TIME OF IMPLANTATION OF THE CONCEPTUS AND LOSS OF PREGNANCY

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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 days 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-

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proximately 13 mIU per nanogram if mass is converted to bioas-
say units.23 For each pregnancy, implantation was defined as having oc-
curred on the first day on which urinary excretion of chorionic
gonadotropin exceeded 0.015 ng per milliliter. The time of im-
plantation 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.24 The day
of ovulation or implantation could not be determined for 10 preg-
nancies (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 preg-
nancies 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 gonad-

tropin 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 im-
plantation were 9.1 and 9.2 days after ovulation, re-
spectively (P=0.59). In contrast, the distribution of
implantation times for the 48 pregnancies that end-

ced 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 oc-
curred over a broader range (6 to 18 days) (Fig. 1).

The estimated risk of early loss was strongly relat-
ed 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 pregnan-
cies) 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 uter-
ine 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 uter-
ine 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 can-
not be observed directly, the best indirect marker of
implantation is chorionic gonadotropin.1 Its produc-
tion 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 implan-
tation 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 treat-
ed with hormones. In the majority of successful
pregnancies (84 percent), the first hormonal evi-
dence 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 de-
pends in part on the precision of the markers of ovu-
lation 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 stand-
ard clinical marker of ovulation, and our marker
appears to be as precise as serum luteinizing hor-

mone.15 Our assay for chorionic gonadotropin is

sensitive enough to detect low concentrations even
among premenopausal women with tubal ligation,8
so it is likely that the assay is able to detect the in-
tial increase associated with pregnancy. Still, no
measure is without error, and the true biologic win-

dow of implantation may be even narrower than we
found.

The only previous study of the timing of implan-
tation in women with no known fertility problems
reported results similar to ours. In a study of 14 preg-
nancies ending in live births, rises in serum chorion-
ic 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 im-
plantation has come from studies of patients with
infertility, especially women treated by in vitro fer-
tilization. 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.8 In our data from nat-
ural 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
agreement with data from smaller studies.\textsuperscript{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,\textsuperscript{1,2} and the corpus luteum is less responsive to chorionic gonadotropin by 11 or 12 days after ovulation.\textsuperscript{30} Factors intrinsic to the zygote could also be at work. Unhealthy zygotes may develop more slowly, or implantation may be abnormal,\textsuperscript{31} resulting in later and weaker production of chorionic gonadotropin.\textsuperscript{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.\textsuperscript{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

\begin{figure}
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\includegraphics[width=\textwidth]{figure1}
\caption{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.}
\end{figure}
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.

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REFERENCES