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From the Laboratory

The Measurement of Human Chorionic Gonadotropin for Pregnancy Testing

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Among many measurable hormones, human chorionic gonadotropin (HCG) is commonly used for pregnancy testing because it is very sensitive and relatively specific. Pregnancy can be identified shortly after implantation. Because some reagents cross-react with luteinizing hormone (LH), the sensitivity of urine tests has been adjusted to maintain specificity. Radioreceptor assays on serum are more sensitive than urine tests but similarly lack specificity. The problems of LH cross-reactivity are eliminated by antisera specific for the beta subunit. Quantitation of HCG provides additional information useful in diagnosing ectopic pregnancy, providing a prognosis in threatened abortion, and following neoplasms. Considerations of cost, availability, accuracy, and sensitivity determine which test should be selected.

The use of human chorionic gonadotropin (HCG) measurements as a screening test to determine pregnancy is a sensitive issue in laboratory service. Both positive and negative results often have significant emotional impact on the patient. In addition, the laboratory and the clinician are under constant scrutiny, since apparently false positive or false negative results soon become evident. A thorough understanding of the various test methods available and their limitations is required to use the service appropriately. Factors such as analytical sensitivity and specificity, turnaround time, personnel time, cost, and the clinical demand all must be considered in choosing the appropriate test.

Endocrinology of Early Pregnancy

It is essential to understand the endocrinology and physiology of early pregnancy in order to select pregnancy tests appropriately. Elevated serum hormones in women come from three sources: the corpus luteum, the placenta, and the maternal pituitary gland.

Corpus luteum

In a typical human non-pregnant cycle, one oocyte—with its surrounding investment of zona pelucida, granulosa cells, basement membrane, and theca cells—establishes dominance over other ovarian steroid-producing elements. In the days just before ovulation, this dominant follicle secretes increasing amounts of estradiol so that the reproductive tract is receptive to sperm and also elicits a surge of luteinizing hormone (LH) from the pituitary gland. The LH surge initiates ovulation: maturation of the oocyte, release of the egg, and luteinization. The dominant follicle, bereft of its oocyte and invaded by blood vessels, become the corpus luteum. In the absence of "rescue" by trophoblastic gonadotropin and under the luteolytic influence of endometrial prostaglandin, the corpus luteum secretes estrogen and progesterone for 12-16 days only. If pregnancy does not occur, progesterone levels drop, the endometrial lining sloughs, and menses ensues.

The normally evanescent life of the corpus luteum is altered by fertilization and subsequent implantation of the cleaving zygote. When the zygote invades the endometrium one week after fertilization, human cho-

rionic gonadotropin (HCG) of embryonic origin enters the maternal circulation. In the corpus luteum HCG occupies LH-HCG receptors and stimulates the enzyme systems necessary for continued estrogen and progesterone production. Whether from a normal pregnancy, an embryonic blighted ovum, or an ectopic gestation, the HCG produced by the viable trophoblast permits the corpus luteum to continue to secrete estrogen and progesterone.

At five to seven weeks after conception, trophoblastic secretion of estrogen and progesterone exceeds that of the corpus luteum. Estrogen and progesterone production in the placenta increases until the third trimester. The waning function of the corpus luteum is marked by a fall in 17-OH progesterone (Fig. 1).

Trophoblastic products

One of the many polypeptide products of the trophoblast is human placental lactogen (HPL). HPL is structurally analogous to prolactin but functionally similar to growth hormone, directing maternal carbohydrate and fat metabolism to provide glucose and fatty acids to the fetus. HPL does not appear in maternal serum in analytically significant amounts until the fourth week after conception.

The trophoblast also secretes several other pregnancy-specific proteins. Some are thought to protect the embryo from immune rejection, while others such as alkaline phosphatase are isomers of common enzymes. Although they are secreted in large amounts, the functions of these pregnancy-specific proteins are unknown.
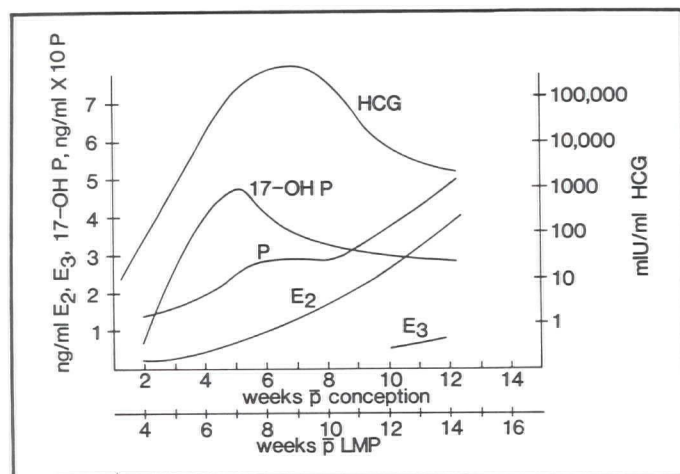
When the corpus luteum dies, the placenta becomes the principal source of steroid hormones in pregnancy. Of these, estriol is secreted in especially prodigious amounts late in pregnancy. Although estriol is normally detected in limited amounts in the non-pregnant woman as a metabolite of estradiol, it increases a thousandfold by the third trimester. The fetal adrenal gland is essential to this increased production of estriol. During the first trimester, when this gland is absent or just forming, estriol levels are not elevated enough to be clinically useful.

Maternal hormones

Maternal pituitary gonadotropins are suppressed by estrogen and progesterone originating in the corpus luteum and placenta. The maternal thyroid and adrenal glands continue to function at normal or minimally increased levels, while maternal prolactin increases significantly. Various experiments indicate that increased prolactin is not essential to the survival of a pregnancy. Rather, elevated prolactin levels reflect increased estrogen stimulation of the pituitary gland. Estrogen also stimulates the liver to increase production of many proteins, including clotting factors, lipoprotein constituents, and binding proteins for cortiocosteroids, thyroxine, and testosterone/estrogen.

Choice of a Pregnancy Marker

Because many hormones are either characteristically elevated or secreted only during pregnancy, measurement of a particular substance depends on clinical and analytical considerations. Clinically, the ideal pregnancy hormone should appear only in pregnancy, change in a characteristic manner throughout pregnancy, and reflect the moment-to-moment status of the pregnancy. Analytically, the assay should be rapid, accurate, precise, and free from interference by cross-reacting substances.

Estrogen and progesterone are not specific enough to identify early pregnancy, because their levels rise slowly in the first trimester and do not differ markedly from the non-pregnant state at that time (Fig. 1).

In established pregnancies, the short half-lives of circulating estrogen and progesterone allow sensitive monitoring of changes. In the first trimester a dropping estradiol level augurs impending miscarriage, whereas in the third trimester estriol variations reflect feto-placental well-being.

The measurement of prolactin and estrogen-stimulated liver proteins is subject to limitations similar to those of estrogen, since levels rise only gradually through the first trimester. In addition, several non-pregnant states

Fig. 1

First trimester serum levels of chorionic gonadotropin (HCG), estardiol (E$_2$), estriol (E$_3$), progesterone (P), and 17-hydroxyprogesterone (17-OHP).

(exogenous hormones and pituitary adenoma) can cause specious elevations.

The immunologically distinct polypeptide products of the trophoblast are excellent candidates for pregnancy testing. Placental lactogen is specific but not sensitive, since it is not measurable above background levels until the sixth week after the patient's first missed period. In some cases, the pregnancy-specific proteins have been noted to rise at the same time as HCG. Although it is not popular at the present time, the measurement of these substances might someday complement or supplant the determination of HCG for pregnancy testing.

Of several endocrine markers for biochemical events during pregnancy, HCG has had the widest application for both detecting and monitoring the course of pregnancy during the first two to three months of gestation. It has the desirable characteristic of originating exclusively from trophoblastic tissue, except in the case of certain non-trophoblastic tumors (often of germ cell origin). In addition to its specificity for tissue of origin, HCG rises exponentially during early pregnancy as a reflection of trophoblast function (Figs. 1,2). Finally, analytical methods with increased sensitivity and specificity make HCG an important clinical tool, as discussed below.

Chorionic gonadotropin

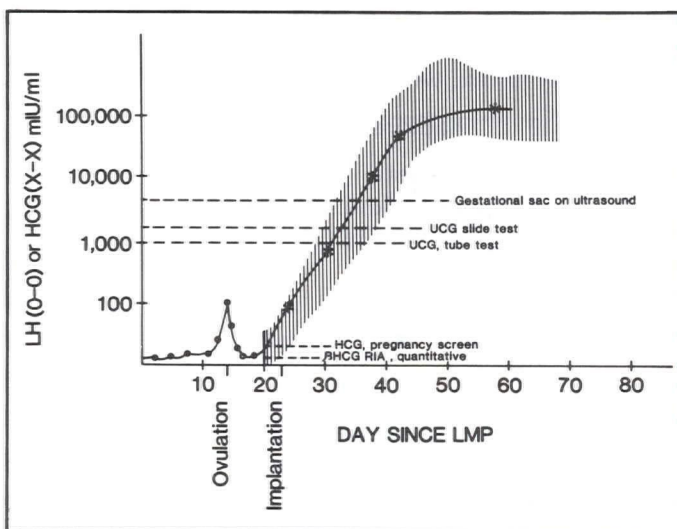HCG is a glycoprotein hormone secreted by the trophoblast and, subsequently, by the chorion and placenta. The hormone, which has a molecular weight of 36,000-40,000 daltons, is composed of two non-covalently bound subunits designated alpha and beta. The alpha subunit is essentially identical to the alpha subunit of the pituitary hormones LH, follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH). Each of these is distinguished biologically and immunologically by the respective, unique beta subunit. Besides possessing a common alpha subunit, LH and HCG exhibit extensive homology in the beta subunit. The beta subunit of HCG possesses 30 additional amino acids at the carboxyl end, but this amino acid "tail" does not appear to affect its biological activity. Since both LH and HCG bind with equal affinity to identical receptors in vivo, they are equivalent in bioassays and ligand binding assays which use membrane receptors. This cross-reactivity is particularly important in interpreting radioreceptor assays which use bovine corpus luteum. Antisera directed against the carboxyl terminus of the beta subunit may distinguish between hormones containing different beta subunits (LH and HCG) or in some cases between the free subunit and intact hormone. The development of these so-called beta HCG antisera has made it possible to measure HCG specifically in the presence of physiological levels of LH.

Normal serum levels of HCG for men and non-pregnant women are less than 10 mIU/ml. The least detectable dose for the radioimmunoassay (RIA) currently in use is 3 mIU/ml. Thus, HCG is detectable by RIA in the non-pregnant state as well as the preimplantation phase of pregnancy. Beginning shortly after implantation, approximately eight days after presumed ovulation, HCG rises rapidly (Fig. 2). Braunstein, et al (1) reported mean serum HCG values during early pregnancy of 21.6 mIU/ml (10.3-33.0) at 3-3.5 weeks after the last menstrual period and of 353 mIU/ml (264-442) at 4-4.5 weeks. An exponential increase with a doubling time of 1.7-2.0 days was evident, reaching a peak of 100,000-200,000 mIU/ml at 8-12 weeks after the last menstrual period. Thereafter, HCG plateaus over a wide range of normal values. For the duration of pregnancy, HCG levels remain above 5,000 mIU/ml.

The measurement of HCG levels for pregnancy testing has several limitations. Values may vary substantially from patient to patient for each day of gestation (Fig. 2, shaded area); thus, false negative results are possible. It is also difficult to interpret whether a single value is "normal for gestational age." Moreover, since the biological half-life of intact HCG, which is the predominant species in serum, is approximately two days, it may be detectable up to four weeks after the pregnancy ends.

Fig. 2

Mean LH and HCG levels in early pregnancy and pregnancy test sensitivities. Shaded area shows patient-to-patient variations in the normal population.

Although the concentration of the hormone in urine parallels its concentration in serum, it is not a one-to-one relationship. Typical graphs depicting analytical and clinical sensitivities (Fig. 2) usually reflect values determined on both urine and serum in different analytical ranges. Urine may also contain variable carbohydrate moieties as well as subunits and fragments, which are variably detectable by different assay methods.

Laboratory Tests for Pregnancy

Bioassay

Essentially replaced by immunologic assays, the bioassay of HCG as a pregnancy test measured various parameters such as sperm discharge, increased prostate weight, or ovarian corpora hemorrhagica in laboratory animals after they had been injected with a urine sample. While the assay was time consuming and imprecise, it detected pregnancy two to four weeks after the missed menses. Biological potency varied with clearance, fragmentation, and desialation.

Immunoassay

Although results of the immunologic tests discussed below (Table I) are expressed as units of biologic activity, measurements by immunoassay reflect hormone mass.

Slide Test

The UCG-slide test (Wampole Laboratories) is a rapid screening test of urine using the principle of latex agglutination inhibition (Fig. 3). Latex beads coated with HCG clump in the presence of rabbit anti-HCG. Sufficient free urinary HCG binds the specific antiserum and inhibits the clumping of the latex beads. The reaction is performed on a glass slide. In a negative test, clumping will be visible in two minutes.

This test is sensitive to 2,000 mIU/ml urine and will reliably detect pregnancy approximately 12 days after the missed period (30 days gestation). Although LH reacts with the HCG antiserum, the test sensitivity is low enough that a normal LH surge should not interfere. Drawbacks to this test are that LH in menopausal urine will occasionally give a positive result. Also, some drugs, including phenothiazines and methadone metabolites, have been reported to cause false positive or false negative results.

Tube Test

Another urine pregnancy screening test is the UCG-tube test (Wampole Laboratories), a hemagglutination inhibition assay (Fig. 4). Red blood cells coated with HCG agglutinate to form a mat in the presence of antiserum to HCG. When urine containing HCG is added, agglutination is inhibited and the red cells settle to the bottom of the test tube in a button or doughnut pattern. The sample is compared to a control, and the test is complete in two hours.

This test is sensitive to 500-1,500 mIU/ml of urine and is positive five to 12 days after the missed menstrual period if the patient has a follicular phase of consistent length. As is true with the slide test, LH cross-reacts with this antiserum. Given the increased sensitivity of this test, a false positive result in a perimenopausal woman is even more likely than with the slide test. Again, certain drugs may interfere.

Slide and tube tests are subject to specific and nonspecific protein interferences, which usually give false positive results. In general, slide tests are more liable to interference by proteins. Caution is required to interpret urine pregnancy tests in conditions (e.g., infections and kidney failure) with elevated levels of urine proteins.

Radioreceptor Assays

The radioreceptor assays (Wampole, Biocept-G) offer sensitivity adequate to detect HCG in serum 14 days after

TABLE I

Pregnancy Tests

Test	Specimen	Analytical Sensitivity	Clinical Sensitivity	Performance Time
UCG-slide test	urine	2,000 mIU/ml	5-12 days after missed period	2 min.
UCG-tube test	urine	500-1,500 mIU/ml	5-12 days after missed period	2 hr.
HCG, pregnancy screen (qualitative RIA)	serum	30 mIU/ml	7-14 days after ovulation	3 hr.
BHCG, quantitative (RIA)	serum	5 mIU/ml	non-pregnant	4½ hr.

conception. In this assay, HCG and LH in serum compete with ^{125}I-labeled HCG for binding sites on bovine corpus luteum membranes. Radioactivity bound to receptor membrane is inversely proportional to total hormone present (Fig. 5). As a pregnancy screening test, the cut-off point for a "positive" result, or its sensitivity, is set at 200 mIU/ml. A normal LH surge does not result in false positive results. This test will detect some pregnancies by seven days of gestation and most pregnancies by the time of the expected menses. Serum proteins and drugs do not significantly interfere.

HCG, Pregnancy Screen (RIA)

At our institution the radioreceptor assay has been replaced as a serum pregnancy screening test by a qualitative radioimmunoassay. Enhanced specificity as well as sensitivity may be obtained by the use of antisera directed toward the unique carboxy-terminal end of the beta subunit of HCG. These antisera bind intact HCG (the predominant circulating species) as well as free beta subunit but show very low cross-reactivity with pituitary hormones including LH.

This HCG pregnancy screening test uses an antibody directed against the beta subunit of HCG (Leeco Diagnostics). HCG in patient serum competes with radio-iodinated intact HCG for binding sites on the specific antibody (Fig. 6). Antibody-bound HCG is then precipitated and counted in a gamma counter. Counts bound are inversely related to HCG concentration. A "positive" result is correctly interpreted as "greater than 30 mIU/ml," measured against a 30mIU/ml standard. This test may detect pregnancy as early as seven to ten days after presumed conception.
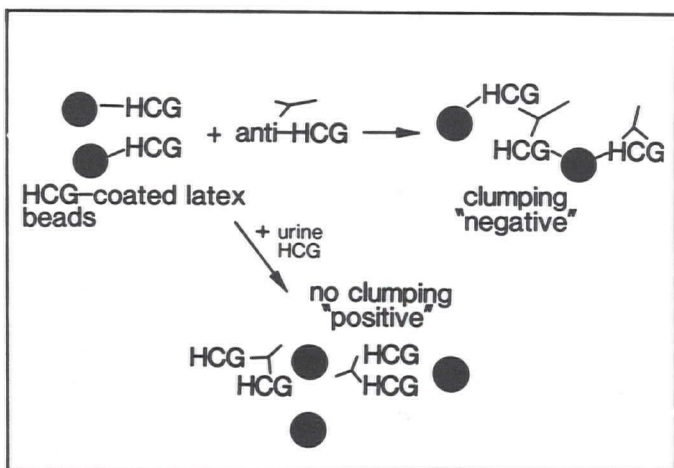
Results are considered "borderline" if they fall ±5% around the cut-off point and represent analytical variation. With a borderline result, the patient is either not pregnant or has a low concentration of HCG. These tests should be repeated after 24-48 hours for confirmation.

Since this antibody cross-reacts with LH at a level of less than 5% (calculated on an IU basis), mid-cycle and menopausal LH concentrations should not give a false positive result. Unusual levels of LH and other sources of HCG must, of course, be considered in the clinical context. Serum proteins and drugs are not significant problems.

Interpretation of Results

Although screening tests are commonly reported as "positive" or "negative," the only correct interpretation is "greater than" or "less than" the stated cut-off value. Cut-off levels are set to maximize clinical reliability while minimizing the effect of known interfering substances.

Negative pregnancy tests

A negative result from a pregnancy screening test does *not* mean "not pregnant." Clearly, there is a concentration below which HCG cannot be detected, varying from time of implantation (five to six days) to two to four weeks after the first missed menstrual period. No currently available pregnancy screening test will reliably detect pregnancy before implantation.

Since HCG concentrations rise rapidly between six and 36 days of gestational age, a negative result can easily become positive in 24-48 hours.

Positive pregnancy tests

Strictly speaking, none of the laboratory screening tests described above measure pregnancy as such. Rather, they use immunologic or biologic methods to measure
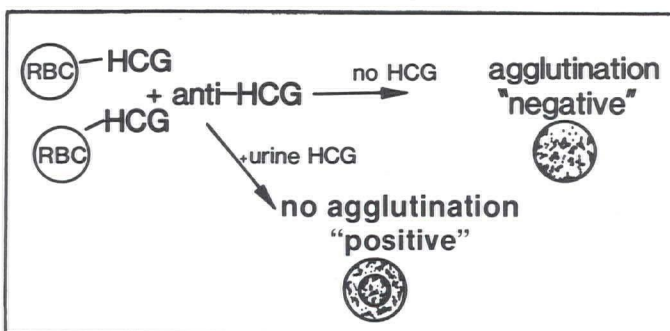

Fig. 3
UCG Slide Test: Latex agglutination inhibition assay.

Fig. 4
UCG Tube Test: Hemagglutination inhibition assay showing patterns for positive and negative results.

HCG. Their primary application is to verify normal pregnancy in patients reasonably expected to be pregnant. In all cases, sources of HCG other than a normal pregnancy must be considered in the clinical context. For less specific tests, the presence of elevated LH must also be ruled out.

HCG, Quantitative (RIA)

The radioimmunoassay called "beta HCG, quantitative" is a sensitive assay specific for both beta subunit and intact HCG (Leeco Diagnostics). As with other radioimmunoassays, radio-iodinated HCG competes with the hormone in the patient's serum for binding sites on a rabbit antibody specific for the beta subunit of HCG. Unknown samples are compared to standards (Fig. 6). The antibody used in our Ligand Assay Laboratory is the same one used in the qualitative screening test described above, but experimental conditions have been changed to increase the sensitivity and precision of the measurement. LH cross-reacts with this antibody by approximately 5% (mIU HCG/mIU LH X 100). Thus, it is possible to detect HCG in the serum of men or non-pregnant women, and usual LH elevations should not raise the apparent HCG above the upper limit of normal. In our experience, a postmenopausal woman with LH of 160 mIU/ml had an HCG value of 8 mIU/ml. Quantitative RIA is not designed as a screening test for normal pregnancies. Because of the time and expense involved and the nature of the clinical applications, HCG beta subunit radioimmunoassay is run twice per week in our laboratory.

Considerations for the Choice of HCG Assays

The cost and turnaround time of pregnancy tests vary inversely with their sensitivity. Thus, in ordering a pregnancy test, the practitioner must ask, "How much time and money should I spend for the information?"

Routine identification of pregnancy can usually wait until two weeks after the missed period when a tube test
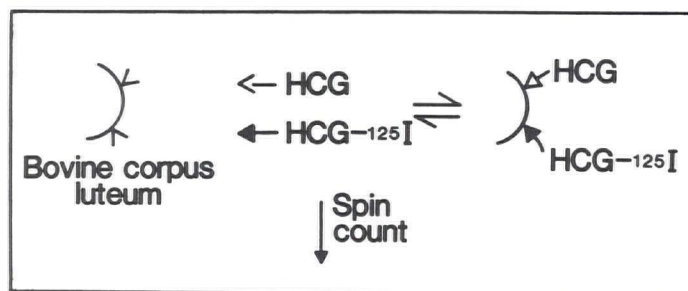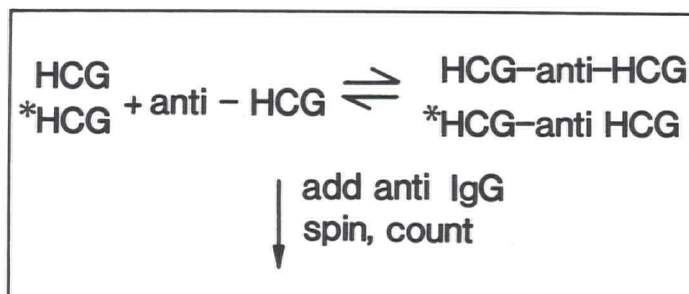
Fig. 6

Radioimmunoassay for HCG using a second antibody separation.

and confirming examination are sufficient. If referral for pregnancy termination is a consideration, immediate identification of a pregnancy provides better information about the decision to have an abortion at a time when complications are low. Here, a serum pregnancy test with increased sensitivity is appropriate. Anxiety about exposure to infection or toxins is another justification for early serum testing.

Economic, medico-legal, and clinical considerations determine whether routine use of sensitive serum pregnancy tests is appropriate. For example, for patients who are to be exposed to radiation, anesthetic gases, surgery, or who are to receive teratogenic medications or undergo radiological or surgical procedures, the early identification of pregnancy is important. The radiologist and surgeon must recognize that no pregnancy test presently available will detect pregnancy between the time of fertilization and implantation. Shortly after implantation, the quantitative beta subunit assay will become positive. About four days later, the pregnancy screening test used in our laboratory will identify pregnancy.

Because of the possibility of an ectopic implantation, identification of pregnancy is desirable in a woman who is experiencing menstrual irregularity and lower abdominal pain. All viable ectopic pregnancies secrete HCG. A negative serum test of sufficient sensitivity (30 mIU/ml) excludes the possibility of tubal pregnancy with over 95% reliability. The distinction between intrauterine and ectopic gestation, however, cannot be made on the basis of a qualitative HCG assay. The use of ultrasound can resolve this diagnostic dilemma by the visualization of the gestational sac approximately five weeks after the last menstrual period. Moreover, in an intrauterine pregnancy with serum HCG above 6,500 mIU/ml, the sac will always be visualized. Therefore, for a patient with lower abdominal pain, an HCG level greater than 6,500 mIU/ml, and no gestational sac visible on ultrasound, the likelihood of ectopic gestation is high. When the HCG level is below 6,500 mIU/ml, the clinician must rely

Fig. 5

Radioreceptor assay for HCG. Patient hormone (◄HCG) competes with ^{125}I-labeled-HCG (◄ HCG) for receptor sites.

on other means to establish the diagnosis of ectopic pregnancy.

In the case of threatened miscarriage (bleeding and/or cramping), HCG levels may help establish the prognosis. If the date of conception is known, a single determination predicts miscarriage with over 80% accuracy when the value is clearly below the normal range. Accurate determination of the date of conception is not necessary if serial HCG values are obtained. As noted above, HCG normally rises with a doubling time of 1.7-2.0 days during the first six weeks of pregnancy. If serial HCG determinations show a doubling time slower than two days, the likelihood of miscarriage is high. The use of serial HCG determinations to follow early pregnancy does not supply information that would allow a clinician to intervene significantly.

HCG may serve as a marker for ovarian germ cell tumors. In addition, very large amounts of HCG are secreted by hydatidiform mole, a non-malignant neoplasm of the placenta. In approximately 7% of cases, hydatidiform mole progresses to trophoblastic choriocarcinoma. When HCG levels fail to decline appropriately after a mole has been evaluated, the patient should be treated by chemotherapy for gestational trophoblastic disease. Continuing HCG measurements are often made to evaluate the effectiveness and adequacy of treatment.

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