

# High stretch cycling inhibits the morphological and biological decidual process in human endometrial stromal cells

Ryohei Saito<sup>1</sup> | Takeshi Kajihara<sup>1</sup>  | Masashi Takamura<sup>1</sup> | Hideno Tochigi<sup>1</sup> | Tsuyoshi Sato<sup>2</sup> | Osamu Ishihara<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Saitama Medical University, Iruma-gun, Japan

<sup>2</sup>Department of Oral and Maxillofacial Surgery, Saitama Medical University, Iruma-gun, Japan

## Correspondence

Takeshi Kajihara, Department of Obstetrics and Gynecology, Saitama Medical University, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-0495, Japan.  
Email: kajihara@saitama-med.ac.jp

## Funding information

Saitama Medical University Hospital for young scientists, Grant/Award Number: 29E108; JSPS KAKENHI Grant, Grant/Award Number: 16K20204

## Abstract

**Purpose:** Subendometrial myometrium exerts wave-like activity throughout the menstrual cycle, and uterine peristalsis is markedly reduced during the implantation phase. We hypothesized that abnormal uterine peristalsis has an adverse effect on the endometrial decidualization process. We conducted an in vitro culture experiment to investigate the effect of cyclic stretch on the morphological and biological endometrial decidual process.

**Methods:** Primary human endometrial stromal cells (HESCs) were isolated from hysterectomy specimens and incubated with or without 8-bromo-cyclic adenosine monophosphate (8-br-cAMP) and medroxyprogesterone acetate (MPA) for 3 days. After decidualization, cultures were continued for 24 hours with or without cyclic stretch using a computer-operated cell tension system.

**Results:** Cyclic stretch significantly repressed expression of decidual markers including insulin-like growth factor-binding protein 1 (*IGFBP1*), prolactin (*PRL*), forkhead box O1 (*FOXO1*), and *WNT4* on decidualized HESCs. In addition, cyclic stretch of decidualized HESCs affected the decidual morphological phenotype to an elongated shape. The alternation of F-actin localization in decidualized HESCs was not observed in response to cyclic stretch.

**Conclusions:** These data suggest that cyclic stretch inhibits the morphological and biological decidual process of HESCs. Our findings imply that uterine abnormal contractions during the implantation period impair endometrial decidualization and contribute to infertility.

## KEYWORDS

cyclic stretch, endometrial decidualization, forkhead box O1, insulin-like growth factor-binding protein 1, prolactin

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Reproductive Medicine and Biology* published by John Wiley & Sons Australia, Ltd on behalf of Japan Society for Reproductive Medicine

## 1 | INTRODUCTION

Successful pregnancy requires coordination of three interdependent processes: embryo development, endometrial differentiation, and placenta formation. Decidualization is a morphological and biological transformation of human endometrial stromal cells (HESCs) into decidual cells, and it is essential for blastocyst implantation and subsequent formation of the placenta.<sup>1,2</sup> The most characteristic morphological alteration in the decidual process is the drastic transformation of the endometrial stromal fibroblasts into secretory epithelioid decidual cells.<sup>3</sup> At the molecular level, decidual transformation involves extensive reprogramming of many cell functions mediated by the activation of various key transcription factors. The convergence of these transcription factors on promoters of key genes drives the expression and secretion of major decidual factors including prolactin (PRL), *WNT4*, and insulin-like growth factor-binding protein 1 (*IGFBP1*).<sup>3,4</sup> Impairment of the decidualizing process is increasingly linked to a variety of pregnancy disorders including infertility, recurrent implantation failure, recurrent miscarriages, preeclampsia, intrauterine growth restriction, endometriosis, and endometrial malignancy.<sup>5-8</sup>

It has been reported that subendometrial myometrium exerts wave-like activity (uterine peristalsis) throughout the menstrual cycle,<sup>9</sup> and uterine peristalsis is markedly reduced during the implantation phase. The unique feature of uterine peristalsis may facilitate implantation of the embryo to the endometrium. A clinical study reported that none of the enrolled patients with intramural myomas in a high-frequency peristalsis group achieved pregnancy, whereas one-third of patients in the low-frequency peristalsis group became pregnant.<sup>10</sup> Furthermore, myomectomy reduced the frequency of uterine peristalsis in patients who had exhibited an abnormally high-frequency of peristalsis prior to surgery. In addition, myomectomy increased the pregnancy rate in patients who had exhibited high-frequency peristalsis.<sup>11</sup> These observations suggest that abnormal uterine peristalsis during the implantation period influences endometrial functions and contributes to infertility. Harada et al<sup>12</sup> found that endometrial peristalsis elevates *IGFBP-1* levels and promotes the decidualization of HESCs. The effect of physiological stretching conditions had been already investigated in that study. However, the effect of abnormal stretching conditions on HESCs is unclear. Therefore, we hypothesized that abnormal uterine peristalsis has an adverse effect on the endometrial decidual process. We conducted an *in vitro* culture experiment to investigate the effect of cyclic stretch on the morphological and biological endometrial decidualization process.

## 2 | MATERIALS AND METHODS

### 2.1 | Tissue collection and isolation of HESCs

Human endometrium was collected from patients undergoing hysterectomy for uterine fibroids at Saitama Medical University Hospital. Institutional Review Board approval (17-085) was obtained for this research project. All women had regular menstrual cycles and were not

receiving hormonal treatment at the time of surgery. Informed consent was obtained from all patients before tissue collection. The age of participants was  $44.5 \pm 4.9$ . All the endometrial tissues were obtained from women in proliferative phase. HESCs were isolated as previously described.<sup>6,13-15</sup> Harvested HESCs were cultured in maintenance medium of DMEM/F-12 (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% dextran-coated charcoal-treated FBS supplemented with 2  $\mu\text{g}/\text{mL}$  insulin from bovine pancreas (Sigma-Aldrich, St. Louis, MO, USA),  $1 \times 10^{-9}$  M beta-estradiol (Sigma-Aldrich), 1% antibiotic-antimycotic solution (Thermo Fisher Scientific), and 1% L-glutamine solution (Thermo Fisher Scientific) according to the previous study.<sup>6</sup>

### 2.2 | Mechanical loading experiment

Confluent monolayers on a BioFlex six-well plate (Flexcell International Corporation, Hillsborough, NC, USA) were treated with or without 0.5 mmol/L 8-bromo-cAMP (8-br-cAMP; Sigma-Aldrich) and  $10^{-6}$  M medroxyprogesterone acetate (MPA; Sigma-Aldrich) as decidual stimulation for three days. Decidualized or non-decidualized HESCs were then subjected to cyclic sinusoidal equibiaxial tensile strain (amplitude, 0% to 8%; frequency, 0.1 Hz: 6 cycles/min; sine waveform) for 24 hours using the Flexcell FX-4000™ Tension System (Flexcell® Tension Plus System; Flexcell Corporation, McKeesport, PA, USA) according to the manufacturer's instructions. Culture medium had not been changed after the start of decidual stimuli. All experiments were performed before the fourth cell passage.

### 2.3 | The aspect ratio

Because cell elongation is a morphologic indicator of abnormal cyclic stretch, we determined the aspect ratio of each cell's major axis of cell length over the minor axis of cell width (with an aspect ratio of 1.0 yielding a circle) for the HESCs in culture using ImageJ software. The shape indexes were calculated from 20 cells from each field to quantify morphologic changes.

### 2.4 | Prolactin measurement

The concentration of PRL was measured by the electrogenerated chemiluminescence immunoassay (ECLIA) method using ECLusys Prolactin III reagent (Roche Diagnostics, Basel, Switzerland) and Cobas 6000 (Roche Diagnostics). HESC culture media was collected on day three of decidualization for PRL measurement.

### 2.5 | Total RNA extraction and quantitative real-time reverse transcriptase PCR

Total RNA extraction from HESCs was performed using miRNeasy Mini Kit (Qiagen, Hilden, Germany). The reverse transcription

for synthesis of cDNA from extracted total RNA was performed using BioScript reverse transcriptase (Bioline, London, UK). *PRL*, *IGFBP1*, forkhead box O1 (*FOXO1*), and *WNT4* mRNA expression were analyzed by real-time reverse transcriptase PCR (RT-qPCR). RT-qPCR was performed with PowerUP SYBR Green PCR Master Mix (Thermo Fisher Scientific) and the PikoReal 96 Real-Time PCR system (Thermo Fisher Scientific). Primer sequences for each gene are shown in Table 1. The mRNA expression levels relative to those of *GAPDH* were calculated by the  $2^{-\Delta\Delta C_t}$  method.<sup>16</sup>

## 2.6 | Actin staining analysis

Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS; Nacalai Tesque, Inc, Kyoto, Japan) for 10 minutes, washed in PBS, and permeabilized with 0.5% Triton X-100 at room temperature. Actin was stained with the fluorescent marker Acti-stain™ 555 phalloidin (Cytoskeleton, Inc, Denver, CO, USA). Cells were mounted with mounting media containing 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Inc, Burlingame, CA, USA) and visualized using a fluorescent laser microscope (AxioCam MRm; Carl Zeiss AG, Oberkochen, Germany).

## 2.7 | Statistical analysis

PRL or RT-qPCR analysis was conducted on six sets of measurements. Statistical analyses were performed by two-tailed Student's *t* test for comparisons within two groups. All graphs with error ranges denote mean  $\pm$  standard error of the mean (SEM). A *P*-value of  $<.05$  was considered significant.

## 3 | RESULTS

### 3.1 | Cyclic stretch inhibits morphological change and expression of decidual markers on decidualized HESCs

In the absence of hormonal treatment with or without cyclic stretch, confluent primary HESCs have a spindle-shaped

fibroblast-like appearance via light microscopy. In agreement with our previous study,<sup>8</sup> treatment of decidualizing HESCs with 8-br-cAMP and MPA without cyclic stretch resulted in typical morphology of decidual cells characterized by the presence of larger and rounder cells with larger nuclei and abundant cytoplasm. By contrast, cyclic stretch of decidualized HESCs failed to show typical decidual transformation and instead showed an elongated shape (Figure 1A). The aspect ratio of the major axis (length) over the minor axis (width) of each cell was used to quantify the changes in cell morphology upon decidualization in the presence or absence of cyclic stretch. For non-decidualized HESCs, cyclic stretch had no effect on the aspect ratio. By contrast, the aspect ratio of decidualized HESCs with cyclic stretch was significantly higher, indicating the relative abundance of spindle-shaped cells compared with decidualized HESCs without cyclic stretch (Figure 1B). Furthermore, PRL secretion of decidualized HESCs with cyclic stretch was significantly reduced in comparison with decidualized HESCs kept stationary (Figure 2).

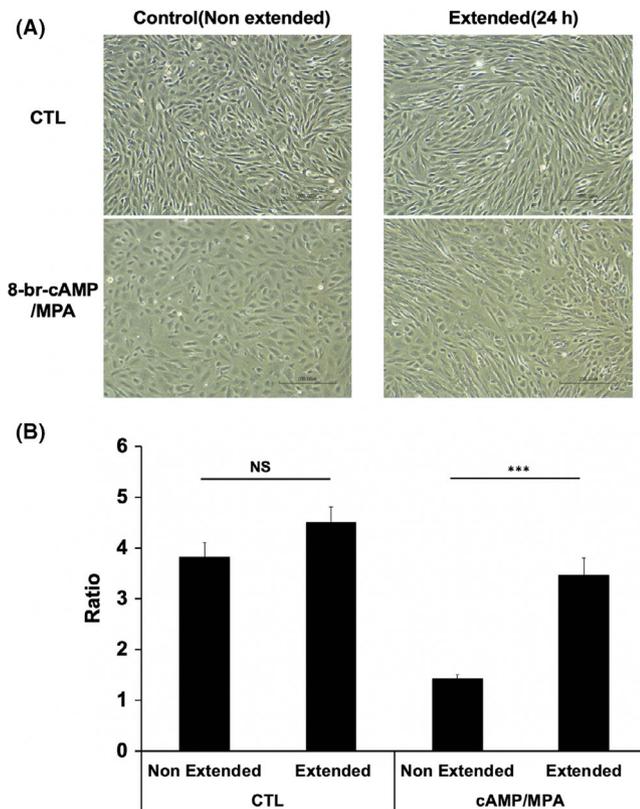
Next, we analyzed the effect of cyclic stretch on the expression of major decidual makers on decidualized HESCs. Cyclic stretch significantly repressed expression of *PRL*, *IGFBP1*, *FOXO1*, and *WNT4* on decidualized HESCs as compared with decidualized HESCs kept stationary (Figure 3).

### 3.2 | Alteration of F-actin localization and cytoskeleton of decidualized of HESCs with cyclic stretch

A previous study reported that the actin-based cytoskeleton contributes to both functional and morphological endometrial decidualization.<sup>17</sup> Therefore, we investigated the subcellular distribution of actin stress fibers in non-decidualized and decidualized HESCs treated with or without to cyclic stretch using Acti-stain™ 555 phalloidin. Well-stretched F-actin was distributed throughout the cytoplasm in undifferentiated HESCs during both stationary and cyclic stretch conditions. In response to decidual stimulation, F-actin was located in the periphery of decidualized HESCs kept stationary. However, we were unable to observe an alteration in F-actin localization in decidualized HESCs with cyclic stretch (Figure 4).

|  |  |
|--|--|
| Glyceraldehyde phosphate dehydrogenase ( <i>GAPDH</i> )        | Forward: 5'-CGACCACTTTGTCAAGCTCA-3'<br>Reverse: 5'-AGGGGTCTACATGGCAACTG-3'           |
| Insulin-like growth factor-binding protein 1 ( <i>IGFBP1</i> ) | Forward: 5'-CTGCGTGCAGGAGTCTGA-3'<br>Reverse: 5'-CCCAAAGGATGGAATGATCC-3'             |
| Prolactin ( <i>PRL</i> )                                       | Forward: 5'-CTACATCCATAACCTC TCCTCA-3'<br>Reverse: 5'-GGGCTTGCTCCTTGCTTC-3'          |
| Wnt family member 4 ( <i>WNT4</i> )                            | Forward: 5'-CAT GCAACAAGACGTCCAAG-3'<br>Reverse: 5'-AAGCAGCACCAGTGAATTT-3'           |
| Forkhead box O1 ( <i>FOXO1</i> )                               | Forward: 5'-ATTCCGGAATGACCTCATGGA-3'<br>Reverse:<br>5'-TTTAAAGTGTAACCTGCTCACTAACC-3' |

**TABLE 1** Primer sequence used in real-time RT-PCR

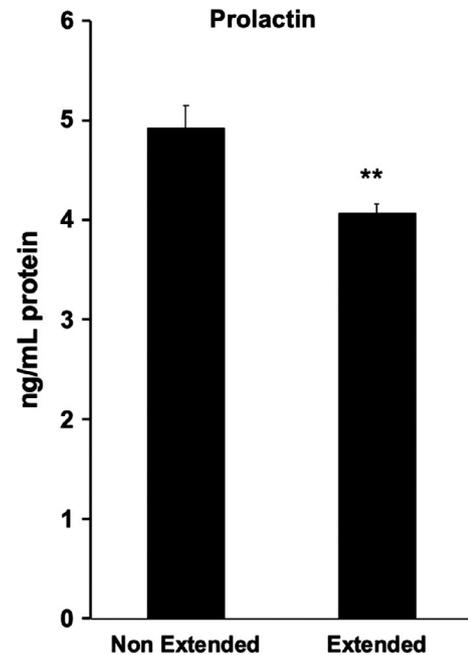


**FIGURE 1** The effect of cyclic stretch on the phenotype of HESCs. HESCs were treated with or without 8-bromo-cAMP and  $10^{-6}$  M MPA as decidual stimulation for three days. Decidualized and non-decidualized HESCs were then cultured with or without cyclic stretch for 24 h. Scale bar indicates 200  $\mu$ m (A). The aspect ratio of cell major axes (lengths) over minor axes (widths), with an aspect ratio of 1.0 yielding a circle, was determined for the HESC culture system by using ImageJ software. The shape indexes were calculated from 20 cells from each field of cells to quantify morphologic changes. Data shown are mean  $\pm$  SEM of five biological repeats. \*\*\* $P < .001$  (B)

## 4 | DISCUSSION

The findings of the present study demonstrate that cyclic stretch hinders the morphological alteration of HESCs upon decidualization. Cyclic stretch repressed expression of decidual markers in decidualized HESCs including *PRL*, *IGFBP1*, *FOXO1*, and *WNT4*. In addition, cyclic stretch inhibited the change in F-actin localization in decidualized HESCs. These observations suggest that cyclic stretch inhibits morphological change through biological decidual process. Therefore, abnormal uterine peristalsis may affect the implantation of the embryo.

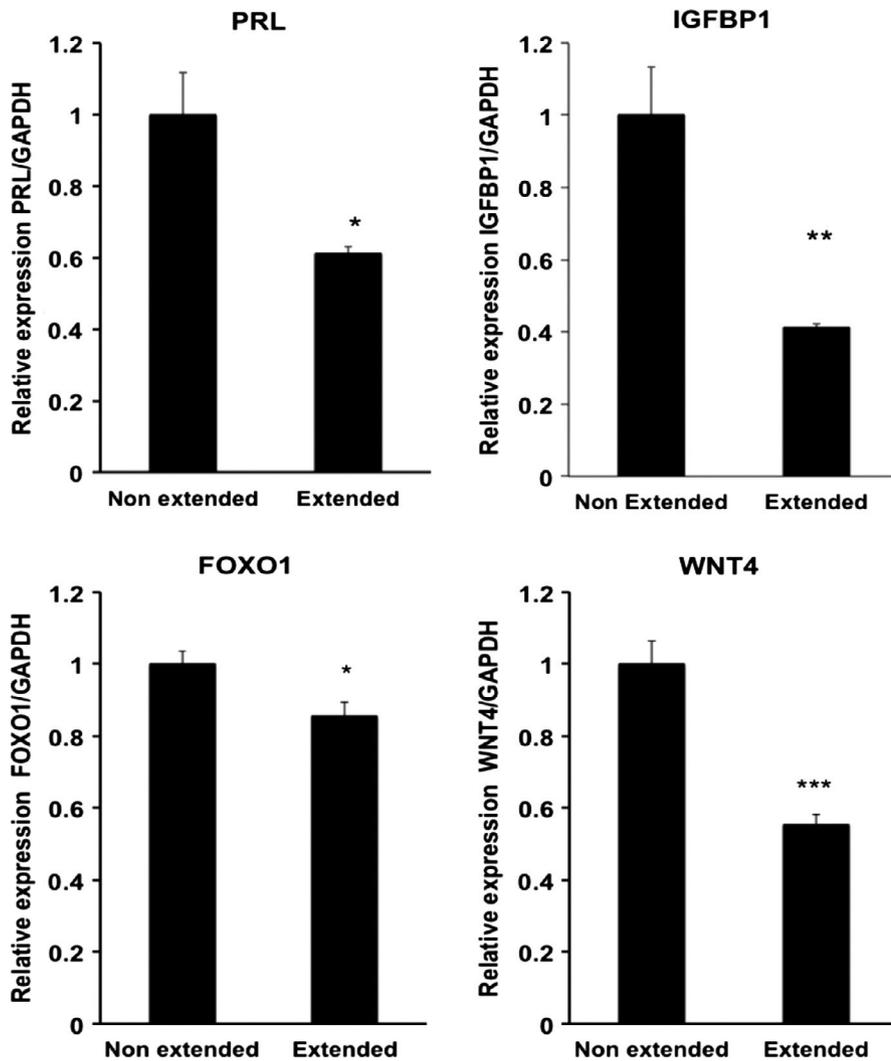
Decidualization represents the transformation of endometrial stromal cells into specialized secretory epithelioid cells. This differentiation process is initiated in the mid-to-late secretory phase of the menstrual cycle and coincides with vascular remodeling and influx of specialized immune cells, mainly uterine natural killer cells and macrophages, into the stroma. The decidual process is essential for formation of a functional feto-maternal interface as it controls



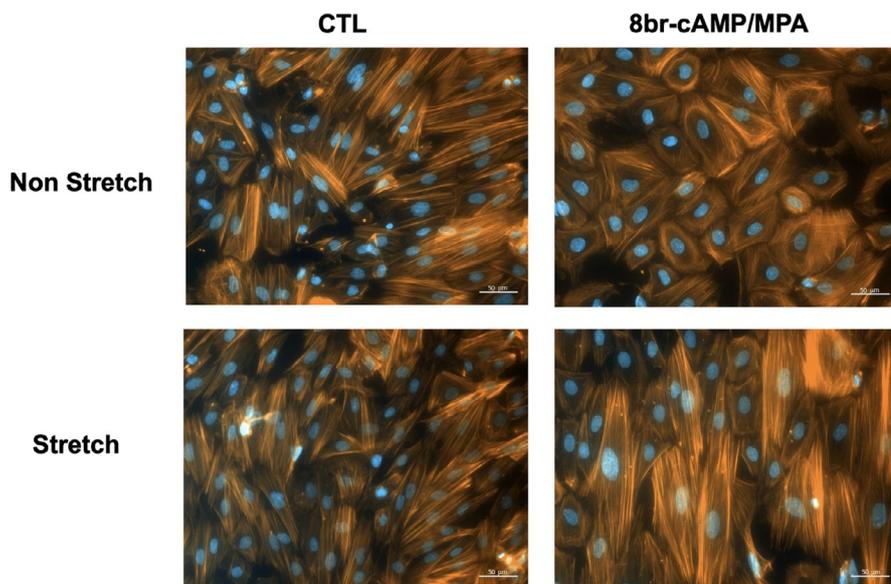
**FIGURE 2** The effect of cyclic stretch on the secretion for PRL of decidualized HESCs. HESCs were treated with or without 8-bromo-cAMP and  $10^{-6}$  M MPA as decidual stimulation for three days. Then decidualized and non-decidualized HESCs were cultured with or without cyclic stretch for 24 h. These data represent the mean of PRL protein concentration in the supernatant, normalized by the total protein content. Data are shown as mean  $\pm$  SEM of five individuals. \*\* $P < .01$

endovascular trophoblast invasion, ensures tissue homeostasis, and confers resistance to oxidative stress signals.<sup>1</sup> Impairment of the decidualizing process is increasingly linked to a variety of pregnancy disorders including infertility, recurrent implantation failure, recurrent miscarriages, preeclampsia, intrauterine growth restriction, endometriosis, and endometrial malignancy.<sup>5-8</sup>

It has been reported that subendometrial myometrium exerts wave-like activity (uterine peristalsis) throughout the menstrual cycle,<sup>9</sup> and uterine peristalsis is markedly reduced during the implantation phase. Previous observations suggest that abnormal uterine peristalsis during the implantation period influences endometrial functions and contributes to infertility.<sup>10,11</sup> The present study demonstrated that cyclic stretch significantly repressed expression of decidual markers including *IGFBP1*, *PRL*, *FOXO1*, and *WNT4* on decidualized HESCs. These alterations may be through the reduction of *FOXO1* gene since it has been reported *FOXO1* target genes are involved *PRL*, *IGFBP1*, and *WNT4*.<sup>4</sup> In addition, cyclic stretch of decidualized HESCs impaired decidual morphological phenotype to show a more elongated shape. It has been well-known that decidual change of HESCs is associated with the process of mesenchymal epithelial transition (MET). In this decidual process, HESCs accumulate glycogen and lipid intracellularly, expand the rough endoplasmic reticulum, and reconstruct cytoskeletal actin filaments.<sup>3</sup> In addition, we previously demonstrated that decidualized stimulation down-regulated N-cadherin expression and upregulated E-cadherin expression in HESCs (cadherin



**FIGURE 3** The effect of cyclic stretch on the expression of *PRL*, *IGFBP1*, *FOXO1*, and *WNT4* in decidualized HESCs. HESCs were treated with or without 8-bromo-cAMP and  $10^{-6}$  M MPA as decidual stimulation for three days. Decidualized HESCs were then cultured with or without cyclic stretch for 24 h. RT-qPCR analysis of *PRL*, *IGFBP1*, *FOXO1*, and *WNT4* transcript levels was performed in decidualized HESCs with or without cyclic stretch. Expression levels were normalized to *GAPDH*. Data are the mean  $\pm$  SEM of three independent experiments \*\* $P < .01$ , \* $P < .05$



**FIGURE 4** Alterations to F-actin localization in non-decidualized HESCs and decidualized HESCs with or without cyclic stretch for 24 h. Actin filaments were visualized with Acti-stain™ 555 phalloidin (Cytoskeleton). Scale bar, 50  $\mu$ m

switch).<sup>18</sup> It is possible that high stretch cycling may inhibit this cadherin switch. However, further studies need to clarify this mechanism of impairment for decidualization. The alteration of F-actin

localization in decidualized HESCs was lost in response to cyclic stretch. These observations together suggest that cyclic stretch inhibits the morphological and functional process of decidualization.

Therefore, these findings raise the possibility that uterine abnormal contraction during the implantation period leads to an impaired endometrial decidualization process and thereby contributes to the development of infertility. However, a previous report showed that cyclic stretch upregulated IGFBP1 secretion from decidualized HESCs.<sup>12</sup> One possible explanation for this discrepancy could lie in different cyclic stretch conditions (a cyclic fashion at a rate of 2 vs 6 cycle/min). It has been reported that uterine movements undergo cycling at a rate of approximately 2 cycles/min in the luteal phase.<sup>9</sup> Therefore, we chose this condition as abnormal uterine contraction. Several studies demonstrated different stretch conditions including the frequency of the cycle resulted in the opposite effect on the same cells.<sup>19,20</sup> For instance, Kubo et al reported that cell proliferation in cultured tenocytes stretched in physiologically frequent condition was augmented. Conversely, those in unphysiologically frequent condition show reduced cell proliferation.<sup>20</sup> Thus, we assume it is reasonable to demonstrate opposite effect on the decidualized cells with the non-physiological conditions compared to those with physiological conditions in Harada's study.<sup>12</sup>

The cytoskeleton plays a critical role in various cellular processes including migration.<sup>21</sup> The organization and plasticity of the cytoskeleton are characterized primarily by forces generated by actin-myosin interactions.<sup>17</sup> Previous studies demonstrated that F-actin was localized in the periphery of decidualized HESCs. By contrast, well-stretched F-actin was distributed throughout the cytoplasm in non-decidualized HESCs.<sup>21,22</sup> These observations are consistent with our present microscopic findings. Furthermore, the present study demonstrated that cyclic stretch reversed the distribution of F-actin in decidualized HESCs. Interestingly, it has been reported that in women with endometriosis, the endometrium has reduced decidualization capacity compared with that of women without endometriosis.<sup>23</sup> Therefore, it is possible that abnormal uterine contractions may actively contribute to sustaining the mesenchymal phenotype, retaining cell motility of HESCs against decidualization stimulation, and establishing endometriosis lesions.

In conclusion, the present study demonstrates that cyclic stretch inhibits the morphological and functional decidual process of HESCs. Our findings suggest that uterine abnormal contractions during the implantation period of fertilization impair endometrial decidualization and contribute to infertility.

## ACKNOWLEDGMENTS

The authors would like to thank Dr Yumi Mizuno for her excellent experiment's support. This work was supported by an internal grant of Saitama Medical University Hospital for young scientists (29E108) and JSPS KAKENHI Grant Number 16K20204. The authors would like to thank Enago (www.enago.jp) for the English language review.

## DISCLOSURE

**Conflict of interest:** The authors declare no conflict of interest. **Human rights statement and informed consent:** All procedures followed were in accordance with the ethical standards of the responsible

committee on human experimentation (institution and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients included in the study. The Institutional Review Board of the Saitama Medical University Hospital approved this study.

## ORCID

Takeshi Kajihara  <https://orcid.org/0000-0002-4853-9297>

## REFERENCES

- Kajihara T, Brosens JJ, Ishihara O. The role of FOXO1 in the decidual transformation of the endometrium and early pregnancy. *Med Mol Morphol.* 2013;46:61-68.
- Gellersen B, Brosens J. Cyclic AMP and progesterone receptor cross-talk in human endometrium: a decidualizing affair. *J Endocrinol.* 2003;178:357-372.
- Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. *Semin Reprod Med.* 2007;25:445-453.
- Takano M, Lu Z, Goto T, et al. Transcriptional cross talk between the forkhead transcription factor forkhead box O1A and the progesterone receptor coordinates cell cycle regulation and differentiation in human endometrial stromal cells. *Mol Endocrinol.* 2007;21:2334-2349.
- Maruyama T, Yoshimura Y. Molecular and cellular mechanisms for differentiation and regeneration of the uterine endometrium. *Endocr J.* 2008;55:795-810.
- Brosens JJ, Hayashi N, White JO. Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. *Endocrinology.* 1999;140:4809-4820.
- Bulun SE, Noble LS, Takayama K, et al. Endocrine disorders associated with inappropriately high aromatase expression. *J Steroid Biochem Mol Biol.* 1997;61:133-139.
- Daly DC, Maslar IA, Rosenberg SM, Tohan N, Riddick DH. Prolactin production by luteal phase defect endometrium. *Am J Obstet Gynecol.* 1981;140:587-591.
- Orisaka M, Kurokawa T, Shukunami K, et al. A comparison of uterine peristalsis in women with normal uteri and uterine leiomyoma by cine magnetic resonance imaging. *Eur J Obstet Gynecol Reprod Biol.* 2007;135:111-115.
- Yoshino O, Hayashi T, Osuga Y, et al. Decreased pregnancy rate is linked to abnormal uterine peristalsis caused by intramural fibroids. *Hum Reprod.* 2010;25:2475-2479.
- Yoshino O, Nishii O, Osuga Y, et al. Myomectomy decreases abnormal uterine peristalsis and increases pregnancy rate. *J Minim Invasive Gynecol.* 2012;19:63-67.
- Harada M, Osuga Y, Takemura Y, et al. Mechanical stretch upregulates IGFBP-1 secretion from decidualized endometrial stromal cells. *Am J Physiol Endocrinol Metab.* 2006;90:E268-E272.
- Labied S, Kajihara T, Madureira PA, et al. Brosens JJ. Progestins regulate the expression and activity of the forkhead transcription factor FOXO1 in differentiating human endometrium. *Mol Endocrinol.* 2006;20:35-44.
- Christian M, Zhang X, Schneider-Merck T, et al. Cyclic AMP-induced forkhead transcription factor, FKHR, cooperates with CCAAT/enhancer-binding protein b in differentiating human endometrial stromal cells. *J Biol Chem.* 2002;277:20825-20832.
- Tochigi H, Kajihara T, Mizuno Y, et al. Loss of miR-542-3p enhances IGFBP-1 expression in decidualizing human endometrial stromal cells. *Sci Rep.* 2017;7:40001.
- Livak K, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 2001;25:402-408.

17. Elson EL. Cellular mechanics as an indicator of cytoskeletal structure and function. *Annu Rev Biophys Biophys Chem.* 1998;17:397-430.
18. Sultana S, Kajihara T, Mizuno Y, et al. Overexpression of microRNA-542-3p attenuates the differentiating capacity of endometriotic stromal cells. *Reprod Med Biol.* 2017;16:170-178.
19. Wilkins JR, Pike DB, Gibson CC, Kubota A, Shiu YT. Differential effects of cyclic stretch on bFGF- and VEGF-induced sprouting angiogenesis. *Biotechnol Prog.* 2014;30:879–888.
20. Kubo Y, Hoffmann B, Goltz K, et al. Different frequency of cyclic tensile strain relates to anabolic/catabolic conditions consistent with immunohistochemical staining intensity in tenocytes. *Int J Mol Sci.* 2020;21:32041254.
21. Maruyama T, Yoshimura Y, Sabe H. Tyrosine phosphorylation and subcellular localization of focal adhesion proteins during in vitro decidualization of human endometrial stromal cells. *Endocrinology.* 1999;140:5982-5990.
22. Ihnatovych I, Hu W, Martin JL, Fazleabas AT, de Lanerolle P, Strakova Z. Increased phosphorylation of myosin light chain prevents in vitro decidualization. *Endocrinology.* 2007;148:3176-3184.
23. Klemmt PA, Carver JG, Kennedy SH, Koninckx PR, Mardon HJ. Stromal cells from endometriotic lesions and endometrium from women with endometriosis have reduced decidualization capacity. *Fertil Steril.* 2006;85:564-572.

**How to cite this article:** Saito R, Kajihara T, Takamura M, Tochigi H, Sato T, Ishihara O. High stretch cycling inhibits the morphological and biological decidual process in human endometrial stromal cells. *Reprod Med Biol.* 2020;19:378–384. <https://doi.org/10.1002/rmb2.12341>