Mouse ascites Golgi mucin expression abnormalities in natural cycle endometrial biopsies predict subsequent in vitro fertilization–embryo transfer (IVF-ET) failure in patients with previous IVF-ET failures

We performed a double-blinded retrospective cohort study to determine whether endometrial expression of mouse ascites Golgi mucin in a natural, unmedicated cycle predicts subsequent IVF-ET outcome among women with prior IVF-ET failure and poor pre-embryo quality. We found a statistically significant decrease in clinical pregnancy rate among women with abnormal mouse ascites Golgi expression, a test which was found to have high positive predictive value for failed IVF-ET. (Fertil Steril® 2006;85:255–8. ©2006 by American Society for Reproductive Medicine.)

Implantation is a major hurdle for successful human reproduction. There is only a brief window of time, normally between cycle days 17 and 19, during which an embryo might successfully initiate implantation (1, 2). There are three main phases of implantation: apposition, adhesion, and penetration (3). Studies in the mouse have shown that at the time of apposition and adhesion, mucin oligosaccharides appear on the endometrial surface, whereas lectin-like oligosaccharide receptors appear on the blastocyst. A receptor–ligand interaction between these two components is thought to represent the earliest molecular event in the implantation process and might be necessary for implantation to occur (4).

Mouse ascites Golgi (MAG) mucin, a MUC1-associated (5, 6), blood group A–related epitope that is expressed in human endometrial cells in a menstrual cycle–dependent fashion, might be the human analog of this interaction (2). Immunohistochemical staining with anti-MAG antibodies has identified a high-molecular-weight mucin first appearing in the Golgi of endometrial glandular cells on cycle day 5, where the epitope is likely posttranslationally added to MUC1 (2). This modified mucin is then secreted into the lumen, a process visualized as increased surface staining, which is greatest on days 17–19, during the implantation window.

We proposed a correlation between abnormal MAG expression and subsequent IVF-ET failure. To test this hypothesis, we performed a retrospective, double-blinded analysis of MAG expression in natural, unmedicated cycle endometrial biopsies and subsequent pregnancy outcomes among women with at least one previous failed IVF-ET attempt with poor pre-embryo quality (having fewer than six cells or less than grade 2 morphology at transfer on day 3 [7]). Two hundred thirty-six women, aged 25–44 years, who were being treated with IVF for infertility secondary to a variety of etiologies, were selected for our study. All participants signed a consent form approved by the New York Hospital–Cornell Medical Center Institutional Review Board. Analysis of residual biopsy specimens was also approved by the Human Investigation Committee of the Yale University School of Medicine.

All participants underwent luteal phase Pipelle endometrial biopsy during a baseline natural cycle. A small amount of each biopsy specimen was fixed in 10% neutral buffered formalin solution and paraffin embedded to be sent to Yale University. With hematoxylin and eosin–stained sections, biopsies were dated according to Hendrickson and Kempson's decision tree for endometrial dating (8). Slides were stained with anti-MAG antibodies, anti-ABO blood group antibodies, anti-MUC1 antibodies, and nonimmune mouse ascites, as previously described (2).

Only those biopsies that were reactive against blood group A antibody were evaluated for MAG expression because MAG reactivity only occurs in blood group A or AB patients (2). Expression of MAG mucin was quantified microscopically by determining the percentage of glandular epithelial cells expressing MAG. Biopsies were categorized as MAG normal ($>10\%$ glandular MAG expression on cycle days 5–18, or $<10\%$ on cycle days 19–25) or MAG abnormal ($<10\%$ glandular MAG expression on cycle days 5–18, or $\geq 10\%$ on cycle days 19–25).

The majority of each biopsy sample was used clinically to prepare an autologous endometrial coculture medium at the Center for Reproductive Medicine and Infertility (9).

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Briefly, stromal and glandular cells were isolated by enzymatic digestion and differential centrifugation. Cells were cryopreserved until 1 day before administration of hCG in the patient's IVF-ET treatment (on average, 3 months later), at which time an approximately equal number of stromal and glandular cells were thawed to create an autologous coculture medium.

Selection of one of three ovarian stimulation protocols (short- or long-acting leuprolide acetate with GnRH agonists or clomiphene citrate with GnRH agonists) was based on the individual patient's clinical scenario, as outlined in detail elsewhere (10). When at least two follicles measured ≥17 mm by transvaginal ultrasound, patients were given hCG (5,000–10,000 IU). Transvaginal oocyte retrieval was performed 35 hours later, followed by conventional insemination or intracytoplasmic sperm injection.

Fertilization was confirmed after 12–18 hours by the presence of two pronuclei. Up to six pre-embryos per patient were transferred to the autologous endometrial coculture medium, where they were grown for 72 hours before intrauterine transfer (11). Selective assisted hatching was performed (12), and only the morphologically best pre-embryos were transferred. Clinical pregnancy was defined as the presence of an intrauterine sac with a fetal heartbeat at gestation of 49 days.

The MAG expression results were compared with clinical outcomes by the Fisher exact probability test with two-tailed probability to examine the proposed correlation between abnormal MAG expression and subsequent negative pregnancy outcomes. Significance was defined as $P<.05$.

Of the 236 biopsies examined, 114 were from blood group A or AB patients and therefore could be used for MAG assessment. Pregnancy outcomes were available for 90 of these patients. Data analysis was performed with only those biopsies that had hematoxylin and eosin stromal dating between days 5 and 25 (n = 78), because this is the period for which reliable characterization of normal MAG expression is known (2).

Of the 78 biopsies used in the final analysis, 17 had abnormal MAG expression (Fig. 1). All biopsies, regardless of MAG expression, showed strong MUC1 staining and negative nonimmune mouse ascites staining, suggesting that the defect in abnormal expression is a lack of posttranslational addition of the MAG epitope to MUC1, rather than a lack of MUC1 itself (2). Notably, there was no significant difference between the mean age of women with abnormal MAG expression (mean 39.4 years, SD 4.83 years) and women with normal MAG expression (mean 37.8 years, SD 3.86 years) ($P=.86$). Thus, abnormal MAG expression is not a marker for advanced maternal age.

Only 1 woman out of 17 who had abnormal MAG expression successfully became pregnant (5.9%). In contrast, among the 61 women with normal MAG expression, 24 became pregnant (39.3%), revealing a highly statistically significant difference in clinical pregnancy rates between the normal and abnormal MAG expression groups ($P=.0164$). Abnormal MAG expression correlated with failed IVF-ET with a specificity of 96.0%. The positive predictive value of abnormal MAG expression for negative pregnancy outcome was 94.1%, with a likelihood ratio (weighted by prevalence) of 16 (95% confidence interval 9.8–26.1). However, abnormal MAG expression showed low sensitivity (30.2%) because even among women with normal MAG expression, more than half did not become pregnant.

Our data indicate that abnormal MAG expression in a natural, unmedicated cycle in patients with at least one previous IVF failure is an effective predictor of subsequent failed IVF-ET. Patients with abnormal MAG expression are 16 times more likely to have failed attempts than patients with normal MAG expression. The high positive predictive value (94.1%) of abnormal MAG expression renders it useful as a preliminary test for patients with previous IVF failure(s) and poor embryo quality who are contemplating IVF-ET. Analysis of MAG is highly specific (96.0%)—patients with abnormal MAG expression are likely to have a failed IVF-ET. However, normal MAG expression does not ensure a successful IVF-ET cycle.

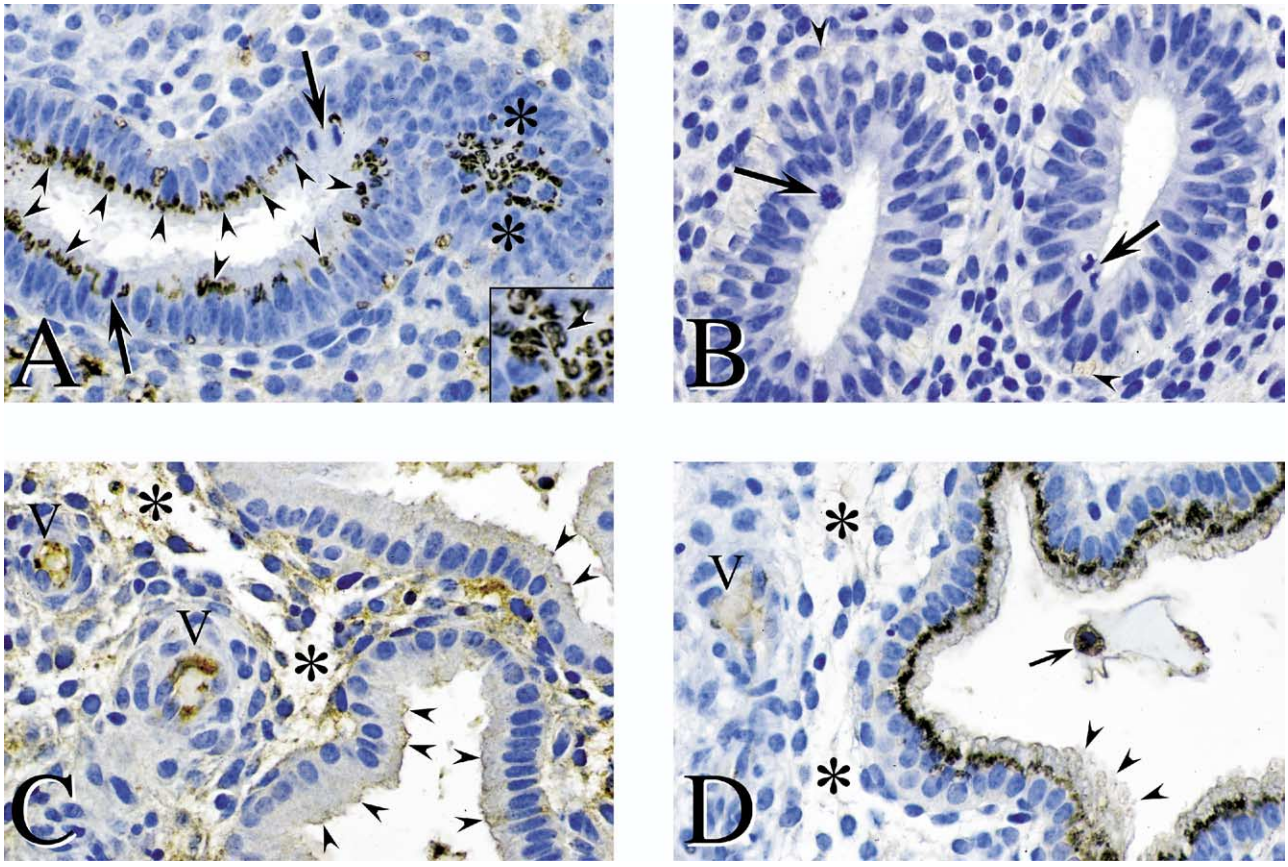
Although the patient population in this study was limited to women with prior IVF-ET failure and poor pre-embryo quality, we believe our findings are highly generalizable because we included women with between 1 and 11 prior IVF attempts and various etiologies of infertility, including male and female factors. In addition, previous work with MAG expression among donor embryo recipients showed similar results (13).

Although natural and IVF cycles are very different, we were surprised to find that MAG expression during natural cycles seems to predict endometrial receptivity in hormonally stimulated cycles. Absence or delayed expression of MAG on MUC1 might impede the complex mechanism of endometrium–trophoblast interaction required for successful implantation and pregnancy, as has been described in mouse implantation (4). Whereas it is possible that such a functional relationship for MAG does not exist, and, in fact, that administration of exogenous hormones might correct abnormal MAG expression, it is clear that abnormal MAG expression is either a direct or surrogate marker strongly associated with failed pregnancy outcomes.

Owing to limitations of our data, we were unable to examine whether MAG expression remains a predictor when placed in a model with other factors associated with poor outcomes, such as ovarian reserve, age, number of embryos transferred, and duration of infertility.

FIGURE 1

Normal (**A, C**) and abnormal (**B, D**) MAG expression in follicular (**A, B**) and luteal (**C, D**) biopsies. (**A**) Normal MAG-positive day-15 endometrial gland with obvious mitotic figures (*arrows*) and exhibiting many immunohistochemically positive Golgi bodies (*arrowheads*). In a tangential section through the perinuclear zone (*between the asterisks*), complex Golgi bodies (note especially *arrowhead in inset*) can be identified. (**B**) Day-16 MAG-absent endometrial glands with obvious mitotic figures (*arrows*) but no Golgi staining. Only occasional, light staining in the basal portions of some cells can be seen, which most likely represents background staining (*arrowheads*). (**C**) Normal MAG-negative day-24 biopsy with decidual cuffing around the stromal vessels (V), residual stromal edema (*asterisks*), and glandular epithelium without any evidence of vacuolization. No Golgi staining is noted in the glandular epithelium, but residual apical MAG staining can be seen (*arrowheads*). The vascular endothelial cells (V) exhibit some MAG reactivity and serve as an internal positive control. (**D**) Abnormal MAG-positive day-24 biopsy with decidual cuffing around the stromal vessel (V), residual stromal edema (*asterisks*), but inappropriately persistent Golgi reactivity in virtually all of the glandular epithelial cells. Occasional cells show some evidence of MAG secretion (*arrowheads*) with, in this example, ingestion by a luminal macrophage (*arrow*), but the majority of the staining is in the typical perinuclear Golgi region. Original magnification, ×200.



Catalanotti. MAG expression predicts IVF-ET outcome. Fertil Steril 2006.

Despite the promising results presented here, it is not likely that MAG testing alone will be clinically useful because it can only be used in blood group A or AB patients (approximately 44% of the U.S. population). An ideal alternative marker might be cyclin E, a mitotic regulator expressed in the proliferative phase, which is believed to play a critical role in the hormonal mediation of proliferation and differentiation of the endometrium (14). Abnormal cyclin E expression might identify defects in endometrial development causing an unreceptive endometrium in some women with idiopathic infertility (14). Therefore, cyclin E, as part of an endometrial function test, might be an ideal way to evaluate women of all blood types before IVF-ET. The full potential of this approach remains to be established with large, prospective, multicenter trials.

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REFERENCES

1. Acosta AA, Elberger L, Borghi M, Calamera JC, Chemes H, Doncel GF, et al. Endometrial dating and determination of the window of implantation in healthy fertile women. Fertil Steril 2000;73:788–98.
2. Kliman HJ, Feinberg RF, Schwartz LB, Feinman MA, Lavi E, Meaddough EL. A mucin-like glycoprotein identified by MAG (mouse ascites Golgi) antibodies. Menstrual cycle-dependent localization in human endometrium. Am J Pathol 1995;146:166–81.
3. Lindenberg S. Ultrastructure in human implantation: transmission and scanning electron microscopy. Baillieres Clin Obstet Gynaecol 1991; 5:1–14.
4. Lindenberg S, Sundberg K, Kimber SJ, Lundblad A. The milk oligosaccharide, lacto-N-fucopentaose I, inhibits attachment of mouse blastocysts on endometrial monolayers. J Reprod Fert 1988;83:149–58.
5. Lindhard A, Bentin-Ley U, Ravn V, Islin H, Hviid T, Rex S, et al. Biochemical evaluation of endometrial function at the time of implantation. Fertil Steril 2002;78:221–33.
6. Aplin JD. MUC-1 glycosylation in endometrium: possible roles of the apical glycocalyx at implantation. Hum Reprod 1999;14:17–25.
7. Veeck L, editor. Atlas of the human oocyte and early conceptus. Baltimore: Williams & Wilkins, 1991.
8. Hendrickson MR, Kempson RL. Surgical pathology of the uterine corpus. Major Probl Pathol 1980;12:36–98.
9. Barmat LI, Liu HC, Spandorfer SD, Xu K, Veeck L, Damario MA, et al. Human preembryo development on autologous endometrial coculture versus conventional medium. Fertil Steril 1998;70:1109–13.
10. Palermo GD, Cohen J, Alikani M, Adler A, Rosenwaks Z. Intracytoplasmic sperm injection: a novel treatment for all forms of male factor infertility. Fertil Steril 1995;63:1231–40.
11. Barmat LI, Liu HC, Spandorfer SD, Kowalik A, Mele C, Xu K, et al. Autologous endometrial co-culture in patients with repeated failures of implantation after in vitro fertilization-embryo transfer. J Assist Reprod Genet 1999;16:121–7.
12. Cohen J, Alikani M, Trowbridge J, Rosenwaks Z. Implantation enhancement by selective assisted hatching using zona drilling of human embryos with poor prognosis. Hum Reprod 1992;7:685–91.
13. Kliman HJ, Keefe DL. MAG mucin expression predicts pregnancy outcome in donor egg patients and when absent may identify those patients who can benefit from high dose luteal phase progesterone [abstract no. O-063]. In: Abstracts of the Scientific Oral and Poster Sessions of the 51st Annual Meeting of the American Society for Reproductive Medicine; 1995; Seattle, WA, October 9–11, 1995.
14. Dubowy RL, Feinberg RF, Keefe DL, Doncel GF, Williams SC, McSweet JC, et al. Improved endometrial assessment using cyclin E and p27. Fertil Steril 2003;80:146–56.