Uterine and placental expression of TRPV6 gene is regulated via progesterone receptor- or estrogen receptor-mediated pathways during pregnancy in rodents

Bo-Mi Lee, Geun-Shik Lee, Eui-Man Jung, Kyung-Chul Choi and Eui-Bae Jeung*

Address: Laboratory of Veterinary Biochemistry and Molecular Biology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, 361-763, Republic of Korea

Email: Bo-Mi Lee - bongmae@naver.com; Geun-Shik Lee - geunshiklee@paran.com; Eui-Man Jung - jemman@hanmail.net; Kyung-Chul Choi - kchoi@cbu.ac.kr; Eui-Bae Jeung* - ebjeung@chungbuk.ac.kr

* Corresponding author

Abstract

Transient receptor potential cation channel, subfamily V, member 6 (TRPV6) is an epithelial Ca\textsuperscript{2+} channel protein expressed in calcium absorbing organs. In the present study, we investigated the expression and regulation of uterine and placental TRPV6 during gestation in rodents. Uterine TRPV6 peaked at pregnancy day (P) 0.5, P5.5 and, P13.5 and was detected in uterine epithelium and glands of rats, while placental TRPV6 mRNA levels increased in mid-gestation. Uterine and placental TRPV6 mRNA levels in rats appear to cyclically change during pregnancy, suggesting that TRPV6 may participate in the implantation process. In addition, uterine TRPV6 mRNA is only expressed in placenta-unattached areas of the uterus, and uterine TRPV6 immunoreactivity was observed in luminal and glandular epithelial cells. In the placenta, TRPV6 was detected in the labyrinth and spongy zone. These results may indicate that TRPV6 has at least two functions: implantation of the embryo and maintenance of pregnancy. To investigate the pathway(s) mediating TRPV6 expression in rodents, anti-steroid hormone antagonists were injected prior to maximal TRPV6 expression. In rats, TRPV6 expression was reduced by RU486 (an anti-progesterone) through progesterone receptors, and ICI 182,780 (an anti-estrogen) blocked TRPV6 expression via estrogen receptors in mice. The juxtaposition of uterine and placental TRPV6 expressed in these tissues supports the notion that TRPV6 participates in transferring calcium ions between the maternal and fetal compartments. Taken together, TRPV6 gene may function as a key element in controlling calcium transport in the uterus between the embryo and the placenta during pregnancy.

Background

Uterine calcium ions are considered to be a critical factor for smooth muscle contraction and embryo implantation. At the implantation stage, these ions may help in secreting uterine extra-cellular matrix components at the site of implantation, and during pregnancy and labor calcium ions may regulate uterine tension [1,2]. Despite these critical roles of calcium, the regulation of uterine calcium levels is not yet fully understood. In calcium absorbing organs, i.e., intestine and kidney which express several calcium processing proteins, the regulation and function of calcium ions and the associated genes are relatively well

Published: 21 May 2009
Received: 17 October 2008
Accepted: 21 May 2009

This article is available from: http://www.rbej.com/content/7/1/49

© 2009 Lee et al; licensee BioMed Central Ltd.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
understood. Calcium ions are processed via entering, transporting, and extruding proteins [3-5]. The transient receptor potential cation channel, subfamily V, member (TRPV) 5 and 6 were found in apical membranes of intestinal and renal epithelial cells, and are proposed as mediators for calcium uptake during trans-cellular transport [6]. Cytosolic calbindin-D9k and -28 k transport calcium ions from apical to basolateral layers, and these ions are finally extruded from the cell membrane via plasma membrane Ca2+ ATPase and sodium-calcium exchanger 1 [4,5].

In the past decade, uterine calcium-binding proteins have been studied to elucidate the function of uterine calcium ions, however their regulation and mechanisms of action are not completely resolved [7-9].

In female reproductive organs, TRPV6 is expressed in the uterus as well as in the placenta [10-12]. In calcium absorbing organs, TRPV6 expression is regulated by vitamin D, estrogen and dietary calcium. An active form of vitamin D increases duodenal calcium absorption, and abnormally low calcium absorption has been observed in vitamin D receptor-knockout mice [4,13]. Dietary calcium can also induce duodenal and renal TRPV6 mRNA expression [5,14], and estrogen therapy in menopausal women induces duodenal TRPV6 mRNA, suggesting that this hormone independently modulates TRPV6 expression [15].

TRPV6 of the female reproductive organs is robustly expressed; however its exact role is reproduction is not clearly understood. In the previous study, we reported that rat TRPV6 is expressed in the uterine endometrium and glandular endometrium [11,12] and is up-regulated at diestrus in matured animals and by progesterone supplementation in immature or ovariectomized animals [11]. Recently, the physiological significance of TRPV6/Ca2+ channel in maternal-fetal Ca2+ transport was investigated using TRPV6 knockout mice [16], demonstrating that Ca2+ concentration in fetal blood and amniotic fluid was significantly lower and the transport activity of radioactive Ca2+ from mother to fetuses was 40% lower in TRPV6 KO fetuses than in WT. Progesterone appears to be a dominant regulator of TRPV6 via progesterone receptor activation [11]. In a mouse model, uterine TRPV6 expression varied during estrous cycles, however it was dominantly observed at the estrous stage [12]. In addition, estrogen seemed to be a major factor in the regulation of uterine TRPV6 transcription via estrogen receptor α pathway [12]. However, the role of TRPV6 in the implantation process during pregnancy is not yet determined.

Thus, in the present study, we employed a rat model to examine the expression of TRPV6 mRNA in the uterus and placenta during pregnancy. In addition, we investigated whether sex hormones regulate uterine TRPV6 expression in rats and mice during pregnancy, using antagonists for estrogen and progesterone. Finally, we further determined the localization of TRPV6 protein in the uterus and placenta to elucidate the role of this protein during pregnancy.

Methods

Animals and treatments

Sprague-Dawley rats (male, 12-week-old; female, 10-week-old) and pregnant ICR mice (12 to 15-week-old) were obtained from KOATECH (Pyeongtaek, Gyeonggi, Korea). All animals were housed in polycarbonate cages, and acclimatized to an environmentally controlled room before experimentation (temperature, 23 ± 2°C; relative humidity, 50 ± 10%; frequent ventilation and 12 h light cycle). Experiments were performed with the approval of the Animal Ethics Committee at the College of Veterinary Medicine, Chungbuk National University. Adult female rats were mated with adult males overnight [17], then examined the following morning for the presence of a vaginal plug. This time point was designated as day 0.5 of pregnancy (P0.5) when the morning a plug is found. The rats (n = 3 per group) were euthanized on each day of pregnancy (P0.5 to P21.5).

Pregnant rats (P4.5 for uterine RNA preparation, P19.5 for placental RNA preparation) received subcutaneous injections of RU486 (RU, 2.5 mg per rat; Sigma-Aldrich Corp, St. Louis, MO), ICI 182,780 (ICI, 0.5 mg per rat; Tocris, Eslisvill, Missouri, USA) or 50% ethanol as a vehicle [11,18]. In addition, ICR mice at P9.5 were injected with RU (25 μg per mouse) or ICI (2 μg per mouse) [19]. The chemicals were dissolved in 0.2 ml ethanol and saline mixture (1:1), and 0.2 ml and 0.05 ml were administered to rats and mice, respectively. The pregnant rats were treated with the antagonists one day before TRPV6 mRNA is maximally expressed depending on the tissues and species [12].

Total RNA extraction and reverse transcriptase (RT)-PCR

Animals were euthanized, and the uteri and placentas were rapidly excised and washed in cold sterile saline (0.9% NaCl). Total RNA was prepared with TRIzol reagent (Invitrogen Life Technologies, Inc., Carlsbad, California, USA), and the concentration was determined by measuring light absorbance at 260 nm. Before RT-PCR, total RNA samples were electrophoresed on 1% formaldehyde denaturing agarose gels to validate the quality and purity. RT-PCR was performed, and the resulting products were visualized by agarose gel electrophoresis as described previously [20]. In brief, total RNA (1 μg) was reverse transcribed to first strand complementary DNA (cDNA) using mMLV reverse transcriptase (Invitrogen Life Technologies, Inc.) and random primers (9 mers; TaKaRa Bio, Inc., Otsu, Shiga, Japan). TRPV6 and IA (cytochrome oxidase subunit I, a house keeping gene) were amplified in a
20 μl PCR reaction containing 1 U Taq polymerase (iNtRON Bio Inc, Sungnam, Kyungki-Do, Korea), 1.5 mM MgCl₂, 2 mM dNTP, and 50 pmol TRPV6- or 1A specific primers [12]. The oligonucleotide sequences for TRPV6 were 5'-GTT CTC GGT GCC ATC TAC GT-3' (sense) and 5'-CAA TGA CAT GGA ATG GCC CGG-3' (antisense). The primer sequences for 1A were 5'-CCA GGG TTT GGA ATT TC-3' (sense) and 5'-GAA GAT AAA CCC TAA GGC ATT TC-3' (antisense). PCR reactions were denatured at 95°C for 30 s, annealed at 60°C for 30 s and extended at 72°C for 45 s. TRPV6 and 1A were quantified after 25 and 18 cycles, respectively. PCR products (10 μl) were separated on a 2% agarose gel, stained with ethidium bromide, and photographed under UV illumination. Photographs were taken using a Gel Doc EQ (Bio-Rad, Hercules, California, USA).

Real-time PCR using TaqMan™ probe

Real-time PCR was performed in 20 μl reactions containing 10 μl TaqMan Universal PCR Master Mix (Applied Biosystems, Foster, CA, USA), and 1 μl of 20× Assays-on-Demand™ Gene Expression Assay Mix (Applied Biosystems; rat TRPV6, Rn00586673_m1 [Assay ID in the Applied Biosystems]; mouse TRPV6, Mm00499069_m1; rat HPRT1, Rn01527840_m1; mouse HPRT1, Mm00446968_m1) and 2 μl cDNA. PCR amplification was conducted using a 7300 Real-Time PCR System (Applied Biosystems), with initial enzyme activation at 50°C for 2 min, followed by 90°C for 10 min. Each of 40 amplification cycles consisted of denaturation at 95°C for 15 sec, followed by annealing and extension at 60°C for 1 min. Relative expression levels for each sample were determined using RQ software (Applied Biosystems). The expression of TRPV6 was normalized relative to that of HPRT1 [21,22].

Immunohistochemistry

The tissue localization of TRPV6 protein was examined by immunohistochemistry by using Vectastain Universal Elite ABC Kit (Cat. No. PK-6200, Vactor Laboratories, Inc., Burlingame, California, USA) as previously described [12]. The uterus from P5.5 to P 10.5 and placenta from P11.5 to P13.5 were embedded in paraffin. Sections (5 μm) were deparaffinized in xylene and hydrated in descending grades of ethanol. TRPV6 staining involved immersion of the uterus and placenta sections in boiled citrate target retrieval buffer (0.01 M sodium citrate and 0.01 M citric acid, pH 6.0) at 100°C for 5 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in PBS-T (PBS containing 0.05% tween20) for 30 min. Incubation of the section in 10% normal goat serum (NGS) for 2 h at room temperature blocked nonspecific reaction. Sections were subsequently incubated with a polyclonal rabbit antibody specific to TRPV6 (#ACC-036, 0.8 mg/ml, diluted 1:100; Alomone Labs Ltd., Jerusalem, Israel) dissolved in 10% NGS, at room temperature for 2 h. A negative control was incubated with in 10% normal serum for 2 h at room temperature. After washing with PBS-T, the sections were incubated with biotinylated secondary antibody (anti-rabbit IgG; Vector Laboratories, Inc.) for 30 min at 37°C and further incubated with FAST™ 3,3′-Diaminobenzidine Tablets (#D4293, Sigma Aldrich) for 30 min at 37°C. The sections were counterstained with hematoxylin followed by mounting with a coverslip.

Data analysis

Data were analyzed by nonparametric one-way analysis of variance using the Kruskal-Wallis test, followed by Dunnnett’s test for multiple comparisons to vehicle. All statistical analyses were performed with SPSS for Windows Edition (SPSS, Chicago, Illinois, USA). p < 0.05 was considered statistically significant.

Results

Expression of TRPV6 mRNA in the uterus and placenta during pregnancy

The expression of rat TRPV6 mRNA during pregnancy was examined by RT- and real-time PCR. The uteri were collected from rats at P0.5 to P21.5, and fetuses were removed. TRPV6 mRNA was moderately expressed at P0.5, decreased through P4.5, and then suddenly increased to the highest level at P5.5 (Figure 1). Following

![Figure 1](http://www.rbej.com/content/7/1/49)
this at mid-gestation, TRPV6 expression was moderate and then increased again at P13.5. From P12.5 to P21.5, the expression of TRPV6 in the placenta steadily increased and expressed at the highest level at P20.5 in rats (Figure 2).

**Localization of TRPV6 protein in the uterus and placenta**

The spatial distribution of TRPV6 protein in the rat uterus and placenta was investigated by using an anti-TRPV6 antibody, respectively, when its transcripts were shown to be expressed at relatively high levels. The rat uterus was longitudinally sectioned. TRPV6 protein was detected on the endometrial layer and glandular epithelium of the gestating uterus. TRPV6 was observed on the glandular epithelial apical layer but not on the basolateral membrane, implying that this protein may control luminal calcium ion transport (Figure 3). In addition, we observed that the site of implantation was marked by swollen uterine tissue (Figure 3). The site was strongly stained by anti-TRPV6 antibodies, supporting the notion that early embryo settlement might require the expression of TRPV6 protein (Figure 3). Between swollen areas in the rat uterus (Figure 3), TRPV6 was observed in the endometrial apical layer, consistent with our previous reports [11,12].

TRPV6 protein was detected throughout the rat placenta as seen in Figure 4. The placenta was separated from fetus at P20.5 and the tissue was crossly sectioned. Three areas were probed for TRPV6 expression: the inner and middle labyrinth layers, and the spongy outer layer (Figure 4A, B and 4C). Although the tissue layer on the embryo-attached-side dominantly expressed TRPV6 protein, the middle labyrinth area was also shown to express this protein. In the outer spongy layer containing giant cells, TRPV6 immuno-positive cells were also detected as demonstrated in Figure 4C.

In a time-dependent manner, uterine TRPV6 was mainly detected in the epithelial and glandular cells of the non-attached uteri of rats (Figure 5A). The TRPV6 positive cells were observed on the attached uterus, while modest TRPV6 signal was shown in the placental-like structure regarded as the implantation site (Figure 5A). In addition, TRPV6 positive cells were broadly observed through the placenta containing the labyrinth and spongy zones in a time-dependent manner (Figure 5B). The strong signals of TRPV6 protein were detected on the fetal membranes as shown.

**Effects of sex steroid receptor antagonists on uterine and placental TRPV6 mRNA in a rat and mouse model**

Recently, uterine TRPV6 was shown to be regulated by progesterone in immature and non-pregnant rats [11], whereas its expression in a mouse model was shown to be controlled by estrogen [12]. To clarify which hormone mediates uterine TRPV6 expression, the rats and mice were treated with a single treatment of RU or ICI to invoke progesterone and estrogen receptor antagonism conditions, respectively. In the uterus at P5.5 (maximum expression levels), RU treatment resulted in a significant inhibition of TRPV6 mRNA expression in the rat uterus. In addition, ICI significantly reduced TRPV6 transcription in this tissue (Figure 6A). In the rat placenta, hormonal regulation was tested at P19.5 after a single treatment with RU or ICI. Treatment with RU significantly down-regulated the expression of placental TRPV6 at P20.5, while ICI did not alter its expression in the rat placenta (Figure 6B), suggesting that placental TRPV6 transcription in gestating rats may be controlled by progesterone and its receptors.

Previously, we reported that uterine TRPV6 transcription in mice was regulated by estrogen via estrogen receptor α [12], but its placental regulation during pregnancy has not been investigated. In the previous study, steroid hormone-induced regulation of murine TRPV6 transcription was shown by using ICI or RU at P10, a point in gestation when TRPV6 was previously reported to be highly expressed [12]. As shown in Figure 6C, treatment with ICI significantly reduced uterine TRPV6 expression, whereas RU did not affect uterine TRPV6 transcription levels in the mouse uterus. In addition, TRPV6 gene was significantly down-regulated by ICI, while RU did not alter its mRNA levels in the mouse placenta (Figure 6D). Taken together,
these data indicate that uterine and placental TRPV6 expression may be primarily regulated by estrogen during non-pregnancy and pregnancy in mice. This regulation of TRPV6 expression by estrogen in mice is in contrast to progesterone-mediated regulation in rats.

**Discussion**

Several uterine calcium-regulating genes are alternatively expressed during estrous cycle and pregnancy [23]. In the implantation period, calcium-binding proteins appear to play a central role [8,12,24-33]. Calbindins are required during the early phase of embryo implantation, implying that the regulation of calcium availability in the vicinity of the implanting embryo is critical for successful implantation [8,9]. Although the importance of calbindins in implantation has been established, the precise role of calcium ions during implantation remains unclear. Other calcium processing genes have recently been identified in female reproductive organs, and TRPV6 is an interesting protein in elucidating uterine process of calcium [11,12,34].

Uterine expression of TRPV6 varies during the estrous cycle in rodents. For instance, rat TRPV6 transcripts in the uterus were highly expressed at diestrus [11], while mouse TRPV6 mRNA was highly expressed at estrus [12]. Distinct their expression patterns indicate that uterine TRPV6 is differentially regulated during the estrous cycle in rats and mice. During pregnancy, mouse TRPV6 mRNA is actively expressed with maximal expression observed in the middle of gestation, followed by a reduction in the late period. Immediately prior to birth, another high level of TRPV6 expression is observed, which disappears during lactation [12]. In the present study, rat uterine TRPV6 was modestly expressed at P0.5, which corroborates previous reports; and this gene was up-regulated by progesterone at...
the diestrous stage in a rat model [11]. In days after P0.5, TRPV6 expression gradually decreased and was undetectable at P4.5. However, at P5.5 a substantial increase and peak in TRPV6 expression was observed. This time point coincides with the earliest sign of the implantation attachment reaction which occurs around P4 or P5 in rodents [35]. Therefore, the maximal expression of TRPV6 gene at P5.5 may be related to the regulation of uterine calcium ion concentration required for successful implantation.

Calbindin-D9k, another uterine calcium associated protein, gradually increases at pregnancy day 16.5, peaks at day 18.5, and declines at birth and the beginning of lactation [17]. A comparison of uterine TRPV6 and calbindin-D9k time-dependent expressions in pregnant rats suggests that these proteins may share in the function for maintaining uterine calcium concentrations during pregnancy.

In mice, uterine calbindin-D9k showed a gradual increase in mRNA expression during late pregnancy (from day 12.5 to 18.5), followed by a decline at birth and during lactation [17]. It has also been reported that serum estrogen levels increase at the end of pregnancy and decline sharply following birth and during lactation, whereas serum progesterone levels remain constant throughout late gestation and lactation [17]. With regard to these studies, it is possible that increased serum estrogen levels induce uterine TRPV6 transcription at birth and during pregnancy, and that TRPV6 ceases during lactation when secreted estrogen is no longer present. It was previously shown that mouse TRPV6 expression in the placenta initially follows the pattern of uterine expression, with an induction in the middle of gestation (P10.5 and P14.5) but not at the end of gestation [12]. TRPV6 seems to have a positive influence on transplacental calcium ion concentrations and to be a crucial factor in fetal development, because it is highly expressed in human placenta as compared to levels in other organs [36]. In the present study, placental expression of TRPV6 gene steadily increased from P12.5 to P20.5 before labor in rats. Taken together, these results imply that constitutive expressions of TRPV6 and/or other calcium processing genes may play a crucial role in transporting calcium ions from maternal to fetal.
Figure 5
Spatial expression of uterine (A) and placental (B) TRPV6 expression in a time-dependent manner. A, Rat uteri from P5.5 to P10.5 were separately shown into the non-attached or attached uteri using anti-TRPV6 serum. u, attached uterus; f, placenta-like structure; arrows, epithelial and glandular cells. B, Rat placentas from P11.5 to P13.5 were presented in a time-dependent manner. la, labyrinth zone; sp, spongy zone; gc, giant cells; arrows, fetal membrane.
Effects of steroid receptor antagonists on uterine and placental TRPV6 mRNA expressions. Panel A (uteri at P5.5) and B (placenta at P20.5) presented the rat TRPV6 mRNA levels. Four groups of pregnant rats (n = 4 per group) were treated with ethanol as a negative control (VE), progesterone receptor antagonist (RU, 2.5 mg per rat), or estrogen receptor antagonist (ICI, 0.5 mg per mouse). Panel C (uteri at P10.5) and D (placenta at P10.5) showed the mouse TRPV6 mRNA expressions. Four groups of pregnant mice (n = 4 per group) were treated with ethanol as a negative control (VE), progesterone receptor antagonist (RU, 25 μg per mouse) or estrogen receptor antagonist (ICI, 2 μg per mouse). Murine TRPV6 mRNA levels were examined by real-time PCR. The bar graph represents the analysis of real-time PCR data expressed as a percentage of TRPV6/HPRT1 (mean ± SEM of duplicates). a, statistically significant compared to a vehicle (P < 0.05).
TRPV6 is involved in maternal-fetal Ca\(^{2+}\) transport, posing that TRPV6 functions as a Ca\(^{2+}\) entry pathway, which is critical for fetal Ca\(^{2+}\) homeostasis [16]. In addition, a phenotype of mice with targeted disruption of TRPV6 gene was reported. TRPV6 knockout (KO) mice showed disordered Ca\(^{2+}\) homeostasis and reduced fertility, and deficient maternal-fetal transfer of calcium ions caused abnormal calcium ion deficiency during embryonic development [38]. In this study, placental TRPV6 expression is expected to enhance the rate of maternal transfer of calcium ions to the fetus during pregnancy [38]. In the present study, the expression of placental TRPV6 mRNA in rats was blocked by RU treatment, but no significant inhibitory effect was observed for ICI treatments, suggesting that placental TRPV6 expression is under the sole control of progesterone via a progesterone receptor-mediated pathway. However we cannot rule out the possibility that the difference in their doses may account for the observed difference between rats and mice.

In contrast to rats, it was previously reported that murine uterine TRPV6 transcription at normal estrous cycles was totally dependent on estrogen [12]. Uterine TRPV6 mRNA levels increased at mid and late pregnancy, and were strongly induced at mid-pregnancy in the labyrinth and spongy zone of the placenta, and in the fetal membrane [12]. To examine an expression pattern of TRPV6 transcripts by steroids compared to its level in rats during pregnancy, we treated the mice with steroids, E2 and P4, and isolated the identical tissues from them to measure TRPV6 levels. In the present study, sex steroids controlled uterine and placental TRPV6 expression during pregnancy. The expression of uterine TRPV6 mRNA at P10 was blocked by ICI, and placental TRPV6 transcription was blocked by ICI, suggesting that the expression of TRPV6 was primarily regulated by estrogen in gestating uterus and placenta during pregnancy in mice. These results suggest that TRPV6 may be distinctly regulated by different sex steroids, progesterone in rats and estrogen in mice, in spite of shared similarities in their chromosomal make-up [12,20,39].

These results show that uterine and placental TRPV6 mRNA levels in rats appear to cyclically change during pregnancy, suggesting that TRPV6 protein may participate in the implantation process. It was of interest to note that uterine TRPV6 mRNA is only expressed in placenta-unattached areas of the uterus, indicating that TRPV6 has at least two functions: implantation of the embryo and maintenance of pregnancy. In rats, TRPV6 transcription appeared to be solely controlled by P4 through PRs, while its level was regulated by E2 via ERs in mice. In both rodents, uterine TRPV6 immunoreactivity was observed in luminal and glandular epithelial cells, and in the placenta TRPV6 was detected in the labyrinth and spongy zone. The juxtaposition of uterine and placental TRPV6 expressed in these tissues can support a novel concept that TRPV6 participates in transporting calcium ions between the maternal and fetal compartments. In conclusion, TRPV6 gene may function as a key element in controlling calcium transport in the uterus between the embryo and the placenta during pregnancy.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
BL carried out the overall experiments including animal treatments and molecular experiments. GL carried out the animal treatments and molecular experiments with BL, and drafted the manuscript. EJ participated in the real-
References

1. Wang J, Mayernik L, Arment DR: Integumentary signaling regulates blastocyst adhesion to flask uterine epithelium: intracellular calcium transients and vesicle trafficking in primary trophoblast cells. Developmental biology 2002, 245(2):270-279.

2. Wang J, Mayernik L, Arment DR: Trophoblast adhesion of the peri-implantation mouse blastocyst is regulated by integrin signaling that targets phospholipase C. Developmental biology 2007, 302(1):143-153.

3. Hoenderop JG, Nilius B, Bindels RJ: Epithelial calcium channels: from identification to function and regulation. Pflugers Arch 2003, 446(3):304-309.

4. van Abeelen Huybers S, Hoenderop JG, Kem AW van der, van Leeuwen JP, Bindels RJ: Age-dependent alterations in Ca2+ homeostasis: role of TRPV5 and TRPV6. Am J Physiol Renal Physiol 2006, 291(6):F177-183.

5. Van Cromphaut S, Rummens K, Stockmans I, Van Herck E, Djicka FA, Edewein AG, Carmeliet P, Verhaeghe J, Bouillon R, Carmeliet G: Intestinal calcium transporter genes are upregulated by estrogens and the reproductive cycle through the expression of vitamin D receptor-independent mechanisms. J Bone Miner Res 2003, 18(10):1725-1736.

6. den Dekker E, Hoenderop JG, Nilius B, Bindels RJ: The epithelial calcium channels, TRPV5 & TRPV6: from identification to function. Mol Cell Endocrinol 1992, 88(1-3):119-128.

7. Loo KC, Nie GY, Samalonsen LA. Endometrial calbindins are critical for embryo implantation: evidence from in vivo use of morpholino antisense oligonucleotides. Proc Natl Acad Sci USA 2004, 101(21):8028-8033.

8. Mathieu CL, Burnett SH, Mills SE, Overpeck JG, Bruns DE, Bruns ME: Gestational changes in calbindin-D9k in rat uterus, yolk sac, and placenta: implications for maternal-fetal calcium transport and uterine muscle function. Proc Natl Acad Sci USA 1989, 86(9):3433-3437.

9. Bernucci L, Henriquez M, Diaz P, Riquelme G: Diverse calcium channel types are present in the human placental syncytiotrophoblast basal membrane. Placenta 2006, 27(1-2):1082-1095.

10. Kim HJ, Lee GS, Ji YK, Choi KC, Jeung EB: Differential expression of uterine calcium transporter I and plasma membrane Ca2+ ATPase Iib during rat estrous cycle. Am J Physiol Endocrinol Metab 2006, 291(2):E234-241.

11. Lee GS, Jeung EB: Uterine TRPV6 expression during the estrous cycle and pregnancy in a mouse model. Am J Physiol Endocrinol Metab 2007, 293(1):E123-138.

12. Bailey NF, Howard A, O’Callaghan D, Legon S, Walters JR: Epithelial calcium transporter expression in human duodenum. Am J Physiol Gastrointest Liver Physiol 2001, 280(2):G285-290.

13. Van Cromphaut S, Dewerchin M, Hoenderop JG, Stockmans I, Van Herck E, Kato S, Bindels RJ, Collen D, Carmeliet P, Bouillon R, Carmeliet G: Duodenal calcium absorption in vitamin D receptor-knockout mice: functional and molecular aspects. Proc Natl Acad Sci USA 2001, 98(23):13234-13239.

14. Weber K, Erber I, Rump A, Adamski J: Gene structure and regulation of the murine epithelial calcium channels ECaC1 and 2. Biochem Biophys Res Commun 2001, 289(5):1287-1294.

15. Suzuki Y, Kovacs CS, Takanaga H, Peng JB, Landowski CP, Hediger MA: Calcium channel TRPV6 is involved in murine maternal-fetal calcium transport. Bone Miner Res 2000, 15(2):E187-196.

16. An BS, Choi KC, Kang SK, Lee GS, Hong EJ, Hwang WS, Jeung EB: Mouse calbindin-D(9k) gene expression in the uterus during late pregnancy and lactation. Mol Cell Endocrinol 2003, 205(1-2):79-86.

17. Fujik X, Yang S, Mitchell BF: Effects of RU486 on estrogen, progesterone, oxytocin, and their receptors in the rat uterus during late gestation. Endocrinology 1997, 138(7):2763-2768.

18. Ji YK, Lee GS, Choi KC, Jeung EB: Anti-progestogenic effect of flutamide on uterine expression of calbindin-D9k mRNA and protein in immature mice. Reprod Toxicol 2006, 22(4):69-701.

19. Lee KY, Oh GT, Kang JH, Shin SM, Heo BE, Yun YW, Paik SG, Krisinger J, Leung PC, Jeung EB: Transcriptional regulation of the mouse calbindin-D9k gene by the ovarian sex hormone. Mol Cells 2003, 16(1):48-53.

20. van Abel M, Huybers S, Hoenderop JG, Kemp AW van der, van Leeuwen JP, Bindels RJ: Age-dependent alterations in Ca2+ homeostasis: role of TRPV5 and TRPV6. Am J Physiol Renal Physiol 2006, 291(6):F177-183.

21. Simmen RC, Simmen FA: Progesterone receptors and Sp1/Sp3 element-mediated family members in the uterine endometrium. Front Biosci 2002, 7d:1556-1565.

22. An BS, Choi KC, Kang SK, Hwang WS, Jeung EB: Novel Calbindin-D(9k) protein as a useful biomarker for environmental estrogenic compounds in the uterus of immature rats. Reprod Toxicol 2003, 17(3):311-319.

23. An BS, Kang SK, Shin JH, Jeung EB: Stimulation of calbindin-D(9k) mRNA expression in the rat uterus by ocyt-phenol, nonylphenol and bisphenol. Mol Cell Endocrinol 2002, 191(3):177-186.

24. Salamonsen LA, Simmen RC, Simmen FA: Progesterone receptors and Sp1/Sp3 element-mediated family members in the uterine endometrium. Front Biosci 2002, 7d:1556-1565.

25. An BS, Choi KC, Kang SK, Hwang WS, Jeung EB: Mouse calbindin-D(9k) gene expression in the uterus during late gestation. Mol Cell Endocrinol 2003, 212(1-2):63-72.

26. Hong EJ, Choi KC, Jeung EB: Induction of calbindin-D9k messenger RNA and protein by maternal exposure to alkylphenols during late pregnancy in maternal and neonatal uteri of rats. Biol Reprod 2004, 71(2):669-675.

27. Hong EJ, Choi KC, Jung YW, Leung PC, Jeung EB: Transfer of maternally injected endocrine disruptors through breast milk during lactation induces neonatal Calbindin-D(9k) in the rat model. Reprod Toxicol 2004, 18(5):661-668.

28. Lee GS, Kim HJ, Jung YW, Choi KC, Jeung EB: Estrogen receptor alpha pathway is involved in the regulation of Calbindin-D9k in the uterus of immature rats. Toxicol Sci 2005, 84(2):270-277.

29. Nguyen TH, Lee GS, Ji YK, Choi KC, Lee CK, Jeung EB: A calcium binding protein, calbindin-D9k, is mainly regulated by estrogen in the pituitary gland of rats during estrous cycle. Brain Res Mol Brain Res 2005, 141(2):166-173.

30. Yan SM, Choi KC, Kim IH, An BS, Lee GS, Hong EJ, Oh GT, Jeung EB: Dominant expression of porcine Calbindin-D9k in the uterus during a luteal phase. Mol Reprod Dev 2004, 69(3):251-256.

31. Moeure L, Hamel A, Daoud G, Simonneau L, Lafond J: Expression of calcium channels along the differentiation of cultured trophoblast cells from human term placenta. Biol Cells 2002, 75(5):1473-1479.

32. Dey SK, Lim H, Das SK, Reese J, Pari BA, Daikoku T, Wang H: Molecular cues to implantation. Endocr Rev 2004, 25(3):341-373.

33. Peng JB, Brown EM, Hediger MA: Epithelial Ca2+ entry channels: transcellular Ca2+ transport and beyond. J Physiol 2003, 551(Pt 1):729-740.

34. An BS, Choi KC, Lee GS, Leung PC, Jeung EB: Complex regulation of Calbindin-D(9k) in the mouse placenta and extra-embry...
38. Bianco SD, Peng JB, Takanaga H, Suzuki Y, Crescenzi A, Kos CH, Zhuang L, Freeman MR, Gouveia CH, Wu J, Luo H, Mauro T, Brown EM, Hediger MA: Marked disturbance of calcium homeostasis in mice with targeted disruption of the Trpv6 calcium channel gene. J Bone Miner Res 2007, 22(2):274-285.

39. Lee GS, Choi KC, Kim HJ, Jeung EB: Effect of genistein as a selective estrogen receptor beta agonist on the expression of Calbindin-D9k in the uterus of immature rats. Toxicol Sci 2004, 82(2):451-457.