Endometrial markers of uterine receptivity utilizing the donor oocyte model

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BACKGROUND: Ethical constraints limit the ability to study peri-implantation phase human endometrium. In this study, the donor oocyte model was used to study candidate endometrial markers of uterine receptivity.

METHODS: Archived, paraffin-embedded tissue obtained by endometrial biopsy during cycle days 21–23 of patients undergoing ‘mock’ hormonal treatment cycles were evaluated by standard histological criteria and immunohistochemical staining for αvβ3 integrin and glycodelin. All of these patients (n = 101) had undergone a donor oocyte embryo transfer cycle utilizing the exact same hormonal protocol.

RESULTS: Histological evaluation revealed 62 (61.3%) in-phase, 34 (33.7%) dyssynchronous, 2 (2.0%) immature and 3 (3.0%) advanced endometria. The clinical outcomes of patients with either in-phase or dyssynchronous endometria were similar. Very strong correlations were noted between endometrial glandular dating and either αvβ3 integrin or glycodelin immunostaining intensity (P < 0.001 for both). Glycodelin and αvβ3 integrin immunostaining intensities were also highly correlated with each other (P < 0.001).

CONCLUSIONS: Throughout the time period corresponding to the putative window of maximal endometrial receptivity (cycle days 21–23) a dynamic process was observed in exogenous hormonal replacement cycles characterized by a rapid histological advancement of endometrial glandular elements as well as progressive αvβ3 integrin and glycodelin expression.

Key words: endometrial receptivity/integrins/glycodelin/implantation/oocyte donation

Introduction

Oocyte donation affords a scientific opportunity to study the biological participation of the uterus in the process of human embryo implantation. Oocyte donation involves synchronization strategies intended to enable embryonic and endometrial development to coincide (De Ziegler et al., 1994; Younis et al., 1996). From previous work involving the oocyte donation model, strong evidence for a temporal window of maximal endometrial receptivity has been observed. Implantation efficiency appears to decrease significantly when embryo transfer is performed outside this window (Navot et al., 1991; Bergh and Navot, 1992). Although controversial, many investigators have promoted the use of ‘mock’ treatment cycles to ensure adequacy of hormonal replacement cycles for oocyte donation. Traditional evaluation of ‘mock’ hormonal cycles, however, defines endometrial receptivity only in terms of gross histology.

Recent investigations have suggested that immunohistochemical analyses of certain endometrial proteins may provide better information about endometrial function during the putative window of implantation. The epithelial endometrial integrin αvβ3, for instance, appears during the mid-secretory phase, coincident with the opening of this window (Lessey, 1994; Lessey et al., 2000). Decreased mid-luteal αvβ3 expression has been reported in connection with luteal phase deficiency (LPD) (Lessey et al., 1992), endometriosis (Lessey et al., 1994b), hydrosalpinges (Meyer et al., 1997) and unexplained infertility (Lessey et al., 1995). Glycodelin, a glycoprotein with immunosuppressive properties, has similar temporal characteristics. In the endometrium, glycodelin is absent during the periovulatory period, and usually appears in the glands on the fifth postovulatory day (Seppälä et al., 1988). A possible role of endometrial glycodelin in the local inhibition of natural killer cell activity and the immune tolerance of the conceptus during the implantation process has also been proposed (Seppälä et al., 1998).

In this study, mid-luteal αvβ3 integrin and glycodelin immunohistochemical staining as well as endometrial histology were evaluated in ‘mock’ hormonal cycles in order to determine their utility in predicting clinical outcomes of subsequent oocyte donation treatment.
Materials and methods

Study design
All cycles of oocyte donation performed at The Center for Reproductive Medicine and Infertility, The New York Presbyterian-Weill Medical College of Cornell University from January 1, 1992 to December 31, 1995 were reviewed. Clinical management of this programme was monitored under the Institutional Review Board Protocol number 0695–96 of the Committee for Human Rights in Research of The New York Presbyterian-Hospital-Cornell University Medical College. Only those cycles of oocyte donation in which a preceding mid-luteal (cycle days 21–23) ‘mock’ treatment cycle endometrial biopsy had been performed at The New York Presbyterian Hospital-Cornell University Medical College were selected. Archived endometrial tissue was processed for standard endometrial histology and immunohistochemical analysis. From 119 initial cases, 18 cases were excluded due to either failure to locate paraffin blocks or insufficient tissue for histological or immunohistochemical analysis. All exclusions were made prior to clinical outcome analysis. A total of 101 cases was studied further.

Oocyte donors
Oocyte donors consisted of anonymous volunteers (n = 77), sisters of recipients (n = 15) and IVF patients (n = 9) who agreed to donate half their oocytes anonymously. The anonymous donors underwent a careful screening process involving medical, genetic and psychological evaluation as described previously (Moomjy et al., 1995). All donors were 21–35 years of age. In most instances, each anonymous donor was matched phenotypically with two potential recipients. Shared donation occurred successfully in the majority of cases, as reported previously (Moomjy et al., 2000). In this process of shared matching, each recipient received an average of ~8 oocytes, which were divided evenly according to quality and maturity. A minority of the anonymous oocyte donors were converted to single recipients either because of low oocyte yield, history of prior compromised fertilization or lack of another phenotypically appropriate match.

Recipients
Potential oocyte recipients also underwent a comprehensive evaluation. Indications for oocyte donation included primary ovarian failure (n = 4), premature ovarian failure (n = 17), prior bilateral oophorectomy (n = 1), diminished ovarian reserve (n = 62), prior IVF treatment failure (n = 10) and advanced maternal age with associated ovarian failure (n = 7). Oocyte donation therapy was reserved for women under the age of 52. Preparation of recipients included confirmation of a normal uterine cavity by means of hysterosalpingography or hysteroscopy. A trial transfer procedure was performed to determine uterine cavity depth and direction. Semen analyses were performed on recipients’ husbands and, when indicated, antisperm antibody testing was included. In addition, all recipients completed a ‘mock’ preparatory cycle to assess endometrial response to exogenous oestradiol and progesterone. During ‘mock’ treatment cycles, oocyte recipients underwent endometrial biopsies during standardized cycle days 21–23 (with initiation of progesterone designated as cycle day 15). Endometrial biopsies were obtained from the uterine fundus with a standard endometrial suction curette (Pipelle; Unimar Inc., Wilton, CT, USA). The tissue was fixed in 10% neutral-buffered formalin and prepared for histological analysis utilizing conventional methods.

Treatment
Oocyte donors underwent a standard controlled ovarian hyperstimulation protocol. Leuprolide acetate (Lupron; TAP Pharmaceuticals, Deerfield, IL, USA) was begun in the mid-luteal phase at a dosage of 1.0 mg s.c. daily followed by a decrease to 0.5 mg daily on the day of gonadotrophin commencement. Some donors started an overlapping long leuprolide acetate protocol following a cycle of oral contraceptives (Damario et al., 1997). Following determination of adequate down-regulation (serum oestradiol <50 pg/ml and no ultrasonically visualized ovarian cysts >2 cm), gonadotrophins [purified FSH (Metrodin; Serono Laboratories, Norwell, MA, USA) and/or HMG (Pergonal; Serono Laboratories)] were generally started at a dosage of 75 IU ampoules i.m. daily for the first two days, with further adjustments of the dose according to ovarian response. In general, HCG was administered in a dosage of 5000 to 10 000 IU i.m. once the mean lead follicular diameters were ≥17 mm. Oocyte retrievals were scheduled 35 h after HCG administration. All donors underwent transvaginal ultrasound-guided oocyte retrieval. All oocytes were inseminated with 0.15–0.25×10⁶ washed motile spermatozoa ~5 h after oocyte aspiration. If a significant male factor was present, intracytoplasmic sperm injection (ICSI) was performed.

Oocyte recipients underwent identical hormonal replacement protocols for both the ‘mock’ treatment cycle as well as the actual oocyte donation transfer cycle. Women with ovarian function underwent down-regulation with the gonadotrophin-releasing hormone (GnRH) agonist, leuprolide acetate, initiated in the mid-luteal phase at a dosage of 1.0 mg s.c. daily and continued until commencement of progesterone therapy. Women with no evidence of ovarian function did not receive GnRH agonist therapy. Oestrogen replacement utilized transdermal oestradiol (Estraderm; Ciba Pharmaceutical Co., Summit, NJ, USA) applied to deliver a dose of 0.1 mg on days 1–5, 0.2 mg on days 6–9, and 0.4 mg on days 10–13. Thereafter, the dosage was decreased to 0.2 mg/day. Daily i.m. progesterone-in-oil injections (Steris Laboratories, Phoenix, AZ, USA) were started at a dose of 25 mg on day 15 and increased to 50 mg on day 16 onwards. During actual embryo transfer cycles, progesterone was initiated the day before the donor’s retrieval, thus permitting embryo transfer on cycle day 18 (for 3-day old embryos). If pregnant, recipient hormonal replacement therapy continued until ~12 weeks gestation.

Tissue evaluation
Endometrial histological dating was performed according to standard criteria (Noyes et al., 1950) by a single investigator (B.A.L.) who was blinded to both the immunohistochemical staining results and clinical outcomes. In each case, endometrial glandular and stromal elements were dated independently. Endometrial dysynchrony was defined as endometrial glandular dating lagging both chronological and stromal dating by ≥3 days. Endometrial immaturity was deemed when both glandular and stromal elements were judged to be lagging the chronological date by ≥3 days. Patients with endometrial glandular dating exceeding the chronological date by ≥3 days were described as advanced. Patients with appropriate dating of both glandular and stromal endometrial elements were said to have ‘in-phase’ endometrium.

Immunoperoxidase staining was performed for the β3 integrin subunit, using the monoclonal antibody, SSA6, as described previously (Lessey et al., 1994a). Prior to performance of the immunohistochemical staining on the study samples, extensive verification of the immunohistochemical staining method on fixed, paraffin-embedded samples was undertaken utilizing endometrial tissue with known frozen-tissue staining characteristics. Formalin-fixed, paraffin-embedded tissue was then sectioned 8 µm thick and affixed to slides. Immunoperoxidase staining was performed using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). Diaminobenzidine (DAB; Sigma Chemicals, St Louis, MO, USA) was used as the chromogen. Primary antibody (SSA6) was placed on sections follow-
ing blocking with 2% normal goat serum (Vector Laboratories) in phosphate buffered saline (PBS) pH 7.2–7.4, and allowed to bind at 4°C overnight. A rinse with PBS was followed by secondary antibody consisting of biotinylated goat anti-mouse antibody (Vector Laboratories) for 30 min at room temperature. Avidin-biotin complex (ABC) was added for 1 h at room temperature followed by three PBS rinses, each of 3 min duration. DAB was added for 10 min, rinsed, and the slides were then dehydrated and coverslips placed.

Similar immunohistochemical methods were utilized for glycolelin. Sections (5μm) of formalin-fixed, paraffin-embedded endometrial tissues were examined with an affinity-purified polyclonal anticygcolelin IgG as described (Kämäräinen et al., 1996). Briefly, the sections were cleared and subjected to microwave heat treatment to enhance immunoreactivity. Rabbit anti-glycolelin IgG was used as the first antibody (Koistinen et al., 1996), and normal rabbit IgG (Vector Laboratories) was used as a control. The second antibody was biotinylated porcine anti-rabbit immunoglobulin (Dako A/S, Glostrup, Denmark). Endogenous peroxidase activity was blocked by treating the samples with 0.6% perhydrol in methanol. Immunostaining was carried out using Vectastain ABC kit (Vector Laboratories) and 3-amino-9-ethylcarbazole as a substrate. The sections were counterstained with haematoxylin.

The resultant staining of both endometrial markers was evaluated on a Nikon microscope utilizing both 100× and 400× magnifications by a single blinded observer. The histological score (HSCORE) was calculated using the following equation: $HSCORE = \sum P_i (I + 1)$, where $I$ is the intensity of staining with a value of 1, 2 or 3 (weak, moderate or strong respectively), $Pi$ is the percentage of stained epithelial cells for each intensity, varying from 0–100% and 1 is a correction for optical density. This yields a range of results from 0 for no staining to 4 for maximal staining. The HSCORE has been previously utilized clinically with low inter- and intra-observer variation (Budwit-Novotny et al., 1986; Lessey et al., 2000).

**Clinical outcomes**

Clinical outcomes from just the first oocyte donation embryo transfer cycle were included in order to provide a similar treatment exposure for each patient. Clinical pregnancies excluded biochemical as well as ectopic pregnancies and required a detectable intrauterine gestational sac on ultrasound. Deliveries required the presence of a liveborn child. Implantation rate was determined by dividing the number of intrauterine gestational sacs with embryonic cardiac activity by the total number of embryos transferred.

**Statistical analysis**

Continuous data was reported as mean ± SD. Pearson correlation coefficients were used to identify relationships between continuous variables. Wilcoxon rank sum tests and Kruskal-Wallis tests were utilized as appropriate for comparisons of continuous variables with categorical clinical outcome variables. $\chi^2$ analyses were used in 2×2 tables for comparisons of categorical variables. Extensive multivariate modelling was performed between endometrial markers, histological characteristics and clinical outcome parameters utilizing both generalized estimating equations and logistic regression. Significance was determined by a $P$ value < 0.05.

**Results**

A total of 101 patients were studied who had undergone a ‘mock’ hormonal treatment with an evaluable cycle day 21–23 endometrial biopsy and at least one oocyte recipient embryo transfer utilizing the same hormonal protocol. Mean age was 40.2 years. Mean numbers of oocytes donated, normally fertilized and embryos transferred were 9.3, 6.0 and 3.2 respectively. Overall implantation rate per embryo transferred was 30.9%. Clinical pregnancy and delivery rates per transfer were 66.3 and 56.4% respectively. 72 cycles were undertaken in women with residual ovarian function, all of whom were treated with GnRH agonist down-regulation in both ‘mock’ hormonal as well as embryo transfer cycles. 29 cycles were undertaken in women with documented ovarian failure who did not require GnRH agonist down-regulation. Clinical outcomes were similar between the ovarian function and ovarian failure patients, except the ovarian failure patients had a statistically younger mean age and a statistically higher mean number of normally fertilized oocytes ($P < 0.01$ for both; Table I). Consequently, there seemed to be a trend towards higher implantation, pregnancy and delivery rates in the ovarian failure subgroup, although this was not statistically significant.

The systematic dating of both the endometrial glandular and stromal elements of the ‘mock’ treatment cycle biopsies resulted in 62 (61.3%) in-phase, 34 (33.7%) dysynchronous, 2 (2.0%) immature, and 3 (3.0%) advanced endometria. Interestingly, the clinical outcomes of patients with either in-phase or dysynchronous endometrium were virtually identical, including implantation rate per embryo transferred (32.5 versus 29.9%), clinical pregnancy (66.1 versus 67.6%) and delivery rates (56.5 versus 55.9%) (Table II). Highly significant differences were noted, however, between the in-phase and dysynchronous endometrial groups for both mean $\alpha\beta\beta3$ integrin and glycolelin immunohistochemical scores ($P < 0.001$ for both).

There were very strong correlations between endometrial glandular dating and both endometrial markers ($P < 0.001$) whether analysing the total group (Figure 1) or just cycle day 22 and 23 patients. Indeed, it appears that both $\alpha\beta\beta3$ integrin and glycolelin are excellent markers of endometrial glandular maturity during the secretory phase. The immunohistochemical stainings of both markers were also noted to be highly correlated with each other ($P < 0.001$). However, throughout the time period corresponding to the putative window of maximal endometrial receptivity (cycle days 21–23), a dynamic process was observed in these ‘mock’ hormonal treatment cycles (Figure 2). A lag in endometrial glandular development usually associated with normal stromal development was relatively common on cycle day 21, but was rare on cycle day 23. A marked progression of increased immunohistochemical staining for both $\alpha\beta\beta3$ integrin and glycolelin was seen over this time interval. On cycle day 21, $\alpha\beta\beta3$ integrin and glycolelin immunostaining was often absent or relatively weak. By contrast, although the variability of immunostaining increased, mean HSCOREs for both $\alpha\beta\beta3$ integrin and glycolelin trended significantly higher over the time interval ($P = 0.0001$ for both).

Utilizing univariate methodology, there were no associations noted between mean HSCOREs and clinical outcome parameters (implantation rate, clinical pregnancy and delivery rates) for both $\alpha\beta\beta3$ integrin and glycolelin. Due to the fact that immunohistochemical staining was fairly restricted and not evenly distributed on cycle day 21, a repeat univariate
analysis was conducted utilizing just patients who had undergone endometrial biopsy on either cycle days 22 or 23. No associations between mean HSCORES and clinical outcome parameters were again found for both markers. The absence of appropriate statistical power prohibited us from analysing solely the patients with cycle day 23 biopsies. None of the generalized estimating equation models or logistic regressions predicting clinical outcomes using endometrial markers and histological characteristics revealed stable, interpretable associations for either αvβ3 integrin or glycodelin.

Discussion

Our ability to study directly the human endometrium during the putative window of implantation is limited by ethical constraints. Most investigators would defer invasive methods, such as endometrial sampling, in the midst of infertility treatment cycles. Therefore, the ethical study of the peri-implantation phase human endometrium requires a strategy in which the peri-implantation phase endometrium is mimicked or modelled at a time separate from the actual infertility treatment cycle. One model that is proposed in this study is the use of ‘mock’ hormonal treatment cycles, in which the patient undergoes the identical hormonal protocol intended for an actual treatment cycle although embryos are not transferred.

The endometrium is then sampled during the suspected time interval of maximal endometrial receptivity under hormonal conditions similar to actual treatment cycles. An additional advantage of the donor oocyte model is that while there may be some clinical variability, the oocyte factor should be fairly consistent due to the use of careful screening procedures and age requirements. The one apparent disadvantage of this model, however, is its inability to account for endometrial changes which may occur as a result of embryonically-generated signals.

In this study, endometrial sampling was performed during ‘mock’ hormonal treatment cycles during the time interval which is generally felt to be most representative of the window of implantation (cycle days 21–23) (Sarani et al., 1999; Acosta et al., 2000). Hormonal replacement protocols were not altered by the presence of endometrial glandular-stromal dysynchrony, based on prior favourable clinical outcomes in this setting (Rosenwaks, 1987; Droesch et al., 1988). Droesch et al. (1988) performed a study in which a limited number of similarly treated patients underwent endometrial biopsies on day 21 of the first replacement cycle and day 26 of a second replacement cycle. In general, a 3-day glandular/stromal disparity was observed in the early biopsies, whereas the late biopsies were in phase. These results were interpreted as revealing a differential pattern of endometrial glandular development that occurred in response to exogenous hormonal replacement that
Endometrial assessment and oocyte donation outcome

Figure 1. Scatterplots of the relationships between endometrial receptivity markers and endometrial glandular dating. Statistical analyses reveal strong correlations ($P < 0.001$) for both $\alpha\beta_3$ staining (HSCORE) and glycodelin staining (HSCORE) and endometrial glandular date. Glycodelin staining (HSCORE) and $\alpha\beta_3$ staining (HSCORE) were also found to be strongly correlated with each other ($P < 0.001$).

was different from that seen in natural cycles. Since this early report, however, the clinical outcomes of oocyte recipients who demonstrated mid-luteal endometrial glandular-stromal dyssynchrony have not been extensively studied.

This study utilized archived paraffin-embedded endometrial tissue. Only endometrial biopsy samples that were processed in the Department of Pathology at the Weill Medical College of Cornell University were sought. As a requirement, patients had to have the exact hormonal protocol prescribed for both the ‘mock’ hormonal treatment cycle and at least one clinical donor oocyte–embryo transfer cycle. For appropriate statistical analysis, only the first embryo transfer cycle outcomes were analysed. Because of this study design, special precautions were undertaken to ensure that the investigators performing either histological or immunohistochemical analyses were blinded to the clinical outcomes. In fact, all of the endometrial assessments were performed at institutions separate from the clinical centre. As with other studies utilizing archival histological tissue, not all cases sought resulted in either an available paraffin block or adequate tissue for analysis (Hinton et al., 2000). All case exclusions, however, were established prior to analysis. There were no reasons to believe that this method of case selection caused a selection bias.

The clinical outcomes of patients with ovarian failure were compared with those with at least some residual ovarian function. The main reason that we looked at this was due to the fact that the latter patients were treated with a GnRH agonist, whereas the former patients were not. There were no statistically relevant clinical outcome differences between these two groups with the exception of a younger mean age (35.3 versus 42.2 years) and a greater mean number of normally
fertilized oocytes (7.2 versus 5.5) in the ovarian failure group. Our results concur with those of other investigators who have found neither enhancement nor detriment as a result of GnRH agonist use in oocyte donation (Remohi et al., 1994).

An analysis of the clinical outcomes according to endometrial classification, however, reveals several notable findings. First, in confirming previous reports, it appears clear that oocyte recipients who experienced ‘mock’ cycle endometrial glandular-stromal dysynchrony were not disadvantaged in comparison with oocyte recipients who had in-phase endometrium noted during similar biopsy conditions. Clinical pregnancy (66.1 versus 67.6%), delivery (56.5 versus 55.9%) and implantation rates (32.5 versus 29.9%) were quite similar for the in-phase and dysynchronous subgroups respectively. In fact, successful pregnancies were also noted to occur in one of two patients who had immaturity of both glandular and stromal endometrial elements as well as two of three patients who had advanced endometrial glandular elements. Second, in confirmation of previous work (Droesch et al., 1988), this study further suggests a differential rate of secretory phase endometrial glandular development between exogenous hormonal treatment cycles and natural cycles. As a result, endometrial glandular-stromal dysynchrony was fairly common on cycle day 21 but was relatively rare on cycle day 23. This time interval is best characterized as one of transition, in which both rapid histological advancement of endometrial glandular elements as well as rapid appearance of candidate markers of endometrial receptivity occurs.

Both αvβ3 integrin and glycodelin appear to be excellent markers of luteal phase endometrial glandular maturity. Very strong correlations were noted between endometrial glandular date and immunostaining results for either marker (P < 0.001 for both). A very strong correlation was also noted between αvβ3 integrin and glycodelin immunostaining (P < 0.001). Our results concur with those of previous investigators who noted diminished αvβ3 integrin or placental protein 14 (glyco-delin) immunostaining associated with retarded endometrial differentiation (Klentzeris et al., 1994; Krasnow et al., 1996; Meyer et al., 1999). In addition, a recent study has also demonstrated a significantly increased proportion of glycodelin-stained endometrial cells in controlled ovarian hyperstimulation (COH) patients as compared with natural cycling control patients throughout the window of implantation, a finding that was correlated with advanced histology in the COH patients (Brown et al., 2000).

Due to the dynamic endometrial process, our ability to analyse overall clinical outcomes across this time interval in relation to endometrial glandular dating or immunostaining intensities for either marker was limited. Certainly, there did not appear to be any clinical detriment when endometrial glandular-stromal dysynchrony was present early in this time interval and αvβ3 integrin and glycodelin immunostaining intensities were correspondingly weak. Limited patient numbers prohibited us from performing meaningful statistical analyses on the chronological subgroup with limited endometrial glandular-stromal dysynchrony (i.e., cycle day 23 group). Fairly elaborate multivariate models were further not able to differentiate statistically significant clinical effects with either putative endometrial marker.

One might speculate on the seemingly apparent paradox in which retarded endometrial development with limited or no expression of putative markers of endometrial receptivity frequently occurs in ‘mock’ treatment cycles at the suspected opening of the window of implantation without subsequent clinical detriment. Perhaps the window of implantation might be simply shifted in exogenous hormonal replacement cycles. It has been suggested (Krasnow et al., 1996) that the endometrial glandular histology was delayed in ‘mock’ hormonal treatment cycles even further when women were given oral micronized oestradiol rather than transdermal oestradiol. On the other hand, it has been contemplated that a less than optimal endometrial milieu might result in an embryonic diapause (Bergh and Navot, 1992; Tarin and Cano, 1999), namely, a physiological arrest, which is well described in other mammals (Spindler et al., 1995; Song et al., 1998). In both in-vivo studies of natural cycles as well as in-vivo studies of IVF cycles, HCG detection has been noted to vary over an exceptionally wide range, despite the fact that embryonic/endometrial maturational stages were generally felt to be constant (Lenton et al., 1981; Cole et al., 1987).

An alternative hypothesis is that the endometrium only plays a generally permissive role, with endometrial maturation and receptivity and the resultant timing of implantation primarily derived from an active dialogue between the embryo and endometrium. Although our study design will not identify the relative contribution of embryonic signals on endometrial receptivity, it is interesting to note that evidence from either in-vitro or in-vivo models suggests that both αvβ3 integrin and glycodelin may be up-regulated by HCG (Simón et al., 1997; Hauserman et al., 1998).

In conclusion, endometrial glandular-stromal dysynchrony is relatively common at a time interval corresponding to the opening of the putative window of implantation in ‘mock’ hormonal replacement cycles. Endometrial glandular retardation is strongly correlated with reduced immunostaining intensities of two candidate markers of endometrial receptivity, αvβ3 integrin and glycodelin. There is considerable evidence that such endometrial glandular-stromal dysynchrony may be transient and of no apparent clinical consequence during subsequent oocyte donation cycles.

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