Histochem Cell Biol (2013) 139:89–97
DOI 10.1007/s00418-012-1030-4

ORIGINAL PAPER

Expression and localization of nodal in bovine oviduct and uterus during different functional stages of oestrus cycle and pregnancy

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Accepted: 9 September 2012 / Published online: 4 October 2012

Abstract Members of TGF-β superfamily play a major role in the endometrial changes involved in the establishment and maintenance of pregnancy. Their deregulated expression and action could lead to absolute or partial failure of embryo implantation. Nonetheless, the precise function and mechanism of many of these cytokines remain unclear. Nodal, a transforming growth factor beta (TGF-β) superfamily member, was characterized in the human and rodent uterus and implicated in the tissue remodeling events during menstruation and embryo implantation. In order to study its possible role in the cattle reproductive process, we have analyzed Nodal expression pattern and localization in the oviduct and uterine horn during the oestrus cycle and early pregnancy (day 20). Nodal was detected both in oviduct and uterus during either the oestrus cycle or pregnancy; however, it shows a differential expression profile in the uterine horn at dioestrus and pregnancy, decreasing 1.5 and 1.4 folds in comparison with oestrus. Nodal immunostaining intensity was observed in stromal and in epithelial cells of the surface and the glandular epithelium. The staining pattern correlates with the RT-qPCR expression profile. This work is the first to evidence the presence of Nodal in the bovine reproductive tract; our data suggest that Nodal is a novel cytokine that would be involved in the remodelling occurring in the endometrium of cattle during the oestrus cycle and in the embryo implantation. The identification of new molecules that participate in endometrium cycling and/or pregnancy may be useful for predicting the ability of the uterine tissue to establish and maintain pregnancy or for detecting the infertility processes. These results highlight Nodal as a possible novel marker of the fertility process, nevertheless further studies should be done to determine its role in the reproductive system.

Keywords Nodal · Pregnancy · Embryo implantation · Oestrus cycle

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Introduction

The identification of new molecules that participate in endometrium cycling and/or pregnancy may be useful for predicting the ability of the uterine tissue to establish and maintain pregnancy or for detecting the infertility processes, specially problems of unknown etiology (unexplained infertility), many of which are caused by the development of lesions within the molecular repertoire of endometrium during the critical period of endometrial receptivity (Tabibzadeh et al. 2000). It is known that many members of TGF-β superfamily play a major role in the endometrial changes involved in the establishment and maintenance of pregnancy. Their deregulated expression and action could lead to absolute or partial failure of embryo implantation (Park and Dufort 2011; Tabibzadeh 2011).

Nodal is a member of the TGF-β superfamily and has been pointed out to play an important role in many

processes of the vertebrate development and in the uterine function of adult mammals (Papageorgiou et al. 2009; Shen 2007; Torres et al. 2009). Nodal regulates gene expression by activating serine/threonine kinase receptors (Shen 2007; Wu and Hill 2009); the signal is received by type I (ALK4) and II (ActRII and ActRIIB) Activin receptors and EGF-CFC co-receptors. Receptor activation results in the phosphorylation of the transcription factors Smad2 and Smad3. This leads to their binding to Smad4, nuclear translocation, and association with additional transcription factors to regulate target genes (Shier 2009). An important extracellular feedback inhibitor of Nodal signaling is Lefty, whose transcription is directly activated by Nodal signaling (Meno et al. 1996). In addition, Lefty antagonizes Nodal signaling through a competitive binding (Tabibzadeh and Hemmati-Brivanlou 2006).

Components of the Nodal pathway, such as Lefty, EGF-CFC co-receptors and ALK4, ActRII receptors, have been found in human and murine endometrium (Papageorgiou et al. 2009; Park and Dufort 2011; Torres et al. 2009) and oviduct (Argañaraz et al. 2007, 2010). Even more, an oestrus cycle dependent variation has been detected (Argañaraz et al. 2007, 2010; Papageorgiou et al. 2009; Tang et al. 2005a). In endometrium, the Nodal/Lefty pathway is involved in the normal metabolism of the connective tissue, which suffers a remodelling during the oestrus cycle and pregnancy. Specifically, this signaling pathway regulates the production of collagen, connective tissue growth factor (CTGF) and several matrix metalloproteinases (MMPs) (Cornet et al. 2002). The deregulated expression of this pathway factors is associated with infertility (Tabibzadeh et al. 2000); for example in vivo gene transfer of Lefty leads to implantation failure in mice (Tang et al. 2005b).

Although Nodal signaling pathway has been well documented during vertebrate embryogenesis (Brennan et al. 2001; Shen 2007) it has also been recently found and studied in human and mouse endometrium (Papageorgiou et al. 2009; Park and Dufort 2011). Moreover, Nodal presence was not described in the bovine reproductive system (oviduct and uterus), until this work. Here, we provide a detailed profile of Nodal expression in the bovine oviduct and uterus. RT-PCR and immunohistochemistry were utilised to examine Nodal site and temporal expression throughout the oestrus cycle and early pregnancy in the bovine reproductive system.

Materials and methods

Animals and collection of endometrial tissues

Oviducts and uteri were obtained from German Fleckvieh and Argentinean Branford cows at a local abattoir in Munich and Tucuman, Argentina, in accordance with protocols approved by local institutional animal care.

For mRNA determination and immunohistochemistry assays, oviducts and uteri ($n = 5$/stage) were collected from cows at different stages of the oestrus cycle (oestrus, mid-dioestrus and proestrus) and in early pregnancy (about 20th day of pregnancy). The stage of the oestrus cycle was defined by post mortem examination of the ovaries, considering the presence and size of follicles (proestrus or oestrus) or corpora lutea (mid-dioestrus) as described by Ireland et al. (1980). The stage of pregnancy was determined by measuring the conceptus with a caliper. The size of conceptus from artificially inseminated females was used as a reference (Bauersachs et al. 2006), 0.20 cm longitudinally (Rosiles et al. 2005).

Uteri and oviducts were trimmed free of surrounding tissue. In pregnant animals, the conceptus was localised and removed. Ampulla and isthmus from the oviduct, and the cranial portion of the uterine horn ipsilateral to the ovary with dominant follicle were selected and used for all experiments (Bauersachs et al. 2005). The tissues were immediately dissected in two fractions, one was maintained in RNAlater (Ambion, Huntingdon, Cambridgeshire, UK) during 24 h at 4 °C and stored at −80 °C until use; the other was fixed with 4 % formaldehyde (v/v) in PBS for 24 h at 4 °C, respectively.

RNA extraction and gene quantification

Total RNA was extracted from endometrial and oviductal tissue using Trizol reagent (Sigma-Aldrich, Munich, Germany) according to the manufacturer's instructions. Total RNA quality and quantity were determined with an Experion Automated Electrophoresis Station (BioRad, Munich, Germany) by using Experion RNA StdSens Analysis Kit (BioRad, Munich, Germany). Only samples, which RNA quality indicator (RQI) ranged between 5 and 10 were used (Fleige and Pfaffl 2006).

A two-step quantitative real-time RT-qPCR was undertaken as described recently (Sakumoto et al. 2010). Total RNA (200 ng) was reverse transcribed using iScript™ reverse transcription supermix for RT-qPCR (BioRad, Munich, Germany).

The Nodal quantification was performed using an iQ5 Real-Time PCR Analyzing System (BioRad, Munich, Germany) and SsoFast EvaGreen Supermix (BioRad, Munich, Germany). The PCR conditions were: an initial denaturation time of 75 s. at 95 °C, followed by 50 cycles at 95 °C for 15 s; 60 °C for 30 s; denaturing step of 60 s. at 95 °C and for the melting curve 70 cycles of 60 °C for 10 s. It was then held at 25 °C. All the amplified PCR fragments were sequenced to verify the resulting PCR product (GENterprise). Thereafter, the specific melting point of the amplified product served as a verification of the product identity.

In order to quantitate gene expression, the amplification efficiency for each gene was calculated. Different RNA amounts (50, 100, 150, 200, 250, 300, 350 ng) were transcripted and amplified. The efficiency for each primers pair, Nodal (5′-GCTGAGCAGCTGCAACCGGA-3′, 5′-GCGG ACATCATCCGCAGCCT-3′), β-actin (5′-GATCATTGCT CCTCCTGAGC-3′, 5′-ACTCCTGCTTGCTGATCCAC-3′), glyceraldehyde phosphate dehydrogenase (5′-AGATGGT GAAGGTCGGAGTG-3′, 5′-GAAGGTCAATGAAGGGGT CA-3′), and RNA polymerase II (5′-CCAACTTCAACC AACCCATT-3′, 5′-CGGACACACCAGCATAGTGA-3′) were determined and 200 ng of total RNA, in a final volume of 11.5 µl, were used in all the RT-qPCR. The Nodal, β-actin, GAPDH and polymerase had an efficiency of 1.50, 1.85, 1.72, and 1.60 (calculated with the software REST 2009) and amplified a product of 100, 116 and 119 pb, respectively.

Non templates (without the sample and with the specific primers) and non-transcriptional (without the reverse transcriptase and with RNA as sample) controls were run for each sample an gene. All the controls were negative or were almost negative, in all the cases assayed, even though any DNAse digestion step were performed during the ARN purification.

Data analysis of qPCR

Results are presented as mean ± SEM ($n = 5$) of relative mRNA expression levels. The crossing point (CP) determined for the Nodal was normalized against the reference genes β-actin, glyceraldehyde phosphate dehydrogenase (GAPDH) and polymerase II. Results are presented as arbitrary units of relative expression of Nodal versus the reference genes.

For statistical analysis the GenEx 5.0.1 program package (http://www.gene-quantification.de/) was used. The effect of the cycle day on mRNA expression was analyzed using one-way ANOVA. Differences were considered significant at $p < 0.05$. Graphs were plotted with SigmaPlot 8.0 (SPSS Software GmbH, Munich, Germany).

Immunohistochemistry

Briefly, paraffin sections (5 µm) were mounted on glass slides (SuperFrost Ultra Plus; Menzel-Gläser, Braunschweig, Germany) coated with animopropyltriethoxysilane (APES). Sections were dewaxed and heated once at 95 °C in a 600-W microwave oven in citrate buffer (pH 6.1) for 5 min and allowed to cool for 20 min at room temperature (RT); this treatment was done in order to expose masked antigenic sites. Then, the sections were subjected to the following immunohistochemical staining schedule: (a) elimination of endogenous peroxidase activity with 0.3 % H_2O_2 in distilled water for 10 min at RT (b) elimination of nonspecific protein binding by a 10 min incubation at RT with protein block serum-free (XO9O9; DAKO Cytomation, Carpinteria, CA, USA), (c) incubation with Nodal antibody (Abcam, Munich, Germany). Anti-Nodal is a mouse monoclonal antibody, which has cross-reactivity with humans, however the human Nodal antigenic peptide (RCEGECPNPVGEEFHPTNHA YIQSLLKRYQ PHRVPSTCCAPVKTKPLSMLYVDNG RVLLDHHKDMIVEECGC) was compared with bovine Nodal mRNA, presenting a similarity of 100 %, indicating a very high possibility of cross-reactivity between these species. The antibody does not present cross-reactivity with other proteins according the manufacture. The primary antibody was diluted 1:100 (v/v) in an antibody diluent with background reducing components solution (S3022; DAKO Cytomation, Carpinteria, CA, USA). The incubation was performed overnight at 4 °C in a humidified chamber. (d) Then, sections were incubated at RT for 30 min with a biotinylated rabbit anti-mouse IgG (E0413; DAKO; diluted 1:200 (v/v) antibody diluent solution (S0809; DAKO). (e) incubated with streptavidin–biotin horseradish peroxidase complex and diluted 1:300 (v/v) in PBS for 30 min at RT. (f) The bound complex was made visible by reaction with 3,3′-diaminobenzidine tetrahydrochloride (DAB) in buffer solution (KB467; DAKO) for 3–10 min at RT. Sections were left unstained or counterstained in Mayer's haematoxylin, dehydrated, and mounted with DePeX Eukitt (Riedel–de–Haen, Seelze, Germany). The control sections received the antibody dilution buffer (S3022; DAKO) in place of the specific first antibody. For further negative control experiments, non-specific antibodies diluted to the same final protein concentration like the used specific antibodies were substituted for the primary antibodies.The used non-specific antibodies were matched to the host species (mouse) and isotype of the specific antibodies. The following non-specific antibodies for isotype controls in immunohistochemistry were used: Goat IgG Purified, Mouse IgG2b, Mouse IgG3 (Biozol, Eching, Germany).

A minimum of five sections was examined for each sample. Representative tissue sections were viewed, analysed and photographed under light microscope without counterstain.

Bovine oviduct samples were also shock frozen in liquid nitrogen. Cryosections (10 µm) were cut using a Kryostat HM 500 OM (Firma Microm, Walldorf, Germany). The temperature of the microscope stage was kept to −15 °C. The sections were placed onto standard glass slides (Super frost Plus) and dried for 1 h at room temperature. Then the sections were fixed for 2 min in 100 % acetone (at 4 °C) and stored at −20° C until used for the immunohistochemical investigations.

Results

Nodal mRNA expression in the cow oviduct across the oestrus cycle and early pregnancy

The expression of Nodal was found in oviductal segments, ampulla and isthmus throughout the oestrus cycle as well as in the pregnant oviduct. No differences in the expression of reference genes (β-actin, GAPDH and polymerase II) were observed between the groups. Nodal mRNA expression was normalized against these reference genes and presented as relative expression levels. The level of expression of Nodal transcripts did not change significantly between the stages examined, neither in the ampulla nor in the isthmus (Fig. 1).

Nodal mRNA expression in the uterus across the oestrus cycle and early pregnancy

A similar procedure was used for assessment of the presence and pattern of Nodal mRNA in the uterus. RT-qPCR assays revealed that Nodal transcripts were present in the bovine uterine horn at all stages, although its relative expression varied depending on the oestrus cycle and pregnancy. The relatively highest Nodal expression was during oestrus. Nodal was significantly down-regulated during dioestrus stage ($p < 0.029$) 1.5 folds, whereas in the proestrus, the transcripts recovered their initial level of expression, with an increase of 1.3 folds with respect to the dioestrus stage (Fig. 2). Interestingly, the Nodal expression is significantly down-regulated during pregnancy ($p < 0.026$), 1.4 folds lower than oestrus stage.

Immunolocalization of Nodal in the oviduct throughout the oestrus cycle and pregnancy

In ampulla and isthmus, Nodal seems to have the same pattern, showing notable differences between pregnancy and oestrus cycle. Immunostaining of frozen section proved that antigen retrieval used in the paraffin section did not change the staining pattern (Fig. 3b, c). No immunostaining was found when control buffer, mouse serum or an irrelevant mouse antibody was used in the negative controls. During proestrus (Fig. 3a) and oestrus, the cytoplasm of the ampullar epithelium appeared weakly positive for Nodal, whereas the peri-nuclear area displayed a more distinctive immunostaining. Strong immunostaining was seen in the some lymphocytes located in the basal area of the epithelium (Fig. 3d). In the stroma, the peri-nuclear area of many but not all fibroblast and endothelial cells were distinctly positive after incubation with the antibody against Nodal. During dioestrus (Fig. 3b) the pattern of Nodal immunostaining did not change significantly in the stroma and a distinct positivity was seen in the peri-nuclear area of the epithelium, also the immune reaction was weak in the cytoplasm of the cells. In samples taken from pregnant animals the nuclear staining has mostly disappeared from the epithelium and stroma. A moderate Nodal staining was found in the luminal part of the epithelium and the tongue-like apical protrusions. In addition some Nodal positive material was found at luminal levels (Fig. 5a). In the isthmus, the peri-nuclear area of the epithelium and stroma showed a marked positive immunoreaction. In the epithelium small Nodal positive granules occur in its supranuclear

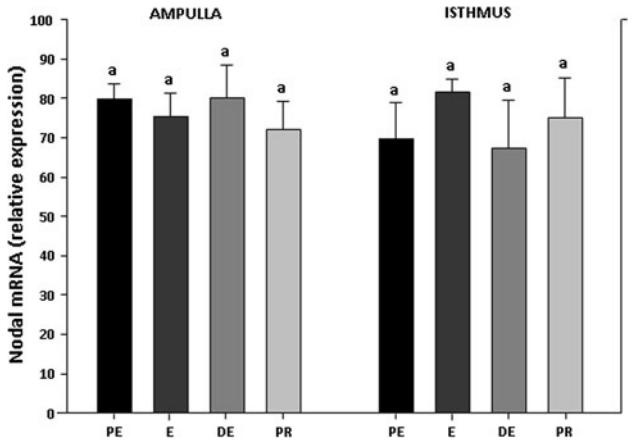

Fig. 1 Quantification of Nodal mRNA expression patterns in the oviduct across the oestrus cycle and pregnancy by RT-qPCR. Histograms illustrating fluctuations in mRNA expression (mean values ± SEM) during the bovine oestrus cycle (*PE* Proestrus, *E* Oestrus, *DE* Dioestrus) and at 20th day of pregnancy (*PR*). No significantly different expression of Nodal mRNA was detected in all cases. *Different letters* indicate significant differences, $P < 0.05$ ($n = 5$ for each stage)

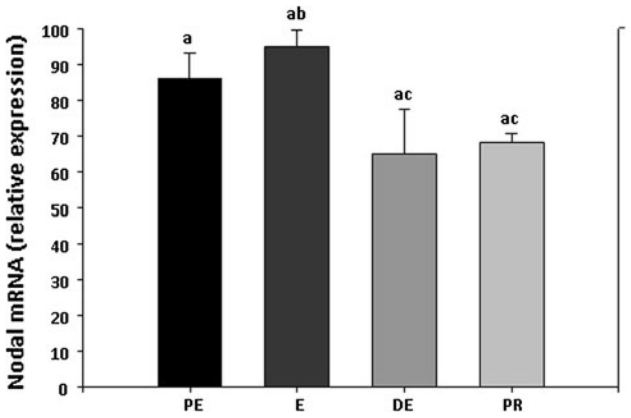

Fig. 2 Quantification of Nodal mRNA expression patterns in the uterus across the oestrus cycle and pregnancy by RT-qPCR. Histograms illustrating fluctuations in mRNA expression (mean values ± SEM) during the bovine oestrus cycle (*PE* Proestrus, *E* Oestrus, *DE* Dioestrus) and at 20th day of pregnancy (*PR*). Significantly decreased expression of Nodal mRNA was detected in the DE and PR stages. *Different letters* indicate significant differences, $P < 0.05$ ($n = 5$ for each stage)

part (Fig. 4a). During dioestrus (Fig. 4b), the nuclear immunostaining has somewhat increased compared to pro-estrus and oestrus and in pregnant animals the immuno-staining, like in the ampulla, appeared considerably reduced and the peri-nuclear stained has disappeared (Fig. 5b).

Immunolocalization of Nodal in the uterus throughout the oestrus cycle and pregnancy

Differences were observed between the cycle and pregnancy in the uterus. During proestrus and oestrus, a distinct staining of stromal and epithelial cells (both surface and glandular epithelium) was found at pro-oestrus (Fig. 6a). In the epithelium, also a weak immunostaining was found in the cytoplasm where it was uniformly distributed showing no differences between the apical and basal zones of the epithelial cells. The peri-nuclear area of stromal cells (fibroblasts) exhibited also a distinct immunostaining. During dioestrus, the intensity of Nodal staining in the peri-nuclear area of the surface and of the glandular epithelium has somewhat increased (Fig. 6b). In pregnancy, a remarkable immunostaining was observed in the cytoplasm of epithelial cells, as well as in the stroma. The immuno-staining of the epithelial and glandular peri-nuclear area appeared significantly reduced and was only weak, whereas the cytoplasmic reactivity remained more or less unchanged compared to the dioestrus. In the stroma, groups of distinctly Nodal positive fibroblast were found, whereas other fibro-blasts only showed a weak immunostaining (Fig. 7a).

Discussion

The mechanism that regulates the anatomical and physio-logical changes that occur during the menstrual shedding and during the sexual cycle in the uterus of humans have been studied thoroughly. Although, most of the signaling pathways that participate in this process have been con-served during evolution, there are substantial differences between species regarding endometrial changes during the

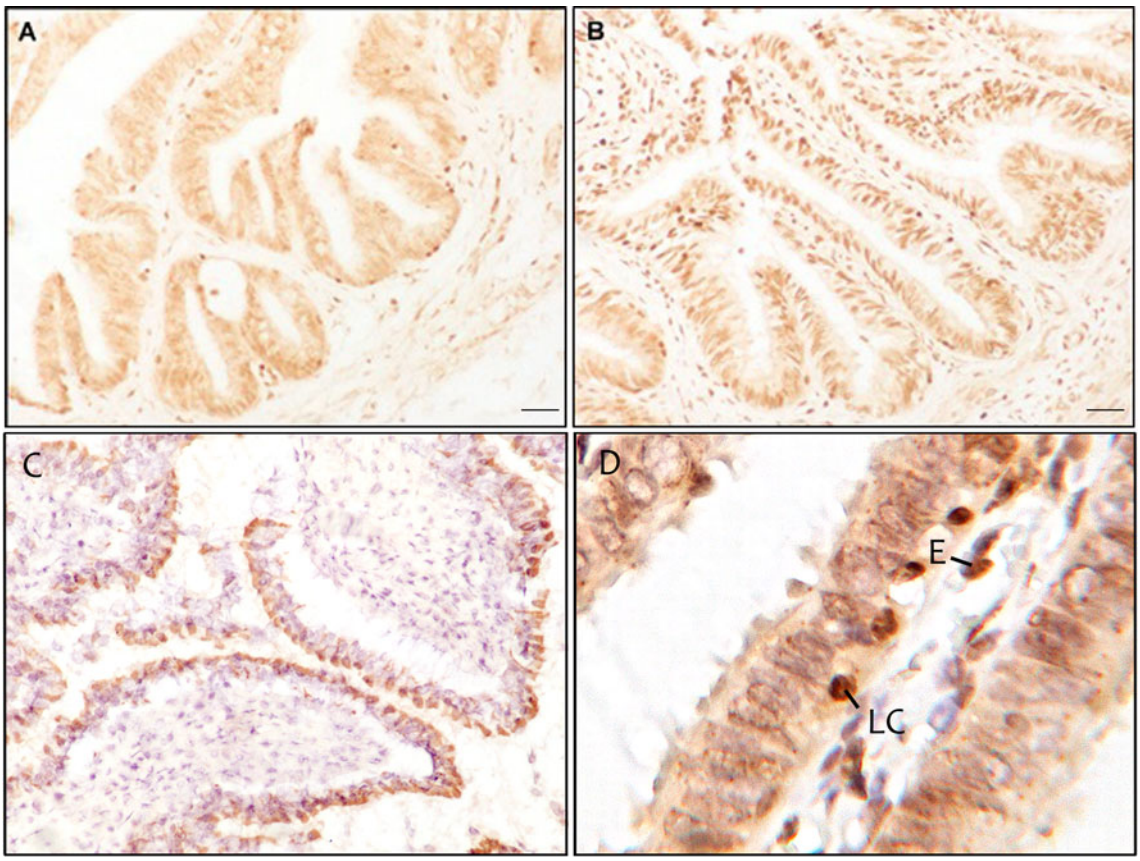

Fig. 3 Immunolocalization of Nodal in the ampulla throughout the oestrus cycle. **a** Proestrus Nodal is mainly located in some fibroblasts and epithelial cells (peri.-nuclear localization). Also the cytoplasm of the epithelium shows a weakly positive immunostaining; **b** Dioestrus: distinct positivity immunostaining can be observed in the peri-nuclear area of the epithelium and stroma. **c** Dioestrus: immunostaining of frozen sections prove that antigen retrieval used in immunostaining of paraffin sections did not significantly influence the staining pattern. **d** Dioestrus: Nuclei of intraepithelial lymphocytes (*LC*), endothelial cells (*EC*) and the nuclei of some fibroblasts are distinctly positive for Nodal

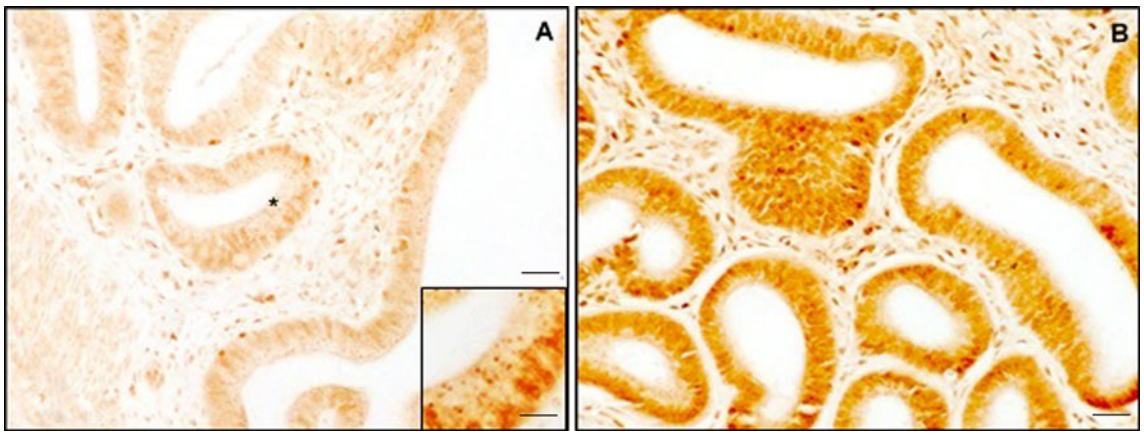

Fig. 4 Immunolocalization of Nodal in the Isthmus throughout the oestrus cycle. Marked presence of Nodal in the peri-nuclear area of the epithelium and stroma. Also small Nodal positive granules occur in the supranuclear part of the epithelium. During dioestrus **b**, the nuclear immunostaining is increased, when compared to proestrus **a** and oestrus (not shown)

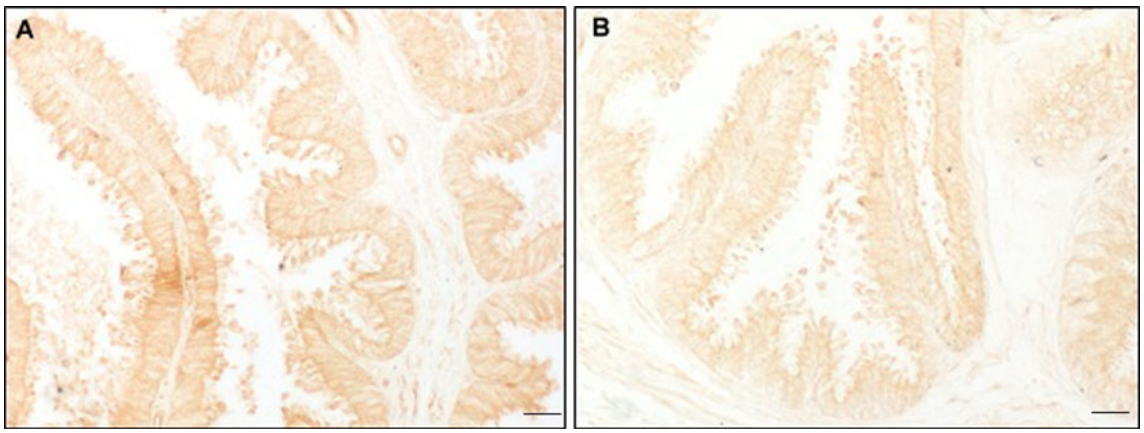

Fig. 5 Immunolocalization of Nodal in the oviduct at 20 days of pregnancy. Moderate Nodal presence in **a** ampulla where apical protusions were observed in the luminal region of the epithelium. A positive immunostaining has also been found in the luminal secretions **b** isthmus, the immunostaining appeared considerably reduced

cycle and the type of embryo implantation. In general, far less tissue remodelling occurs during the oestrus cycle of many mammalian species compared to primates. Therefore, within the uterus of domestic animals, only moderate expression of the genes involved with tissue remodelling has been observed compared to more robust expression during the tissue loss and tissue repair processes that are the hallmark of the menstrual cycle (Blomberg et al. 2008; Curry and Osteen 2003).

Bauersachs et al. (2005) have identified a variety of molecules involved in the marked functional changes of bovine endometrium during the oestrus cycle, such as Wnt, cAMP, MAPK, IGF, EGFR, TGF-β, a.s.o. Among these systems the TGF-β cascade was the most striking one. Nodal/Lefty2 system, a well-known TGF-β signaling pathway in the early embryo development, was recently described to be involved in human and rodent reproductive process (Argañaraz et al. 2007, 2010; Papageorgiou et al. 2009; Park and Dufort 2011). However, their presence in the reproductive system of other mammals was unknown until now. In this work, the presence of Nodal, in the bovine uterus and oviduct and its possible involvement in the reproductive process was addressed by RT-qPCR and immunohistochemistry.

In the oviduct, no statistically significant differences were observed between the oestrus phases (proestrus, oestrus and dioestrus) and pregnancy (day 20). In addition, ampulla and isthmus showed a similar Nodal expression profile, without variations between them. Nodal is localized primarily in the epithelial and some stromal cells. This localization with no notorious differences in the distribution pattern was observed in ampulla as well as in the isthmus during the oestrus cycle. The epithelial localization of Nodal, in addition to the modification of its cellular localization, from being peri-nuclear to ubiquitously distributed in the cytoplasm, and the presence of supra-nuclear

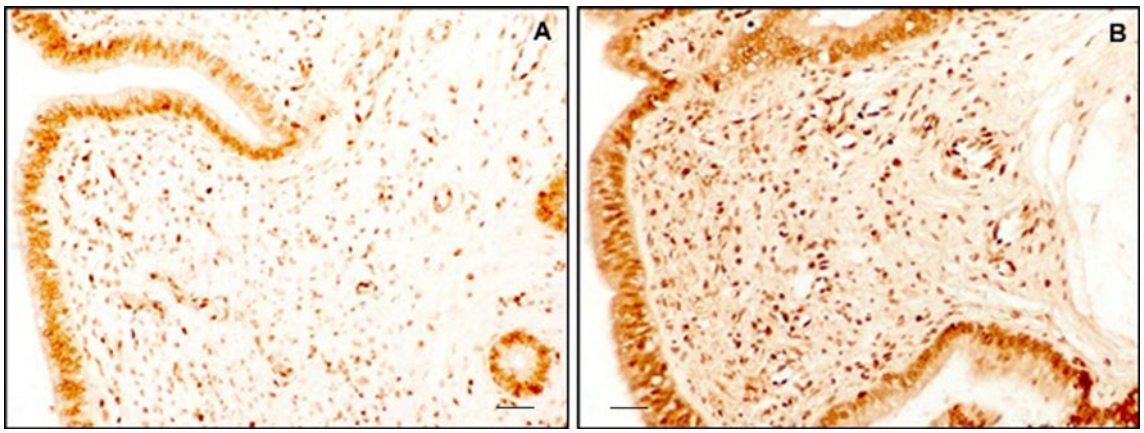

Fig. 6 Immunolocalization of Nodal in the uterus throughout the oestrus cycle. **a** Oestrus: Marked Nodal presence in the peri-nuclear area of stromal and epithelial cells, where also a weak immunostaining can be observed uniformly distributed in the cytoplasm. **b** During dioestrus, the intensity of Nodal staining in the peri-nuclear area of the surface and of the glandular epithelium has increased. Apositive immunostaining can also be seen in the cytoplasm of epithelial cells and stroma

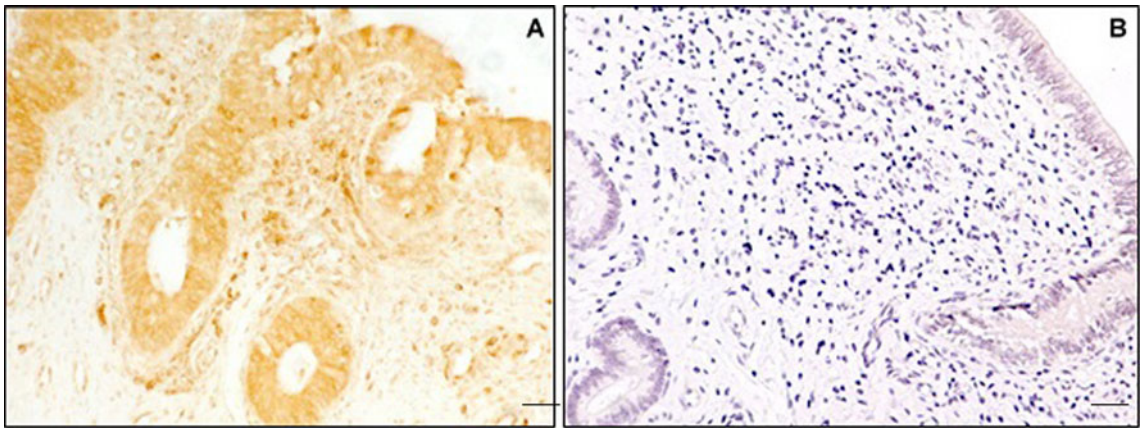

Fig. 7 Immunolocalization of Nodal in the uterus at 20 days of pregnancy. **a** The immunostaining of the epithelial and glandular peri-nuclear area appeared significantly reduced, compared to the oestrus cycle. whereas the cytoplasm of epithelial cells shows a positive reaction. In the stroma groups of distinctly Nodal positive fibroblast peri-nuclear area are scattered between stromal areas. **b** Negative control, sections were incubated without Nodal antibody and counterstained with haematoxylin

positive granules, suggest that Nodal could be secreted into the lumen by these cells. Interestingly, human Nodal proprotein was recently identified in lavage fluid samples from women (Papageorgiou et al. 2009) indicating that Nodal could be secreted to oviductal lumen as well. Blanchet et al. (2008) proposed that Nodal is exocytosed together with Cripto for proteolytic processing and autocrine signaling, or secreted through the trans Golgi network/endosomal system for processing in neighbouring cells. These previous data would explain the Nodal tissue distribution found in the oviduct and uterus of cows.

It has been described that Nodal signaling activates the transcription of many genes, among then Lefty, a Nodal feedback inhibitor, the presence of which was shown in the rat oviduct during the oestrus cycle and early pregnancy. Lefty maintained relatively at steady-state levels across the cycle (Argañaraz et al. 2007; 2010), which could indicate that Nodal would be expressed at steady-state levels in the oviduct. Furthermore, Lefty was up-regulated in the oviduct at the 4th day of pregnancy when the embryo is at the stage of morula and completing its passage through the oviduct (Argañaraz et al. 2007, 2010), meaning that Nodal expression would be down-regulated at that moment in the oviduct. These data are in concordance with our data. Although Nodal decrease during pregnancy was not reflected in the protein levels of the immunohistochemistry, the weak immunostaining is distinctive, indicating a low presence of Nodal during the early pregnancy in cow. Therefore the RNA overexpression detected by RT-qPCR could not result in a protein because of posttranscriptional regulation or that the protein was not detectable because of its short life span (Jonsdottir et al. 2008).

We also detected Nodal in the bovine uterus both in pregnancy and oestrus cycle (proestrus oestrus and dioestrus). Nodal was highly expressed in oestrus and decreased 1.5 folds by the dioestrus, subsiding low at the 20th day of pregnancy. The protein was found mostly in the surface and glandular mucosa in both stromal and epithelial cells. In general no changes in the distribution pattern were observed along the oestrus cycle, meanwhile the protein presence was reduced in pregnancy. Papageorgiou et al. (2009) observed high expression levels of Nodal and the EGF-CFC co-receptor, Cripto, in the human endometrium during the proliferative phase of the menstrual cycle; declining significantly by the mid-secretory phase. In humans Nodal/Lefty cascade has been shown to be involved in remodelling of the endometrium during the sexual cycle and menstruation by stimulating the expression of matrix metalloproteinases (Cornet et al. 2002). Although, the oviduct is a dynamic organ that suffers physiological, transcriptomic and proteomic changes during pregnancy and the oestrus cycle (Bauersachs et al. 2003; Georgiou et al. 2005, 2007; Jiwakanon et al. 2005), there is no marked remodelling of its structure, as does befall with the uterus. Lefty mRNA levels, a Nodal feedback inhibitor, are depressed at oestrus and elevated at metoestrus and dioestrus (Papageorgiou et al. 2009). Similarly, Nodal expression profile was observed in the cow uterus.

During the peri-implantation period of mouse, Nodal was localized throughout the glandular epithelium of the endometrium (Park and Dufort 2011). Alternatively, Lefty mRNA, a Nodal feedback inhibitor, was previously shown to be relatively low during early pregnancy in the mouse before undergoing a significant increase in expression at the implantation site between days 3 and 5, which corresponds with the blastocyst implantation in this species. Lefty and metalloproteases (MMP-3, MMP-7, MMP-12 and MMP-14) were expressed coordinately; increasing on days 3 and/or 5 of pregnancy and decreased by day 9 (Tang et al. 2005a). Interestingly, the attachment and initial implantation period in cattle starts on day 20 of pregnancy (Wooding 1992; Yamada et al. 2002).

Mamo et al. (2011) demonstrated that the genes of MMP-2, MMP-13, MMP-19 and FURIN, among others genes, are up-regulated between the 16th and 19th day of pregnancy in the cow, which suggests a role in maternal recognition and initiation of implantation. FURIN is one of the several proprotein convertase family genes responsible for cleaving intra- and extracellular precursors of various growth factors such as Nodal and Lefty (Mesnard and Constam 2010; Tabibzadeh 2011; Thomas 2002; Ulloa et al. 2001). Moreover; MMPs, Lefty target genes, are responsible for the basement membrane and extracellular matrix degradation required for the invasive processes necessary for embryo implantation and the establishment of pregnancy (Curry and Osteen 2003). The invasion of the endometrium by trophoblast cells during the process of implantation and placentation involves dramatic MMPs mediated cellular mobility, host tissue remodelling, and placental development (Curry and Osteen 2003). It can be assumed that Nodal down-regulation during the pregnancy at the moment of implantation would allow the up-regulation the Lefty, due to the feedback existing between this ligand-inhibitor pair. High levels of Lefty stimulate a proteolytic cascade that produce the remodelling of the endometrium and the decidualization process (Tabibzadeh, 2011), thus supporting embryo implantation in bovine.

Embryo-maternal communication is vital for the successful establishment and maintenance of gestation, yet relatively little knowledge exists for many of the mechanisms and the nature of the embryo signals responsible for this cross-talk (Bazer et al. 2011; Roberts et al. 1996). Nodal stimulated the secretion of tissue inhibitor of metalloproteinase-1 and inhibited MMP-2 and-9 activity, suggesting that the Nodal/ALK7 pathway plays important roles in human placentation and that its abnormal signaling may contribute to the development of preeclampsia (Nadeem et al. 2011). Also, in a subset of infertile patients, the expression Lefty was prematurely increased during the implantation window, indicating that Lefty acts as a molecular switch that modulates both the induction of decidual differentiation and the maintenance of a decidualized state (Tabibzadeh 2011).

Acknowledgments This research was partially supported by grants of Min. C y T. ARG 08/013-BFBFAL0814 and BID 2006 no. 502 (ANPCyT-UNT). Dr. Argañaraz was partially supported by a grant for CONICET-Argentina.

Conflict of interest The authors declare that there is no conflict of interest that prejudices the impartiality of this scientific work.

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