study can have a different cut off point resulting, which in term can affect the data because a raised or lowered cut off point can lead to more cases being detected or not detected. , This is because the data collected dictate the value of the cut off point. (Sasaki et al., 2007 and Cole, 2012).

Conclusion

From the data that I have found I am able to conclude hCG-H is a superior pregnancy detecting molecule comparing to hCG. It can detect almost 73% to 83% of pregnancy failure giving far better results than hCG. The results have also shown one-quarter of pregnancy failure are triggered by other means, which closely matches the theory of only two third of pregnancy failure are caused by improper implantation due to insufficient hCG-H molecule. It can be concluded hCG-H molecule and pregnancy do share a very close correlation. Some of the data do suggest the pregnancy failure isn't mainly caused by insufficient hCG-H

but the % hCG-H of total hCG between termed pregnancy and failed pregnancy can be as great as 70% with almost 100% certainty. It is also worth noting serum sample should always be used for pregnancy confirmation due to a 5 folds lower false positive rate. Moreover, hCG-H molecules can also be used to distinguish spontaneous abortion and biochemical pregnancy.

References

Barnhart, K., Sammel, M.D., Chung, K., Zhou, L., Hummel, A.C., Guo, W. (2004) Decline of serum human chorionic gonadotropin and spontaneous complete abortion: defining the normal curve. Obstet Gynecol **104** (2004) 975-981.
Barnhart. K.T. (2012) Early pregnancy failure beware of the pitfalls of modern management. Fertility and sterility **98** (2012) 1061-1065.
Behery, M.M.E., Siam, S., Seksaka, M.A, Mansour, S.M. (2013) Uterine artery Doppler and urinary hyperglycosylated HCG as predictors of threatened abortion outcome. Middle East Fertility Society Journal **18** (2013) 42–46.
Cervinski, M.A., Lockwood, C.M., Ferguson, A.M., Odem, R.R., Stenman, U.H., Alfthan, H., Grenache, D.G., Gronowski, A.M. (2009) Qualitative point-of-care and over-the-counter urine hCG devices differentially detect the hCG variants of early pregnancy. Clinica Chimica Acta **406** (2009) 81–85.
Cole, L.A, Khanlian, S.A., Sutton, J.M., Davies, S., Stephens, N.D. (2003) Hyperglycosylated hCG (Invasive Trophoblast Antigen, ITA) a key antigen for early pregnancy detection. Clinical Biochemistry **36** (2003) 647–655.
Cole, L.A. (2009) hCG and hyperglycosylated hCG in the establishment and evolution of hemochorial placentation. Journal of Reproductive Immunology **82** (2009) 112–118.
Cole, L.A. (2010) Hyperglycoslated hCG a review. Placental 31 (2010) 653-664.
Cole, L.A. (2012) hCG five independent molecules. Clinica Chimica Acta **413** (2012) 48–65.
Cole, L.A. (2012) Hyperglycosylated hCG and pregnancy failures. Journal of Reproductive Immunology **93** (2012) 119–122
Cole, L.A. Dai, D., Bulter, S.A., Leslie, K.K., Kohorn, E.I. Gestational trophoblastic diseases 1 Pathophysiology of hyperglycosylated hCG. Gynecologic Oncology **102** (2006) 145–150.
Cole, L.A., Butler, S. (2012) Hyperglycosylated hCG, hCGb and Hyperglycosylated hCGb: Interchangeable cancer promoters. Molecular and Cellular Endocrinology **349** (2012) 232–238.
Cole. L.A. (2007) Hyperglycoslated hCG. Placenta 28 (2007) 977-986.
Cole. L.A., Khalian S.A. (2007) Hyperglycosylated hCG A variant with separate biological functions to regular hCG. Molecular and Cellular Endocrinology 260–**262** (2007) 228–236.
Guibounche, J., Handschuh, K., Tsatsaris, V., Gerbaud, P., Leguy, M.C., Muller, F., Brion, D.E., Fournier, T. (2010) Hyperglycosylated hCG is a marker of early human trophoblast invasion. J Clin Endorcrinol Metab **95** (10) 240-244
Jauniaux, E., Poston, L., Burton, G.J. (2006) Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. Hum Reprod Update **12**(6) 747-755.
Kovalevaskaya, G., Genbacev, O., Fisher, S.J., Caceres, E., Connor, J.F. (2002) Trophoblast origin of hCG isoforms: cytotrophoblasts are the primary source of choriocarcinoma-like hCG. Molecular and Cellular Endocrinology **194** (2002) 147-155.
Norwitz, E.R., Schust, D.J., Fisher, S.J. (2001) Implantation and the Survival of Early Pregnancy. N Engl J Med **345** (2001) 1400-1408.
Sasaki, Y, Ladner, D.G., Cole, L.A. (2007) Hyperglycosylated human chorionic gonadotropin and the source of pregnancy failures. Fertility and sterility. **89** (6) 1781-1786.

Sutton-Riley, J.M., Khanlian, S.A., Francis, W.B., Cole, L.A. (2006) A single serum test for measuring early pregnancy out come with high predictive value. Clinical biochemistry. **39** (2006) 682-687.
Ticconi, C., Zicari, A.B., Realacci, M., Rao C.V., Piccione, E. (2007) Pregnancy Promoting Actions of HCG in Human Myometrium and Fetal Membranes. Placental 28 **21** (2007) 137-143.
Urbancsek, J.N., Hauzman, E., Fedorcsak, P.T., Halmos, A., Nyi, N.R.D.V., Papp, Z.N., (2002 Serum human chorionic gonadotropin measurements may predict pregnancy outcome and multiple gestation after in vitro fertilization. Fertility and Sterility **78** (3) 540-542.
Wright, A., Zhou, Y., Weier, J.F., Caceres, E., Kapidzic, M., Tabata, T., Khan, M., Nash, C., Fisher, S.J. (2004) Trisomy 21 is associated with variable defects in cytotrophoblast differentiation along the invasive pathway. Am J Med Genet A **130** (4) 354-364.

Zygmunt, M., Herr, F., Keller-Schoenwetter, S., Kunzi-Rapp, K., Munstedt, K., Rao CV, Lang, U., Preissner, K.T. (2002) Characterization of human chorionic gonadotropin as a novel angiogenic factor. J Clin Endocrinol Metab. **87** (11) 5290-5296.

Appendix 1 – Learning Log

Appendix 2 - Consideration of Ethical Issues and Research Governance

Appendix 3 - NSESC Application Form