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Short communication

High Body Mass Index is associated with an expansion of endometrial T Regulatory cell and macrophage populations

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ABSTRACT

Obesity is associated with implantation failure, miscarriage, low-grade inflammation and a reduction in peripheral immune-suppressive T regulatory (Treg) cells. Therefore, we postulated that this reduction may also extend to the endometrium and cause embryonic loss. In a cohort of 40 infertile women, without implantation failure or recurrent miscarriage, we examined the density of Treg cells, macrophages and natural killer (NK) cells in mid-luteal endometrial biopsies. Significant positive correlations were observed between BMI and endometrial Treg cells and macrophages, but no relationship with NK cells. We postulate that this change may be a positive adaption to minimise adiposity related inflammation.

1. Introduction

Obesity is an increasing public health issue associated with impaired implantation and a significant increase in miscarriage risk. The increased rate of loss of obese women's genetically normal (euploid) pregnancies strongly points to an adverse uterine milieu as the underlying cause (Tremellen et al., 2017). Although it is far from clear what causes this impairment, obese women are known to have chronic low-grade systemic inflammation (Kern et al., 2001), with some studies suggesting that this inflammation spills over into the endometrium (Oróstica et al., 2016).

A deficient immune-permissive (tolerant) endometrial response to the implanting embryo may potentially cause pregnancy failure. Treg cells, a key subset of CD4 + T cells whose function is to supress immune responses and maintain self-tolerance, have been proposed as playing a key role in supporting the establishment of pregnancy (Guerin et al., 2009). Peripheral blood Treg cell numbers increase early in viable human and murine pregnancies, while experimental depletion of Treg cells is associated with pregnancy loss in murine models (Darrasse-Jèze et al., 2006; Shima et al., 2010). Furthermore, recurrent IVF implantation failure and miscarriage has been correlated with a reduction in both peripheral blood (Lashley et al., 2015) and endometrial Treg cell activity (Jasper et al., 2006; Galgani et al., 2015). Since obesity has also been linked with a reduction in peripheral blood Treg cells (Wagner et al., 2013), we proposed that this deficiency in Treg cells may also extend to the endometrium and cause reproductive failure.

The aim of this study was to determine whether maternal BMI has any impact on endometrial peri-implantation (mid-luteal) leukocyte populations, with a primary focus on the impact of obesity on endometrial Treg cell density.

2. Materials and methods

2.1. Study design and participants

Between January 2015 and June 2016, a total of 112 women attending a private IVF clinic (Repromed, Adelaide, Australia) underwent a mid-luteal (7 ± 1 day post ovulation) endometrial biopsy precisely timed by serum LH/progesterone monitoring. Most patients underwent this biopsy procedure for investigation of recurrent implantation failure (three or more failed embryo transfers) or recurrent miscarriage. However, a sub-group of 40 patients underwent this biopsy as an "endometrial scratch", a procedure suggested to assist implantation of embryos in a subsequent transfer cycle (Nastri et al., 2015). This "control" sub-group of 40 patients was chosen to investigate the impact of BMI on endometrial immune cell numbers. The controls had an average of 1.4 ± 0.8 embryos transferred in the past, with 17.5% of them never having undergone a prior embryo transfer. Patients with

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recurrent implantation failure, recurrent miscarriage or any uterine pathology likely to alter endometrial function (polyp, sub-mucosal fibroids, adenomyosis or hydrosalpinx) were excluded from the final study. Patients undergoing programmed artificial hormone replacement cycles or on immune suppressive therapy were also excluded. Finally, if the endometrial biopsy showed evidence of pathology (polyps or plasma cell infiltrate suggestive of endometritis), or histological dating was three or more days out of synchrony with hormone timed ovulation, the subject was excluded from the final analysis.

This study was assessed and approved by the local institutional human research review committee. All subjects had previously given their written informed consent for accessing their clinical notes for this type of low-risk retrospective audit.

2.2. Endometrial biopsy sampling and processing

All endometrial biopsies were performed using a flexible endometrial curette, then fixed in 10% neutral buffered formalin and processed into paraffin by a commercial pathology laboratory (Douglass Hanly Moir Pathology, Sydney, Australia) using previously published protocols (Russell et al., 2011). Briefly, 4 μm endometrial tissue sections were stained with hematoxylin and eosin (H & E), with immunostaining performed on serial sections from the same block and stained in an auto-immunostainer (Ventana OptiView) using ultraview® DAB detection kit (Ventana Medical Systems). All staining batches included appropriate controls and employed commercially available monoclonal antibodies for the detection of Treg cells (FOXP3; Catalog no. AB20034; Abcam, Cambridge) (Fig. 1), and macrophages (CD163; Novocastra; Leica, Germany) plus NK cells (CD56; Novocastra; Leica, Germany) as previously published by our group (Russell et al., 2011). The density of immune cells was calculated from 5 high power (×400) fields of endometrial stromal tissue.

3. Results and discussion

3.1. Clinical characteristics

The median BMI of the 40 participants was 24.05 kg/m^2, with a range between 19 and 41.6 kg/m^2, with 17.5% of subjects being obese. Their average age was 34.9 ± 4.6 years, with male factor (27.5%) and advanced maternal age (22.5%) being the two most common infertility diagnoses. Eleven of the cohort (27.5%) had at least one previous live-born child.

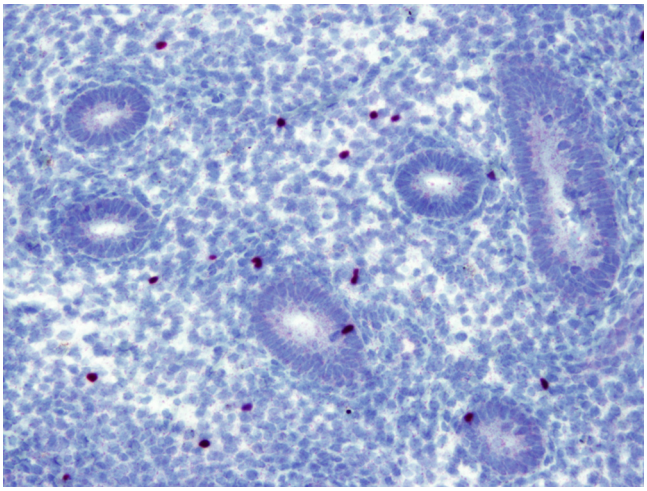

Fig. 1. Foxp3 immunohistochemistry identification of scattered T Reg cells in a secretory endometrial biopsy sample. H and E section, 200×.

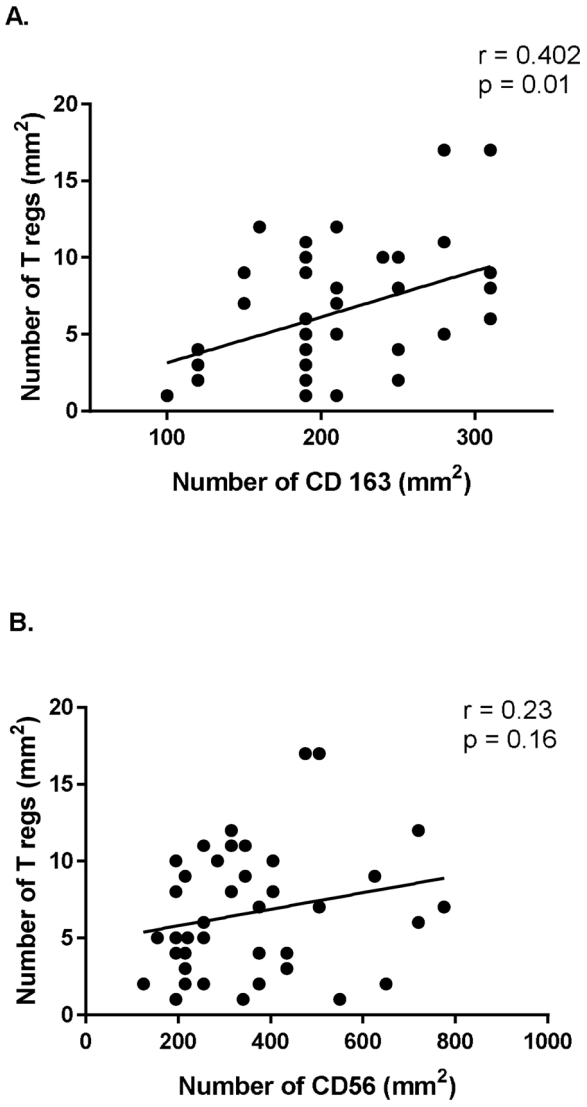

Fig. 2. Relationship between endometrial immune cell populations. T reg cell and macrophage (CD163) density (A), T reg cells and NK (CD56) density (B). Statistical analysis is expressed as Pearson correlation coefficient and p value.

3.2. Endometrial histology and leukocyte populations

Good correlations were observed between hormonal based ovulation timing and endometrial luteal secretory change development using traditional Noyes based criteria (Russell et al., 2011), with the average difference between the two being only 0.8 ± 1 days and unrelated to women's BMI status.

Treg cell density was normally distributed, with an average (± SD) of 6.6 ± 4.1 cells per mm^2 observed within the endometrial stromal tissue. In contrast, macrophage and NK cell densities were not normally distributed, with a median (IQR) density of 210 per mm^2 (190–250) and 315 per mm^2 (215–435) respectively. A significant positive correlation was observed between endometrial Treg cell and macrophage density, but nonewith NK cell density (Fig. 2). The CD163 macrophage marker used in this study is known to be associated with the M2 "alternative" macrophage immune-phenotype, with M2 macrophages being reported to produce cytokines (IL-4, IL-10 and IL-13) supportive of a tolerant immune response (Tiemessen, 2007). Interestingly, Treg cells are reported to induce an M2 phenotype in naïve monocytes/macrophages (Tiemessen, 2007). As such, given the observed positive correlation between Treg cells and macrophage density it is possible that endometrial Treg cells may play a positive role in the expansion of