It starts before they can talk.

Does human chorionic gonadotropin (hCG) indicate parent offspring conflict during pregnancy?

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A thesis submitted in partial fulfillment of the Honours Thesis (05.4111/6) Course

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2007

Abstract

Natural selection predicts that the presence of maternal offspring screening will result in resistance by the offspring. Human chorionic gonadotropin (hCG) produced by the offspring is essential in maintaining uterine receptivity and the corpus luteum. If hCG is the result of parent-offspring conflict (POC), then uncomplicated twin pregnancies will produce double the amount of hCG as gestational age matched uncomplicated singleton pregnancies. No POC should result in hCG levels in twins that are only slightly higher than singletons since high hCG levels are linked to adverse effects for both the offspring and the parent. The average twin: singleton hCG ratio (obtained from a comparison of 36 studies) was 1.985 (SD $\pm$ 0.393), with a p-value of 7.89×10^{-17}. This 2:1 ratio may not be an indication of POC if differences in twin: singleton placental sizes are 2:1. However, high rates of pregnancy problems associated with high hCG and early spontaneous abortions (vanishing twins) associated with low hCG indicates involvement in POC. Confounding factors in the data itself could be comparisons between assisted reproduction and spontaneous conceptions. Obtaining results from urine vs. serum on hCG levels may affect the twin: singleton hCG ratio. The 2:1 hCG ratio between twins and singletons may be an indication of POC.

Acknowledgements

I would like to thank Dr. Moodie for his effort on behalf of all of the thesis students. I would like to thank Dr. Hubner, and Dr. Young for their help and their willingness to be my committee members. Thank you Dr. Forbes for being my supervisor and for your help with statistics and editing. I would also like to thank my family for their constant love and support and my Dad especially for his help in editing.

Thank you very much.

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Introduction

Parents and offspring have shared but not identical genetic interests. This is at the core of Robert Trivers concept of parent-offspring conflict, introduced in his 1974 paper in the American Zoologist. Trivers suggested that parents and offspring should be expected to disagree over decisions about the level of parental investment including conflict about the duration and quantity of parental care. The concept of parent-offspring conflict has been widely applied to address such evolutionary questions as sex ratios among offspring in social insects, infanticide in fish, plants, birds and even humans (reviews in Godfray 1995, Mock and Parker 1997, Burt and Trivers 2006).

Even human pregnancy is not exempt from genetic conflict as Haig (1993, 1996) has noted. He argues that puzzling features of human pregnancy such as pregnancy sickness, pre-eclampsia and gestational diabetes arise from conflicts between the offspring and mother. These phenomena are linked to the production of offspring hormones that affect the maternal endocrine system, and serve to enhance the offspring's access to maternally provided nutrients (Haig 1993, 1996, Forbes 2005). But why should POC occur during pregnancy? The explanation for this comes (ironically) from a theory of how altruistic behavior evolved through natural selection. According to this theory, altruistic behavior will only occur if the benefit (b) to copies of an individual's genes (r for relatedness since copies of you genes are most likely to be found in relatives) is greater than the cost (c, measured in loss of fitness) to the individual (Stearns and Hoekstra, 2005).

$$b \times r > c$$

An additional implication of this theory is that if the benefit of cooperation does not outweigh the cost in fitness, it is likely that conflict will occur. And since mother and

offspring are genetically different individuals, it is possible that situations will arise where the cost of cooperation is greater than the benefits gained through cooperation.

For a mother, continuing a pregnancy can be disadvantageous in a number of circumstances. The most obvious is when the offspring has chromosomal defects that will prevent it from surviving to maturity. The resources spent on maintaining such a pregnancy are lost since this offspring will not survive to reproduce, resulting in the mother's genes not being passed on. In this case, it would be best for the mother to simply cut off resources before the offspring is born, which conserves resources that would otherwise be lost not only during pregnancy but also post-partum.

The fact that the mother needs to spend energy on an offspring not only during pregnancy but post partum as well also lead to conflicts between mother and offspring. If a chromosomally normal offspring is conceived during a time when resources are few, then a continued pregnancy will be very costly to the mother not only in terms of immediate survival (resources spent on the developing offspring are ones lost to the mother), but possibly also in terms of reproductive success. A less fit mother is less likely to have the resources to spend on the production of additional offspring. She is also likely to have fewer surviving offspring. The reduction in available resources may result in a less fit infant (due to lack of resources during gestation), as well as a subsequent reduction in offspring fitness due to lack of resources after birth or a less fit mother's inability to provide them, and possibly a reduction in the fitness of previous offspring (due to a division of resources). In situations like these, natural selection would select for a mechanism that would allow the mother to abort the pregnancy and try again under better conditions, a situation that creates an opposing selective pressure on the offspring.

How is it possible to detect whether this type of conflict occurs, and how does it manifest itself? On the maternal side of this potential conflict, selection should favor maternal choice about which offspring she has and when to have them. This would lead to maternal tests of offspring quality and a mechanism for terminating the pregnancy if the offspring was found wanting. Evidence of this type of maternal offspring selection would indicate that natural selection is selecting not for compromise, but for advantage. And if natural selection is not selecting for compromise in the mother, it is creating a selective pressure on the offspring. The selective pressures created by a maternal offspring screening process and maternal pregnancy choice should lead the offspring to develop both a mechanism for passing the test and a mechanism for removing the mother's choice of whether a pregnancy will continue or not. A hormone, human chorionic gonadotropin, appears to provide evidence of both a maternal screening system and a survival mechanism on the part of the offspring that usurps maternal control of the pregnancy.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) belongs to a family of glycoprotein hormones, consisting of luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid simulating hormone (TSH) (Ren and Braunstein 1992). hCG consists of two sub-units, an alpha and a beta chain. The alpha sub-unit is identical throughout this family, and the beta sub-unit varies, determining the specific function of each glycoprotein hormones (Lapthorn *et. al.*, 1994, Stenman *et. al.*, 2006). The beta sub-unit of hCG developed from a duplication of the beta sub-unit of luteinizing hormone (Maston and Ruvolo 2001). It consist of 145 amino acids, 115 of which are homologous to the beta

sub-unit of LH. The remaining amino acids located on the carboxy-terminal differentiate hCG from the other glycoproteins (Ren and Braunstein 1992). The gene for the alpha sub-unit is encoded on chromosome six while the gene for the beta sub-unit is located on chromosome nine.

Attached to hCG are eight carbohydrate functional groups, two N-linked carbohydrate chains on the alpha sub-unit and six O-linked oligosaccharides on the beta sub-unit. Differences in where these carbohydrate chains are linked create different isoforms of hCG, which have slightly different biological activity (Stenman *et. al.*, 2006). The other major hCG isoform has hyperglycosylated carbohydrate chains, and is called the hyperglycosylated isoform or HhCG (Stenman *et. al.*, 2006). This isoform is produced early in the pregnancy by the cytotrophoblast cells of the blastocyst but is eventually replaced by the hCG produced by the syncytiotrophoblast (Kovalevskaya *et. al.*, 2002).

hCG is a pregnancy hormone synthesized by the offspring from the eight cell stage until birth. During an uncomplicated singleton pregnancy, hCG production begins to increase four to seven days after implantation and the amount in maternal serum begins to double every one and a half days after that until the seventh to tenth week of pregnancy. Once this peak level of hCG is achieved, concentrations drop slightly until week 13 to 15 and remain relatively constant until birth (Stenman *et. al.*, 2006).

Most hCG is produced is by the syncytiotrophoblast, a mass of cytoplasm with many nuclei that develops from the fusion of cells derived from the trophoblast of the blastocyst (Jones and Lopez 2006). This structure is responsible for implantation (the invasion of the blastocyst into the maternal endometrial stroma) and forms the interface between the maternal and fetal circulatory systems in the placenta. Cytotrophoblastic

cells are the other type of cells that develop from the trophoblast. These cells (which also produce hCG) are encased by the syncytiotrophoblast and form the chorionic villi, responsible for the exchange of nutrients, wastes and hormones between the maternal and fetal circulatory systems (Jones and Lopez 2006).

Human Chorionic Gonadotropin and Parent-Offspring Conflict

Natural selection favors reproductive mechanisms that increase fitness. One common method of increasing reproductive fitness is progeny choice (Kozlowski and Stearns 1987, Forbes 2005). Progeny choice allows a parent to determine which offspring to invest its energy in. For instance, where only one offspring is produced at a time (as opposed to litters), there is no sibling competition. In this situation, testing each offspring individually by setting a standard of quality test is the best way for mothers to screen prospective offspring. This type of screening is called sequential offspring screening (Forbes 2005).

Studies done on spontaneous abortion rates and rates of chromosomal abnormalities at conception, compared to rates of chromosomal abnormalities at birth provide evidence that offspring screening occurs in humans. The rate of spontaneous abortions in clinically recognizable pregnancies (miscarriages) is about 15 percent (Forbes 1997). However, many more spontaneous abortions take place before a pregnancy is clinically recognized (Forbes 1997). Mathematical models predict a spontaneous abortion rate in clinically unrecognized pregnancies to be about 78% (the high estimate) while other studies, using sensitive hormone assays to detect very early pregnancy, have determined that the rate of spontaneous abortion is close to 31% (the

most conservative estimate). Even the average of these two rates would mean that over half of all conceptions end in spontaneous abortions (Forbes 1997).

Given this high rate of spontaneous abortions, one might expect a successful pregnancy to be a matter of chance, but research on chromosomal defects at conception and at birth suggest it is not. Trisomies are the most commonly occurring chromosomal defects in newborns. Trisomies occur when an extra chromosome is present; that is a person has 47 rather than 46 chromosomes. Trisomy 21 or Down syndrome, which is the most commonly occurring trisomy. While there are many other kinds of birth defects, Down syndrome is the most common chromosomal defect with a 0.00125% rate in live births. Even taking other trisomies into account, very few blastocysts with chromosomal defects complete gestation. This is not because there is a low rate of chromosomal defects in human conceptions. Blastocysts fertilized in vitro show about a 20-25% rate of chromosomal abnormalities (Forbes 1997). This indicates that the rate of chromosomal abnormalities occurring in both in vivo and in vitro conceptions is significantly higher than the rate of chromosomal abnormalities at birth would suggest.

Over half of clinically recognized pregnancies that are spontaneously aborted involve an offspring with a chromosomal defect and analysis of early pregnancy losses indicates that as many as 77% of pre-clinical spontaneous abortions have chromosomal abnormalities (Forbes 1997). This indicates the existence of a maternal screen that efficiently removes defective offspring early in gestation.

A maternal screen is a feature of the mothers' physiology which can interfere with offspring development. Only a fit offspring should be able to overcome this influence and continue to develop. In sequential offspring screening, the earlier an offspring can be screened the better. Implantation competence provides an early test of offspring quality.

Implantation requires that the developing offspring be able to prevent the mother from spontaneously aborting it and gain access to maternal resources. In order to do so, the offspring must maintain the corpus luteum, create a receptive environment in the uterus and begin to develop a placenta.

The multiple functions of the hCG in pregnancy

The evolutionary history of hCG suggests that this hormone developed specifically for parent-offspring conflict (POC), since hCG production has not developed simply as a mechanism for maternal-fetal communication (Maston and Ruvolo 2001). Natural selection has made use of hCG production by the offspring only in mammals with hemochorial placentas. In this type of placenta fetal tissues are directly bathed in maternal blood, which makes the secretion of fetal macromolecules into the maternal blood stream possible (Maston and Ruvolo 2001).

hCG is an allocrine hormone (Haig 1993), which means it is a hormone produced by one individual to influence another. Because of its homology with LH, hCG acts on the same receptor as LH, a large cell surface receptor that is a member of the G protein coupled receptor family (Fillicori *et al.*, 2005). But while the gene for the beta subunit of hCG originally evolved due to a duplication of the gene for the beta subunit of LH, these subunits have not remained identical (Maston and Ruvolo 2001). Changes made to the beta subunit have increased the circulatory half-life of hCG, from the 30 minuets for beta LH to almost twelve hours for beta hCG. This extension of half-life increases hCG's effectiveness on LH/hCG receptors over that of an equivalent dose of LH (Maston and Ruvolo 2001).

LH/hCG receptors occur in a variety of maternal tissues, including the corpus luteum, endometrial epithelium/stroma, myometrium and smooth muscle cells of the spiral arteries. While LH/hCG receptors are found in other maternal tissues (Fillicori *et al.*, 2005, Reshef *et al.*, 1990), it is hCG's influence on the previously mentioned tissues that makes it possible for offspring survival mechanisms to combat maternal fitness strategies (Fillicori 2005, Jones and Lopez 2006).

Maintaining the corpus luteum is essential for the maintenance of the early stages of pregnancy. The corpus luteum is a temporary endocrine gland that develops on the ovary from follicular cells left behind when a mature egg is ovulated (Jones and Lopez 2006). These cells differentiate into luteal cells and begin to secrete progesterone, a hormone essential for the development of a fertile uterine lining. Progesterone is a major contributor to the development of the secretory endometrium, the layer of uterine cells that contain the spiral arteries and in which a developing embryo implants and which forms the maternal component of the placenta. If a pregnancy does not occur, the corpus luteum regresses ten to twelve days after ovulation (Jones and Lopez 2006), resulting in the degeneration of the uterine lining. If a pregnancy occurs, degradation of the corpus luteum will be prevented by the action of hCG. LH/hCG receptors on the corpus luteum respond to hCG which prevents the degeneration of the corpus luteum long enough for the blastocyst to implant in the uterine lining. Embryonic production of hCG therefore prevents the mother from entering another menstrual cycle and prematurely terminating the pregnancy.

hCG also plays a critical role in implantation. A blastocyst can only implant in the uterus during a narrow window of time, between eight and ten days after the peak of luteinizing hormone (about six to nine days after ovulation). During this time, glandular

cells in the epithelium produce secretions that will provide nutrients to the offspring until the placenta develops and can absorb nutrients from the maternal blood stream (Lobo *et. al.*, 2001). It is also during this time that the stromal cells begin the process of decidualization; a process which makes it possible for the blastocyst to implant in these cells (Lobo *et. al.*, 2001).

hCG stimulates decidualization of uterine stromal cells and prevents their premature apoptosis (Lobo *et. al.*, 2001). Apoptosis of decidualized cells begins about ten days after the LH peak and before implantation of the blastocyst. Apoptosis of stromal cells is triggered by the binding of insulin-like growth factor (IGF) to insulin-like growth factor binding proteins (IGFBP, specifically IGFBP-1) that the decidualized cells begin to produce in response to progesterone at day ten after the LH peak (Seppala *et. al.*, 1992, Licht *et. al.*, 2001). The production of IGFBP-1 marks the closing of the fertile window. hCG prolongs the fertile window by inhibiting the production of IGFBP-1 in decidualized cells in spite of increasing progesterone levels. This extends the fertile window until the blastocyst has a chance to implant (Licht *et. al.*, 1998).

hCG acts on the uterine wall to maintain a receptive environment for implantation and development. The uterus contains high concentrations of LH/hCG receptors (Kurtzman *et. al.*, 2001). In the uterus, the endometrial cells contain the most LH/hCG receptors, the myometrium the second most, and the vascular smooth muscle of the spiral arteries the least (Reshef *et. al.*, 1990). hCG acts on the myometrium to prevent muscle contractions. Contractions of the uterus help expel the necrotic endometrial tissue during menstruation, and would be detrimental to the blastocyst if it occurred either before or after implantation. hCG prevents the expulsion of both the uterine lining and the developing offspring during the implantation process and decreases muscle contractility

until the completion of the pregnancy (Ticconi *et. al.*, 2006). The number of LH/hCG receptors in the myometrium changes during the course of a pregnancy. The level of LH/hCG receptors declines during both term and pre-term labor, indicating that the myometrium's reduced sensitivity to hCG is a major contributing factor to uterine contractions during labor (Kurtzman *et. al.*, 2001).

hCG also influences angiogenesis (the development of new capillaries from existing blood vessels), by promoting the secretion of VEGF (vascular endothelial growth factor). VEGF plays an important role in vascular remodeling in placental development and in the proliferation of capillaries in the uterine stroma. The effect of hCG on VEGF causes maternal blood flow in the uterus to concentrate at the site of implantation. hCG also appears to be involved in developing the hyper-permeability of maternal placental blood vessels through its effects on the vascular smooth muscle (Lobo *et. al.*, 2001, Sherera and Abulafia 2001).

Parent-offspring conflict and hCG in production in pregnancy

This thesis examines the pattern of hCG production in human pregnancy for evidence of genetic conflict between mother and offspring. It began by examining whether twin pregnancies are associated with higher levels of hCG production than singletons, and if so by how much? Since hCG production is linked to the incidence of pregnancy sickness and preeclampsia, levels of hCG production above the singleton norm indicate a *potential* for conflict between the mother and offspring. Given that a single offspring is capable of producing sufficient hCG to maintain corpus luteum function and hence pregnancy in the first trimester, one must ask what is the function of supranormal levels of hCG?

The final line of inquiry reviewed the link between growth of the placenta and hCG production. A possible proximate explanation for a higher level of hCG production in twin pregnancies is simply that the placental mass is greater than in singleton pregnancies, given that hCG is produced by the placenta. If this is true, then there should be a link direct correlation of hCG levels to placental mass, as well as a to placenta chroionicity (more hCG produced in dichorionic than monochorionic pregnancies).

Methods

The experiment examined the levels of hCG produced in twin and singleton pregnancies using data obtained from the published biomedical literature. hCG levels for uncomplicated twin pregnancies were compared to uncomplicated gestational age matched uncomplicated singleton pregnancies derived from the same study (i.e. values were compared within individual studies and not between studies). All hCG levels compared were between gestational age matched pregnancies since the concentrations of hCG are dependant on gestational age. hCG levels were taken from either maternal serum or maternal urine and the concentrations were measured using immunoassays, most commonly radio and florescent labeled. Different units were used between studies to measure hCG concentrations, but units were consistent within studies. In order to compare hCG levels between studies, all hCG levels were converted into a twin: singleton hCG ratio.

If the studies presented hCG concentrations for more than one gestational age, the hCG levels were often derived from sub-samples of the total number of pregnancies in each category (twins or singletons), possibly because not every subject in the study contributed a urine or serum sample for each gestational age. In order to avoid pseudo-replication of data within these experiments, only data from the gestational age with the highest sub-sample of twin pregnancies was used in these cases (see Table 1).

Table 1: Refined data. Twin and singleton hCG values from one comparison per experiment.

Reference	*Variable measured*	*Units*	*Singleton (n)*	*Twin (n)*	*Singleton value*	*Twin value*	*T/S ratio*	*Age (weeks)*
1	free βhCG	MoM	270	93	1.00	2.27	2.270	12
2	hCG	MoM	34740	410	1.02	2.17	2.127	17
3	free βhCG	MoM	370	150	1.00	2.01	2.010	12
4	free βhCG	MoM	6585	52	1.00	2.54	2.540	17
5	free βhCG	U/I	17	13	65500.00	171000.00	2.611	10
6	free βhCG	ng/mL	4181	136	34.00	65.00	1.912	12
7	hCG	ng/mL	15	9	10000.00	34500.00	3.450	10
8	hCG	UI/mL	362	39	14.50	39.10	2.697	36
9	free βhCG	MoM	3466	159	1.00	2.11	2.100	12
10	hCG	KIU/L	51517	798	27.80	52.60	1.892	18
11	free βhCG	MoM	65489	145	1.00	1.83	1.830	15
12	free βhCG	MoM	4279	67	1.08	1.85	1.713	13
13	free βhCG	MoM	6661	420	0.99	2.17	2.198	17
14	free βhCG	MoM	10	225	0.99	1.99	2.010	AM
15	free αhCG	MoM	600	199	1.01	1.66	1.644	19
16	free βhCG	MoM	600	199	0.99	1.90	1.919	AM
17	hCG	MoM	600	200	0.99	1.84	1.859	19
18	free βhCG	MoM	4181	148	1.00	1.94	1.940	12
19	hCG	UI x 1000/l	60	24	41.00	74.00	1.805	7
20	hCG	mUI/ml	7	5	25237.00	34680.00	1.374	31
21	hCG	UI/ml	452	92	28.70	52.91	1.844	16
22	hCG	UI x 1000/l	13	13	91.00	132.00	1.451	11
23	hCG	IU/l	12	12	90.60	138.60	1.530	11
24	hCG	IU/l	362	96	115.00	201.00	1.748	11
25	hCG	IU/l	9	11	178.00	252.00	1.416	2
26	hCG	mIU/ml	107	23	253.00	421.00	1.664	3
27	hCG	mIU/ml	24	14	98420.00	164800.00	1.674	9
28	hCG	MoM	2272	36	1.00	2.02	2.020	NG
29	free βhCG	MoM	1616	62	1.03	1.98	1.922	12
30	free βhCG	mUI/ml	45	15	490.00	874.00	1.784	2
31	hCG	IU/l	30	15	96.30	214.00	2.222	2
32	hCG	MoM	x	890	1.00	2.06	2.060	NG
33	free βhCG	MoM	x	206	1.00	2.15	2.150	10
34	hCG	MoM	x	24	1.00	1.98	1.980	17
35	free βhCG	MoM	x	3043	1.00	2.11	2.110	15
36	free βhCG	MoM	x	50	1.00	1.97	1.970	15

AM = age matched. NG = not given. MoM = multiples of the median

1. Mashiach et al. 2004; 2. Neveux et al. 1996; 3. Orlandi et al. 2002; 4. Cuckel et al. 1999; 5. Grun et al. 1997; 6. Noble et al. 1997; 7. Jovanovic et al. 1977; 8. Thiery et al. 1976; 9. Spencer 2000; 10. O'Brien et al. 1997; 11. Raty et al. 2000; 12. Niemimaa et al. 2002; 13. Spencer et al. 1994; 14. Barnabei et al. 1995; 15. Wald and Densem. 1994b; 16. Wald and Densem. 1994a; 17. Wald et al. 1991; 18. Sebire and Nicolaides. 1998; 19. Johnson et al. 1993; 20. Keith et al. 1993; 21. Nebiolo et al. 1991; 22. Johnson et al. 1994; 23. Ogueh et al. 2000; 24. Poikkeus et al. 2002; 25. Korhonen et al. 1996; 26. Tong et al. 2004; 27. Suzuki and Okudaira. 2004; 28. Crossley et al. 2002; 29. Ardawi et al. 2007. 30. Kuo et al. 2005. 31. Klein et al. 2005. 32. Cuckle 1998; 33. Gonce et al. 200; 34. Groutz et al. 1996; 35. Muller et al. 2003; 36. Berry et al. 1995.

Statistical analysis

The twin:singleton ratio for each study was derived by dividing the twin hCG level by its corresponding singleton hCG level. The mean, standard deviation and standard error were determined for the twin:singleton ratios. Statistical significance of the mean was calculated using a one-sample t-test, testing the mean ratio for statistically significant differences from one (twin hCG levels equal to singleton hCG levels) and two (twin hCG values double those of singleton hCG values).

Since there were a wide range of twin sample sizes between experiments, the effect of twin sample size on the twin: singleton ratio was also analyzed using regression analysis. This method was also used to analyze the effect of gestational age on the twin: singleton ratio.

Results

Statistical analysis of twin: singleton hCG ratios

The average twin:singleton ratio for the 36 studies used was 1.985 (s.e. = 0.066, range = 1.374 to 3.450). A one-tailed t-test comparing this ratio to an expected value of 1.00 (twin value = singleton value), indicated that the difference between the observed value for twins and 1.00 was highly significant ($t = 15.021$, $df = 35$, $P=7.89 \times 10^{-17}$). An additional one-tailed t-test was preformed to determine the statistical difference of the average ratio from 2.0, and this indicated that the twin:singleton hCG ratio was not significantly different from 2.0 ($t=0.229$, $df = 35$, $P=0.82$). Therefore twin pregnancies were producing double the level of measurable hCG as singletons.

This experiment also examined whether differences in sample size across studies had any effect on the estimated ratio of hCG in singleton and twin pregnancies (Figure 1). Although there is some evidence that studies with smaller sample sizes yielded poorer quality estimates of the hCG levels in twins, there is no evidence of bias – all the data were centered on the ratio of 2.0 and the overall regression was not significant ($R^2 = 0.004$, $F=0.133$, $df = 1,35$, $P=0.717$).

The effect of gestational age (weeks of gestation) on the average twin:singleton ratio was examined using linear regression (Figure 2). Although the overall level of hCG varied dramatically according to the stage of gestation, the ratio of twins to singletons was virtually constant, and the regression equation was not significant ($R^2 = 0.004$, $F=0.304$, $df = 1,24$, $P=0.587$).

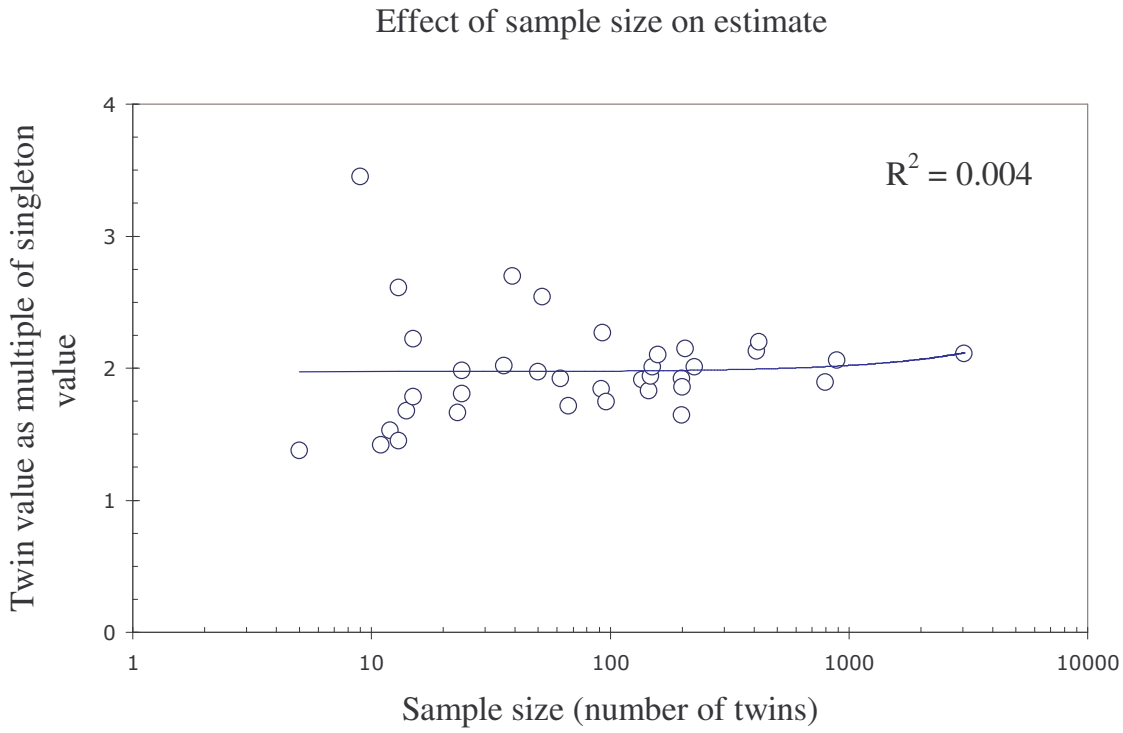

Figure 1. Data quality and estimates of hCG production in singletons and twins. The twin:singleton ratio is shown in relation to the number of subjects with twin pregnancies in the study.

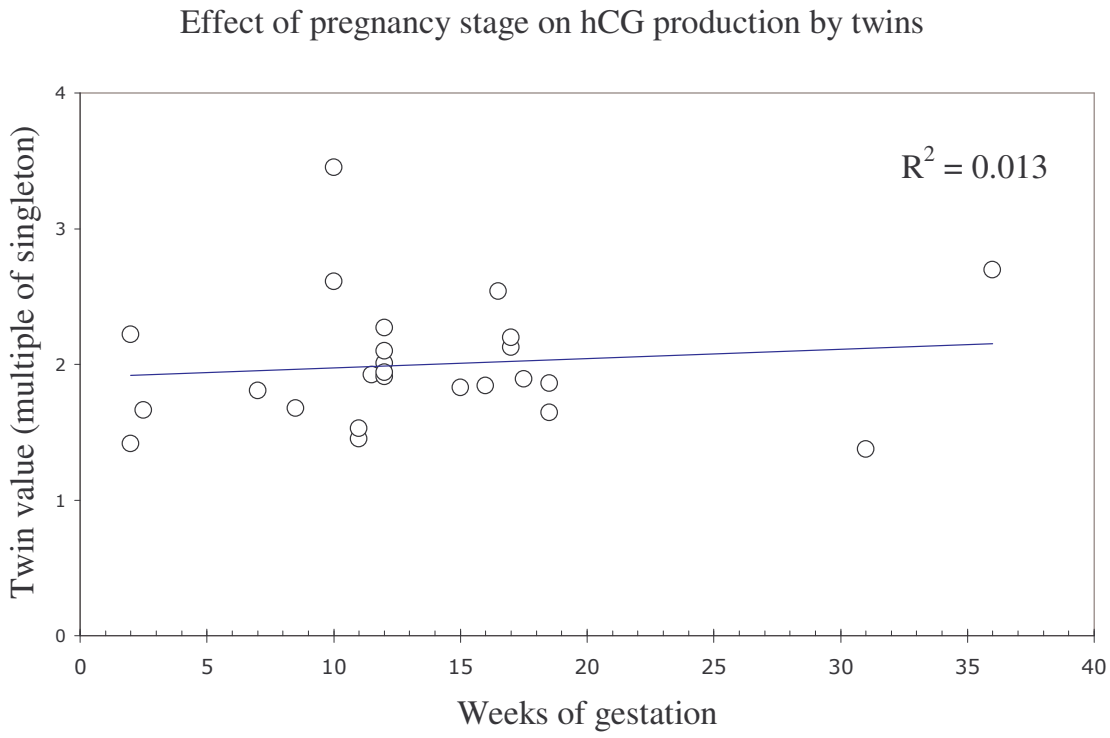

Figure 2. Data quality and estimates of hCG production in singletons and twins. The twin: singleton ratio is shown in relation to the gestational age of the twin pregnancies in the study.

Discussion

Twins clearly produce more hCG than singletons in human pregnancy, and high hCG production is associated with maternal morbidity, and in particular pregnancy sickness and preeclampsia. Do these problems arise as a by-product of a parent-offspring conflict between mother and offspring? Low hCG levels in early pregnancy are linked to early pregnancy failures (Kurtzman *et al.*, 2001, Kovalevskaya *et al.*, 2002, Barnhart *et al.*, 2004), demonstrating that a minimum level of hCG is needed to maintain the corpus luteum and prevent the developing offspring from being lost in the menstrual flow. Higher hCG levels are an indication of POC if the increase in the concentration of hCG results in a gain of fitness for the offspring at the expense of maternal fitness. This can be demonstrated by the two major causes of double the normal levels of hCG in singleton pregnancies; chromosomal defects and placental insufficiency.

As previously stated, Down syndrome is the most common chromosomal defect in live births, most other offspring with chromosomal defects are spontaneously aborted very early in pregnancy. This may result from insufficient action of the maternal screen in screening out conceptions with trisomy 21 as a result of elevated levels of hCG secreted by these offspring (Haig 1993, Forbes 1997). A singleton Down syndrome fetus produces concentrations of hCG comparable to those of twin pregnancies (Berry 1995, Wald *et al.*, 1991, Wald and Densem 1994a, 1994b) which suggests that the high levels of hCG are interfering with the maternal screen, benefiting the offspring while lowering the reproductive fitness of the mother.

The other major cause of elevated hCG levels is placental insufficiency. The placenta is an organ developed by the offspring in order to gain access to maternal resources. The placenta develops as cytotrophoblast cells and their syncytiotrophoblast

covering extend into the stroma. These projections are called chorionic villi and contain fetal capillaries. These villi project into sinusoids filled with maternal blood (Whittle *et al.*, 2006). Placental insufficiency describes a variety of problems associated with placental development. These problems arise from insufficient maternal blood flow to the implantation site, small placental size or the improper development of chorionic villi. Placental insufficiency is detrimental to offspring fitness, resulting in reduced growth or even death due to nutrient insufficiency and an inability to dispose of wastes. The offspring would clearly benefit from increased maternal blood flow to the placenta and an increase in the amount of fetal contact with the maternal blood supply. As stated in the introduction, hCG causes a proliferation of uterine capillaries at the site of implantation as well as causing them to become hyper-permeable. An increase in hCG levels will thus increase maternal blood flow to the placenta by enhancing capillary growth and by increasing the permeability of existing blood vessels. The increase in hCG levels will increase the area of contact with the maternal blood supply since hCG is involved in the development of chorionic villi. LH/hCG receptors are present in fetal placental tissues (Whittle *et al.*, 2006 and Reshef *et al.*, 1990), and hCG is involved in the differentiation of the trophoblast into the cytotrophoblast and syncytiotrophoblast (Licht *et al.*, 2001). An increase in hCG causes cytotrophoblast cells to differentiate into syncytiotrophoblast (Licht *et al.*, 2001), the primary structure for the invasion of maternal tissues. Increased hCG affects the fetal tissues by causing more chorionic villi to develop and by stimulating fetal angiogenesis that causes blood vessels to develop in the new villi (Whittle *et al.*, 2006).

hCG levels in mothers with preeclampsia (an accumulation of toxins caused by placental insufficiency) are similar to those of uncomplicated twin pregnancies, or even

higher in more severe cases (Heinonen *et al.*, 1996, Luckas *et al.*, 1996). This can be detrimental to maternal fitness because high levels of hCG result in side-effects created because of homology between hCG to thyroid stimulating hormone (TSH) and their respective receptors. Fetal hCG acts on the maternal thyroid by increasing the amount of mRNA synthesis for the sodium/iodide channels (Hershman *et al.*, 2004); hCG is only $1/10^4$ as effective as TSH at stimulating the TSH receptor. At the normal levels of hCG production in singleton pregnancy there is usually little impact on thyroid function. However, supranormal levels of hCG can and often do cause abnormal thyroid stimulation during pregnancy, which is linked to pregnancy sickness and its extreme form hypermesis gravidarum (Goodwin *et al.*, 1992, Hershman *et al.*, 2004). Hypermesis gravidarum is normally begins in the first trimester and lasts until late in a pregnancy, and is characterized by continuous nausea and vomiting that often leads to hospitalization due to dehydration, electrolyte and acid/base imbalance, rapid weigh loss and liver problems (Verberg *et al.*, 2005).

hCG production, twins and placental growth

There is evidence that higher levels of hCG production than normally occur in singleton pregnancy are required for the maintenance of twin pregnancy. Recent studies have shown that twin pregnancies with less than twice the hCG production of singleton pregnancies are spontaneously reduced to singleton pregnancies, the so-called 'vanishing twins' phenomenon. In cases of vanishing twins, hCG levels in a pregnancy that begins as a twin pregnancy rises to only 1.135 times higher than normal singleton pregnancies (Chasen *et al.*, 2006). This leads to the spontaneous abortion of one of the twins, usually

within the first four weeks of pregnancy, after which hCG levels fall to normal singleton levels (Kelly *et al.*, 1991).

The vanishing twin phenomenon shows that twin hCG levels cannot be lower than double those of singleton pregnancies if both twins are to survive. Does this mean that hCG levels in twin pregnancies are due to POC? POC would only be evident if the two to one ratio is not due to developing and maintaining a placenta that is twice as large as that of a singleton. hCG is involved in POC in a singleton pregnancy when hCG levels than exceed the normal range lead to a reduction in maternal fitness. It could be that uncomplicated singleton pregnancies produce just enough hCG to pass the initial test of fitness (the maternal screen) and then only enough to develop and maintain the placenta. If this is the case, then the two to one hCG ratio could just be an artifact, rather than an indication of POC. However, two facts suggest that this is not the case; the average twin: singleton placenta size and hCG levels produced by monochorionic and dichorionic placentas.

Twin placentas are on average only 1.6 times larger than those of singletons (Steier *et al.*, 1989, Pinar *et al.*, 1996). It appears that twin placentas produce more hCG per gram of placental weight than singletons. That the two to one hCG ratio does not translate into a two to one ratio for placental size indicates that hCG is being used for more than placental development and maintenance. Twins are smaller at birth probably be due to growth restrictions from having a small placenta (Bleker *et al.*, 2006).

That hCG levels in twin pregnancies exceed the level just required for placental development as shown through a comparison of hCG levels from monochorionic and dichorionic placentas. In a monochorionic placenta (characteristic of monozygotic twins), both twins share a single fused placental mass whereas in a dichorionic placenta,

each twin has a separate placenta. Dichorionic placentas tend to be larger and stem from separate implantation events, but there is little evidence of any difference in hCG production in monochorionic and dichorionic placentas (Matias *et al.*, 2005, Gonce *et al.*, 2005, Ardawi *et al.*, 2006). In the one study (Muller *et al.*, 2003) reporting a difference, it was small (2.16 for monochorionic vs. 2.07 dichorionic) and in the opposite direction of that expected on the basis of placental size.

If hCG levels in twins were due only to the necessity of building and maintaining a larger placenta, then the twin hCG level from the twin:singleton ratio should be more closely correlated to placental size (be only about 1.6 times higher than those of comparable singletons) and there should be a difference between the amounts of hCG produced between monochorionic and dichorionic placentas.

Fetal hCG produced during implantation is essential to offspring fitness and hCG produced during the remainder of the pregnancy is involved in the development of offspring access to maternal resources. If over-production of hCG is an attempt by the offspring to gain a fitness advantage at the expense of the mother, hCG levels higher than absolutely necessary should be an indication of hCG's involvement in POC. hCG levels in uncomplicated twin pregnancies are two-fold higher than those of gestational age matched uncomplicated singleton pregnancies, and this ratio cannot be explained simply through the necessity of producing a larger placenta. High hCG production in twins, and its role in pregnancy sickness and preeclampsia may be manifestations of a parent-offspring conflict.

Conclusions

1. hCG is used to prevent the mother from spontaneously aborting the offspring and to gain access to maternal resources (via its influence on placental development) regardless of the affect on maternal fitness.

2. hCG levels in twin pregnancies are, on average 1.985 times higher than gestational age matched singletons.

3. This two to one twin:singleton hCG ratio cannot be explained by a larger placenta, inflation of the twin:singleton ratio by artificial reproductive techniques or the effects of twin sample size or gestational age.

4. The two to one twin:singleton hCG ratio may represent a parent offspring conflict.

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