Immunohistochemical analysis of selected stem cell markers including stage-specific mouse embryonic antigen 1 (SSEA-1) in porcine and bovine endometrium throughout the oestrous cycle

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Abstract

There has been a lack of research regarding endometrial stem cells in farm animals, and particularly, regarding epithelial stem/progenitor cells. We aimed to identify potential stem cell subpopulations in porcine and bovine endometrium by measuring the expression of selected stem cell markers (OCT3/4, CD44, SSEA-1, SOX10, CD73, and CD117) during the follicular and luteal phases of the oestrous cycle. We studied 28 endometrial tissue samples collected from 14 healthy, cycling pigs and cows. The endometrial mucosa of each sample was divided into basal, middle, and luminal portions, and the immunohistochemical staining intensity and percentages of cells that were marker-positive were recorded for each portion of the endometrium. Expression of OCT-3/4 was identified in the glands and stroma, and that of SOX10 and CD117 was identified in stroma of both porcine and bovine endometrium. In porcine endometrium, CD44 was only expressed in the glands, whereas SSEA-1 was expressed in the glands and stroma. In bovine endometrium, CD73 was only expressed in the glands. Differences in the expression of most of these markers were found between phases of the oestrous cycle and/or portions of the endometrial mucosa. Our data indicate the existence of both epithelial and mesenchymal stem cell subpopulations in the endometrium of pigs and cows during their oestrous cycles. The expression pattern of the stem cell marker SSEA-1 in porcine endometrium suggests a possible alternative location of the epithelial stem/progenitor cell population in the middle portion of the endometrial glands.

Endometrium, farm animal, pig, cow, SSEA-1, immunohistochemistry

Stem cells are present in both adult organisms and embryos. In adult tissues, including the endometrium, they form a minor subpopulation of unspecialized cells that are capable of self-renewal and differentiation into various cell types (He et al. 2009).

Progress has recently been made in the identification of markers of the glandular epithelium of the zona basalis (the basalis glandular epithelium), the putative site of epithelial progenitor cells, in the human endometrium. The first such marker to be identified was stage-specific embryonic antigen 1 (SSEA-1), also known as CD15, which was originally described in the human endometrium by Valentijn et al. (2013). Among the other reported markers are leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) and N-cadherin (cadherin-2 or CD235) (Gil-Sanchis et al. 2013; Nguyen et al. 2017). A population of endometrial mesenchymal stem cells (eMSCs) has also been identified using several specific markers that are expressed by these cells individually or in combination: CD146, platelet-derived growth factor receptor beta (PDGFRβ),

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mesenchymal stromal cell antigen-1 (MSCA-1), and sushi domain-containing 2 (SUSD2). The expression of other stem cell markers, including octamer-binding transcription factor 3/4 (OCT3/4) and CD117, has also been characterized in the endometrial tissue of both humans and farm animals in several studies (Cabezas et al. 2014; Wiater et al. 2018).

There has been a lack of research regarding endometrial stem cells in farm animals. In addition, most studies have focused on endometrial mesenchymal stem cells, and the endometrial epithelial stem cell population has not been well characterized. Therefore, we aimed to identify an endometrial stem cell subpopulation in pigs and cows by immunohistochemically assessing the expression of selected stem cell markers (OCT3/4, CD44, SSEA-1, SOX10, CD73, and CD117) during the oestrous cycle. We also aimed to characterize the differences in expression between animals of various ages and those that had previously been pregnant.

Materials and Methods

Animals and tissue samples

We studied 28 samples, comprising the uterus and both ovaries that were collected at an abattoir from 14 healthy, cycling pigs and cows. For each, 10 mm^3 tissue samples were dissected from the middle third of both uterine horns. Longitudinal sections of both ovaries were also prepared. These tissue samples were placed in 10% neutral-buffered formalin and fixed for 48 h. Further tissue processing was performed using standard procedures.

We studied samples from animals of different ages. Eight of the 14 pigs were ~1 year old, and six were approximately 3 years old. Of the 14 cows studied, seven were 2–2.5 years old, and seven cows were 4.5–5 years old. Seven of the pigs and eight of the cows had had multiple pregnancies, according to their veterinary records.

The cycling status of all the animals was assessed by macroscopic examination of both ovaries, according to the criteria reported by Ireland et al. (1980), and verified by microscopic evaluation of the endometrium and both ovaries to identify folliculogenesis and luteogenesis (Ginther et al. 1989). To facilitate comparisons with the levels of SOX2 expression identified in our previous study (Lenz et al. 2022), the endometrial mucosa was divided into basal, middle, and luminal portions (the thickness of the basal endometrial portion was limited to 0.5 mm), with the latter including the subepithelial area located below the surface epithelium. The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Veterinary Sciences, Brno.

Immunohistochemistry

Six antibodies were used for the immunohistochemical analysis (Table 1). Immunohistochemical staining was performed using an automated immunostainer (Benchmark Ultra, Ventana) and an ultraView Universal DAB Detection Kit (Ventana). Endometrial tissue samples were incubated with the anti-OCT3/4, anti-CD44, and anti-SSEA-1 antibodies for 32 min, and with the anti-SOX-10, anti-CD73, and anti-c-KIT antibodies for 16 min. Prior to immunohistochemical staining, heat-induced antigen retrieval was performed in an EDTA-based buffer (pH 8.4) for 36 min (OCT3/4), 64 min (SOX-10, CD73, c-KIT, and SSEA-1), or 76 min (CD44) at a temperature of 95 °C (SOX-10, CD73, c-KIT, and SSEA-1) or 99 °C (OCT3/4 and CD44) (Ventana). External positive controls were used for all the antibodies. Negative controls were prepared by incubating samples with diluted rabbit serum (dilution 1:25 for SSEA-1; 1:50 for OCT3/4; and 1:100 for c-KIT, CD73, and SOX-10) or diluted mouse serum (dilution 1:100 for CD44).

Table 1. Summary of antibodies used for immunohistochemistry.

Antibody	Clone	Dilution	Manufacturer
OCT3/4	Polyclonal (rabbit)	1:50	DCS Innovative Diagnostik-Systeme, Hamburg, Germany
CD44	DCM-13	1:100	DCS Innovative Diagnostik-Systeme, Hamburg, Germany
SOX-10	SP267	Predilute	Cell Marque, Rocklin, CA, USA
CD73	Polyclonal (rabbit)	1:100	Elabscience, Houston, TX, USA
c-KIT	9.7	Predilute	Ventana Medical System Inc., Oro Valley, AZ, USA
CD15/SSEA-1	SP159	1:25	Abcam, Cambridge, UK

Evaluation of immunostaining

For the endometrial tissue, nuclear staining for OCT3/4 and SOX-10; cytoplasmic staining for c-KIT, membranous and cytoplasmic staining for CD44 and CD73; and membranous, cytoplasmic, or Golgi staining for SSEA-1 were considered to be positive. All the markers were assessed in both the endometrial glands and

stroma. At least ten areas of each endometrial tissue sample were analysed, and the percentages of the cells that were marker-positive across the studied areas were calculated. Differences in expression between portions of the endometrial mucosa and the intensity of the immunoreaction for each marker were also recorded as weak, moderate, or strong. Furthermore, we evaluated the differences in immunostaining between the follicular and luteal phases of the oestrous cycle, as well as between animals of different ages and those that had previously undergone pregnancy.

Statistical analysis

Differences in marker expression between animals of the same species in different phases of the oestrous cycle were compared using Pearson's Chi-squared test with simulated *P* value (based on 2,000 replicates) and proved/disproved to be significant at the level of significance $\alpha = 0.05$. Differences in marker expression between pigs and cows (in the corresponding phases of the oestrous cycle) were compared using Pearson's Chi-squared test with simulated *P* value (based on 2,000 replicates) and proved/disproved to be significant at the level of significance $\alpha = 0.05$.

Results

Histological features of porcine and bovine endometrium and ovaries

Eight of the endometrial tissue samples from the pigs corresponded to the follicular phase (three samples were evaluated as prooestrus and five as oestrus), and six samples corresponded to the luteal phase (all samples were evaluated as metoestrus), of the oestrous cycle. Seven of the fourteen bovine endometrial samples corresponded to the follicular phase (two samples were evaluated as prooestrus and five as oestrus), while the histological appearance of the remaining seven samples corresponded to the luteal phase (all samples were evaluated as metoestrus) of the oestrous cycle. In all instances, the histological findings in the ovaries were consistent with those in the endometrial tissue.

Immunohistochemical expression of OCT3/4 in porcine endometrium

Nuclear expression of OCT3/4 was present in both the endometrial glands and stroma. Strong staining was present in > 95% of glandular cells in all portions of the endometrial glands, while a mean 25% of the stromal cells (range 10–40%) demonstrated weak, moderate, or strong staining throughout the endometrial mucosa (Plate III, Fig. 1A, B). In porcine endometrium, OCT3/4 expression did not vary according to the phase of the oestrous cycle, age, or previous pregnancy status.

Immunohistochemical expression of OCT3/4 in bovine endometrium

In the follicular phase, moderate-to-strong staining intensity was found in 60–75% of glandular cells. In two samples, differences in the staining intensity and the percentages of OCT3/4-positive glandular cells were identified between the basal portion and luminal two-thirds of the endometrial glands (approximately 90% of the cells showed strong expression in the basal portion, compared to 30–40% showing moderate expression in the remaining luminal glandular portion) (Fig. 1C). Significantly lower OCT3/4 expression was found during the luteal phase of the oestrous cycle. Specifically, uniform weak-to-moderate expression was observed in 15–50% of endometrial glandular cells (mean 40%) (Fig. 1D). Overall, lower expression in endometrial glands was present in older cows, including those that had undergone pregnancy (expression was present in ≤ 15–20% of cells). The percentage of stromal cells that were OCT3/4 weakly to moderately positive averaged 20% (range 10–35%) (Fig. 1C, D), and no differences were identified among endometrial stromal cells from animals in different phases of the oestrous cycle, of differing age, or with differing previous pregnancy status.

Immunohistochemical expression of CD44 in porcine and bovine endometrium

CD44 immunopositivity was only present in porcine endometrium. Weak-to-(at most)-moderate staining intensity was identified in approximately 70% of the epithelial cells

in the basal and middle portions of the endometrial glands, and no immunopositivity was present in the luminal glandular portions (Plate IV, Fig. 2A). Positive immunostaining was only present during the luteal phase of the oestrous cycle. Endometrial stromal cells were immunonegative, regardless of the phase of the oestrous cycle. There were no differences between pigs of different ages, nor between those that had or had not undergone pregnancy.

Immunohistochemical expression of SSEA-1 in porcine and bovine endometrium

SSEA-1 expression was identified only in pigs. The pattern of staining was cytoplasmic with a paranuclear, dotlike, Golgi localization. During the follicular phase, there was moderate, sporadic expression in < 1% of glandular cells in the luminal third of the endometrial mucosa, and at the interface between the middle and luminal portions of the endometrial glands (Fig. 2B). During the luteal phase, there was SSEA-1 expression in the middle portion and, to a lesser extent, in the luminal glandular portion of the endometrium, with approximately 2–3% of glandular cells showing moderate-to-strong immunopositivity (Fig. 2C, D). Differences between the follicular and luteal phases did not achieve statistical significance. Regarding the endometrial stroma, moderate (in the follicular phase) or strong (in the luteal phase) expression was identified in < 1% of cells, mainly in the subepithelial area. Very slightly higher SSEA-1 expression (up to 2–3% of cells) was present only in the endometrial glands of older pigs, including those that had undergone pregnancy.

Immunohistochemical expression of SOX10 in porcine and bovine endometrium

The immunostaining pattern in both species indicated sporadic and strong nuclear expression that was restricted to the endometrial stromal cells. SOX10-positive stromal cells were present throughout the endometrial mucosa, with apparent clustering in subepithelial areas. Overall, the number of SOX10-positive cells was low, with the largest number being identified during the follicular phase (~8% in cows and ~1% in pigs) and noticeably fewer being present during the luteal phase (~2% in cows and < 1% in pigs); differences between the individual phases of the statistically significant ($P < 0.001$ (Plate V, Fig. 3A-D). Both porcine and bovine endometrium contained small areas with larger numbers of SOX10-positive stromal cells, some of which were present in poorly formed vessel walls (Fig. 3E, F). Slightly lower SOX10 immunopositivity was present in older cows, including those that had undergone pregnancy (< 2% of cells overall).

Immunohistochemical expression of CD73 in porcine and bovine endometrium

Immunostaining with the CD73 antibody achieved positive results only in bovine endometrial samples (only in glands). In the follicular phase, there was weak-to-moderate staining intensity in approximately 4% (basal portion) and < 1% (middle and luminal portions) of the glandular cells (Plate VI, Fig. 4A, B). During the luteal phase, there was an increase in the number of CD73-positive glandular cells and in the intensity of staining. In the basal glandular portion, CD73 was present in 10–20% (mean 15%) of cells, and in a significantly lower 5% of cells in the luminal two-thirds of the endometrial glands ($P < 0.001$) (Fig. 4C, D). There was strong immunoreactivity across the endometrial glands. There was slightly higher expression of CD73 in older cows, including those that had undergone pregnancy (~12% of cells overall).

Immunohistochemical expression of CD117 in porcine endometrium

In pigs, regardless of the oestrous cycle phase, age, or previous pregnancy status, immunostaining for CD117 revealed sporadic weak-to-moderate (follicular phase) or strong (luteal phase) expression only in < 1% of endometrial stromal cells located in the subepithelial area (Plate VII, Fig. 5A, B).

Immunohistochemical expression of CD117 in bovine endometrium

During the follicular phase, moderate-to-strong expression was present in approximately 8% of stromal cells (range 4–11%) (Fig. 5C), whereas in the luteal phase, this was significantly lower (approximately 1%) ($P < 0.001$), and the staining intensity was weak to (at most) moderate (Fig. 5D) (glands were negative). We found a slightly lower percentage of CD117 positive stromal cells in older cows, including those that had undergone pregnancy (< 5% overall). Negative controls are also illustrated (Plate VIII, Fig. 6A-D).

Discussion

In the present study, we have characterized the immunohistochemical expression of selected stem cell markers in the endometria of cycling pigs and cows, during both the follicular and luteal phases of the oestrous cycle, indicating the existence of endometrial stem cells of both epithelial and mesenchymal origin. We have made several novel findings regarding stem cell protein markers in both porcine and bovine endometrium.

OCT-3/4 plays a crucial role in maintaining the pluripotency and self-renewal of embryonic stem cells (Niwa et al. 2000). A literature search revealed only two publications reporting OCT-3/4 positivity in both pigs (Subbarao et al. 2015; Wiater et al. 2018) and cows (Cabezas et al. 2014; Lara et al. 2017). The present study is the first to identify OCT-3/4 positivity in porcine endometrial glandular cells. We found differences in the OCT-3/4 expression between the porcine and bovine endometrium. Compared to pigs, the bovine endometrial samples showed less OCT-3/4 expression in both the glands and stroma, and there was a significant decrease between the follicular and luteal phases of the oestrous cycle. Interestingly, during the follicular phase, two bovine samples showed a similar expression pattern to that of SOX2 identified in our previous study (Lenz et al. 2022). OCT-3/4 has been reported to be expressed not only in stem cells but also in differentiated cells (Zangrossi et al. 2007), which could explain the large number of OCT-3/4-positive glandular cells identified in the current study.

CD44 is considered a marker of eMSCs (Miernik and Karasinski 2012). The present study is only the third to show CD44 expression in porcine endometrium and the first to show that CD44 is expressed in porcine endometrial glands. Interestingly, CD44 expression was identified only during the luteal phase of the oestrous cycle and only in glandular cells, with an expression pattern that was similar to those of OCT-3/4 and SOX2.

There have been few studies of the expression of SSEA-1 in the human endometrium. In cycling women, the highest expression of SSEA-1 has been shown in the basalis glandular epithelium (Valentijn et al. 2013). SSEA-1-positive glandular cells were found to express both oestrogen and progesterone receptors at a lower level than SSEA-1-negative cells, which is indicative of a less differentiated cell phenotype (Valentijn et al. 2013). Another study showed differences in oestrogen receptor 1 expression between the stratum basalis (expression consistent throughout the menstrual cycle) and the stratum functionalis (expression restricted to the proliferative phase) (Leyendecker et al. 2002). Taken together, these findings suggest that endometrial epithelial progenitor cells may be a subset of SSEA-1-positive cells that are located in the lower portion of the functionalis, near its interface with the basalis. To our knowledge, the present study is the first to demonstrate SSEA-1 expression in endometrial cells during the oestrous cycle in pigs. These SSEA-1-positive glandular cells were principally located in the middle glandular portion of the endometrium, which may suggest that this is the location of porcine endometrial epithelial progenitor cells, and this would be similar to the presumed location of SSEA-1-positive epithelial progenitor cells in human endometrium.

SOX10 is a transcription factor that plays an important role in neural crest and peripheral nervous system development. Its expression has been found in various tissues, including

blood vessel walls and the surrounding connective tissue (Tang et al. 2012). Currently, SOX10 is considered to be an adult stem cell marker. One previous study showed coexpression of SOX10 with that of several mesenchymal stem cell (CD29, CD44, CD73, CD90, and Snai) and neural crest stem cell (nestin and p75) markers (Wang et al. 2017). However, to date, SOX10 expression has not been characterized in the endometrium of farm animals. Thus, the present study is the first to show SOX10 expression in both porcine and bovine endometrium. We found that only a minority of stromal cells were SOX10-positive, with little (pigs) or a more noticeable (cows) difference in its expression between the follicular (1% in pigs and 8% in cows) and luteal (< 1% in pigs and 2% in cows) phases of the oestrous cycle. In older cows and those that had previously undergone pregnancy, there was slightly lower expression of SOX10 (in pigs, the associations with these two factors could not be assessed because of the very low expression levels). Most of the SOX10-positive stromal cells were located in the luminal portion of the endometrium, with some showing a perivascular distribution and others being scattered throughout this portion. This finding is consistent with that of a previous study showing both perivascular and interstitial distribution of SOX10-positive mesenchymal cells in subcutaneous connective tissue (Wang et al. 2017). Interestingly, both porcine and bovine endometrium contained small areas with much larger numbers of SOX10-positive stromal cells, some of which had elongated nuclei and resembled stromal fibroblasts. In addition, some of these cells were present in poorly formed vessel walls. Careful microscopic examination of these areas also revealed sparse deposits of haemosiderin, indicating that they were sites of previous haemorrhage. In general, bleeding is followed by tissue repair, during which fibroblasts proliferate, and blood vessel formation occurs. Thus, SOX10-positive stromal cells may contribute to endometrial regeneration. This hypothesis is supported by previous findings of an important role of SOX10-positive stem cells in tissue remodeling. Furthermore, SOX10-positive mesenchymal cells have been shown to differentiate into fibroblasts and perivascular cells (myofibroblasts), thereby contributing to tissue repair (Tang et al. 2013; Wang et al. 2017).

CD73 is a classical mesenchymal stem cell marker and an enzyme that plays a key role in the production of extracellular adenosine, which is involved in cell signalling pathways in various tissues. CD73 is present on the cell surface of a number of tissues, including epithelial cells, and its expression is increased by proinflammatory cytokines, oxidative stress, and hypoxia (Synnestvedt et al. 2002).

Populations of CD73-positive MSCs have been identified in several bovine tissues, including in pancreatic tissue, adult adipose tissue, foetal lung tissue, the ovaries, umbilical cord, amnion, placenta, foetal liver, and bone marrow (Lu et al. 2014; Gao et al. 2019), unlike in humans (Cheng et al. 2017). Thus, the present study is the first one conducted on bovine endometrium to show an increase in CD73 expression during the oestrous cycle, higher expression in the basal glandular portion (an expression pattern similar to that of OCT-3/4), and higher expression in older cows and those that have undergone pregnancy. Interestingly, the endometrial stromal cell population was immunonegative, as were the porcine endometrial samples. The differences in CD73 expression between the individual phases of the oestrous cycle may be related to differences in the proinflammatory cytokine milieu. A previous human study showed correlations between circulating cytokine concentrations and those of reproductive hormones and their variability during the menstrual cycle (Whitcomb et al. 2014). The authors reported increasing concentrations of the proinflammatory cytokines IL-1β, IL-6, and IL-8 from the late follicular phase to the menses. As in humans, the oestrous cycle in farm animals is controlled by reproductive hormones. Therefore, it is possible that the higher CD73 expression detected during the luteal phase of the oestrous cycle in the present study could be explained by increasing proinflammatory cytokine concentrations.

CD117, also known as c-KIT, is expressed in various cell types, including haematopoietic stem cells (Stankov et al. 2014). The present study represents the first description in porcine endometrium and the first description of CD117 expression at the protein level in bovine endometrium. Consistent but sporadic expression (< 1% of cells) was found in the porcine endometrium, whereas its expression in the bovine endometrium decreased during the oestrous cycle (from 8% during the follicular phase to 1% during the luteal phase), with slightly lower expression levels in older cows and those that had undergone pregnancy previously.

In conclusion, data obtained from our study indicate the existence of both epithelial and mesenchymal stem cell subpopulations in the endometria of cycling pigs and cows during the oestrous cycle. The age of the animals and previous pregnancy appear to have little effect on stem cell protein marker expression in either the porcine or bovine endometrium. The expression pattern of the stem cell marker SSEA-1 in porcine endometrium suggests a possible alternative location of the epithelial stem/progenitor cell population in the middle portion of the endometrial glands. To our knowledge, this study is the most in-depth investigation of stem cell protein/marker expression in both porcine and bovine endometrium undertaken to date.

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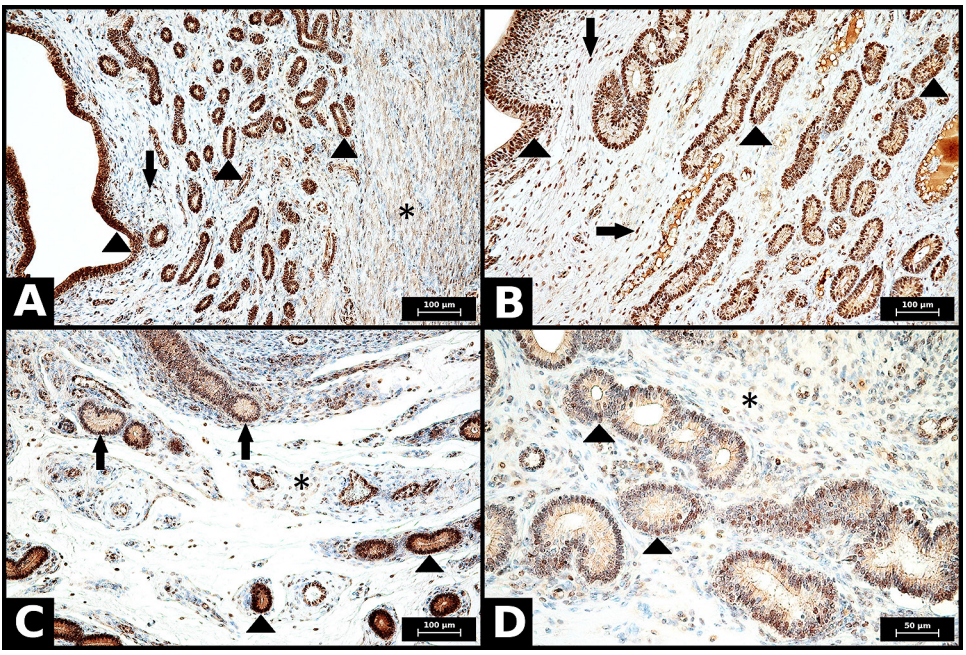

Fig. 1. Immunohistochemical expression of OCT3/4 in porcine and bovine endometrium during the oestrous cycle (all images immunohistochemistry).

Diffuse strong nuclear expression of OCT3/4 in all portions of endometrial glands (arrowheads) and focal expression (less than 40%) of variable intensity in endometrial stromal cells (arrows) during both the follicular (A) and luteal phase (B) of the oestrous cycle in porcine endometrium; myometrium (*) (× 200 magnification, scale bar 100 µm). Strong and diffuse expression in the basal glandular portions (arrowheads) and a moderate focal expression (around 35%) in the remaining luminal portions of the endometrial glands (arrows) in the bovine endometrium during the follicular phase (C) (× 200 magnification, scale bar 100 µm). Approximately 40% weak/moderate positivity across endometrial glands (arrowheads) during the luteal phase in bovine endometrium (D) (× 400 magnification, scale bar 50 µm). Weak/moderate positivity in less than 30% of stromal cells during both phases of the estrous cycle in bovine endometrium (C, D). NA of the objective lens: 0.40 for × 200 magnification and 0.65 for × 400 magnification.

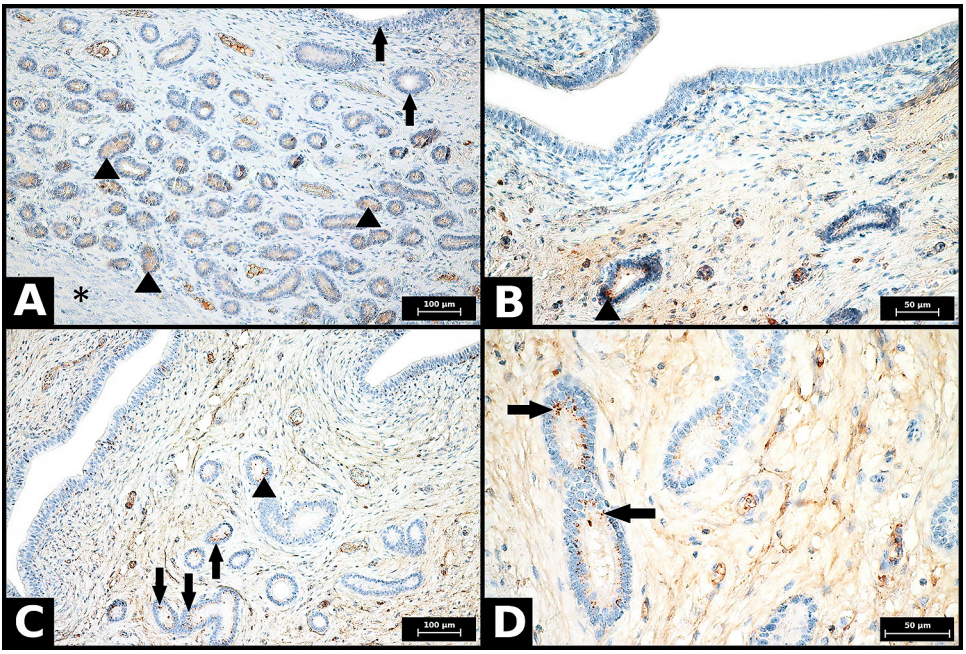

Fig. 2. Immunohistochemical expression of CD44 and SSEA-1 in glandular cells in porcine endometrium during the oestrous cycle (all images immunohistochemistry).

Weak/moderate membranous and cytoplasmic expression of CD44 in approximately 70% of epithelial cells in the basal and middle glandular portions (arrowheads) and no expression in the luminal portions of the endometrial glands (arrows) during the luteal phase (A); myometrium (*) (× 200 magnification, scale bar 100 µm). Less than 1% of glandular cells were SSEA-1 positive in a paranuclear dotlike fashion (Golgi area) in the luminal glandular portions (arrowhead) during the follicular phase (B) and < 3% of cells were positive in the middle glandular portions (arrows) and to a lesser extent at the interface between the middle and luminal glandular portions (arrowhead) during the luteal phase of the oestrous cycle (C, D) (× 400 magnification, scale bar 50 µm [B, D], × 200 magnification, scale bar 100 µm [C]). NA of the objective lens: 0.40 for × 200 magnification and 0.65 for × 400 magnification.

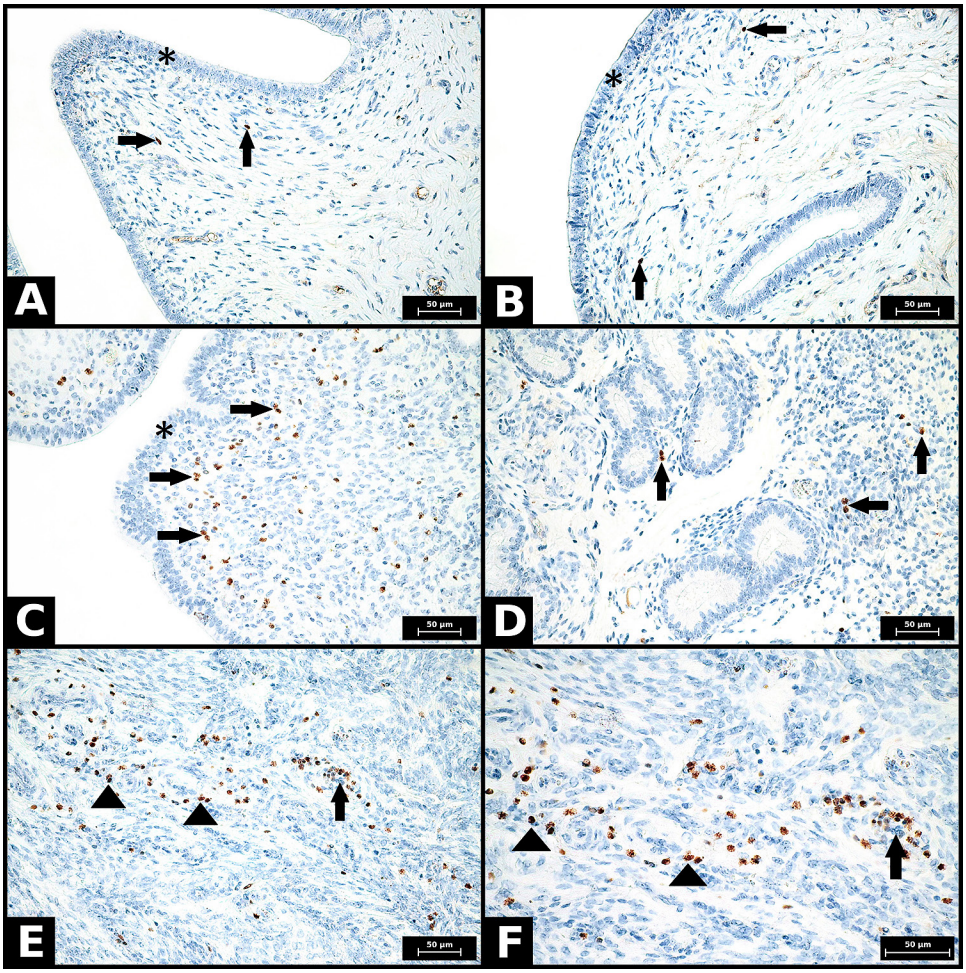

Fig. 3. Immunohistochemical expression of SOX10 in stromal cells in porcine and bovine endometrium during the oestrous cycle (all images immunohistochemistry).

Porcine endometrial samples showed sporadic and strong SOX10 expression in < 2% of endometrial stromal cells (arrows) located in these images in the subepithelial area during both the follicular (A) and luteal phases (B) of the oestrous cycle; surface epithelium (*). Bovine endometrial samples showed strong SOX10 expression in approximately 8% of endometrial stromal cells (arrows) in this image in the subepithelial area (* surface epithelium) during the follicular phase (C) and expression of approximately 2% during the luteal phase (D) of the oestrous cycle. Small aggregates of SOX10-positive endometrial stromal cells (arrowheads) (E, F), some of which are present in poorly formed vessel walls (arrows); bovine endometrium (all images × 400 magnification, scale bar 50 µm). NA of the objective lens: 0.65 for × 400 magnification.

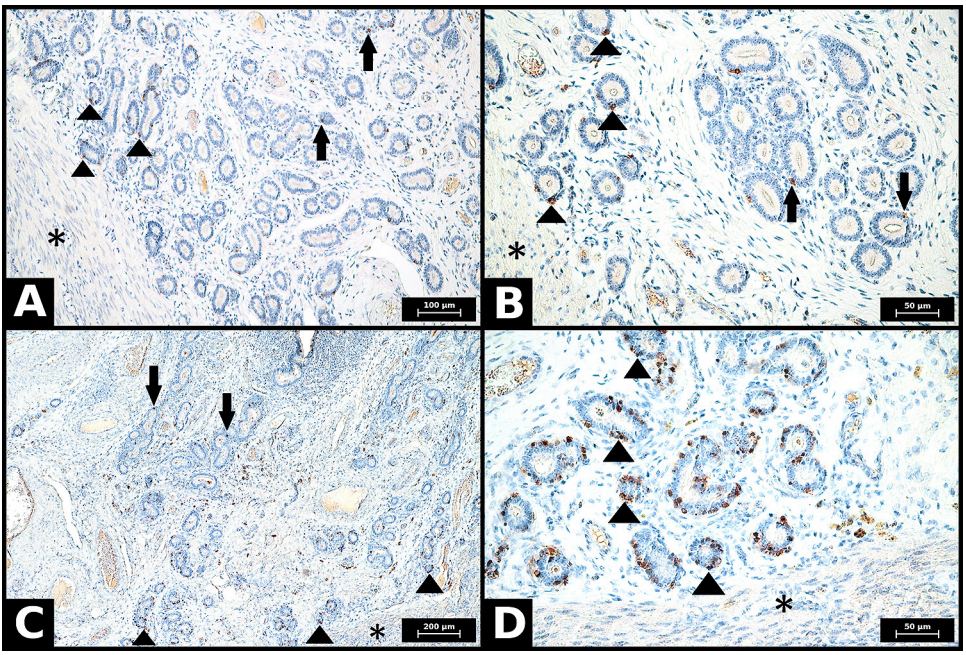

Fig. 4. Immunohistochemical expression of CD73 in glandular cells in bovine endometrium during the oestrous cycle (all images immunohistochemistry).

Weak/moderate expression of approximately 4% in the basal portions (arrowheads) and < 1% in the middle and luminal portions (arrows) during the follicular phase (A, B) and strong expression approximately of 15–20% in the basal portions (arrowheads) and < 4% in the middle and luminal portions of the endometrial glands (arrows) during the luteal phase (C, D) of the oestrous cycle; myometrium (*)(× 200 magnification, scale bar 100 µm [A], × 400 magnification, scale bar 50 µm [B, D], × 100 magnification, scale bar 200 µm [C]). NA of the objective lens: 0.25, 0.40 and 0.65 for × 100, × 200 and × 400 magnification, respectively.

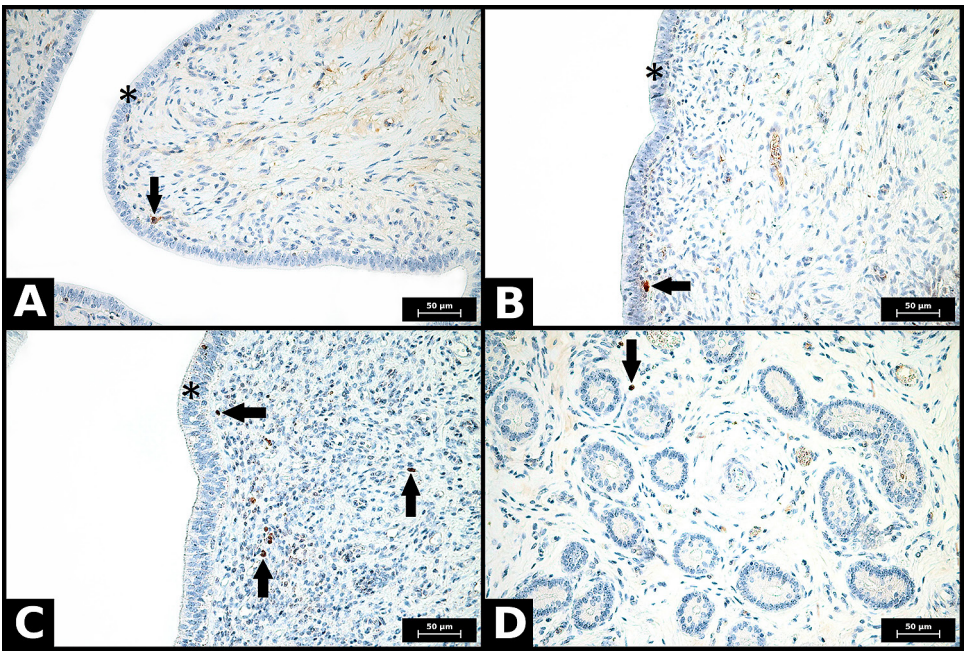

Fig. 5. Immunohistochemical expression of CD117 in stromal cells in porcine and bovine endometrium during the oestrous cycle (all images immunohistochemistry).

Sporadic (< 1%) moderate (follicular phase [A]) or strong (luteal phase [B]) expression in endometrial stromal cells (arrows) located in the subepithelial area in porcine endometrium. In bovine endometrium, moderate/strong expression was detected in approximately 8% of stromal cells (arrows) during the follicular phase (C) and weak/moderate expression was found in < 1% of stromal cells (arrow) during the luteal phase (D) of the oestrous cycle (all images × 400 magnification, scale bar 50 µm). Surface epithelium (*). NA of the objective lens: 0.65 for × 400 magnification.

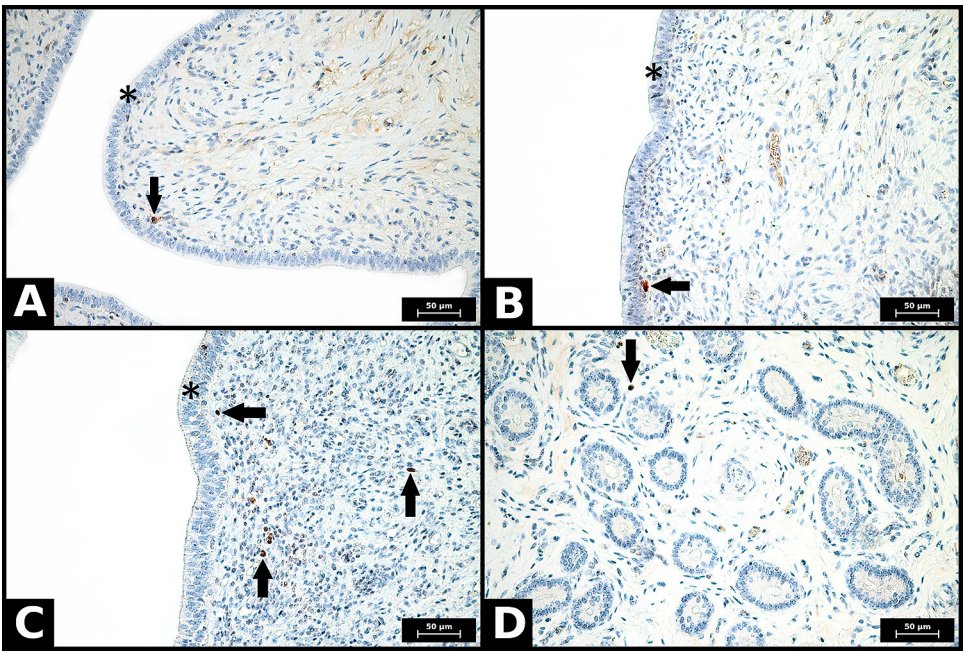

Fig. 6. Negative controls in porcine and bovine endometrium (all images immunohistochemistry).

Negative controls in which the OCT3/4, SOX-10, CD73, CD117, SSEA1 antibodies were replaced by diluted rabbit serum in bovine (A) and porcine (B) endometrium and negative controls in which the CD73 antibody was replaced by diluted mouse serum in bovine (C) and porcine (D) endometrium showing completely negative endometrial glands (arrowheads) and stroma (arrows), myometrium (*) (all images × 100 magnification, scale bar 200 µm). NA of the objective lens: 0.40 for × 200 magnification.