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Differential Expression of Serum Glycodelin and Insulin-Like Growth Factor Binding Protein 1 in Early Pregnancy

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Abstract

This prospective study evaluated whether serum glycodelin and insulin-like growth factor binding protein 1 (IGFBP-1) predict the likelihood of embryo implantation in recipients undergoing donor egg in vitro fertilization. We measured glycodelin and IGFBP-1 at 6 points from lining check to lutenizing hormone (LH) + 31. β-Human chorionic gonadotropin levels were first measured at LH + 17. The recipients were divided into those without embryo implantation (group 1, n = 6) and those with successful implantation (group 2, n = 30). Although this is a negative study in that neither glycodelin nor IGFBP-1 alone reflected endometrial (EM) receptivity, the glycodelin/IGFBP-1 ratio on the day of blastocyst transfer was higher in recipients who achieved pregnancy ($P = .05$). At LH + 17, glycodelin was higher ($P = .04$), and IGFBP-1 was lower ($P = .004$) in recipients who achieved pregnancy when compared to those who did not. These observations are likely due to EM changes induced by successful embryo implantation.

Keywords

glycodelin, IGFBP-1, in vitro fertilization, implantation, pregnancy

Introduction

Successful human embryo implantation depends upon both embryo quality and endometrial (EM) receptivity. During the window of implantation (WOI), typically defined as days 20 to 24 of a 28-day menstrual cycle, the embryo and endometrium engage in a molecular dialog.[1] Although the embryonic factors are the primary determinants of implantation and pregnancy, failed implantation of high-quality embryos produced by in vitro fertilization (IVF) remains an important clinical problem in assisted reproduction. Appropriately timed exposure to estrogen and progesterone alters gene transcription in the endometrium resulting in a "receptive endometrium" during the WOI. Several proteins, including glycodelin, insulin-like growth factor binding protein 1 (IGFBP-1), homeobox A10, and leukemia inhibitory factor, are expressed by secretory phase endometrium and may play integral roles in EM function.[2] We initiated this pilot study to determine whether serum glycodelin and IGFBP-1 levels in donor egg IVF recipients reflect a "receptive endometrium" and predict the likelihood of embryo implantation.

Glycodelin is the major glycoprotein produced by secretory phase EM glands and gestational decidua.[3-5] Studies using both in situ hybridization and immunohistochemistry demonstrate peak glycodelin expression during the secretory phase of normal menstrual cycles.[6] Significantly lower levels of glycodelin were detected in uterine flushings on days lutenizing hormone (LH) + 10 and LH + 12 from women with unexplained infertility[7] and recurrent miscarriage,[8] when compared to normal controls. Decreased serum concentrations of glycodelin have been associated with delayed EM development, early pregnancy failure, and recurrent miscarriage.[9,10]

IGFBP-1 is one of the major secretory proteins of decidualized endometrium believed to mediate embryo–endometrium interactions during the peri-implantation period.[11-13] IGFBP-1 is expressed in stromal cells in the mid-late secretory phase, and secretion of IGFBP-1 is increased by progesterone.[14] Peri-implantation messenger RNA and protein levels of IGFBP-1 were reduced after administration of mifepristone, a progesterone receptor antagonist.[15] In addition, serum IGFBP-1 levels, measured from 3 to 11 weeks of gestation, were lower in patients with polycystic ovary syndrome, a group of patients

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with an increased rate of pregnancy loss, when compared to controls.[9]

Compared to EM biopsy and uterine flushings, the maternal serum provides a weaker reflection of the molecular cross talk between the implanting embryo and the endometrium. However, serum sampling is the least invasive method to measure the biomarkers during conception cycles. To reduce variability in embryo quality and lessen concerns that ovarian hyperstimulation may produce supraphysiologic estradiol (E2) levels believed to impair embryo implantation, we measured serum glycodelin and IGFBP-1 levels in recipients undergoing donor egg IVF cycles. Furthermore, with ovarian suppression and exogenous administration of controlled amounts of prescribed E2 and progesterone, any changes in serum glycodelin and IGFBP-1 levels would reflect EM production. We hypothesized that peri-implantation EM production of glycodelin and IGFBP-1 would be higher in those recipients who had successful embryo implantation.

Materials and Methods

Thirty-nine patients enrolled in the egg donation program at the Center for Women's Reproductive Care (CWRC) at Columbia University were prospectively consented for this study. These recipients (median age, 42 years; range, 35-48 years) were healthy, received no medications except those prescribed for the recipient cycle, and underwent age appropriate medical testing for pregnancy. Recipients of oocyte donation undergoing treatment cycles with frozen embryos were excluded. A total of 43 fresh donor egg cycles were completed; 4 patients completed 2 treatment cycles. The institutional review board at Columbia University Medical Center approved the study protocol, and all participants gave their signed informed consent.

Recipients were synchronized for embryo transfer (ET) with their egg donor as previously described.[16] Briefly, this included pituitary desensitization with leuprolide acetate followed by hormone replacement with oral E2, 4 mg daily, given in divided doses to achieve measured EM thickness values greater than 7 mm prior to initiating progesterone (P4) supplementation. If EM thickness was less than 7 mm after 8 to 10 days of oral E2 supplementation, additional estrogen was prescribed via a transdermal 0.1 mg E2 patch. Vaginal micronized P4, 200 mg daily, was started on the day prior to the donor's egg retrieval. P4 supplementation was then increased to 600 mg daily (200 mg three times daily) on the day of egg retrieval. The E2 and P4 supplementation was continued until 14 weeks gestation in patients achieving pregnancy. All transvaginal ultrasounds (TVUS) to measure EM thickness and characterize EM morphology were performed by the same physician. Additionally, EM thickness was measured immediately prior to ET and was performed using abdominal ultrasound guidance.

All egg donors underwent a standard antagonist IVF protocol with gonadotropin stimulation. Gonadotropin dosage adjustments were made according to physician discretion, based on serum E2 levels and/or follicular response. The method of fertilization was determined by semen analysis parameters, and ET occurred at the cleavage stage or blastocyst stage of embryo development depending upon embryo quality. Supernumerary high-quality embryos that were not transferred were cryopreserved. Grading of embryos was based on visual assessment of morphological characteristics.[17] The overall appearance of the blastocyst-stage embryo, taking into account the degree of expansion of the blastocoele and the morphology of inner cell mass (ICM) and trophectoderm (TE), was assigned a grade of good, fair, or poor. The ICM and TE of each blastocyst were assigned grades ranging from A to D, with A being the best. Embryos characterized as "poor" or "D" quality were not transferred.

Table 1. Study Design: The 6 Points of Assessment for Recipients of Egg Donation.

	LC	ET	WOI	LH + 17	LH + 19	LH + 31
Ultrasound	x	x				x
Glycodelin	x	x	x	x	x	x
IGFBP-1	x	x	x	x	x	x
β-hCG	x	x	x	x	x	x
E2	x	x	x	x	x	x
P4	x	x	x	x	x	x

Abbreviations: β-hCG; β-human chorionic gonadotropin; E2, estradiol; ET, embryo transfer; IGFBP-1; insulin-like growth factor binding protein 1; LC, lining check; LH, luteinizing hormone; P4, progesterone; WOI, window of implantation.

The study design is summarized in Table 1 and Figure 1. Serum glycodelin, IGFBP-1, E2, and P4 levels were measured at the following 6 time points during donor egg IVF treatment cycles: (1) lining check (LC), after 8 to 10 days of E2; (2) blastocyst ET, after 6 to 7 days of P4 exposure; (3) WOI, 9 or 10 days after oocyte pick up and the equivalent of LH surge + 11 days (LH + 11) or LH + 12; (4) LH + 17; and in pregnant recipients (5) LH + 19 and (6) LH + 31 or 6 weeks of gestation. Serum β-human chorionic gonadotropin (β-hCG) levels were determined at WOI, LH + 17, LH + 19, and LH + 31. The initial TVUS to document pregnancy was performed at LH + 31.

Recipient cycles were divided into 2 groups: those without successful embryo implantation (group 1) and those with successful implantation (group 2). There were no statistically significant differences in donor egg IVF recipients in groups 1 and 2 with respect to age, body mass index, menopausal status, and reproductive gynecologic history including prior uterine surgery and prior fertility treatment (Table 2). Blastocyst-stage ET was performed in 36 donor egg IVF cycles, and cleavage stage ET was performed in 7 cycles (which were excluded from the analyses). Serum glycodelin, IGFBP-1, E2 and P4 levels, EM thickness and morphology, the numbers of embryos transferred, and embryo scores were compared for cycles with blastocyst ET, group 1 (n = 6) and group 2 (n = 30). Cycles resulting in biochemical pregnancy (n = 4) or early pregnancy failure after documentation of a clinical pregnancy (n = 4) were included in the analyses, as serum glycodelin, IGFBP-1, E2, and P4 levels were similar to those cycles in which pregnancies resulted in live births.

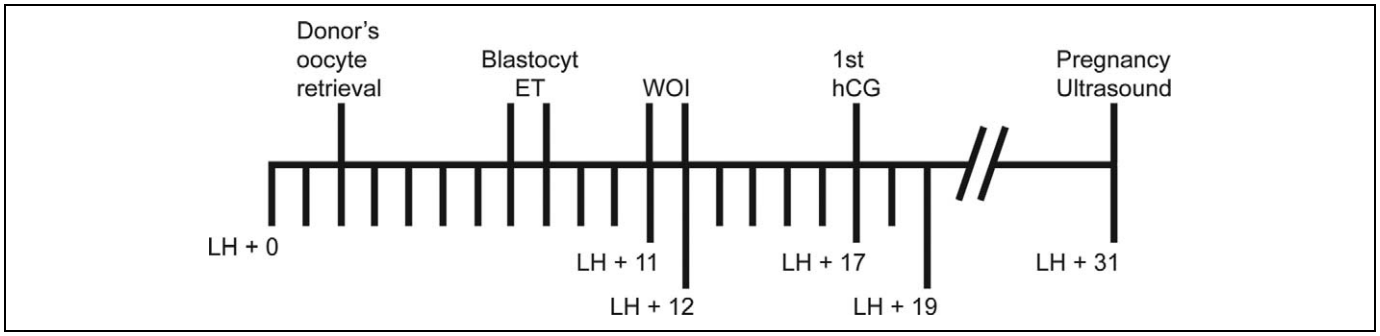

Figure 1. An illustration of the study design with respect to the lutenizing hormone (LH) surge.

Table 2. Baseline Patient Characteristics.[a]

	Group 1 (n = 7)	Group 2 (n = 32)
Age, years	42 (40.8-43)	43 (42-44)
BMI, kg/m^2	22.4 (20.5-23.1)	23.8 (21.1-28.0)
Menopausal status	28.6	12.1
Endometriosis	14.3	18.8
Prior uterine surgery	57.1	51.6
Prior pregnancy	57.1 (n = 4)	71.0 (n = 22)
Prior delivery	25 (1/4)	31.8 (7/22)
Prior miscarriage	100 (4/4)	72.7 (16/22)
Prior fertility treatment	71.4	80
Previous IVF	100	83.3

Abbreviations: BMI, body mass index; IVF, in vitro fertilization.
[a] Values are median and interquartile range or percentage. Fisher exact probability test was used to compare the demographic data. There were no statistically significant differences in any of the above parameters.

Assays

Blood samples were collected in a nonheparinized tube and centrifuged at 4°C. Serum was divided into 5 aliquots and stored at −20°C until assayed. All assays were performed at the CWRC. Serum glycodelin (GdA ELISA; Bioserv Diagnostics, Rostock, Germany[18,19]) and IGFBP-1 (*ACTIVE* Total IGFBP-1 ELISA; Diagnostics Systems Laboratories, Brea, California[20,21]) levels were determined in duplicate. The lower limit of detection for glycodelin was 6 ng/mL, and intra- and interassay coefficients of variation were 9.0% and 10.1%, respectively. The lower limit of detection for IGFBP-1 was 1.86 ng/mL, and the intra- and interassay coefficients of variation were 3.5% and 3.0%, respectively. Concentration values of glycodelin below the limit of detection (LOD) were imputed a concentration equal to LOD/$\sqrt{2}$ for the calculations.[22] All concentration values of IGFBP-1 were above the LOD. Serum levels of E2, P4, and β-hCG were measured in duplicate with chemiluminescence assays using IMMULITE 1000 Systems (Seimens, Los Angeles, California). Intra- and interassay coefficients of variation did not exceed 10%.

Statistics

Nonparametric statistical analyses were used for all comparisons. Medians for nonpregnant (group 1) and pregnant (group 2) recipients were compared using the Mann-Whitney *U* test or the Kruskal-Wallis test for analysis of variance. Fisher exact probability test was used to compare the demographic data, EM morphology, and embryo scores. Spearman rank correlation was calculated to determine whether there were relationships between glycodelin and EM thickness, glycodelin and the number of implantations, and glycodelin or IGFBP-1 and β-hCG. The Kruskal-Wallis test for analysis of variance was used to determine that glycodelin, IGFBP-1, E2, and P4 levels were similar in cycles resulting in biochemical pregnancies, pregnancy failures, and live births after blastocyst stage ET. Statistical analyses were performed using GraphPad Prism Version 5.0d (GraphPad Software, Inc, San Diego, California).

Results

Implantation occurred in 81% (35 of 43) of all donor egg IVF cycles, with 88.6% resulting in clinical pregnancies and 11.4% in biochemical pregnancies. In all, 14.3% of clinical pregnancies ended in a first trimester miscarriage, and the remaining resulted in live births. These clinical pregnancy rates are consistent with pregnancy rates historically seen in recipients of egg donation at CWRC.

Although serum glycodelin was undetected or below the LOD at LC in all recipients without embryo implantation (group 1), glycodelin was above the LOD at LC in 25% of the samples from recipients with successful embryo implantation (group 2; Table 3). In all recipients, serum glycodelin was undetected or below the LOD at ET. Glycodelin levels increased from ET to LH + 17 in both groups 1 and 2 and continued to rise from LH + 17 to LH + 31 in group 2. Median glycodelin levels were similar at WOI but significantly lower ($P = .0429$) in group 1 when compared to group 2 at LH + 17 (Table 3). The percentage increase in serum glycodelin from WOI to LH + 17 was significantly greater in group 2 when compared to group 1 (Figure 2A). In pregnant recipients (group 2), there was no correlation between glycodelin at WOI and EM thickness at LC ($r = .314$, $P = .110$), EM thickness at ET ($r = .077$, $P = .814$), or number of gestational sacs ($r = .026$, $P = .899$). In recipients achieving pregnancy, there was no correlation between glycodelin and β-hCG at WOI ($r = -.061$, $P = .764$), LH + 17 ($r = -.277$, $P = .161$), LH + 19 ($r = .169$, $P = .420$), or LH + 31 ($r = .307$, $P = .229$).

Table 3. A Comparison of Serum Glycodelin and IGFBP-1 and the Glycodelin/IGFBP-1 Ratio in Recipients Without (Group 1, n = 6) and With (Group 2, n = 30) Embryo Implantation After Donor Egg IVF.[a]

	Glycodelin, ng/mL			IGFBP-1, ng/mL			Glycodelin/IGFBP-1 ratio		
	Group 1	Group 2	*P* Value[b]	Group 1	Group 2	*P* Value[b]	Group 1	Group 2	*P* Value[b]
LC	4.2[c] (1.1-4.2)	4.2[c] (4.2-7.7)	.2274	37.4 (23.4-60.1)	38.9 (24.0-58.1)	.7953	0.1 (0.0-0.3)	0.1 (0.1-0.2)	.4513
ET	4.2[c] (2.1-4.2)	4.2[c] (4.2-4.2)	.1321	39.2 (28.0-46.8)	18.4 (9.5-29.2)	.0236	0.1 (0.1-0.2)	0.3 (0.1-0.7)	.0499
WOI	11.5 (7.0-15.4)	15.6 (7.4-28.0)	.3903	62.7 (56.6-70.9)	34.9 (11.8-55.3)	.0162	0.2 (0.1-0.3)	0.7 (0.2-2.0)	.0379
LH + 17	24.7 (8.0-36.6)	93.8 (21.7-172)	.0429	83.1 (67.8-94.5)	40.3 (17.7-60.0)	.0035	0.4 (0.1-0.5)	2.4 (0.7-8.3)	.0092
LH + 19		130.5 (83.7-247.5)			40.8 (17.9-59.7)			3.6 (1.6-12.1)	
Pregnancy scan		514.0 (168-762)			27.2 (19.0-54.8)			14.2 (4.0-36.7)	

Abbreviations: ET, embryo transfer; IGFBP-1, insulin-like growth factor binding protein 1; IVF, in vitro fertilization; WOI, window of implantation; LC, lining check.
[a] Data are expressed as median and interquartile range.
[b] The Mann-Whitney U test.
[c] Limit of detection LOD/$\sqrt{2}$.

Serum IGFBP-1 was above the LOD in both groups 1 and 2 at all time points. In both the groups, IGFBP-1 increased from ET to LH + 17, with significantly higher levels at LH + 17 when compared to ET (Figure 2B; Table 3). However, median serum levels of IGFBP-1 were significantly higher in group 1 versus group 2 at ET, WOI, and LH + 17 (Table 3). With the progression from a decidualized endometrium to initial trophoblast invasion and early placentation (WOI to LH + 31), IGFBP-1 levels decreased. In recipients achieving pregnancy, there was no correlation between IGFBP-1 and β-hCG at WOI ($r = .334$, $P = .088$), LH + 17 ($r = .049$, $P = .813$), LH + 19 ($r = -.184$, $P = .379$), or LH + 31 ($r = -.378$, $P = .135$).

Normalizing to progesterone levels, the data for both glycodelin and IGFBP-1 are similar with one exception, median glycodelin levels at LH + 17 in group 1 versus group 2 are no longer statistically different. However, since the percentage increase in serum glycodelin from WOI to LH + 17 remained significantly greater in group 2 when compared to group 1, the findings in early pregnancy are the same for both glycodelin and IGFBP-1 after normalizing to progesterone levels.

We calculated the glycodelin/IGFBP-1 ratio in groups 1 and 2. In both the groups, the median glycodelin/IGFBP-1 ratio increased from ET to LH + 17 (Table 3). However, the glycodelin/IGFBP-1 ratio was significantly higher in group 2 (recipients with embryo implantation) as compared to group 1 (recipients without embryo implantation) at ET ($P = .05$), WOI ($P = .04$), and LH + 17 ($P = .009$). In recipients achieving pregnancy, increasing glycodelin and decreasing IGFBP-1 resulted in higher glycodelin/IGFBP-1 ratios as pregnancies progressed to 6 weeks of gestation.

Although median EM thickness at LC and ET and EM morphology at LC were similar for groups 1 and 2 (Table 4), recipients in group 1 required more days of E2 supplementation than those in group 2, 14 days (interquartile range [IQR], 9.8-16.8 days) versus 10 days (IQR, 9-11 days), $P = .0316$. Median serum E2 and P4 levels were similar in groups 1 and 2 at all time points (data not shown). The median number of embryos transferred (n = 2) and blastocyst stage embryo quality were similar in groups 1 and 2 (Table 4). Serum β-hCG was detected at WOI in 44% of the recipients achieving pregnancy.

Discussion

To our knowledge, this is the 1st prospectively designed study to measure serum glycodelin and IGFBP-1 in recipients at multiple time points during an egg donation IVF cycle. The aim of our study was to determine whether measuring serum glycodelin and IGFBP-1 could be clinically useful for predicting EM receptivity and a high likelihood of implantation when embryo quality is thought to be optimal (ie, young aged egg donors). Although we found that the peri-implantation glycodelin/IGFBP-1 ratio was greater in recipients achieving pregnancy, neither glycodelin nor IGFBP-1 alone predicted EM receptivity. Differences in peri-implantation levels of glycodelin and IGFBP-1 were too small to be clinically useful in distinguishing between a receptive and a nonreceptive endometrium. Thus, this is a negative study.

Published data suggest that glycodelin may be involved in the peri-implantation decidual angiogenesis that is required for early pregnancy development and placentation.[23-26] We demonstrated that the rate of glycodelin increase from the WOI to LH + 17 was significantly greater—and at LH + 17, glycodelin was significantly higher—in recipients achieving pregnancy. As we did not see differences in glycodelin at ET or WOI, the higher levels of glycodelin observed at LH + 17 most likely reflect EM changes in response to signals expressed by a normal embryo. Our findings are consistent with the role of glycodelin in mediating embryo implantation.

We unexpectedly found lower serum IGFBP-1 in recipients who experienced successful implantation when compared to those without implantation. These lower levels of IGFBP-1 may reflect IGFBP-1's role in mediating IGF-II availability. IGF-II is expressed in proliferating trophoblasts and is believed to have roles in decidual angiogenesis and placental differentiation.[27,28] Thus, increasing IGF-II levels would result in the lower observed levels of IGFBP-1. However, this theory does not completely explain lower IGFBP-1 at ET in recipients

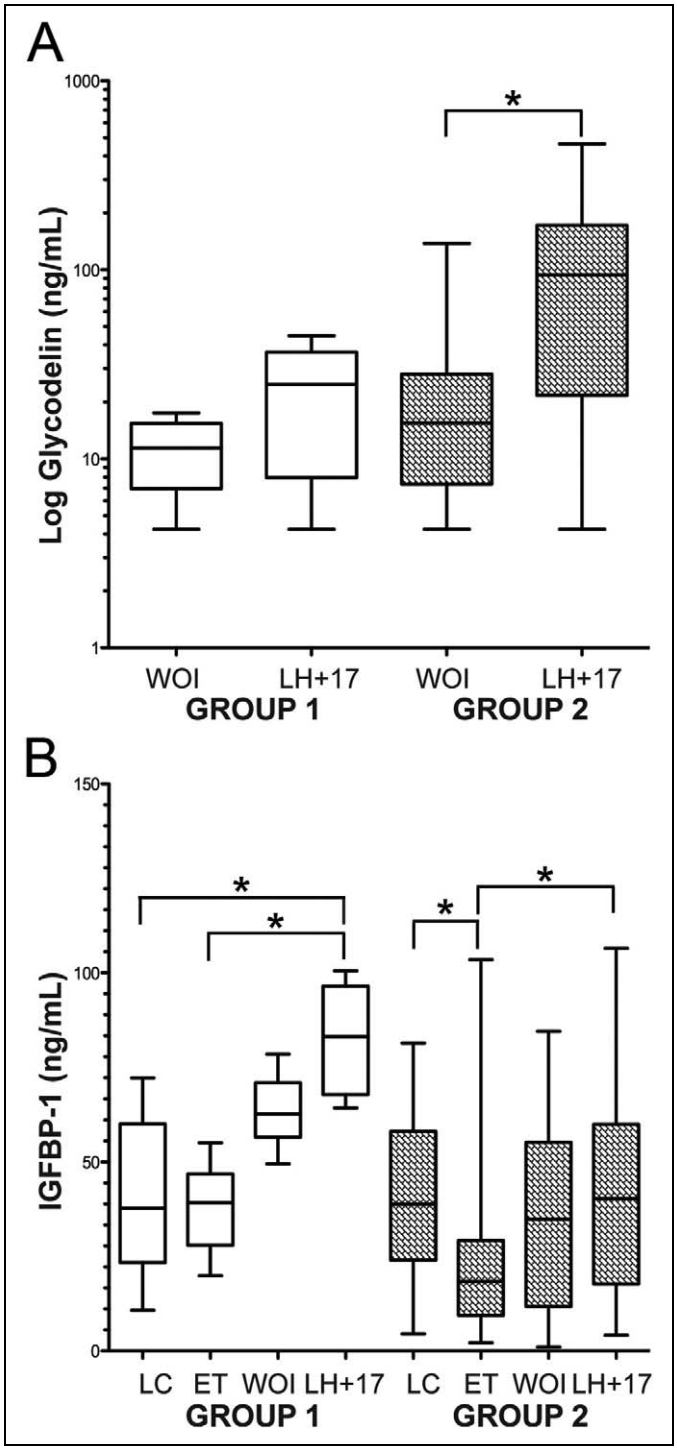

Figure 2. A comparison of glycodelin and IGFBP-1 levels in nonpregnant (group 1) and pregnant (group 2) recipients. A, In group 1, median glycodelin levels were similar at WOI and LH + 17 ($P = .24$). In group 2, median glycodelin levels were higher at LH + 17 when compared to WOI ($P = .0001$). The percentage increase in glycodelin from WOI to LH + 17 in group 2 (311%) was greater than in group 1 (84.7%, $P = .0109$). B, Median IGFBP-1 levels were higher in group 1 when compared to group 2 at ET, WOI, and LH. In both the groups, IGFBP-1 levels increased from ET to LH + 17. Data are presented as the median value with interquartile range. * $P < .05$. ET indicates embryo transfer; IGFBP-1, insulin-like growth factor binding protein 1; LC, lining check; LH, lutenizing hormone; WOI, window of implantation.

Table 4. Donor Egg IVF Cycle Outcomes.[a]

	Group 1 (n = 6)	Group 2 (n = 30)
Lining thickness at LC, mm	8.6 (7.5-9.5)	8.7 (9.0-10.3)
% Trilaminar morphology	33.3	60
Lining thickness at ET, mm	10.6 (10-12.3)	10.0 (9.0-11.7)
Median no. embryos transferred	2	2
Embryo grading		
Good	2/12 (16.7)	11/58 (19)
Fair	10/12 (83.3)	47/58 (81)
ICM score		
A	1/12 (8.3)	2/57 (3.5)
B	5/12 (41.7)	37/57 (64.9)
C	6/12 (50)	18/57 (31.6)
Trophectoderm score		
A	0	4/57 (7)
B	7/12 (58.3)	33/57 (57.9)
C	5/12 (41.7)	20/57 (35.1)
Twin gestations		11/30 (36.7)

Abbreviations: ET, embryo transfer; IVF, in vitro fertilization; LC, lining check.
[a] Values are median and interquartile range or number/total (percentage).

achieving pregnancy, as blastocyst implantation occurs prior to the upregulation of IGF-II.[27]

Our study is limited by its small size, the high pregnancy rate (ie, greater than 80%) in recipients with blastocyst stage ET, and our lack of preimplantation genetic screening to assess embryo aneuploidy. Our study may also be subject to selection bias in that a pseudopregnant state is created in all recipients of donor egg IVF. It is possible that recipients are uniformly receptive to embryo implantation, and that pregnancy is not achieved because abnormal embryos do not provide appropriate signals for implantation. If this were true, recipients would have similar glycodelin levels, as we saw, until after implantation.

To our knowledge, this is the first study to report differential expression of glycodelin and IGFBP-1 in early pregnancy. Future studies with more frequent secretory phase sampling of these proteins in "mock" hormonal cycles and in donor IVF treatment cycles are needed to determine whether differences in serum glycodelin levels and/or the glycodelin/IGFBP-1 ratio reflect EM receptivity. We anticipate that such differences would be found and that measuring serum glycodelin and IGFBP-1 at multiple points in a "mock" luteal phase would be useful in predicting a recipient's likelihood of successful embryo implantation.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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