Embryo Quality and Endometrial Receptivity: Lessons Learned from the ART Experience

The critical contributions of oocyte and embryo quality to successful pregnancies in the assisted reproductive technology (ART) setting have been elucidated during the past decade. The identification and establishment of uterine receptivity remain relatively obscure. Despite nearly 20 years of experience with *in vitro* fertilization and embryo transfer

(IVF-ET), implantation rates for most centers remain at 10 to 15%, similar to that reported for the first successful IVF pregnancy (1). The human uterus is receptive to embryonic nidation only during a narrow "window of implantation." Classic studies by Hertig and colleagues demonstrated the opening of the window on cycle day 20 by performing luteal-

phase hysterectomies on pregnant women (2). No embryos implanted before cycle day 19. The closure of the window has been demonstrated to occur on cycle day 24 based on donor oocyte transfers to hormonally prepared recipients (3,4) and the use of supersensitive β -human chorionic gonadotropin (β hCG) assays (5). ARTs, in general, and oocyte donation, specifically, have been used as models to investigate the relative importance of embryo and endometrium in implantation. Few have questioned their use for this purpose despite the significant differences between ART and natural cycles. The interventions used as part of a typical ART cycle may surmount endometrial dysfunction that might otherwise impede implantation. Furthermore, the increasing availability of biochemical markers suggests that certain conditions such as endometriosis (6) and hydrosalpinges (7) may produce defects in endometrial receptivity that do not require IVF-ET, but, rather, might be successfully treated with simpler, less expensive, and perhaps less invasive modalities.

Studies using donor oocytes have generally demonstrated equivalent implantation and pregnancy rates among reproductive young and older recipients, suggesting that oocyte and embryo quality are paramount in successful implantation. Little light has been shed using such studies to optimize uterine receptivity (8). Reports of successful pregnancies using donor oocytes for women in their sixties has implied that the endometrium is merely a hormonally compliant participant in implantation (9). Donor oocyte cycles typically result in a 40 to 50% pregnancy rate. What is responsible for patients who do not achieve pregnancy? Implantation rates lingering at 10 to 15% suggest that further progress may not be possible until other factors, including occult defects in endometrial receptivity, can be better defined. The transfer of supernumerary embryos undoubtedly overcomes many of these defects—but at the cost of multiple gestations in many patients.

Insights concerning endometrial receptivity drawn from ART cycles are flawed because of the failure to consider the differences between natural and hyperstimulation cycles. The corpus luteum (CL) arguably plays a defining role in pregnancy success in natural cycles. As the third "window of receptivity" (the first and second belonging to the embryo and endometrium, respectively) the timing of embryonic attachment and invasion and the early release of hCG rescues the CL from its ultimate

demise to maintain the early pregnancy until placental steroids take over. As described by Fritz *et al.* (10), the CL has a limited life span and a diminishing capacity to recognize and respond to embryo-derived hCG as the cycle proceeds. In animal models the asynchrony between embryo and endometrium contributes to pregnancy loss, perhaps on this basis (11). Forced to implant late by a histologically delayed endometrium, embryonic signals to the waiting CL occur later than normal resulting in miscarriage or infertility. Based on serum hormone levels, Baird *et al.* have shown that early miscarriages are associated with later-than-normal implantation (12). The sequelae of a delayed endometrial histology on CL function would be minimized in the ART cycle provided with pharmacologic levels of steroid replacement and/or the use of hCG to stimulate ovulation and provide luteal-phase support. Indeed, an expanding body of literature suggests that such histologic delay is commonplace in ovulation induction cycles (13) as well as in donor oocyte recipients who receive estrogen and progesterone in preparation for transfer (14). The fact that pregnancies occur despite these abnormalities argue that ART is able to overcome these constraints; at the same time it leads to speculation about whether success rates would be even higher if endometrial function were normalized in such cycles.

Critical examination of the endometrium to detect occult defects of endometrial function has not been systematically studied. Traditional endometrial histology using the criteria of Noyes *et al.* is, at best, a crude index of endometrial receptivity (15). IVF-ET is increasingly used in patients with polycystic ovarian syndrome (PCOS), tubal disease, endometriosis, and otherwise unexplained infertility with the assumption that, if endometrial defects exist, they can be overcome by the procedure itself. Based on clinical data such assumptions may not be valid. Women with PCOS have a miscarriage rate nearly twice that of women without PCOS in ART cycles (16). The disparity in cycle fecundity of women with PCOS compared to normal controls or to patients with hypothalamic amenorrhea was demonstrated as early as 1980 (17). These data are consistent with defects in endometrial receptivity likely attributable to hyperandrogenism found in such women. Similarly, more than 10 papers have recently appeared in the literature suggesting that women with hydrosalpinges have a decreased implantation rate compared to patients without hydrosalpinges

(for review, see Ref. 7). We demonstrated that women with hydrosalpinges have occult defects in uterine receptivity that are correctable with surgical intervention (7). Investigators have suggested that women with endometriosis also have such defects (6,18,19), although not all studies agree (20). Problems with implantation in women with endometriosis may be more difficult to demonstrate because fewer than half of women with endometriosis appear to be affected and adverse effects on endometrial receptivity appear to occur primarily in women with minimal or mild disease (6). Olivenness *et al.*, who failed to demonstrate differences in outcome between endometriosis and tubal patients in IVF-ET, may have included women with hydrosalpinges, thus comparing two populations which each have potential defects in endometrial receptivity. When hydrosalpinges were specifically excluded from the tubal population, significant differences in IVF-ET outcome could be found in endometriosis patients (21).

The use of ART to compare the quality of the endometrium vs the embryo may be further confused by the common use of GnRH analogues (GnRHa) to down-regulate women entering an ART cycle. Prolonged use of GnRHa has been shown to have a favorable effect on pregnancy outcome in endometriosis (22) as well as PCOS (23) in ART cycles. Protocols to synchronize donors and recipients using long periods of GnRHa treatment to produce amenorrhea may have a similar effect (24), masking a potential preexisting endometrial defect and thus underestimating the effect of endometrial function on pregnancy outcome.

The catheter used to transfer embryos in IVF and donor cycles may inadvertently stimulate the endometrium or remove luminal barriers to implantation as shown in the mouse model (25). The placement of embryos into the uterine cavity at a time prior to normal implantation may trigger endometrial changes that might not occur naturally (26,27). While a single embryo may not be able to improve a nonreceptive or defective endometrium, a collection of embryos placed together in the uterine cavity for a longer period may have a more substantive effect on endometrial receptivity and alter outcome on this basis. With the advent of coculture of embryos with heterotypic cells, the addition of bioactive substances to the transfer media may alter the receptivity of the endometrium.

Studies using donor oocytes have clearly demonstrated that implantation and pregnancy rates

are equivalent for young women and those already menopausal. These studies suggest that, using conventional endometrial priming, there is no endometrial "defect" among older recipients. The unanswered question is, Why are implantation rates so low in both groups? We hypothesize that otherwise occult defects in uterine receptivity or acquired defects as a result of abnormal endometrial stimulation account for many cases of ART failure. We have recently identified a marker of endometrial receptivity, the $\alpha v \beta 3$ endometrial epithelial integrin (28). This marker appears coincident with the opening of the window of implantation and is absent in women with luteal-phase defect (28) and in some women with endometriosis (6), hydrosalpinges (7), and unexplained infertility (29). Whereas ethical constraints preclude endometrial biopsies during IVF-ET cycles, we suspect that many patients in whom IVF-ET fails might lack this or other critical markers. More likely, a better understanding of the cascade of events leading to successful implantation will be derived from natural cycles as well as in vitro laboratory systems.

In summary, as papers that address the respective roles for the embryo versus the endometrium using ART and donor oocyte cycles continue to appear, it is prudent to pause and reflect on the potential limitations of this model. There is a large number of variables that may impact on the physiologic processes of implantation and the establishment of "receptivity." Differences between natural and ART cycles make a direct comparison impossible. Failure to appreciate potential defects in endometrial receptivity may relegate women to IVF-ET who might otherwise be treated effectively using conventional approaches or medical management alone. Furthermore, the relatively low implantation rates seen in most ART cycles may dramatically improve if a greater understanding of the endometrium and the determinants of receptivity can be established. High-order multiple gestations would also be significantly reduced, as fewer embryos would need to be transferred. We still have far to go.

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