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ORIGINAL ARTICLE

Unexplained recurrent pregnancy loss and unexplained infertility: twins in disguise

Chelsea W. Fox[1], Ricardo F. Savaris[3], Jae-Wook Jeong[4], Tae Hoon Kim[4], Paul B. Miller[2], Creighton E. Likes[2], David P. Schammel[5], Steven L. Young[6], and Bruce A. Lessey[7,*]

[1]University of San Diego, Department of Obstetrics and Gynecology, San Diego, CA, USA [2]Obstetrics and Gynecology, Greenville Health System, Greenville, SC 29605, USA [3]Departamento de Ginecologia e Obstetrícia, Universidade Federal do Rio Grande do Sul, Porto Alegre 90035-903, Brazil [4]Obstetrics, Gynecology and Reproductive Biology of Michigan State University, Grand Rapids, MI 49503, USA. [5]Pathology Associates, Greenville Health System, Greenville, SC, USA [6]Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA [7]Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Wake Forest Health, Winston-Salem, NC 27157, USA

*Correspondence address. Wake Forest School of Medicine, 1 Medical Center Blvd, Winston-Salem, NC 27157, USA.
Tel: +1-336-716-6476; E-mail: blessey@wakehealth.edu https://orcid.org/0000-0002-8451-2817

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STUDY QUESTION: Is B-cell CLL/lymphoma 6 (BCL6) endometrial expression, a surrogate biomarker of endometriosis, elevated in women with unexplained recurrent pregnancy loss (uRPL) and unexplained infertility (UI) compared to fertile subjects?

SUMMARY ANSWER: Endometrial BCL6 expression is elevated to a similar degree in women with uRPL and UI compared to fertile controls.

WHAT IS KNOWN ALREADY: Endometriosis has been linked to the genesis of endometrial progesterone resistance and to specific nuclear proteins, including endometrial BCL6. BCL6 overexpression (immune histologic score > 1.4) has been strongly associated with poor reproductive outcomes in IVF cycles in women with UI. Our previous data have demonstrated an accuracy of 94% for diagnosing endometriosis, and BCL6 protein is elevated in the decidua of women with uRPL.

STUDY DESIGN, SIZE, DURATION: In this case-control study, at a tertiary university teaching hospital, 110 samples (control $n = 28$; uRPL $n = 29$; UI $n = 53$) from pathological archives were analyzed. Timed endometrial biopsies were obtained between 2 January 2002 and 31 December 2016.

PARTICIPANTS/MATERIALS, SETTING, METHOD: LH-timed endometrial biopsies were obtained from women with UI, uRPL (two or more consecutive losses) and normal fertile subjects during the mid-secretory phase of the menstrual cycle. Endometrial BCL6 protein levels were compared in women with UI and uRPL and fertile controls using western blot analysis and immunohistochemistry (HSCORE).

MAIN RESULTS AND THE ROLE OF CHANCE: The mean age of the uRPL group was significantly higher than the others [mean (SD)] control = 32.7 (2.6); uRPL = 35.8 (3.7); UI = 32.7 (4.4); $P = 0.002$, ANOVA]. Seventy-nine percent of women in both subfertile groups (uRPL and UI, 65 out of 82) displayed elevated BCL6 protein levels. From these, a subset of cases with abnormal BCL6 went to laparoscopy and endometriosis was found in 9 out of 11 cases of uRPL and in 20 out of 21 cases of UI. Median BCL6 HSCORE for controls versus uRPL and UI was significantly different [median (interquartile); control = 0.3 (0.02 to 0.5); uRPL = 3 (1.9 to 3.6); UI = 2.9 (1.6 to 3.1); $P < 0.0001$, Kruskal-Wallis]. A significant trend in the association between the degree of infertility (fertile, uRPL and UI) and the HSCORE level (negative, medium and high) was found ($P < 0.001$; x^2 for trend). Western blot of representative samples from each group demonstrated similar findings based on protein levels in the whole endometrium. After running ANCOVA analysis for age difference, the BCL6 difference among groups was still significant (P-value < 0.0001).

LIMITATIONS, REASONS FOR CAUTION: We studied subjects with two consecutive pregnancy losses rather than the definition adopted in Europe of three losses. The findings may lack external validity in other clinical settings (e.g. low prevalence of endometriosis).

WIDER IMPLICATIONS OF THE FINDINGS: Based on the data presented here, we postulate that the degree of BCL6 expression may represent a continuum of progesterone resistance and response to inflammation that occurs in women with endometriosis, yielding different degrees of infertility, from uRPL to UI.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported by NICHD/NIH R01 HD067721 (SLY and BAL), by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior: Grant 99999.003035/2015–08 (BAL) and by CAPES/PROAP (RFS). Two authors (BAL, SLY) have licensed intellectual property for the detection of endometriosis. Dr Bruce Lessey is an unpaid scientific Advisor for CiceroDx. The other authors report no conflict of interest.

Key words: pregnancy loss / endometriosis / infertility / endometrial biopsy / unexplained

WHAT DOES THIS MEAN FOR PATIENTS?

Women who have multiple miscarriages may have something in common with those who are unable to conceive but have no abnormalities on routine testing. A protein called BCL6 has been found in samples from the lining of the womb (the endometrium) from women with endometriosis (a painful condition in which this tissue grows outside the uterus) as well as from women who are infertile but in whom all the common fertility tests are normal.

We compared levels of BCL6 in samples of endometrium from women with repeated miscarriage, those whose infertility is unexplained and fertile women: we found that BCL6 levels were higher in the infertile and pregnancy loss groups.

We feel the same pathway may be contributing to recurrent pregnancy loss as well as UI. Women with unexplained pregnancy loss have few treatment options at present, but this research may open new avenues for both treatment and future investigation into the mechanisms involved in unexplained pregnancy loss.

Introduction

Compromised fertility is a common problem among couples. In 2010, it was estimated that 48.5 million couples worldwide were unable to have a child after 5 years (Mascarenhas *et al.*, 2012). Different causes of reduced fecundity include a male factor, tubal disease, ovulatory dysfunction and endometriosis. Within the category of sub-fertility, unexplained infertility (UI) and unexplained recurrent pregnancy loss (uRPL) are recognized subsets (Practice Committee of American Society for Reproductive Medicine, 2013a). The proportion of unexplained fertility to all infertility patients is similar to the proportion of women with recurrent pregnancy losses that are unexplained. Although UI overall is more common (15–30%) than uRPL (1–3%), both diagnoses are devastating to the couples who encounter these problems (Practice Committee of American Society for Reproductive Medicine, 2013b; Gelbaya *et al.*, 2014). Based on the characteristics of these two entities, some authors suggest they may be causally related (Bristow *et al.*, 2014; Giuliani *et al.*, 2014; Patel *et al.*, 2015; Campitiello *et al.*, 2016).

The definition of RPL varies but is defined as either two (Practice Committee of American Society for Reproductive Medicine, 2013a) or three consecutive losses (National Collaborating Centre for Women's and Children's Health (UK), 2013). Known causes of RPL include chromosomal or other genetic abnormalities (Daya and Stephenson, 1996), endocrine abnormalities such as poorly controlled diabetes (Mills *et al.*, 1988), thyroid disease (Abalovich *et al.*, 2002), acquired or structural uterine anomalies (such as a uterine septum), thrombophilia, immunological and psychological causes (Rai and Regan, 2006). However, in ~50% of cases, the cause remains unknown (Practice Committee of American Society for Reproductive Medicine, 2013a). While attention has been focused on defects in oocyte, sperm, embryo and on systemic or local factors, the role of endometrial receptivity defects in uRPL has not been as well studied, and treatment strategies are often not addressed (Kutteh, 2014; Saravelos and Regan, 2014). While some find little evidence that endometriosis is associated with RPL (Vercammen and D'Hooghe, 2000), more recent studies support an association (Kohl Schwartz *et al.*, 2017; Minebois *et al.*, 2017; Zullo *et al.*, 2017). Once diagnosed with endometriosis, most women receive treatment for the disease, while a larger reservoir of undiagnosed endometriosis likely continues to affect pregnancy outcomes in those with UI or pregnancy loss. For this reason, a biological biomarker of endometriosis prior to laparoscopic confirmation would be particularly useful.

Endometriosis is an immune-regulated, inflammatory condition known to alter endometrial function (Kao *et al.*, 2003; Bohler *et al.*, 2007; Lessey and Kim, 2017) with specific changes in endometrial gene expression (Mathyk *et al.*, 2017). Endometriosis has been linked to the genesis of endometrial progesterone resistance (Bulun *et al.*, 2006; Burney *et al.*, 2007; Aghajanova *et al.*, 2010; Joshi *et al.*, 2017) and to specific nuclear proteins, including overexpression of endometrial B-cell CLL/lymphoma 6 (BCL6). By using immunohistochemistry (IHC) analysis, BCL6 overexpression (histologic score, >1.4) has been strongly associated with poor reproductive outcomes in IVF cycles in women with UI (Almquist *et al.*, 2017) and it has an accuracy of 94% for diagnosing endometriosis (Evans-Hoeker *et al.*, 2016). BCL6 was also reported to be elevated in the decidua of women with uRPL (Gong *et al.*, 2016). BCL6, with the assistance of the histone deacetylase sirtuin 1, which is activated by the oncogene KRAS, is thought to epigenetically target genes involved in progesterone signaling, leading to progesterone resistance (Yoo *et al.*, 2017).

Based on this mounting evidence, we wanted to determine whether uRPL may exhibit defects in endometrial receptivity similar to those observed in UI. To answer this question, we analyzed the expression of endometrial BCL6, as a surrogate biomarker of endometriosis, in women with uRPL compared to UI and normal fertile women.

Materials and Methods

Study design and settings

This is a case-control study performed at Greenville Health System (GHS), Greenville, SC, USA, between 18 April 2008 and 31 December 2016 using 110 samples (control = 28; uRPL = 29; UI = 53) from the pathology archives.

Analysis of individual samples was performed at GHS and Michigan State University, East Lansing, MI, USA. Samples for this study were obtained using Institute Review Board (IRB)-approved protocols and included women previously diagnosed with UI and elevated endometrial BCL6. Likewise, the controls were taken from a previous archival set of normal fertile women without signs or symptoms of endometriosis prospectively obtained at the University of North Carolina, NC, USA (Murray *et al.*, 2002) and GHS, and they were selected for this current analysis. The IRBs at the University of North Carolina and GHS approved all protocols for collection and use of human samples (Pro00013885, Pro00000006). Written informed consent was obtained from each subject prior to enrollment. All subjects had a single mid-luteal endometrial biopsy performed between 6 and 10 days after detection of a urinary LH surge. Other normal controls were also prospectively recruited for the acquisition of tissue for western blot analysis at the University of North Carolina, Chapel Hill.

Participants

Three different populations were examined in this study: normal fertile women without suspected endometriosis (controls); women with UI and women with uRPL.

Endometrial tissue biopsies

BCL6 protein was assessed in human endometrium either from frozen sections stored at −80°C (western blot) or from parallel formalin-fixed paraffin-embedded tissue (IHC). Endometrium from normal fertile women without suspected endometriosis was obtained using a pipelle device (Cooper Surgical, Trumbull, CT, USA) during the window of implantation (WOI; cycle day LH + 6 to LH + 10), determined by urinary LH surge monitoring (Ovuquick® ; Quidel, San Diego, CA, USA) in paid volunteers. These control subjects were between 19 and 34 years old, healthy, with proven fertility and with a normal menstrual cycle (21 and 35 days). Those with a BMI $\geq$ 30 kg/m^2, who took medications known to affect reproductive hormones during the previous 3 months, and those who had known anatomic or functional reproductive tract abnormalities were excluded. Samples from controls were included only if endometrial dating agreed with the cycle day of the biopsy (±2 days).

Mid-secretory phase endometrial samples were prospectively obtained in Greenville, SC, USA at the Fertility Center of the Carolinas, as part of their evaluation for either UI or uRPL. UI was considered when women had normal ovulatory menstrual cycles (25−35 days), had at least one patent fallopian tube, and their partners had normal semen analysis. Exclusion criteria included age > 40 years, known tubal disease or polycystic ovary syndrome (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), the presence of significant fibroid tumors (>4 cm) or adenomyosis suspected on ultrasound. Subjects with uRPL were also <40 years of age. For the purposes of this study, uRPL was defined as two or more unexplained consecutive first trimester losses. We used a standardized workup for uRPL that included sequential evaluation of endocrine (thyroid-stimulating factor, prolactin, testosterone, dehydroepiandrosterone sulphate, day 3 FSH and estradiol) and structural disorders (hysterosalpingogram and/or sonohysterogram). Immunologic assessment consisted of lupus anticoagulant and anticardiolipin testing. While parental karyotype testing was recommended for some couples with uRPL, it was not performed in all patients due to cost-effectiveness evidences, since aneuploidy is an unlikely cause of primary uRPL in a group of women with a mean age of 35 years old (Demko *et al.*, 2016). As thrombophilia is not commonly associated with first trimester losses (Krabbendam *et al.*, 2005), this was not a requirement for the workup of uRPL.

Patients were excluded if they had known genetic mutations, including balanced translocation, or immunological abnormalities such as Sjörgens syndrome, thrombophilias, positive anti-phospholipids or significant uterine anomalies including septate uterus or fibroids.

BCL6 protein expression levels were compared in all subjects with UI and uRPL and compared to fertile controls.

Protein isolation and western blot

Western blot analyses were performed as described previously (Kim *et al.*, 2014). Briefly, endometrial tissues were lysed with lysis buffer (150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 2.5 mM EDTA, 0.125% Nonidet P-40 (vol/vol) with both a protease inhibitor cocktail (Roche, Indianapolis, IN, USA) and a phosphatase inhibitor cocktail (Sigma Aldrich, St. Louis, MO, USA). Proteins (20 ug per lane) were separated by electrophoresis using SDS-PAGE and transferred onto polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA, USA). Casein (0.5% v/v) was used to block the membrane prior to exposure to antibodies against BCL6 (561 520; BD Pharmingen, San Jose, CA, USA) and β-actin (used as a loading control) (sc1616; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunoreactivity was visualized by incubation with a horse-radish peroxidase (HRP)-linked secondary antibody [PI-2000 (anti-mouse for BCL6) and PI-9500 (anti-goat for β-actin), Vector Laboratories, Burlingame, CA, USA] followed by exposure to enhanced chemiluminescence reagents, according to manufacturer's instructions (GE Healthcare Biosciences, Piscataway, NJ, USA).

IHC

Formalin-fixed, paraffin-embedded tissue blocks were sectioned at 4 µm. Slides were stained with hematoxylin-eosin and consecutive sections stained with ready-to-use antibodies against BCL6 (clone LN22, Leica Biosystems, Buffalo Grove, IL, USA). IHC was performed on an automated system by a certified Pathology Laboratory at GHS (Pathology Associates, Greenville Health System, Greenville SC) using the Bond immunostainer platform (Leica Biosystems). Following exposure to the HRP-conjugated streptavidin substrate, positive immunoreactivity (red precipitate) was detected using the Vectastain Elite DAB kit (Vector Laboratories, Burlingame, CA, USA). Negative control sections included proliferative phase endometrium from normal fertile individuals, and positive external controls included lymph node sections.

Bias

The use of an automated system for immunostaining reduced a potential operator bias in the immunohistochemical analysis. HSCOREs were read in a blinded fashion by an experienced pathologist (DPS).

Study size

At least 11 patients per group are required to have a 90% chance of detecting, as significant at the 2.5% level, an increase in HSCORE for BCL6 from 0.43 in the control group to 2.44 in the infertile group.

Table I Demographics of fertile controls, and women with uRPL or UI.

Characteristic	Controls ($n = 28$)	uRPL ($n = 29$)	UI ($n = 53$)	*P*-value
Age, years -	32.7 (2.6)	35.8 (3.7)	32.7 (4.4)	0.002[a]
BMI, kg/m^2	25.6 (4.7)	25.5 (7.3)	24.9 (5.7)	0.8
Gravidity	1.9 (0.7)	4.1 (2.4)	0.5 (0.8)	<0.0001
Parity	1.6 (0.5)	0.5 (0.5)	0.2 (0.5)	<0.0001

[a]ANOVA, comparison uRPL versus controls $P = 0.01$; uRPL versus UI $P = 0.002$.
Data are mean (SD).
UI: unexplained infertility, uRPL: unexplained recurrent pregnancy loss.

Quantitative variables

The semi-quantitative assessment of protein staining was made using the HSCORE (0—4), calculated using the following equation: HSCORE = $\sum$ Pi $(i + 1)/100$, where i = intensity of staining with a value of 1, 2 or 3 (weak, moderate or strong, respectively), and Pi is the percentage of stained epithelial cells for each intensity, in the range 0–100%. The use of HSCORE has previously been validated as a semi-quantitative assay for immunohistochemical staining (Budwit-Novotny *et al.*, 1986). Negative, medium and higher staining were defined as <1.4, ≥ 1.4 to <2.7 and ≥ 2.7, respectively, based on our previous report (Evans-Hoeker *et al.*, 2016).

Statistical methods

Statistical analysis was performed using GraphPad Prism version 8 for Macintosh (GraphPad Software, Inc, San Diego, CA, USA). Gaussian distribution was verified using the D'Agostino and Pearson omnibus normality test. ANOVA test was used to compare means among groups. If normality of data was not confirmed, the Kruskal-Wallis nonparametric tests were used to compare medians. Chi-square for trend was used for comparing the degree of infertility with staining level (fertile, uRPL and UI versus negative, medium and high levels of BCL6). ANCOVA was used for adjusting HSCOREs between groups since age differed significantly between control and uRPL groups. ANCOVA online calculator (http://vassarstats.net/ancova2L.html) was used for statistical analysis. Parity and gestations were not considered as confounders because they were part of the definition of the groups.

Results

Participants and descriptive data

Demographic data of the studied populations are depicted in Table I. The uRPL group was older than the others ($P = 0.002$). As expected, gravidity was higher in the uRPL group and lowest in the UI group ($P < 0.0001$). Table II depicts the evidence of endometriosis in participants that had a positive BCL6 result and underwent laparoscopy.

IHC and western blot analysis

Examples of low and high IHC levels of expression of BCL6 are shown in Fig. 1A and B, respectively. Levels of BCL6 were determined by western blot (Fig. 1C) and IHC (Fig. 1D). BCL6 was overexpressed in both UI and uRPL samples compared to historical fertile controls ($P < 0.0001$). The percentage of abnormal (high) BCL6 immunostaining was 79% in both sub-fertile groups, but only 7% in the fertile control group based on the HSCORE cut-off for BCL6, of ≤ 1.4 being normal. There was a significant trend in the association between the degree of infertility (fertile, uRPL and UI) and the HSCORE level (negative, medium and high), as shown in Table II.

Other analyses

We utilized ANCOVA to determine if the age differences noted between group (uRPL versus control) had an influence on BCL6 expression. The result of Levene's test of equality of error variances was 0.4 and 0.3 for uRPL and control groups, respectively. After running ANCOVA analysis, age was adjusted, and the *P*-value between groups was <0.0001 in both analyses, confirming the significant difference in BCL6 levels, despite the age difference between groups.

Discussion

The inflammasome has been implicated as a new direction for study of pregnancy loss and defects in endometrial receptivity (D'Ippolito *et al.*, 2016). Inflammation has been linked to RPL in both human and animal models (Erpenbeck *et al.*, 2016; Kushnir *et al.*, 2016; Alijotas-Reig *et al.*, 2017) and in women with UI (Jasper *et al.*, 2006; Dimitriadis *et al.*, 2007). BCL6 is associated with inflammation as reported in other tissues (Arima *et al.*, 2008; Seto *et al.*, 2011).

Progesterone resistance is the *sine quo non* in endometriosis (Joshi *et al.*, 2017; Mathyk *et al.*, 2017). BCL6 is a biomarker associated with progesterone resistance, mediated by inflammation (Yoo et al., 2017). BCL6 is expressed at low levels in the endometrium of normal fertile women (Burney *et al.*, 2007) and dramatically overexpressed in women with of all stages of endometriosis (Evans-Hoeker *et al.*, 2016) and unexplained IVF failure (Almquist *et al.*, 2017). BCL6 was also recently shown to be overexpressed in the decidua of women with RPL (Gong *et al.*, 2016). Here, we report that mid-luteal epithelial BCL6 is markedly elevated in women with both uRPL and UI.

Endometrial histology is more likely to be delayed in uRPL compared to normal controls (Tuckerman *et al.*, 2004), and inflammatory or immune changes associated with progesterone resistance have been implicated by others in the phenomenon of pregnancy loss (Kwak-Kim *et al.*, 2009). In the landmark study by Wilcox *et al.* (1999), it was

Table II BCL6 staining in endometrium samples from controls and women with uRPL or UI.

Characteristics	Control ($n = 28$)	uRPL ($n = 29$)	UI ($n = 53$)	*P*-value*
BCL6 < 1.4 (negative)	26 (92)	6 (20.7)	11 (20.8)	
BCL6 ≥ 1.4 to 2.7 (medium)	1 (4)	4 (13.8)	13 (24.5)	<0.001
BCL6 ≥ 2.7 (high)	1 (4)	19 (65.5)	29 (54.7)	
**Subset who were positive for endometriosis at laparoscopy		9 out of 11 (81)	20 out of 21 (95)	
Stage of endometriosis				
I		2 (22.2)	7 (35)	
II		4 (44.4)	8 (40)	0.4
III		3 (33.3)	5 (25)	

*Chi-square for trend. Data are *n* (%).
**A subset of patients with positive staining for B-cell CLL/lymphoma 6 (BCL6: >1.4 HSCORE) and with uRPL and UI underwent diagnostic laparoscopy.

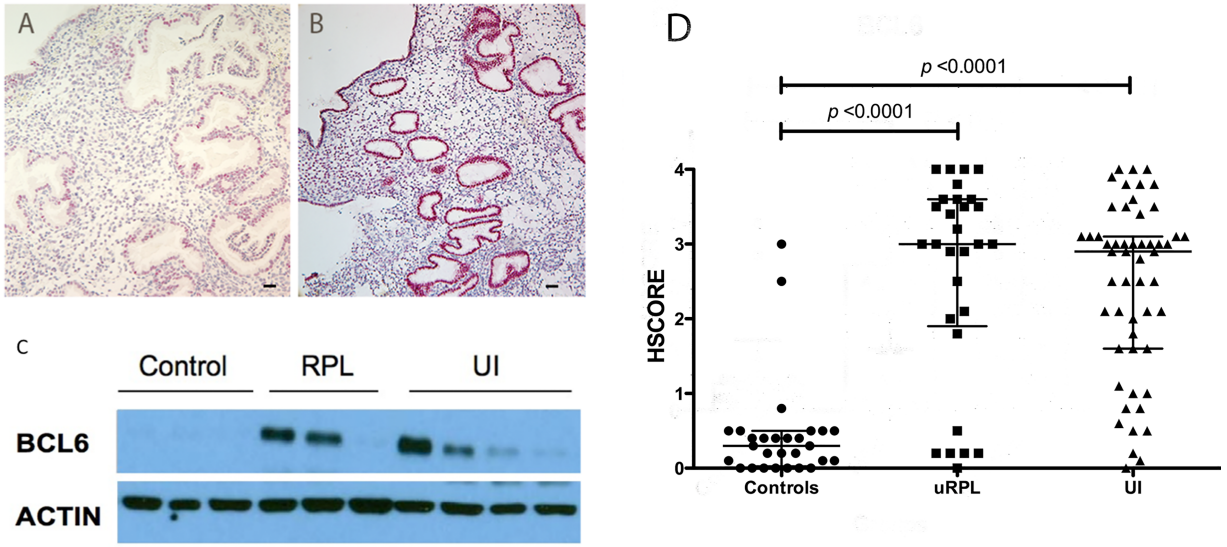

Figure 1 Endometrial BCL6 is overexpressed in uRPL and UI. Examples of immunostaining (see Methods) in eutopic endometrium from women with low (**A**) and high (**B**) B-cell CLL/lymphoma 6 (BCL6). Scale bars +50 μM. (**C**) A representative western blot for BCL6 in normal controls, uRPL and UI samples. The positive control was beta-actin. The western blot independently corroborates the HSCORE technique and was not quantified. (**D**) Distribution of HSCOREs among fertile women (controls, $n = 28$) and women with uRPL ($n = 29$) or UI ($n = 53$). Horizontal bars represent median with interquartile range. Kruskal-Wallis with Dunn's post-hoc test was performed for statistical analysis.

shown that the degree of delay in implantation was associated with an ever-greater miscarriage rate in women trying to conceive. As progesterone is essential for pregnancy, one response to progesterone resistance could be a shift or narrowing in the WOI, which logically might vary between individuals. Based on the data presented here, we postulate that a continuum of progesterone resistance and inflammation occurs in women with endometriosis and that individual differences in response might account for the women experiencing infertility versus recurrent loss. A greater inflammatory response might mediate a greater progesterone resistance leading to a greater degree of implantation failure. We hypothesized (Fig. 2) that a shorter and less delayed WOI might result in pregnancy loss, while a greater shift or narrowing could cause infertility. Such a paradigm could also account for the progression that is sometimes observed over time, between pregnancy loss and infertility, as well as the ability to rescue uRPL patients from loss by providing timely luteal hCG administration (Fox *et al.*, 2017).

The high prevalence of BCL6 overexpression may be considered a limitation of the study. We previously reported that BCL6 expression has an accuracy of 94% for diagnosing endometriosis, using a cut-off of 1.4 in HSCORE analysis (Evans-Hoeker *et al.*, 2016). Further studies are needed to determine if there are other causes of elevated BCL6 in women with infertility. A second concern may be that we included subjects with two consecutive pregnancy losses rather than using the definition adopted in Europe of three losses. One could argue that higher embryonic aneuploidy rates might be seen in the uRPL group,

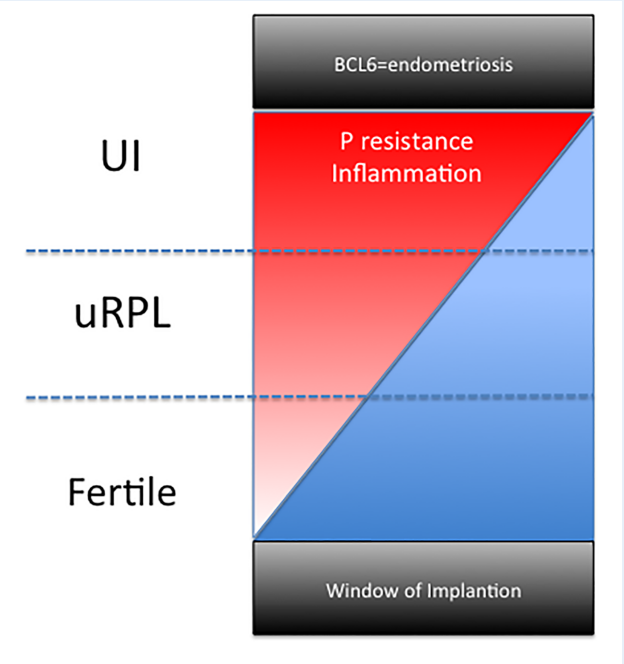

Figure 2 A proposed relationship between the WOI and the continuum of subfertility related to progesterone resistance. Normal fertile women have normal (low) expression of BCL6 and are unlikely to have endometriosis. The WOI is properly opened in a timely fashion (20–24 days after ovulation). Women with aberrant expression of BCL6 often have endometriosis as the cause of their subfertility. However, the degree of the inflammatory process and progesterone (P) resistance likely varies by individual. The greater inflammatory response and P resistance the greater the degree of implantation failure, shifting and narrowing the WOI to later in the menstrual cycle. As the shift and brevity in the WOI progresses, the degree of infertility and implantation failure also increases in severity.

since they were older. However, at a mean age of 35 years, aneuploidy is unlikely to be a primary cause of recurrent loss (Demko *et al.*, 2016).

The question of endometrium versus embryo has previously been addressed by Prapas *et al.* (2012), in the context of endometriosis. They prospectively compared a population of menopausal recipients with and without endometriosis sharing sibling oocytes coming from the same donor. Those with endometriosis were significantly younger (mean 37.28 years) compared to those without endometriosis (45.42 years). Despite this age difference, they found that pregnancy and implantation rates were significantly lower in the younger endometriosis group compared to the older control group (45.00 versus 58.33%, $P = 0.039$) and (23.81 versus 31.48%, $P = 0.019$), respectively. We recently reported that women who conceived during IVF with higher endometrial BCL6 levels had a high miscarriage rate that corrected with medical or surgical treatment of endometriosis (Likes et al., 2019a, 2019b). Likewise, thrombophilia is not commonly associated with first trimester losses (Krabbendam *et al.*, 2005), and this was not a requirement for the workup of uRPL. We used established fertile controls without symptoms of endometriosis: these women were randomly assigned endometrial biopsy on various days of the WOI and were part of a larger study, published previously (Murray *et al.*, 2002). While we assumed that our fertile controls would have a background endometriosis rate of 5–10%, we did find that two out of 28 (7%) tested positive for BCL6.

There are several strengths of this study. The IHC results were read by an experienced blinded observer without knowledge of the status of the subject. The number of subjects in our study was large enough to allow for a post-hoc power analysis, which revealed our trial had the ability to detect a true difference among groups (100%), according to a calculation described in the literature (Levine and Ensom, 2001). Laparoscopy was performed that showed endometriosis was common in both the UI and uRPL groups.

In conclusion, we demonstrate that uRPL and UI are similar in terms of elevated BCL6 protein levels and therefore may be related in terms of the expected finding of endometriosis in a considerable percentage of women, i.e. 90% (95%CI = 75–96%). Endometriosis may cause uRPL, similar to what has been shown for UI. Thus, it is plausible to consider a continuum of endometrial receptivity defects associated with endometriosis that may contribute to both types of subfertility.

Further studies are needed to confirm our findings and to evaluate whether treatment of endometriosis can improve outcomes in both UI and uRPL. Why some women with endometriosis are infertile while others can conceive remains a question to be answered. Perhaps the degree of inflammation, or individual inflammatory response to endometriosis, could provide answers to that question.

Authors' roles

Biopsy and recruitment of subjects was performed by Drs Miller, Likes and Lessey. Data collection was performed by Drs Lessey and Fox. Study design and execution was performed by Drs Lessey, Young and Fox. Immunostaining and reading of slides was performed by Drs Lessey nad Schammel. Jae-Wook Jeong and Tae Hoon Kim performed western blot and critical discussion. Data analysis was performed by Drs Fox, Savaris, Lessey and manuscript drafting and critical discussion performed by all of the authors.

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Conflict of interest

Two authors (BAL, SLY) have licensed intellectual property for the detection of endometriosis. Dr Bruce Lessey is an unpaid scientific Advisor for CiceroDx. The other authors report no conflict of interest.

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