

TIME OF IMPLANTATION OF THE CONCEPTUS AND LOSS OF PREGNANCY

ALLEN J. WILCOX, M.D., PH.D., DONNA DAY BAIRD, PH.D., AND CLARICE R. WEINBERG, PH.D.

ABSTRACT

Background Implantation of the conceptus is a key step in pregnancy, but little is known about the time of implantation or the relation between the time of implantation and the outcome of pregnancy.

Methods We collected daily urine samples for up to six months from 221 women attempting to conceive after ceasing to use contraception. Ovulation was identified on the basis of the ratio of urinary estrogen metabolites to progesterone metabolites, which changes rapidly with luteinization of the ovarian follicle. The time of implantation was defined by the appearance of chorionic gonadotropin in maternal urine.

Results There were 199 conceptions, for 95 percent of which (189) we had sufficient data for analysis. Of these 189 pregnancies, 141 (75 percent) lasted at least six weeks past the last menstrual period, and the remaining 48 pregnancies (25 percent) ended in early loss. Among the pregnancies that lasted 6 weeks or more, the first appearance of chorionic gonadotropin occurred 6 to 12 days after ovulation; 118 women (84 percent) had implantation on day 8, 9, or 10. The risk of early pregnancy loss increased with later implantation ($P < 0.001$). Among the 102 conceptuses that implanted by the ninth day, 13 percent ended in early loss. This proportion rose to 26 percent with implantation on day 10, to 52 percent on day 11, and to 82 percent after day 11.

Conclusions In most successful human pregnancies, the conceptus implants 8 to 10 days after ovulation. The risk of early pregnancy loss increases with later implantation. (N Engl J Med 1999;340:1796-9.)

©1999, Massachusetts Medical Society.

A CONCEPTUS must successfully attach itself to maternal tissue in order to survive. The process of implantation has never been directly observed in humans, and its timing remains uncertain.^{1,2} In 1959, results were published of a study of 210 fertile women who had undergone hysterectomy within three weeks after the estimated day of ovulation.³ In the examination of the uteri, a total of 26 implanted blastocysts were identified. Two blastocysts were identified as being recently implanted (well attached but still on the surface of the endometrium) in uteri removed seven to eight days after the estimated day of ovulation. The remaining blastocysts were found at later stages of implantation and in uteri removed later after ovulation. Subsequent textbooks have described human implantation as taking place by the seventh day after

ovulation.^{4,5} More recent data are based on the detection of chorionic gonadotropin in maternal serum or urine, often in women undergoing treatment for infertility. Among women who conceive as a result of in vitro fertilization, the successful implantation of a conceptus may be detected as late as 14 days after egg retrieval.⁶ However, fertility treatment may distort reproductive function, including the timing of implantation.⁷ We present data on implantation from a large sample of healthy women who conceived naturally.

METHODS

We studied 221 couples who had no history of fertility problems and who planned to have children. The women began to collect daily first morning urine specimens at the time they discontinued their method of birth control, and they continued to collect daily specimens through the eighth week of clinical pregnancy or for up to six months if no clinical pregnancy occurred. The specimens were stored in home freezers for up to two weeks and then transferred to permanent storage at -20°C . Specimens were collected on 98 percent of possible woman-days. More detailed descriptions of the study design, study population, and field methods have been published previously.^{8,9} The study was approved by the institutional review board of the National Institute of Environmental Health Sciences, and informed consent was obtained from all participants.

The day of ovulation was defined on the basis of changes in urinary excretion of the estradiol metabolite estrone 3-glucuronide and the progesterone metabolite pregnanediol 3-glucuronide, which were measured in duplicate or triplicate by radioimmunoassay.^{10,11} All of each woman's urine specimens were analyzed at one time. There is a rapid fall in the ratio of estrone 3-glucuronide to pregnanediol 3-glucuronide in urine at the time of luteinization of the ovarian follicle. An algorithm had been developed to identify the day of ovulation on the basis of the ratio of these urinary hormone metabolites.¹² We refined this algorithm, validating it against the peak urinary excretion of luteinizing hormone,¹³ which corresponds approximately to the day of ovulation.¹⁴ Further analysis has suggested that this method is as precise as methods based on the measurement of serum luteinizing hormone.¹⁵

Pregnancy was detected by means of a sensitive and specific immunoradiometric assay for urinary chorionic gonadotropin, with a detection limit of 0.01 ng per milliliter.¹⁶ Assays were performed in triplicate. In early pregnancy, the concentrations of intact chorionic gonadotropin are similar in serum and urine.¹⁷ Our criterion for pregnancy was the urinary excretion of chorionic gonadotropin in concentrations higher than 0.025 ng per milliliter for at least three consecutive days. (Chorionic gonadotropin values are reported as a function of the mass of chorionic gonadotropin because the biologic potency of chorionic gonadotropin varies with its sialic acid content. Purified reference preparations contain ap-

From the Epidemiology Branch (A.J.W., D.D.B.) and the Biostatistics Branch (C.R.W.), National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, N.C. Address reprint requests to Dr. Wilcox at the Epidemiology Branch, MD A3-05, NIEHS, Research Triangle Park, NC 27709.

proximately 13 mIU per nanogram if mass is converted to bioassay units.¹⁸)

For each pregnancy, implantation was defined as having occurred on the first day on which urinary excretion of chorionic gonadotropin exceeded 0.015 ng per milliliter. The time of implantation was measured as the number of days from the day of ovulation, which was designated day 0.

A total of 199 pregnancies were detected by measurement of maternal urinary excretion of chorionic gonadotropin.¹⁹ The day of ovulation or implantation could not be determined for 10 pregnancies (5 percent) because of missing data; these pregnancies were excluded from our analysis. The remaining 189 pregnancies include all 48 that ended in early loss (loss within six weeks after the last menstrual period), all 15 clinical losses (those occurring after six weeks), and 126 pregnancies ending in live birth.

We compared the distributions of implantation times for pregnancies that continued past six weeks and for those that ended in early loss by means of a contingency-table chi-square statistic. A relation between a later rise in the urinary excretion of chorionic gonadotropin and the early loss of pregnancy was tested for trend by logistic-regression analysis, which yielded a chi-square statistic with one degree of freedom. Two-sided P values are provided.

RESULTS

Among the 126 conceptions that culminated in live birth, the initial rise in urinary chorionic gonadotropin occurred 6 to 12 days after ovulation, with the rise in 106 (84 percent) occurring on day 8, 9, or 10. Similarly, in the case of the 15 conceptions that ended in loss more than 6 weeks after the last menstrual period (clinical losses), urinary chorionic gonadotropin was detectable by 7 to 11 days after ovulation, with the rise detected in 12 (80 percent) on day 8, 9, or 10 (Fig. 1). The mean times of implantation were 9.1 and 9.2 days after ovulation, respectively ($P=0.59$). In contrast, the distribution of implantation times for the 48 pregnancies that ended within 6 weeks after the last menstrual period (early losses) was statistically different ($P<0.001$); in these pregnancies implantation tended to occur later (mean, 10.5 days), and the times of implantation occurred over a broader range (6 to 18 days) (Fig. 1).

The estimated risk of early loss was strongly related to the time of implantation (Fig. 1). Early loss was least likely when implantation occurred by the 9th day (13 early losses among 102 pregnancies, or 13 percent), rising to 26 percent (14 of 53 pregnancies) when implantation occurred on the 10th day, 52 percent (12 of 23) on the 11th day, and 82 percent (9 of 11) with implantation after day 11 (P for trend, <0.001). The three pregnancies in which the initial rise in urinary chorionic gonadotropin occurred after day 12 ended in early loss.

DISCUSSION

In laboratory animals, there are three phases of endometrial development after ovulation: the uterine lining is initially neutral toward the implanting blastocyst, then receptive, and finally resistant.^{20,21} Although specific mechanisms of implantation vary widely among species,²² these three phases of uterine receptivity are also thought to occur in humans.²

There are no undisputed markers in humans of uterine receptivity to a fertilized ovum other than implantation itself.^{23,24} Given that implantation cannot be observed directly, the best indirect marker of implantation is chorionic gonadotropin.¹ Its production by the conceptus begins early, with expression of messenger RNA reported at the eight-cell stage.²⁵ The abrupt appearance of chorionic gonadotropin and its exponential rise in maternal serum or urine may not mark the very earliest steps in the implantation process, but they do mark the point at which the conceptus has successfully invaded the maternal tissue.

In our study, the couples had no known fertility problems, and none of the women were being treated with hormones. In the majority of successful pregnancies (84 percent), the first hormonal evidence of implantation was detected 8, 9, or 10 days after ovulation; the earliest time was 6 days and the latest 12 days. The range of implantation times depends in part on the precision of the markers of ovulation and of chorionic gonadotropin. Any random errors in these measures would tend to spread the distribution of implantation times. Our measure of ovulation has been validated against the surge in the secretion of luteinizing hormone, which is a standard clinical marker of ovulation, and our marker appears to be as precise as serum luteinizing hormone.¹⁵ Our assay for chorionic gonadotropin is sensitive enough to detect low concentrations even among premenopausal women with tubal ligation,⁹ so it is likely that the assay is able to detect the initial increase associated with pregnancy. Still, no measure is without error, and the true biologic window of implantation may be even narrower than we found.

The only previous study of the timing of implantation in women with no known fertility problems reported results similar to ours. In a study of 14 pregnancies ending in live births, rises in serum chorionic gonadotropin were detected as early as 8 days and as late as 12 days after the peak serum concentration of luteinizing hormone.²⁶ More information on implantation has come from studies of patients with infertility, especially women treated by in vitro fertilization. In one of the largest studies, implantation was reported in relation to egg retrieval for 140 clinical pregnancies in which conception occurred in vitro.²⁷ Implantation was detected 6 to 13 days after egg retrieval. In another report of 76 term pregnancies with in vitro conception, implantation occurred as early as 7 or 8 days after egg retrieval and as late as 13 or 14 days.⁶ In our data from natural conception cycles, no conceptus resulting in a clinical pregnancy implanted later than 12 days after ovulation.

We found a strong increase in the risk of early pregnancy loss with late implantation, a finding in

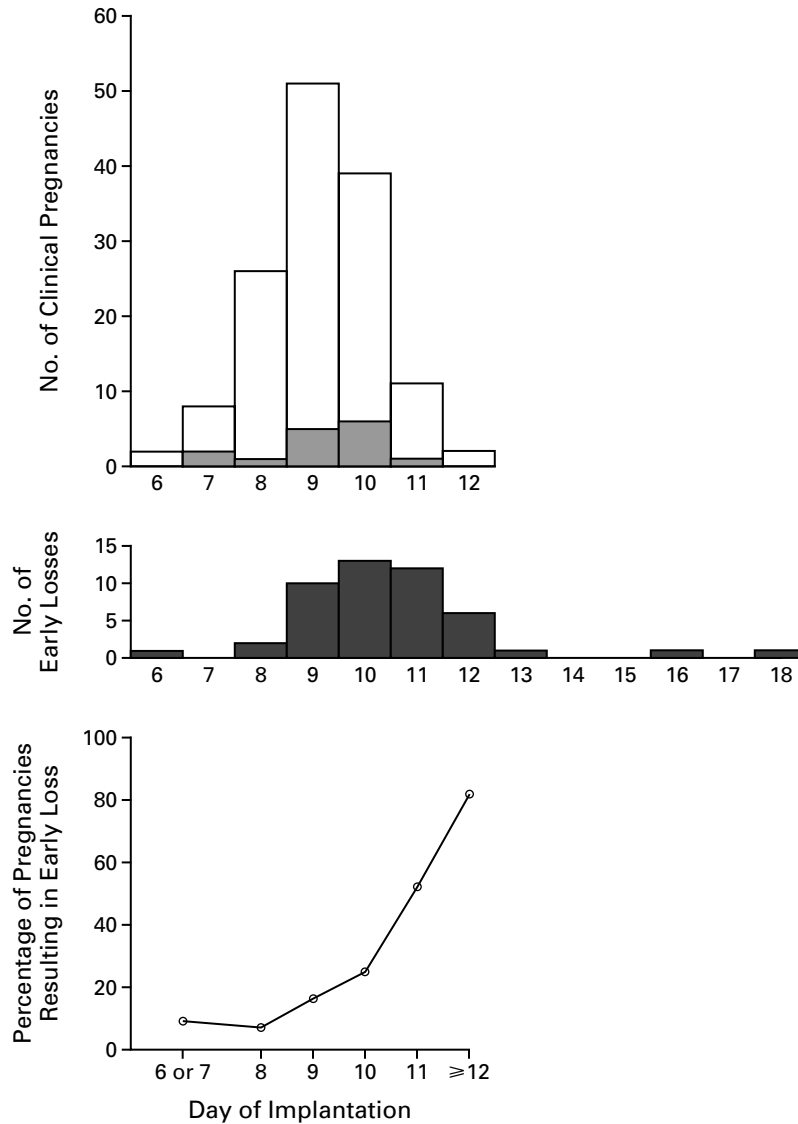


Figure 1. Timing of Implantation in 189 Naturally Occurring Pregnancies and the Risk of Early Loss. Overall, 141 pregnancies lasted at least six weeks after the last menstrual period to become clinically recognized (top panel). Fifteen of these clinical pregnancies ended in miscarriage (shaded area, top panel). The other 48 pregnancies ended in early loss (loss within six weeks after the last menstrual period) (middle panel). The bottom panel shows the increasing proportion of early loss with later implantation (P for trend, <0.001). The day of ovulation was defined as day 0.

agreement with data from smaller studies.^{6,26,28,29} Pregnancies with late-implanting conceptuses may fail for several reasons. The receptivity of the endometrium decreases during the late luteal phase,^{1,2} and the corpus luteum is less responsive to chorionic gonadotropin by 11 or 12 days after ovulation.³⁰ Factors intrinsic to the zygote could also be at work. Unhealthy zygotes may develop more slowly, or implantation may be abnormal,³¹ resulting in later and weaker production of chorionic gonadotropin.³² To the degree

that imperfect embryos develop or are implanted more slowly, a limited window of receptivity may provide a gating mechanism that helps screen out impaired embryos.

The data may have implications for efforts to manipulate uterine receptivity.^{33,34} Some women may be subfertile because of an unusually short window of implantation. There may be opportunities to increase fertility by extending the time during which implantation can occur. Such interventions should

be approached cautiously, however, because they may have unintended consequences with respect to the quality of surviving embryos.

In summary, implantation occurred 8 to 10 days after ovulation in most healthy pregnancies. The proportion ending in early loss increased when implantation occurred later. A refractory period after the time of uterine receptivity may provide a natural mechanism by which impaired embryos are eliminated.

We are indebted to Drs. John O'Connor, Robert Canfield, Paul Musey, and Del Collins for analyses of urine specimens; to Ms. Joy Pierce for her management of the field phase of the study; to the 221 women who so conscientiously provided data and urine specimens; and to Dr. D. Robert McConaughy for assistance with the graphic display of the data.

REFERENCES

- Hearn JP, Webley GE, Gidley-Baird AA. Chorionic gonadotropin and embryo-maternal recognition during the peri-implantation period in primates. *J Reprod Fertil* 1991;92:497-509.
- Rogers PAW. Current studies on human implantation: a brief overview. *Reprod Fertil Dev* 1995;7:1395-9.
- Hertig AT, Rock J, Adams EC, Menkin MC. Thirty-four fertilized human ova, good, bad and indifferent, recovered from 210 women of known fertility: a study of biologic wastage in early human pregnancy. *Pediatrics* 1959;23:202-11.
- Speroff L, Glass RH, Kase NG. *Clinical gynecologic endocrinology and infertility*. 5th ed. Baltimore: Williams & Wilkins, 1994.
- England MA. *Life before birth*. 2nd ed. London: Mosby-Wolfe, 1996.
- Liu H-C, Rosenwaks Z. Early pregnancy wastage in IVF (in vitro fertilization) patients. *J In Vitro Fert Embryo Transf* 1991;8:65-72.
- Tur-Kaspa I, Confino E, Dudkiewicz AB, Myers SA, Friberg J, Gleicher N. Ovarian stimulation protocol for in vitro fertilization with gonadotropin-releasing hormone agonist widens the implantation window. *Fertil Steril* 1990;53:859-64.
- Wilcox AJ, Weinberg CR, Wehmann RE, Armstrong EG, Canfield RE, Nisula BC. Measuring early pregnancy loss: laboratory and field methods. *Fertil Steril* 1985;44:366-74.
- Wilcox AJ, Weinberg CR, O'Connor JE, et al. Incidence of early loss of pregnancy. *N Engl J Med* 1988;319:189-94.
- Wright K, Collins DC, Musey PI, Preedy JRK. Direct radioimmunoassay of specific urinary estrogen glucosiduronates in normal men and nonpregnant women. *Steroids* 1978;31:407-26.
- Samarajeewa P, Cooley G, Kellie AE. The radioimmunoassay of pregnanediol-3 alpha-glucuronide. *J Steroid Biochem* 1979;11:1165-71.
- Royston JP. Statistical approaches to the prediction and detection of ovulation: detecting the signal among the noise. In: Jeffcoate SL, ed. *Ovulation: methods for its prediction and detection*. Vol. 3 of Current topics in reproductive endocrinology. Chichester, England: John Wiley, 1983:19-32.
- Baird DD, Weinberg CR, Wilcox AJ, McConaughy DR, Musey PI. Using the ratio of urinary estrogen and progesterone metabolites to estimate day of ovulation. *Stat Med* 1991;10:255-66.
- Collins WP. Biochemical approaches to ovulation prediction and detection and the location of the fertile period in women. In: Jeffcoate SL, ed. *Ovulation: methods for its prediction and detection*. Vol. 3 of Current topics in reproductive endocrinology. Chichester, England: John Wiley, 1983:49-66.
- Baird DD, McConaughy DR, Weinberg CR, et al. Application of a method for estimating day of ovulation using urinary estrogen and progesterone metabolites. *Epidemiology* 1995;6:547-50.
- Armstrong EG, Ehrlich PH, Birken S, et al. Use of a highly sensitive and specific immunoradiometric assay for detection of human chorionic gonadotropin in urine of normal, nonpregnant, and pregnant individuals. *J Clin Endocrinol Metab* 1984;59:867-74.
- Norman RJ, Menabawey M, Lowings C, Buck RH, Chard T. Relationship between blood and urine concentrations of intact human chorionic gonadotropin and its free subunits in early pregnancy. *Obstet Gynecol* 1987;69:590-3.
- Canfield RE, Ross GT. A new reference preparation of human chorionic gonadotropin and its subunits. *Bull World Health Organ* 1976;54:463-72.
- Wilcox AJ, Weinberg CR, Baird DD. Risk factors for early pregnancy loss. *Epidemiology* 1990;1:382-5.
- Psychoyos A. Hormonal control of uterine receptivity for nidation. *J Reprod Fertil Suppl* 1976;25:17-28.
- Yoshinaga K. Uterine receptivity for blastocyst implantation. *Ann N Y Acad Sci* 1988;541:424-31.
- Ringler GE, Strauss JF III. Recent advances in understanding the process of implantation. *Curr Opin Obstet Gynecol* 1990;2:703-8.
- Younis JS, Simon A, Laufer N. Endometrial preparation: lessons from oocyte donation. *Fertil Steril* 1996;66:873-84.
- Klентzeris LD. The role of endometrium in implantation. *Hum Reprod* 1997;12:Suppl:170-5.
- Bonduelle ML, Dodd R, Liebaers I, Van Steirteghem A, Williamson R, Akhurst R. Chorionic gonadotrophin- β mRNA, a trophoblast marker, is expressed in human 8-cell embryos derived from tripronucleate zygotes. *Hum Reprod* 1988;3:909-14.
- Stewart DR, Overstreet JW, Celniker AC, et al. The relationship between hCG and relaxin secretion in normal pregnancies vs peri-implantation spontaneous abortions. *Clin Endocrinol (Oxf)* 1993;38:379-85.
- Liu H-C, Cohen J, Alikani M, Noyes N, Rosenwaks Z. Assisted hatching facilitates earlier implantation. *Fertil Steril* 1993;60:871-5.
- Lenton EA, Hooper M, King H, et al. Normal and abnormal implantation in spontaneous in-vivo and in-vitro human pregnancies. *J Reprod Fertil* 1991;92:555-65.
- Baird DD, Weinberg CR, Wilcox AJ, McConaughy DR, Musey PI, Collins DC. Hormonal profiles of natural conception cycles ending in early, unrecognized pregnancy loss. *J Clin Endocrinol Metab* 1991;72:793-800.
- Woodward AJ, Lenton EA. Differential responses to a simulated implantation signal at various stages of the luteal phase in women. *J Clin Endocrinol Metab* 1992;74:999-1004.
- Bolton VN, Hawes SM, Taylor CT, Parsons JH. Development of spare human preimplantation embryos in vitro: an analysis of the correlations among gross morphology, cleavage rates, and development to the blastocyst. *J In Vitro Fert Embryo Transf* 1989;6:30-5.
- Woodward BJ, Lenton EA, Turner K. Human chorionic gonadotrophin: embryonic secretion is a time-dependent phenomenon. *Hum Reprod* 1993;8:1463-8.
- Beier HM, Hegele-Hartung C, Mootz U, Beier-Hellwig K. Modification of endometrial cell biology using progesterone antagonists to manipulate the implantation window. *Hum Reprod* 1994;9:Suppl 1:98-115.
- Lessey BA, Ilesanmi AO, Lessey MA, Riben M, Harris JE, Chwalisz K. Luminal and glandular endometrial epithelium express integrins differentially throughout the menstrual cycle: implications for implantation, contraception, and infertility. *Am J Reprod Immunol* 1996;35:195-204.