Expression and regulation of Foxa2 in the rat uterus during early pregnancy

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Abstract. The forkhead box a (Foxa) protein family has been found to play important roles in mammals. Recently, the expression of Foxa2 was reported in the mouse uterus, and it was reported to be involved in regulation of implantation. However, the regulation of Foxa2 expression in the uterus is still poorly understood. Therefore, the present study was conducted to investigate the expression profiles of Foxa2 in the rat uterus during the estrus cycle and pregnancy. Furthermore, the effect of steroid hormones and Hedgehog protein on the expression of Foxa2 was analyzed in vivo and in vitro. In this study, the level of expression of Foxa2 was low in the rat uterus during the different stages of the estrus cycle. However, the expression increased transiently during early pregnancy at 3.5 days post coitus (dpc) and decreased at 5.5 dpc. In ovariectomized rats, P4 treatment had no effect on the expression of Foxa2 compared with the expression in control animals. Moreover, the expression of Foxa2 in cultured epithelial cells was not increased by P4 treatment in vitro. However, Foxa2 expression was significantly decreased in the rat uterus after 24 h of E2 treatment. Treatment of cells with a recombinant Hedgehog protein significantly increased the expression of Foxa2. These results suggest that the expression of Foxa2 may transiently increase just before the implantation and it may be regulated by E2 and Hedgehog protein.

Key words: Foxa2, Ihh, Rat, Steroidal regulation, Uterus

In mammalian reproduction, implantation of the embryo into the maternal uterus is a crucial step and requires spatiotemporally regulated suitable surroundings prepared by many complicated factors and processes involved with materno-fetal interaction. Successful implantation requires dramatic uterine tissue remodeling, which is strictly regulated by two sex steroid hormones, progesterone (P4) and 17β-estradiol (E2). P4 and E2 stimulate endometrial epithelial and stromal cells collectively to induce many cytokines and hormones for proliferation and differentiation for embryo attachment and acceptance. As a result, the endometrium acquires embryonic receptivity, which is even called the “window of implantation”. Although the mechanism of its establishment is still poorly understood, it was recently suggested that a transcription factor, Forkhead box a 2 (Foa2), participates in this process.

The Foxa protein family was first identified in liver nuclear extracts as transcription factors essential for regulation of the hepatocyte-specific expression of several target genes [1]. The Foxa protein contains a 110-amino acid motif that is conserved from yeast to humans [2]. The Foxa family has been found to have important roles in multiple stages of mammalian life, including early development, organogenesis, metabolism and homeostasis. The Foxa family includes Foxa1, Foxa2 and Foxa3 genes. In the early embryo, Foxa2 is expressed in the node, notochord and floor plate, whereas Foxa1 is detected only in the notochord and floor plate. Targeted disruption of Foxa2 leads to an embryonic lethal phenotype in mice, with impaired formation of the node, notochord and foregut endoderm [3]. Recently, it was reported that the Foxa2 gene and protein were localized in the mouse uterine glands [4]. Furthermore, Foxa2 expression gradually increased until its peak at day 2.5 of pseudopregnancy (the first day a vaginal plug was observed was designated day 0.5) and then sharply decreased throughout the remaining period of pseudopregnancy. Interestingly, in Foxa2 conditional ablation mice, implantation sites were significantly decreased compared with in normal pregnant mice [5]. Additionally, the number of uterine glands and the expression of leukemia inhibitory factor (Lif) were decreased in the Foxa2 conditional ablation mice [5]. In mice, the highest levels of Lif were found prior to implantation in the glandular epithelium following the E2 surge in the morning of day 3.5 of pregnancy [6]. Since Lif null mice are infertile, it is considered that LIF is one of the essential cytokines for successful implantation [7]. These observations suggest...
that Foxa2 plays an important role for implantation by inducing \textit{Lif} expression in the mouse uterine gland.

In previous studies, the expression of Foxa2 in the uterus was reported in the mouse [4], human [8] and bovine [9]. Although it was reported that Foxa2 played an important role for implantation in mice [5], the regulatory mechanism of Foxa2 expression remained unexplained. In the floor plate, initiation of Foxa2 expression was induced by Sonic hedgehog (Shh), a member of the Hedgehog (Hh) family, from the notochord [10]. Although Shh was not detected in the rat endometrium, Indian hedgehog (Ihh), a member of the Hh family, was detected in the luminal and glandular epithelium by in \textit{situ} hybridization [11]. Since Ihh conditional ablation mice are infertile, it is considered that Ihh is one of the essential cytokines for successful implantation [12]. The deduced amino acid sequences of SHH and IHH have a high degree of similarity, particularly in the Hh signaling ligand peptide region [11]. Ihh peaked at day 2.5–3.5 of pseudopregnancy and decreased thereafter [13]. Takamoto \textit{et al.} [13] also reported that the expressional profile of Ihh was similar to that of Foxa2, which transiently increased just before the implantation period. Thus, Foxa2 may be promoted by Ihh in the rat uterus. However, the regulation of Foxa2 expression by the Hedgehog protein has not yet been examined.

In the present study, we therefore investigated the expressional profiles of Foxa2 during different stages of the estrus cycle as well as during pregnancy in the rat uterus using quantitative real-time PCR and immunohistochemistry. Then the effect of steroid hormones on the expression of Foxa2 in the rat uterus was analyzed using ovarioctomized (OVX) rats, ICI 182,780 (ICI; a pure estrogen receptor antagonist) treated rats and delayed implantation rats \textit{in vivo}. Finally, the regulation Foxa2 expression by the Hedgehog protein was analyzed using a recombinant Hedgehog protein in a cell culture system of endometrial epithelial cells.

**Materials and Methods**

**Animals**

This research was conducted using Wistar rats raised in our laboratory (Laboratory of Reproductive Physiology and Biotechnology, Department of Animal and Marine Bioresource Sciences, Graduate School of Agriculture, Kyushu University). The rats were housed under temperature- and light-controlled conditions (lights on at 0800 h, off at 2000 h) with free access to food and water. The stages of the estrus cycles in each rat were determined by the vaginal smear method. Adult female rats were mated with males, and the day on which spermatozoa were found in the vaginal smear was designated 0.5 days post coitus (dpc). All experiments were conducted according to the Guidelines for the Care and Use of the Laboratory Animals (Graduate School of Agriculture, Kyushu University) and with the approval of the Kyushu University Laboratory Animal Care and Use Committee.

For studies on steroid hormonal action, 7-week-old female rats were ovarioctomized and subsequently rested for 2 weeks. Progesterone (P4, 10 mg/kg body weight) and 17β-estradiol (E2, 50 µg/kg body weight) were purchased from Steroids Inc. (Wilton, NH, USA) and injected subcutaneously with 200 µl sesame oil. Controls were given an equal volume of vehicle (sesame oil) only. Rats were killed after 6 h and 24 h of treatment, and uteri were rapidly isolated.

To inhibit implantation-initiating estrogenic stimuli, the pregnant rats at 3.5 dpc were treated daily with ICI 182,780 (1.5 mg/kg body weight), a pure estrogen receptor antagonist (Sigma, St. Louis, MO, USA), by subcutaneous injection. Rats were killed at 5.5 dpc, and uteri were isolated. To induce delayed implantation, the pregnant rats at 3.5 dpc were ovarioctomized at 0830–0900 h. P4 (10 mg/kg body weight) was injected daily from 3.5 dpc to 6.5 dpc to maintain a delayed implantation state. At 7.5 dpc, P4 treatment was continued for half of the rats, and the remaining half was treated with P4 and E2 (1 µg/kg) to initiate implantation. Rats were euthanized at 8.5 dpc (24 h after treatment), and uteri were isolated. The number of rats used for each experimental group was three.

**RNA extraction, reverse transcription (RT), polymerase chain reaction (PCR) and real-time PCR**

Total RNA was extracted from homogenized uteri using a Sepasol-RNA I Super (Nacalai Tesque, Kyoto, Japan) with RQ1 RNase-Free DNase treatment (Promega, Madison, WI, USA), according to the manufacturers’ protocols. RNA quality was assessed by ethidium bromide agarose gel electrophoresis and spectrophotometric UV absorbance at 260/280 nm. One microgram of each RNA sample was reverse transcribed with a MMLV High Performance Reverse Transcriptase (Epicentre, Madison, WI, USA) using an oligo-dt primer in a 20 µl solution. Real-time PCR was performed with iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) using a Chromo4 System (Bio-Rad Laboratories). The specific primers for real-time PCR were as follows: 5′-GGCATGAACACTTACATGAGC-3′ (forward) and 5′-GCGCCCACATAGGATGAC-3′ (reverse) for Foxa2 (NM_012743, product length: 98 bp) and 5′-GACGGGTTCTGTCATGTCG-3′ (forward) and 5′-ACCTGGTTTCATCATACTACAC-3′ (reverse) for \textit{Hprt} (NM_012574, product length: 61 bp). The PCR cycle parameters were an initial denaturation step at 95°C for 10 min and then 40 cycles at 95°C for 10 sec and 60°C for 30 sec. Hprt served as an internal control and was used to normalize for differences in each sample.

**In situ hybridization of Foxa2**

The Foxa2 PCR products were subcloned into a pGEM-T Easy Vector. Their antisense and sense RNA probes were synthesized using PCR-amplified templates and RNA polymerase promoter sequences. Digoxigenin (DIG)-labeled antisense and sense RNA probes were prepared with T7 or SP6 RNA polymerase using a DIG RNA Labeling Mix kit (Roche, Tokyo, Japan). The same primers as mentioned for real-time PCR were used for the preparation of DIG-labeled probes. Cryosections (10 µm thick) of the uterus at 3.5 dpc were processed in O.C.T. Compound (Sakura Finetechnical Co., Ltd., Tokyo, Japan) and fixed with 4% paraformaldehyde. After washing with PBS, the sections were acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride. They were then hybridized with eRNA probes in 3 × SSC containing 50% formamide, 125 µg/ml yeast RNA, 100 µg/ml salmon sperm, 10% dextran sulfate, 1 × Denhardt’s solution and 0.12 M phosphate at 60°C overnight. The next day, the sections were serially washed at 42°C with post-hybridization solution, i.e., 5 × SSC and 2 × SSC containing 50% formamide. They were then washed with 2 × SSC, 0.2 × SSC and 100 mM Tris-HCl (pH 7.5)
Culture of the endometrial epithelial cells, real-time PCR and immunocytochemistry

Endometrial epithelial cells were isolated from uterine horns at 1.5 dpc. The uterine lumens were filled with phosphate buffered saline (PBS) containing 0.1% collagenase (Worthington Biochemical Corporation, Lakewood, NJ, USA) and incubated at 37 °C for 45 min in a shaking water bath. The dissociated cells were washed and plated onto 4-well dishes coated with BD Matrigel (BD Biosciences, San Jose, CA, USA). After washing with PBS, they were incubated for 1 h at room temperature with the secondary Goat Anti-Mouse IgG (H+L), F(ab’)2 Fragment (Alexa Fluor 488 Conjugate) antibody (1:500; Cell Signaling Technology) and Alexa Fluor 594 Goat Anti-Rabbit IgG (H+L), F(ab’)2 Fragment (Alexa Fluor 488 Conjugate) antibody (1:500; Invitrogen, Carlsbad, CA, USA) diluted in blocking buffer. Sections were subsequently washed in PBS and mounted with Mount-Quick Aqueous (Daido Sangyo, Tokyo, Japan). Immunostaining was detected under a fluorescence microscope (Nikon, Tokyo, Japan).

Expression of Foxa2 mRNA in the rat uterus

We first examined the expression of Foxa2 in the rat uterus at different stages of the estrous cycle and during early pregnancy using quantitative real-time PCR. Although the expression level of the gene showed some changing patterns in different stages of estrus cycle, the values were not significantly different. On the other hand, expression of Foxa2 significantly increased at 3.5 dpc (P < 0.05) during early pregnancy. The value was almost 7 times higher than that of 1.5 dpc. The elevation in the expression was transient, and the expression decreased significantly to the basal level at 5.5 dpc (P < 0.05) (Fig. 1A). Foxa2 was detected using the antisense probe in the glandular epithelium at 3.5 dpc by in situ hybridization (Fig. 1B). Almost no signal was detected in the luminal epithelium or stroma in the endometrium. The control slide did not show any positive signals when the sense probe was used (Fig. 1C).

Localization of FOXA2 in the rat uterus

Dual-label immunohistochemical analysis of Cytokeratin and FOXA2 was performed to investigate the localization of FOXA2 in the rat uterus. Cytokeratins are proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue and were used as a marker of epithelial cells. Interestingly, FOXA2 was detected in the glandular epithelium but not in the luminal epithelium, stroma, myometrium or vessel in the rat uterus (Fig. 2). FOXA2 was detected at all stages of the estrous cycle and during early pregnancy (data not shown). To analyze the intracellular localization of the FOXA2 in glandular epithelial cells, double staining of FOXA2 and Cytokeratin with counterstaining with Hoechst was performed (Fig. 3). Keratin-containing intermediate filaments were stained as a green (Fig. 3A), and the nucleus of the glandular epithelial cells and surrounding stromal cells were stained blue (Fig. 3B). FOXA2 in the epithelial cells was stained red (Fig. 3C), and it was located in the nucleus, as shown in merged images (Fig. 3D, E).

Effect of steroid hormones on the expression of Foxa2 mRNA

To analyze the effects of steroidal regulation on Foxa2 expression in the uterus, we used OVX rats to avoid the influence of the endogenous hormone. After 6 h of treatment, there was no significant difference in Foxa2 expression in the uterus among the groups treated with the
The expression of Foxa2 has been reported in several animal species including the mouse [4], human [8] and bovine [9]. Although an important role of Foxa2 has been suggested for implantation in mice [5], the regulation of Foxa2 expression is poorly understood in the uterus during early pregnancy. In the present study, we therefore examined the expressional profiles of Foxa2 at different stages of the estrus cycle and during early pregnancy in rat uterus. We demonstrated that the expression of Foxa2 transiently increased just before implantation and that it is regulated by E2 and Hedgehog protein. Thus, the results indicate that transient elevation of Foxa2 expression may have an important role for implantation in the rat uterus.

In the rat uterus, embryo implantation occurs at 4.5 dpc following an E2 surge. Our results showed that the expression of Foxa2 increased just before implantation and decreased thereafter, initiating of implantation. Similar results were obtained for the expression profile of Foxa2 in the mouse uterus, which showed a transient elevation at 2.5 dpc following downregulation at 3.5 dpc [5]. Although the function has not yet been clarified sufficiently, it is suggested that Foxa2 is necessary for development of the glandular epithelium in the uterus [14]. It has also been reported that the glandular epithelium does not develop in the uterus of the Foxa2-deficient mouse [5]. Thus, formation of the uterine glands during implantation should be inhibited as the expression of Foxa2 decreases during this period. It is believed that the uterine endometrial glands have an essential role in uterine function and fertility, including implantation [15]. If so, the decrease in Foxa2 during implantation period may result in inhibition of uterine gland formation, which seems contradictory for the establishment of implantation. Although the importance of the uterine glands in implantation has been recognized, it has been reported that the glandular hyperplasia resulted in fertility defects. Jeong et al. [16] generated β-catenin conditional mutation

**Discussion**

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Fig. 2. Immunohistochemical detection of FOXA2 in the rat uterus (proestrus stage). After fixation, sections were stained with hematoxylin and eosin (A, E), anti-FOXA2 antibody (B, F; red) and anti-cytokeratin antibody (C, G; green). FOXA2 and cytokeratin staining merged image (D, H). GE, glandular epithelium; LE, luminal epithelium; S, stroma; M, myometrium; V, vessel. The scale bar represents 100 μm.

Fig. 3. Intracellular localization of FOXA2 in the glandular epithelium. Sections were stained with anti-cytokeratin antibody (A; green) and anti-FOXA2 antibody (C; red) or without a first antibody (negative control) (F). Counterstaining was performed with Hoechst, showing the location of the nuclei (B; blue). Merged images of FOXA2 and cytokeratin staining (D) and FOXA2 and Hoechst staining (E), respectively. The scale bar represents 50 μm.
mice to investigate the role of β-catenin in uterine development. They reported that expression of the dominant stabilized β-catenin resulted in endometrial glandular hyperplasia. Interestingly, β-catenin conditionally stabilized mice had fertility defects, and the ability of

the uterus to undergo a hormonally induced decidual reaction was lost [16]. Thus, it is necessary for the formation of the uterine gland to be controlled closely during pregnancy. The transient expression of Foxa2 before implantation in the uterus may have roles in preparing a suitable environment for implantation by regulating uterine gland development. Foxa2 may play a role through changing its expression during the implantation period or may be involved in the regulation of gene transcription, which needs to be downregulated for implantation.

Foxa2 was detected only in the glandular epithelium of the rat uterus in both mRNA and protein. Additionally, in the immunohistochemical analysis, FOXA2 was localized to the nucleus of the glandular epithelium, although it was not detected in the luminal epithelium. Franco et al. [17] reported that Foxa2 can be used as a marker gene of the glandular epithelium in the mouse uterus. Considering our results along with the above report in the mouse, Foxa2 appears to be a glandular specific factor in the uterus of rodents. It is reported that Foxa2 plays an important role in epithelial budding and morphogenesis in many organs including the pancreas, liver, lung and prostate [18–20]. Since the Foxa family is conserved from yeast to humans [21], it is possible that Foxa2 may play an important role in the uterus of many mammalian species. Further analysis is needed to understand the relationship between Foxa2 and adenogenesis in the uterus for implantation.

Although Foxa2 expression did not differ after 6 h of steroidal hormone treatments in OVX rats, its expression in E2-treated rats was significantly lower than those of the control and P4-treated groups after 24 h of treatment. This result suggested that Foxa2 expression was reduced by E2 action. These results were similar to a report in mice, which showed repression of Foxa2 by E2 injection in OVX mice [5]. To clarify the effect of E2 action on Foxa2 expression during pregnancy, further experiments using an antagonist of the estrogen receptor and delayed implantation model were carried out in the present study. Foxa2 expression in the rats treated with ICI, a selective antagonist of ERα, was significantly higher in comparison with control animals. Furthermore, since delayed implantation starts just after E2 treatment, it is assumed that Foxa2 expression must be downregulated in accordance with the estrogenic stimuli. Indeed the expression level of Foxa2 was lower in the implantation-induced rats treated with E2 compared with the level of the control group. The results in the OVX, ICI treatment and delayed implantation experiments clearly showed that Foxa2 expression in the rat uterus is decreased by E2 action. The profile of Foxa2 expression in this study is consistent with that of Gli1 [22], which is a signal transducer of the Hedgehog pathway. Gli1 expression transiently increases at 3.5 dpc and decreases at 5.5 dpc. Further, Gli1 expression in the rat uterus is decreased by E2 action [22]. The similarity of Foxa2 and

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**Fig. 4.** Effect of steroid hormones on the expression of Foxa2 analyzed by quantitative real-time PCR. A) Ovariectomized rats were treated with progesterone (P4), 17β-estradiol (E2), a combination of P4 and E2 (P4 + E2) or vehicle (C, sesame oil). Total RNA was extracted from the rat uterus after 6 h (●) or 24 h (■) of the treatment. The results were compared individually for each time of treatment. Values with different superscripts and different times of treatment (a vs. b, or x vs. y) are significantly different (P < 0.05). B) The pregnant rats at 3.5 dpc were treated daily with ICI 182,780, a pure estrogen receptor antagonist, by subcutaneous injection (ICI). Sesame oil injection was used as the control (C). Total RNA was extracted from the rat uterus at 5.5 dpc (48 h after the treatment). C) The pregnant rats at 3.5 dpc were ovariectomized and P4 was injected daily from 3.5 to 7.5 dpc. The rats for initiation of implantation (DI) were treated with E2 at 7.5 dpc. E2 was not administrated to the control group (C). Total RNA was extracted from the rat uterus at 8.5 dpc (24 h after E2 treatment). The expression of mRNA was normalized to the expression of Hprt measured in the same RNA preparation. The results are expressed as a ratio against the control and as means ± SEM (n = 3). Values with different superscripts in each panel are significantly different (P < 0.05).

**Fig. 5.** Effect of Hedgehog protein on the expression of Foxa2 in cultured endometrial epithelial cells. A) Endometrial epithelial cells were isolated from uterine horns at 1.5 dpc. The morphology of the cultured cells is shown with a phase contrast image (a). After fixation, cultured cells were stained with anti-Cytokeratin antibody (b), anti-Vimentin antibody (c) or without a first antibody (negative control) (d). The scale bar represents 100 μm. B) Cultured cells were treated with progesterone (P4), treated with recombinant Mouse Sonic Hedgehog N-terminus (H) or not treated (C). Total RNA was extracted after 24 h of treatment. The expression of mRNA was normalized to the expression of Hprt measured in the same RNA preparation. The results are expressed as a ratio against the control and as means ± SEM (n = 3). Values with different superscripts are significantly different (P < 0.05). C) Immunocytochemical detection of FOXA2 in the cells treated with Mouse Sonic Hedgehog N-terminus (H) or not treated (C). Total RNA was extracted from the rat uterus after 6 h (□) or 24 h (■) of the treatment. Values with different superscripts and different times of treatment. The results were compared individually for each time of treatment. The results in the OVX, ICI treatment and delayed implantation groups were compared with the control group (C). Total RNA was extracted from the rat uterus at 5.5 dpc (48 h after the treatment). C) The pregnant rats at 3.5 dpc were treated with progesterone (P4), 17β-estradiol (E2), a combination of P4 and E2 (P4 + E2) or vehicle (C, sesame oil). Total RNA was extracted from the rat uterus after 6 h (□) or 24 h (■) of the treatment. Values with different superscripts and different times of treatment. The results were compared individually for each time of treatment. The results of E2 treatment in OVX rats, its expression in E2-treated rats was significantly lower than those of the control and P4-treated groups after 24 h of treatment. This result suggested that Foxa2 expression was reduced by E2 action. These results were similar to a report in mice, which showed repression of Foxa2 by E2 injection in OVX mice [5]. To clarify the effect of E2 action on Foxa2 expression during pregnancy, further experiments using an antagonist of the estrogen receptor and delayed implantation model were carried out in the present study. Foxa2 expression in the rats treated with ICI, a selective antagonist of ERα, was significantly higher in comparison with control animals. Furthermore, since delayed implantation starts just after E2 treatment, it is assumed that Foxa2 expression must be downregulated in accordance with the estrogenic stimuli. Indeed the expression level of Foxa2 was lower in the implantation-induced rats treated with E2 compared with the level of the control group. The results in the OVX, ICI treatment and delayed implantation experiments clearly showed that Foxa2 expression in the rat uterus is decreased by E2 action. The profile of Foxa2 expression in this study is consistent with that of Gli1 [22], which is a signal transducer of the Hedgehog pathway. Gli1 expression transiently increases at 3.5 dpc and decreases at 5.5 dpc. Further, Gli1 expression in the rat uterus is decreased by E2 action [22]. The similarity of Foxa2 and
Gli1 expression profiles during early pregnancy suggests that Foxa2 is also one of the target genes of the Hedgehog signaling pathway. Further studies are required to elucidate the relationship between decreasing Foxa2 expression and embryo implantation; both are induced by E2 stimulation.

The results of the present study clearly showed the restraint action of E2 for the expression of Foxa2 in OVX rats, while P4 did not affect the expression level of Foxa2 until at least 24 h after treatment. Although we assumed that Foxa2 expression would be promoted by P4 treatment, the result was slightly unexpected. Two possible explanations for this condition are as follows: (1) The expression of Foxa2 in the OVX rat uterus might be higher under the influence of castration, resulting in masking of the effect of P4 action. In the OVX rats, secretion of the endogenous steroidal hormones disappears, including E2. Therefore, it would be predicted that the downregulation of Foxa2 expression caused by E2 might not occur in the OVX rat uterus. (2) It is possible that the processing time of P4 was not sufficient for elevation of Foxa2 expression. In the present study, we demonstrated that the Ihh protein induces expression of Foxa2 in rat endometrial epithelial cells cultured in vitro. It was previously reported that Indian hedgehog (Ihh), which is a unique Ihh protein expressed in the rat uterus, increased at 3.5 dpc [11]. It was also suggested that P4, which gradually increased from 1.5 dpc, indirectly promoted the expression of Ihh [23] after intermediation of the endometrial stroma cells [24]. Considering these results, if expression of Foxa2 was induced by Ihh in the OVX rat uterus such as in our current in vitro study, the processing time of 24 h after P4 administration might be too short to elevate Foxa2 expression. Further studies are required to elucidate the mechanism of Foxa2 expression in the uterus, including the mechanisms of P4 stimulation, endometrial stromal function and Ihh action.

It was further reported that expression of Foxa2 in the floor plate of the vertebrate neural tube was induced by Sonic hedgehog (Shh), which is a member of the Ihh proteins, derived from the notochord [10]. The results indicate the possibility that the Ihh proteins may induce Foxa2 expression also in the uterus. We assumed that Ihh would induce Foxa2 expression in the uterus. To test this hypothesis, we used a recombinant Ihh protein in the culture system of rat endometrial epithelial cells and performed an in vitro analysis. The results clearly demonstrated the effect of the Ihh protein on the elevation of Foxa2 expression in the rat endometrial epithelial cells. To demonstrate the relation between Ihh and Foxa2 expression in the uterus, an analysis of endogenous Ihh using a specific inhibitor or antibody for Ihh is necessary. It is suggested that the unique Ihh protein in the uterus, Ihh, might induce Foxa2 expression. The Ihh knockout mouse is known to be sterile based on results showing incompetence of implantation and decidualization [12]. Considering the expression of Foxa2 located downstream of the Ihh pathway, the cause of pregnancy imperfection in the Ihh knockout mouse might result from the depletion of Foxa2 function. Recently, a genome-wide investigation of FOXA2 binding target regions was performed in the murine uterus by chromatin immunoprecipitation coupled with massively parallel sequencing (ChIP-Seq) [25]. In that report, FOXA2-bound and GE-expressed genes were enriched for functional processes, including focal adhesion and WNT signaling. It is suggested that analysis of the uterine FOXA2 may provide novel insights into mechanisms governing endometrial gland development and function. Further studies are needed to understand the FOXA2-dependent network governing endometrial gland development and function for implantation.

In conclusion, the present study demonstrated the expression profile and localization of Foxa2 in the rat uterus. In addition, we also demonstrated that E2 downregulated the transient expression of Foxa2 and suggested the possibility that Ihh might promote the elevation of Foxa2 during early pregnancy.

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