10th World Congress on IN VITRO FERTILIZATION AND ASSISTED REPRODUCTION

Vancouver, Canada
24-28 May 1997

Physiology of implantation

T.G. KENNEDY

Departments of Physiology and Obstetrics and Gynaecology
The University of Western Ontario, London, ON (CDN)

SUMMARY

Implantation is arguably the most critical stage in the establishment of pregnancy. In humans, it has been estimated that between 30% and 70% of conceptuses are lost before or at the time of implantation, without women being aware that they were pregnant. Of these losses, half are probably a consequence of genetic defects in the conceptus. The etiology of the remaining losses is unknown. In human in vitro fertilization and embryo transfer programs, the low implantation rate after embryo transfer is an important problem. Whether this low rate is a consequence of an inherently low implantation rate in humans, or to an altered physiological state is presently unknown. Based on studies in experimental animals, there are reasons for suspecting that the low implantation rates may be a consequence of an altered physiological state.

INTRODUCTION

Blastocyst implantation involves a complex series of events occurring over time. It requires synchronized development of the conceptus and a receptive uterus, attachment of the conceptus to the uterus, transformation of the endometrium to decidua, and finally formation of the definitive

placenta. It is arguably the most critical stage in the establishment of pregnancy. In humans, it has been estimated that between 30% and 70% of conceptuses are lost before or at the time of implantation, without women being aware of having been pregnant (see Cooke, 1988). Of these losses, about half are probably a consequence of genetic defects in the conceptus; the etiology of the remaining losses is unknown (Cooke, 1988).

Most of our knowledge of the physiology of implantation has come from animal studies; for obvious ethical reasons, it is not possible to study the process of implantation in humans in detail. There are considerable species differences in the events which occur during implantation, most notably in the extent of trophoblastic invasion of the endometrium (Psychoyos, 1973). Consequently, it is difficult to know to what extent findings in animals can be extrapolated to the human. However, there are findings which seem to be common to all animals which have been studied, and given this apparent conservation across species, it would be surprising if the human differed.

In human in vitro fertilization and embryo transfer programs, the low implantation rates following embryo transfer is a concern. It can be argued that the low rates reflect an inherently low implantation rate in humans. Alternatively, the low rates may be a consequence of an altered physiological state resulting from the interventions involved in the procedures of in vitro fertilization and embryo transfer. This review will present a brief overview of possible causes of the low implantation rates.

EMBRYONIC AND ENDOMETRIAL SYNCHRONIZATION

For a large number of species, the requirement for synchronization of embryonic and endometrial development has been demonstrated. For successful implantation, embryo-endometrial interactions must be initiated when the embryo and endometrium have reached precise stages of development; the embryo must be at the blastocyst stage of development, and hormone-dependent changes resulting in the development of a shortlived receptive endometrium must have occurred (Psychoyos, 1973; Weitlauf, 1994). Classically, the requirement for synchronization between embryo and endometrium has been demonstrated in animals by embryo transfer experiments (see Adams 1982). These experiments have shown clearly that implantation rates are highest when the reproductive cycles of the donors of the embryos are synchronized with the recipients' cycle. When asynchronous transfers are conducted where the stages of the cycles of the donors and recipients differ, the implantation rates decrease, especially if the transferred embryos are retarded in development relative to the recipients endometrium. A stage is ultimately reached when embryos transferred to the uterus fail to implant, and this is usually interpreted in terms of the ending of the short-lived period of endometrial receptivity.

Whether there is a short-lived period of endometrial receptivity for implantation in the human has not been definitively established. Based on morphology and the expression of markers (see, for example, Martel et al, 1981; Lessey et al, 1995), it is apparent that the endometrium undergoes

changes during the luteal phase, and these changes have been interpreted in terms of the existence of a distinct period of endometrial receptivity in the human. To obtain direct evidence for or against the existence of a period of endometrial receptivity in the human would require, for example, that mature blastocysts be transferred to the uteri of women at various times during the luteal phase of the menstrual cycle. If there is a short-lived receptive period, then only embryos transferred during that time would implant, although it is possible that embryos transferred at earlier times before the onset of receptivity could become dormant and subsequently be activated and implant when the endometrium becomes receptive. Certainly it would be expected that ultimately there would be a time after which transferred embryos would fail to implant because the receptive phase had ended. However, for obvious ethical reasons, it is not possible to conduct these types of experiments in humans.

Asynchronous development of the embryo and endometrium may arise because oocytes/embryos have to be cultured in human in vitro fertilization - embryo transfer programs to allow the assessment of fertilization and early embryonic development. Animal studies have demonstrated that, depending on the culture conditions, the rate of embryonic development during culture may be retarded or sped up, relative to that which occurs in vivo (Harlow and Quinn, 1982; Holm et al, 1996; Leppens et al, 1996). Whether this applies to the human has not been established. An additional complication is the possibility that supraphysiological concentrations of ovarian steroid hormones which are the result of ovarian stimulation may accelerate endometrial development, thereby increasing the likelihood of asynchrony.

Culture of oocytes/embryos may have adverse effects on their subsequent ability to implant by mechanisms independent of synchronization of embryonic and endometrial development. For example, culture conditions can affect the proportion of cells differentiating into inner cell mass cells and trophectodermal cells (Leppens et al. 1996). In the same study it was reported that outbred mouse embryos co-cultured with bovine kidney epithelial cells had features more akin to in vivo-derived blastocysts than embryos cultured in a complex medium based on mouse tubal fluid. However, both cultured groups had a poor pregnancy outcome relative to in vivo developed blastocysts when transferred to recipients.

CONTROL OF ENDOMETRIAL RECEPTIVITY

In animals, the development of endometrial receptivity is regulated by ovarian hormones. In all mammals, with the possible exception of the guinea pig (Deanesly, 1960), progesterone during the post-ovulatory period is essential for implantation. As determined by experiments in which mated females were ovariectornized and adrenalectornized shortly after mating, the requirement for exogenous estrogen, along with progesterone, varies with species. In some (mouse and rat, for example), exogenous estrogen is essential, while in others (hamsters and rabbits) it is not. In laboratory animals, the artificially-induced decidual cell reaction has been used as a model for implantation. Provided that a relatively non-traumatic artificial deciduogenic stimulus (such as the intrauterine

injection of a small volume of oil) is used, the timing and endocrine requirements for the decidual cell reaction and for successful implantation are similar, if not identical. Using the artificially-induced decidual cell reaction, it has been known since the classical work of Yochim and De Feo (1963) that the amount of estrogen given with progesterone prior to uterine stimulation is crucial. Estrogen in low doses acts synergistically with progesterone to induce endometrial sensitization/receptivity; at high doses, estrogen is inhibitory.

The controlled ovarian hyperstimulation protocols utilized in in vitro fertilization and embryo transfer programs result in concentrations of estrogen and progesterone in the circulation which are substantially higher than normal. Whether these supraphysiological concentrations of steroids, particularly those of estrogen, adversely affect implantation is controversial. Paulson et al (1990) compared implantation rates in a donor oocyte program with those from a standard in vitro fertilization program; the recipients in the donor oocyte program had not undergone controlled ovarian hyper-stimulation. Implantation rates were higher in the donor oocyte program, suggesting that controlled ovarian stimulation results in an inhibitory hormonal milieu. However, based on a review of the literature, Younis et al (1996) recently concluded that oocyte donation studies have shown that both normal and supraphysiological concentrations of estrogen can allow implantation.

Reports relating serum estrogen concentrations to pregnancy rates in in vitro fertilization and embryo transfer programs have come to all possible conclusions. Some have reported that high estrogen concentrations are detrimental (Forman et al, 1988; Simon et al, 1995), others that there is no relationship (Gelety and Buyalos, 1995), and yet others that there is a positive relationship between serum estradiol concentrations and pregnancy rates (Rosenwaks et al, 1986; Gratton et al, 1993; Paulson et al 1994). In general in these studies the serum estrogen concentrations were determined at mid-cycle, and may not reflect estrogen levels in the periimplantation period. Balasch et al (1995) have recently reported that there were no differences in serum estradiol concentrations between in vitro fertilization patients with on-going pregnancies compared to those who aborted or did not become pregnant. However, these results can be interpreted as indicating that some embryos are able to implant despite supraphysiological concentrations of estrogens which may have produced a less than perfectly receptive endometrium. The important question is whether more embryos would be able to implant if estradiol concentrations were in the physiological range in the peri-implantation period.

LOCAL FACTORS

Recently, there has been considerable interest in factors which may act locally in an autocrine/paracrine fashion to mediate implantation and decidualization. In animals there is experimental evidence for local interactions between the blastocyst and endometrium at the time of implantation (see Kennedy, 1994). The initial interaction probably initiates a cascade of events and these can be mimicked, at least in part in some

species, by the application of an artificial deciduogenic stimulus to the sensitized uterus. An ever-increasing number of compounds have been implicated as mediators of the uterine responses; these include prostaglandins, leukotrienes, platelet activating factor, and a variety of growth factors/cytokines. For many of the compounds, much of the evidence for their involvement is circumstantial, and it has not been possible to exclude any of the proposed mediators with certainty. Also, it is likely that these compounds interact to mediate the responses. For example, both prostaglandin E_2 (Kennedy, 1977) and interleukin-I (Simón et al, 1994) have been implicated in implantation. Interleukin-1 stimulates prostaglandin E_2 production by endometrial stromal cells (Bany and Kennedy, 1995) and this, in part, may be the mechanism by which interleukin-1 affects implantation.

At present it is not known if perturbations in locally-acting mediators contribute to the low implantation rates in human in vitro fertilization programs. In general, the production and actions of the local mediators in the uterus are regulated by ovarian hormones, and consequently could be affected by the non-physiological hormonal milieu which results from controlled ovarian hyperstimulation.

CONCLUSIONS

The low implantation rates in human in vitro fertilization programs may be a consequence of a naturally low implantation rate in humans, or of an altered physiological state resulting from the manipulations involved in these programs. Based on the results of studies in experimental animals, the latter seems more likely. For ethical reasons, it has not been possible to conduct studies in humans which would resolve the possibilities.

REFERENCES

Adams CE (1982) Egg transfer: historical aspects. In: *Mammalian Egg Transfer pp* 1-17 Ed CE Adams. CRC Press, Boca Raton.

Balasch J, Creus M, Fabregues F, Carmona F, Casamitjana R, Penarrubia J, Rivera F and Vanrell JA (1995) Hormonal profiles in successful and unsuccessful implantation in EVF-ET after combined GnRH agonist/gonadotropin for superovulation and hCG luteal support *Gynecol Endocrinol 9:51-58.*

Bany BM and Kennedy TG (1995) Interleukin-la regulates prostaglandin production and cyclooxygenase activity in sensitized rat endometrial stromal cells in vitro *Biol Reprod* 53:126-132.

Cooke ID (1988) Failure of implantation and its relevance to subfertility *J Reprod Fertil SuppI* 36:155-159.

Deanesly R (1960) Implantation and early pregnancy in ovariectomized guinea-pigs J *Reprod Fertil* 1:242-248.

Forman R, Belaisch-Allart J, Fries N, Hazout A, Testart J and Frydman R (1988) Evidence for an adverse effect of elevated serum estradiol

concentrations on embryo implantation *Fertil Steril 49:118-122.*

Gelety TJ and Buyalos RP *(1995)* The influence of supraphysiologic estradiol levels on human nidation J *Assist Reprod Gcnet 12:406-412.*

Gratton RJ, Nisker JA, Daniel S, Toth S, Gunter J, Kaplan BR, Tummon IS and Yuzpe AA *(1993)* An aggressive philosophy in controlled ovarian hyperstimulation cycles increases pregnancy rates *Hum Reprod 8:528-53 1.*

Harlow GM and Quinn P *(1982)* Development of preimplantation mouse embryos in vivo and in vitro *Aust J Biol Sci 35:187-193.*

Holm P, Walker SK and Seamark RF *(1996)* Embryo viability, duration of gestation and birth weight in sheep after transfer of in *vitro* matured and in *vitro* fertilized zygotes cultured *in vitro* or *in vivo J Reprod Fertil 107:175-18 1.*

Kennedy TG *(1977)* Evidence for a role for prostaglandins in the initiation of blastocyst implantation in the rat *Biol Reprod 16:286-291.*

Kennedy TG *(1994)* Involvement of local mediators in blastocyst implantation. In: *Endocrinology of Embryo-Endometrial Interactions pp 183-194* Eds SR Glasser, J Mulholland and A Psychoyos. Plenum Press, New York

Leppens G, Gardner DK and Sakkas D *(1996)* Co-culture of 1-cell outbred mouse embryos on bovine kidney epithelial cells: effect on development, glycolytic activity, inner cell mass:trophectoderm ratios and viability *Hum Reprod 11:598-603.*

Lessey BA, Castelbaum AJ, Sawin SW and Sun J *(1995)* Integrins as markers of uterine receptivity in women with primary unexplained infertility *Fertil Steril 63:535-542.*

Martel D, Malet C, Gautray JP and Psychoyos A *(1981)* Surface changes of the luminal epithelium during the human menstrual cycle: a scanning electron microscopic study. In: *The Endometrium: Hormonal Impacts pp 15-29* Eds J de Brux, R Mortel and JP Gautray. Plenum Press, New York

Paulson RJ, Sauer MV and Lobo RA *(1990)* Embryo implantation after human in vitro fertilization: importance of endometrial receptivity *Fertil Steril 53:870-874.*

Paulson RJ, Sauer MV, Francis MM, Macaso T and Lobo RA *(1994)* Factors affecting pregnancy success of human in-vitro fertilization in unstimulated cycles. *Hum Reprod 9:1571-1575.*

Psychoyos A *(1973)* Endocrine control of egg implantation. In: *Handbook of Physiology* Vol II, Part 2 pp 187-215 Eds RO Greep, EG Astwood and SR Geiger. American Physiological Society, Washington DC.

Rosenwaks Z, Muasher SJ, Acosta AA *(1986)* Use of hMG and/or FSH for multiple follicle development *Clin Obstet Gynecol 29:148-157.*

Simón C, Frances A, Piquette GN, El Danasouri 1, Zurawski G, Dang W and Polan ML *(1994)* Embryonic implantation in mice is blocked by interleukin-1 receptor antagonist *Endocrinology 134:521-528.*

Simon C, Cano F, Valbueno D, Remohi J and Pellicer A (1995) Clinical evidence for a detrimental effect on uterine receptivity of high serum oestradiol concentrations in high and normal responder patients *Hum Reprod* 10:2432-2437.

Weitlauf HM (1994) The Biology of implantation. In: *The Physiology of Reproduction pp* 391-440 Eds E Knobil and JD Neill. Raven Press, New York.

Yochirn JM and De Feo VJ (1963) Hormonal control of the onset, magnitude and duration of uterine sensitivity in the rat by steroid hormones of the ovary *Endocrinology* 72:317-326.

Younis JS, Simon A and Laufer N (1996) Endometrial preparation: lessons from oocyte donation. *Fertil Steril* 66:873-884.