Participation of the oviductal s100 calcium binding protein G in the genomic effect of estradiol that accelerates oviductal embryo transport in mated rats

Mariana Ríos1, Alexis Parada-Bustamante1, Luis A Velásquez2,3, Horacio B Croxatto2,3,4 and Pedro A Orihuela2,3*

Abstract

Background: Mating changes the mechanism by which E2 regulates oviductal egg transport, from a non-genomic to a genomic mode. Previously, we found that E2 increased the expression of several genes in the oviduct of mated rats, but not in unmated rats. Among the transcripts that increased its level by E2 only in mated rats was the one coding for an s100 calcium binding protein G (s100 g) whose functional role in the oviduct is unknown.

Methods: Herein, we investigated the participation of s100 g on the E2 genomic effect that accelerates oviductal transport in mated rats. Thus, we determined the effect of E2 on the mRNA and protein level of s100 g in the oviduct of mated and unmated rats. Then, we explored the effect of E2 on egg transport in unmated and mated rats under conditions in which s100 g protein was knockdown in the oviduct by a morpholino oligonucleotide against s100 g (s100 g-MO). In addition, the localization of s100 g in the oviduct of mated and unmated rats following treatment with E2 was also examined.

Results: Expression of s100 g mRNA progressively increased at 3-24 h after E2 treatment in the oviduct of mated rats while in unmated rats s100 g increased only at 12 and 24 hours. Oviductal s100 g protein increased 6 h following E2 and continued elevated at 12 and 24 h in mated rats, whereas in unmated rats s100 g protein increased at the same time points as its transcript. Administration of a morpholino oligonucleotide against s100 g transcript blocked the effect of E2 on egg transport in mated, but not in unmated rats. Finally, immunoreactivity of s100 g was observed only in epithelial cells of the oviducts of mated and unmated rats and it was unchanged after E2 treatment.

Conclusions: Mating affects the kinetic of E2-induced expression of s100 g although it not changed the cellular localization of s100 g in the oviduct after E2. On the other hand, s100 g is a functional component of E2 genomic effect that accelerates egg transport. These findings show a physiological involvement of s100 g in the rat oviduct.

Background

In mammals, the transport of the gametes, fertilization or preimplantation development depends on the oviduct functions [1]. In this context, the participation of ovarian hormones estradiol (E2) or progesterone (P) is crucial for the successful of these events. Oviduct malfunction may result in tubal ectopic pregnancy that still remains a potentially life-threatening condition in women [2]. Therefore, elucidation of the cellular and molecular mechanisms by which E2 and P regulate oviductal egg transport are essentials to fully understand the physiology of the tubal function.

In the rat, the duration of oviductal egg transport is dependent on ovarian hormones and mating-associated signals [3-5]. A single injection of E2 on day 1 of the cycle (unmated) or pregnancy (mated) shortens oviductal transport of eggs from the normal 72-96 h to less than 24 h [4,6]. However, E2 accelerates egg transport to

* Correspondence: pedro.orihuela@usach.cl
1Laboratorio de Inmunología de la Reproducción, Facultad de Química y Biología, Universidad de Santiago de Chile, Chile
Full list of author information is available at the end of the article
the uterus through intraoviductal genomic pathways in mated rats and through nongenomic pathways in unmated rats [3,7]. Interestingly, both pathways require activation of estrogen receptors (ER) [8,9]. The intraoviductal nongenomic-signalling pathway of E2 has been well established and involves conversion of E2 to 2-methoxyestradiol (2ME) and sequential activation of cAMP-PKA and PLC-IP3 signalling pathways [5,8,10]. In contrast, little is known about the components of the E2 genomic signaling that accelerates embryo transport in the rat. Early works have shown that antagonists of Endothelin receptor type A or B inhibited the effect of E2 on embryo transport [11] while Connexin 43 uncouplers blocked the increase in the instant velocity of microsphere movement induced by E2 in mated rats [12]. Thus, the E2 genomic pathway involves participation of endothelin receptors (ETR) and functional integrity of gap junctions in the oviduct.

Previously, we have found by microarray analysis that E2 increased the expression of several genes in the oviduct of mated rats, but not in unmated rats [13]. These genes were s100 calcium binding protein G (s100 g), adenosine monophosphate deaminase 3 (Ampd3), cysteine rich protein 61(Cyr61) and tumour necrosis factor induced protein (Tnflp6) that are involved in remodeling of extracellular matrix, regulation of energetic metabolism or calcium transport in normal tissues or tumour cells [14-16]. In order to identify whether these genes are functional components of the E2 genomic pathway that accelerates embryo transport in mated rats, we first compared the mRNA level of these four transcripts between oviducts of unmated and mated rats treated with E2. We reasoned that genes whose level of expression increases in response to E2 in mated rats, but not in unmated rats are good candidates to further exploration. The results oriented us towards a possible involvement of s100 g transcript previously described as Calbindin-D9k. s100 g belongs to a family of intracellular proteins having high affinity for calcium and is expressed in a variety of mammalian tissues, i.e., intestine, uterus, kidney and bone [17-19] including the female reproductive tract [20,21]. Furthermore, estrogens up-regulates the expression of the mRNA [18] and protein [18] of s100 g in the uterus. Moreover, s100 g may control motile activity of smooth muscle through regulation of intracellular calcium level in the rat uterus [18]. Therefore, we investigated the participation of s100 g on the E2 genomic effect that accelerates oviducal embryo transport. For this purpose, we determined the time-course of the effect of E2 on the mRNA and protein level of s100 g in the oviduct of mated and unmated rats. Then, we explored the effect of E2 on egg transport in unmated and mated rats under conditions in which s100 g protein was knockdown in the oviduct by a morpholino oligonucleotide against s100 g (s100 g-MO). Finally, the localization of s100 g in the oviduct of mated and unmated rats following treatment with E2 was also examined.

Methods

Animals

Locally bred Sprague-Dawley rats were used. Animals were kept under controlled temperature (21-24°C) and lights were on from 07:00 to 21:00 h. Water and pelleted rat chow was supplied ad libitum. Daily vaginal smears were used to verify cycle regularity [22]. Females weighing 200–220 g were selected from among those having 4-day estrous cycles. Females in proestrus were either kept isolated or caged with fertile males. The following day (estrus) was designated as Day 1 of the cycle (C1) in the first instance and Day 1 of pregnancy (P1) in the second, provided spermatozoa were found in the vaginal smear of the later. The protocols on animal manipulation have been approved by the Ethical Committees of our National Fund of Science (CONICYT-FONDECYT 1080523).

Treatments

Systemic administration of E2

Rats on C1 or P1 were injected s.c. with 10 μg of E2 as a single dose dissolved in 0.1 mL of propylene glycol. Control rats received propylene glycol as the vehicle.

Local administration of morpholinos (MOs)

The following MOs were used: s100 g-MO, 5’CGC TCA TTT TTC TGT GCT GCT TGC T 3’; an irrelevant MO (standard control MO) 5’CCT TTT ACC TCA GTT ACA ATT TAT A 3’; and a Fluorescein-labeled standard control MO (FITC-MO). All MO were purchased from Gene Tools, LLC, Philomarsh, OR. The MOs were prepared at a stock concentration of 10 mM in sterile water. For intraoviductal administration (i.o.), 0.2 μl of Endo-Porter (non-toxic delivery mechanism from Gene Tools) was mixed with 2.8 μl of MO stock (28 nmoles). The MO/Endo-Porter mixture was vortexed and incubated for 20 min at room temperature, immediately prior to injection into each oviduct.

Animal surgery

Intraoviductal administration of MOs was done in the morning of C1 or P1 using a surgical microscope (OPMI 6-SDFC; Zeiss, Oberkochen, Germany) as previously described [3,7]. Since ovulation was completed at this time point, this treatment did not affect the number of oocytes that ovulated.

Assessment of egg transport

Animals were euthanized 24 hours after different treatments, and their oviducts were flushed individually with saline. Each flushing was examined under low-power
magnification (25×). The number of eggs in both oviducts was recorded as a single datum. Attempts to recover eggs from the uterus and vagina with or without placing ligatures in the uterine horns have shown that the reduction in the number of oviductal oocytes following treatment with E2 corresponds to premature transport to the uterus [6]. Thus, we refer to it as E2-induced acceleration of oviductal transport.

Analysis of MO Uptake
Animals were euthanized 5 hours after i.o. administration of FITC-MO or standard control MO and their oviducts were excised and frozen in Tissue Freezing Medium (Electron Microscopy Sciences, Washington, PA). Five-micrometer thick frozen sections were fixed in ethanol 70% and mounted with Fluoromount G (Electronic Microscopy Science, Washington, PA), and then analyzed under an Optiphot Epifluorescence Microscope (Olympus, Middlebush, NJ).

Real-Time Polymerase Chain Reaction (qPCR)
Animals were euthanized and their oviducts were collected and flushed with saline. Total RNA was isolated using Trizol Reagent (Invitrogen Co., California, CA) and 1 µg of total RNA of each sample (2 oviducts from 1 rat) was treated with Dnase I Amplification grade (Invitrogen). The single-strand cDNA was synthesized by reverse transcription using the Superscript III Reverse Transcriptase First Strand System for RT-PCR (Invitrogen), according to the manufacturer’s protocol. The Light Cycler instrument (Roche Diagnostics, Mannheim, Germany) was used to quantify the relative transcript level of s100 g, Ampd3, Cyr61 and Tnfip6 while Gapdh was chosen as the housekeeping gene for load control because we have previously demonstrated that E2 or pregnancy did not affect its expression [9,12]. The SYBR® Green I double-strand DNA binding dye (Roche Diagnostics) was the reagent of choice for these assays. The primers used in qPCR are listed in Table 1. All real time PCR assays were performed in duplicate. The thermal cycling conditions included an initial activation step at 95°C for 25 min, followed by 40 cycles of denaturizing and annealing-amplification (95°C for 15 sec, 60°C for 15 sec and 72°C for 30 sec) and finally one cycle of melting (95° to 60°C). To verify specificity of the product, amplified products were subject to melting curve analysis as well as electrophoresis, and product sequencing was performed to confirm identity using an ABI Prism 310 sequencer. The expression of s100 g, Ampd3, Cyr61, or Tnfip6 were determined using the equation: $Y = 2^{-\Delta\text{Cp}}$ where $Y$ is the relative expression, Cp (crossing point) is the cycle in the amplification reaction in which fluorescence begins to be exponential above the background base line, -ΔCp is the result of subtracting Cp value of s100 g, Ampd3, Cyr61, or Tnfip6 from Cp value of Gapdh for each sample. In order to simplify the presentation of the data the relative expression values were multiplied by $10^3$[23].

Western blot
Animals were euthanized and their oviducts were collected and flushed with saline. Total proteins were isolated, resolved by electrophoresis and electroblotted onto nitrocellulose membranes as previously described [8]. Nitrocellulose blots were blocked by incubation overnight at 4°C in TTBS (100 mM Tris/HCl pH 7.5, 150 mM NaCl and 0.05% v/v Tween 20) containing 5% nonfat dry milk. Afterward, blots were incubated with anti-rat antibodies for 1 h with a rabbit anti-rat s100 g (Swant, Bellinzona, Switzerland) or with a mouse anti-rat β-actin (clone JLA20, Calbiochem, La Jolla, CA) as load control because expression level does not change in the rat oviduct after E2 treatment [12] in 1:2500 or 1:5000 dilutions, respectively. Blots were rinsed 5 times for 5 min each in TBS (100 mM Tris/HCl pH 7.5, and 150 mM NaCl) and were incubated for 2 h in TTBS containing 1:5000 dilution of goat anti-rabbit or anti-mouse IgG horseradish peroxidase conjugate (Chemicon International, Temecula, CA). The horseradish peroxidase activity was detected by enhanced chemiluminescence using Western Lightning Chemiluminescence Reagent Plus (Perkin Elmer Life Sciences, Boston, MA). Negative controls consisting of oviductal samples without anti-s100 g or anti-β-actin were included. Protein extracts from immature rat uterus (18 days old) treated with E2 for 3 days were used as positive controls [24].

Immunohistochemistry
Animals were euthanized and their oviducts were fixed in Bouin’s solution overnight. Paraffin embedded samples

| Gene   | Sense | Antisense | Length of band |
|--------|-------|-----------|----------------|
| s100 g | GGCAGCAGTCACTGACAGC | CAGTAGTGTTGTCGGAGC | 307 bp |
| cyr61  | ATCTACCAAGACGGGAGAG | TATTAACCCACCTCCGAGG | 253 bp |
| tnfip6 | TGACAGTGATGAGGATGTC | GCCGTAGTTGAGTTAGTCGC | 167 bp |
| ampd3  | CACCCTAGACATGCTGTG | CAAAGAGAACACCTCCA | 320 bp |
| gapdh  | ACCACAGTGGATGACCAC | TCCACACCCCTGATGTA | 498 bp |
cut in 6 μm serial sections were deparaffinized, rehydrated, and boiled in 10 mM citrate buffer (pH 6.0), for antigen retrieval. Sections were then rinsed in phosphate-buffered saline (PBS) and endogenous peroxidase was blocked by incubation with 3% v/v H2O2 for 20 minutes. After rinsing with PBS, sections were incubated with 1% w/v Bovine Serum Albumin (BSA) in PBS at room temperature to prevent unspecific binding, and then incubated with anti-rat s100 g antibody 1:100 (Swant, Bellinzona, Switzerland) in a humidified chamber overnight. The slides were washed three times for 3 min with PBS and incubated for 1 h with blocking buffer containing goat anti-rabbit IgG conjugated to horseradish peroxidase 1:1000. The antigen-antibody complexes were visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB)/Metal concentrate (Pierce, Rockford, IL). All sections were lightly counter-stained with hematoxylin (Sigma-Aldrich), dehydrated, coverslipped, and observed under a phase contrast microscope (Optiphoto-2, Nikon, Japan) and photographed with a digital camera (CoolPix 4500, Nikon, Japan). The specificity of immunoreactivity was assessed by omitting the first antibody or replacing it by preimmune serum. Negative controls showed no specific immunoreactivity.

**Statistical Analyses**
The results are presented as mean ± SE. Overall analysis was carried out using the Kruskal-Wallis test, followed by the Mann-Whitney test for pairwise comparisons when overall significance was detected. The actual N value in experiments to determine the effects of drugs on oviductal egg transport is the total number of rats used in each experimental group.

**Results**

**Estradiol up-regulates the expression of s100 g and Ampd3 transcripts in the oviduct of mated but not of unmated rats**

Here we determined the mRNA level of s100 g, Ampd3, Cyr61 and Tnfip6 in the oviducts of mated and unmated rats by quantitative RT-PCR. Rats on C1 (N = 5) or P1 (N = 5) were treated with E2 or vehicle and 3 h later oviducts were excised and their total RNA were processed to Real Time PCR.

Figure 1 shows that all four transcripts increased their mRNA level after E2 treatment in mated rats, but in unmated rats s100 g increased only at 12 and 24 hours after treatment the oviducts were collected to measure the level of s100 g mRNA by Real Time PCR. Five rats were used for each time point. Other rats on C1 (N = 3) or P1 (N = 3) were treated with E2 10 μg µg and 0, 3, 6 12 or 24 hours after treatment the oviducts were excised and processed to determine the level of s100 g protein by western blot.

s100 g transcript showed progressively increasing levels in response to E2 throughout all time points examined in mated rats while in unmated rats s100 g protein increased at the same time points as its transcript (Figure 3).

**Assessment of MOs uptake**

In order to confirm that oviductal cells took up MOs, 3 rats on C1 were injected with 3 μl μl of FITC-MO/Endo Porter mixture into one oviduct and with Endo-Porter alone in the contralateral side. Five hours later oviducts were collected, frozen and sectioned for examination in a fluorescence microscope.

Strong fluorescence representing cellular uptake of FITC-MO was observed in the luminal epithelium demonstrating penetration of MO in these cells in vivo.
No fluorescence was observed in the contra lateral oviduct, which was treated with vehicle alone (Figure 4).

Effect of intraoviductal administration of s100 g-MO on E₂-induced egg transport acceleration in mated and unmated rats

This experiment was designed to determine whether i.o. administration of s100 g-MO can inhibit acceleration of oviductal egg transport induced by E₂. Rats on C1 (N = 5) or P1 (N = 7) were injected i.o. with MO/Endo-Porter mixture (s100 g-MO or standard control MO) and three hours later 10 μg of E₂ was injected s.c. This dose of E₂ accelerates oviducal transport consistently in mated and unmated rats [3,6]. A control group was included that was treated with standard control MO and the vehicle of E₂. Twenty-four hours after i.o. administration the animals were autopsied and the number of eggs was determined as described above. To confirm that s100 g-MO effectively knockdown expression of s100 g protein in the oviduct, other rats on C1 or P1 were i.o. injected with s100 g-MO or standard control MO and 3 hours later E₂ 10 μg μg or vehicle was injected s.c to these rats. Twenty-four hours after i.o. injection oviducts were obtained to determine s100 g level by western blot.

The mean number of eggs recovered from the oviducts of the control group (vehicle of E₂ + standard control MO) was 7.6 ± 1,1 and 10.2 ± 1.2 in unmated and mated rats, respectively. As expected, E₂ reduced the number of oviductal eggs in unmated rats (0.6 ± 0.6) and in mated rats (1.6 ± 0.8). Local administration of s100 g-MO partially blocked the effect of E₂ in mated, but not in unmated rats, 4.9 ± 0.8 and 1.7 ± 1.1 respectively (Figure 5). On the other hand, no changes in the rate of preimplantation embryo development were noticed in the oviducts from mated rats treated with the morpholino s100 g-MO (data not shown).

Intraoviductally administration of s100 g-MO partially reduced the increase of s100 g protein level induced by E₂ in mated rats while s100 g-MO had no effect in
unmated rats, as the level of s-100 g 24 hours after E2 was unchanged in comparison with vehicle-treated rats (Figure 6).

Localization of s100 g protein in the oviduct of unmated and mated rats treated with E2
Here we determined whether mating or E2 treatment changes localization of s100 g protein in the oviduct. Rats injected with 10 μg of E2 on C1 (N = 3) or P1 (N = 3) were sacrificed at 12 hours after treatment and their oviducts were collected and fixed in Bouin’s solution and processed by immunohistochemistry as described above.

s100 g immunoreactivity was observed only in epithelial cells of the oviducts of C1 and P1 and it was unchanged following E2 treatment (Figure 7).

Discussion
Mating changes the mechanism of action by which E2 regulates oviductal egg transport, from a non-genomic to a genomic mode. This change in pathways has been denominated “intracellular path shifting” (IPS) and reflects a functional plasticity in well-differentiated cells. Although, microarray analysis showed that s100 g, Ampd3, Cyr61 and Tnfip6 transcripts increased their level only in the E2-treated oviducts of mated, but not unmated rats [13] confirmation by quantitative RT-PCR demonstrated that Cyr61 and Tnfip6 increased their level in response to E2 in both group of rats. Therefore, we consider that s100 g and Ampd3 were only up-regulated by E2 in the oviducts of mated rats while Cyr61 and Tnfip6 are up-regulated in both physiological conditions. On the other hand, these findings show for the first time evidence that E2 regulates expression of Ampd3, Cyr61 and Tnfip6 in the mammalian oviduct and that mating-associated signals stimulate
the response of s100 g and Ampd3 to E2 in the rat oviduct. However, the functional relevance of Ampd3, Cyr61 and Tnfip6 in the oviductal physiology was not explored in this work.

Despite of s100 g and Ampd3 genes are potential candidates to participate in the intraoviductal E2 genomic effect that accelerates egg transport in mated rats we elected explore the participation of s100 g because it is known that is regulated by E2 in several mammalian tissues including female reproductive tract [20,25]. According with this, we found that mRNA and protein level of s100 g increased in response to E2 in the oviducts of mated and unmated rats. However, the effect of E2 was evidenced much earlier in mated rats than in unmated showing that mating-associated signals changes the kinetic of transcription or translation of s100 g. Estradiol exerts its effects after binding to ER in the nucleus, inducing a conformational change and relocation of co-regulators proteins that permits the association of the E2-ER complex to estrogens-responsive elements (ERE) in the DNA [26]. In the rat, the regulation of s100 g is mediated by an ERE located at the first intron of this gene [27]. The mechanism of distinct regulation of oviductal s100 g between mated and unmated rats is not clear. Probably, mating removes some co-regulator proteins that prevent the transcription of s100 g or there are differences in the stability of the s100 g mRNA or protein in the oviductal cells of mated and unmated rats, but all of this needs further exploration. On the other hand, rat oviductal level of s100 g throughout the estrous cycle or early pregnancy remains undetermined.

The administration of s100 g-MO directly into the oviductal lumen revealed that E2-induced expression of s100 g protein is essential for the E2 genomic pathway that accelerates oviductal embryo transport. The effect of s100 g-MO on accelerated egg transport was only partial in accordance with the fact that expression of the s100 g protein in the oviduct of mated rats was partially blocked by the morpholino. This may attributable to insufficiency of the dose administered—a dose chosen because higher doses could not be dissolved in the small volume that can be injected to the oviductal lumen. Furthermore, On the other hand, local injection of s100 g-MO to unmated rats neither affected the E2-induced egg transport acceleration nor the expression of s100 g protein in the oviduct. In contrast to previously
observed (Figure 3) E2 did not increase the level of s100 g protein in the oviduct of unmated rats 24 h after treatment with standard control MO (Figure 6). This may be explained because in this particular group of rats, oviducal cells were more affected or damaged by the intraoviductal injection blunting the effect of E2 on expression of s100 g in the oviduct. Therefore, we cannot discard the participation of s100 g in the E2 non-genomic pathway that accelerates egg transport in unmated rats. Probably, others routes of administration of the morpholino would be required in unmated rats, but this was not done.

Although, it has been shown that in the mouse endometrial s100 g is critical for embryo implantation [25] the role of oviductal s100 g in the embryo development is unknown. Since, we did not notice any change in the development of early embryos flushed out of the oviducts from mated rats treated with E2 and the morpholino s100 g- MO we could speculate that morpholino s100 g did not affect preimplantation embryo development.

Previous works have shown that s100 g protein was localized exclusively in the epithelial cells of the rat oviduct [18]. Here, we report that mating did not affect the cellular localization of this molecule as well as the response to E2 suggesting that the role of s100 g in the E2 genomic pathway is not explained by a change in the localization of s100 g in the oviduct. Since the proportion between ciliated and secretory cells varies in the oviducal epithelial cells it is probable that mating-associated signals could have acted differentially on these two types of cells. Ultrastructural studies using immunoelectron microscopy needs to be done to resolve this issue.

In other tissues s100 g enhance Ca2+ transport and increase in the cells capacity to store Ca2+[28,29]. In the uterus, s100 g may be involved in controlling myometrial activity related to regulation of the Ca2+ level [20,28]. Furthermore, a role of endometrial s100 g is critical for embryo implantation [25] in the oviductal transport [28,29]. In the uterus, s100 g may be involved in controlling myometrial activity related to regulation of the Ca2+ level [20,28]. Furthermore, a role of endometrial s100 g is critical for embryo implantation [25]. Since, E2 genomic pathway that control oviducal egg transport requires participation of calcium-dependent molecules such as ETR and Cx43 [11,12] we postulate that oviducal s100 g signalling initiated in the epithelial cells could modulate expression or activity of ETR and Cx43 to regulate contractile activity in the oviduct necessary for the embryo movement.

Conclusions
Mating changes the kinetic of E2-induced expression of s100 g in the oviduct although cellular localization of s100 g was not affected by mating following E2 treatment. Furthermore, the E2 intraoviductal genomic pathway that accelerates embryo transport in mated rats requires functional participation of s100 g. These findings provide evidence of a physiological involvement of s100 g in the rat oviduct.
effect of estradiol on egg transport in the rat. Program of the 18th annual meeting of the Latinoamerican Association of Investigators in Human Reproduction La Habana, Cuba, 2003, Abstract-R22.

12. Ríos M, Hermoso M, Sánchez TM, Croxatto HB, Villalón MJ. Effect of oestradiol and progesterone on the instant and directional velocity of microsphere movements in the rat oviduct: gap junctions mediate the kinetic effect of oestradiol. Reprod Fertil Dev 2007, 19:634-640.

13. Parada-Bustamante A, Orhuela PA, Ríos M, Cuevas CA, Oróstica ML, Velasquez LA, Villalón MJ, Croxatto HB. A non-genomic signaling pathway shut down by mating changes the estradiol-induced gene expression profile in the rat oviduct. Reproduction 2010, 139:631-644.

14. Szydlowska M, Roszkowska A: Expression patterns of AMP-deaminase isozymes in human hepatocellular carcinoma (HCC). Mol Cell Biochem 2008, 318:1-5.

15. Deng Y, Chang J, Yeh C, Cheng S, Kuo M: Arecoline stimulated Cyr61 production in human gingival epithelial cells: Inhibition by lovastatin. Oral Oncol 2011, 47:256-261.

16. Mukhopadhyay D, Asari A, Rugg MS, Day AJ, Fulop C: Specificity of the tumor necrosis factor-induced protein 6-mediated heavy chain transfer from inter-a-trypsin inhibitor to hyaluronan. J Biol Chem 2004, 279:1119-1128.

17. Ambrichter H, Boltz M, Strong R, Richardson A, Bruns DE, Bruns ME: Christakos S: Expression of calbindin-D decreases with age in intestine and kidney. Endocrinology 1989, 125:2950-2956.

18. Mathieu CL, Mills SE, Burnett SH, Cloney DL, Bruns DE, Bruns ME: The presence and estrogen control of immunoreactive calbindin-D9k in the fallopian tube of the rat. Endocrinology 1989, 125:2745-2750.

19. Selfert MF, Gray RW, Bruns ME: Elevated levels of vitamin D-dependent calcium-binding protein (calbindin-D9k) in the osteosclerotic (oc) mouse. Endocrinology 1988, 122:1067-1073.

20. Choi KC, Leung PCK, Jeung EB: Biology and physiology of calbindin-D9k in female reproductive tissues: Involvement of steroids and endocrine disruptors. Reprod Biol Endocrinol 2005, 3:6.

21. L'Honoré F, Blin C, Breheret A, Thomasset M: 17 beta-estradiol stimulates the calbindin-D9k (CaB9K) gene expression in the rat uterus during the estrous cycle: late antagonistic effect of progesterone. Endocrinology 1990, 127:2891-2897.

22. Tumer CD: Endocrinology of the ovary. General Endocrinology. 3 edition. Saunders WB Company. Philadelphia. 1961, 365-409.

23. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. Methods 2001, 25:402-408.

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

25. Luu KC, Nie QY, Salamonsen LA: Endometrial calbindins are critical for embryo implantation: evidence from in vivo use of morpholino antisense oligonucleotides. Proc Natl Acad Sci USA 2004, 101:8028-8033.

26. O'Malley B: The year in basic science: nuclear receptors and coregulators. Mol Endocrinol 2008, 22:2751-2756.

27. Darwish H, Krisinger J, Furlow JD, Smith C, Murdoch FE, DeLuca HF: An estrogen-responsive element mediates the transcriptional regulation of calbindin D-9k gene in rat uterus. J Biol Chem 1991, 266:551-558.

28. Linse S, Brodin P, Drakenberg T, Thulin E, Sellers P, Eldemar K, Grundstrom T, Forsen S: Structure-function relationships in EF-hand Ca2+-binding proteins. Protein engineering and biophysical studies of calbindin D9k. Biochemistry 1987, 26:6723-6735.

29. Andersson M, Malmendal A, Linse S, Ivarsson I, Forsén S, Svensson LA: Structural basis for the negative allosterism between Ca2+ and Mg2+ binding in the intracellular Ca2+-receptor calbindin D9k. Protein Sci 1997, 6:1139-47.

doi:10.1186/1477-7827-9-69
Cite this article as: Ríos et al. Participation of the oviductal s100 calcium binding protein G in the genomic effect of estradiol that accelerates oviductal embryo transport in mated rats. Reproductive Biology and Endocrinology 2011 9:69.