Suppressor of cytokine signalling (SOCS) genes are expressed in the endometrium and regulated by conceptus signals during early pregnancy in the ewe

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Abstract

It is established that the conceptus–endometrium dialogue involves cytokines, growth factors and hormones. Given the crucial functions of the suppressor of cytokine signaling (SOCS) family proteins in cytokine signalling, we analyzed the expression and the regulation of CIS and SOCSs 1–3 transcripts during early pregnancy in the ovine endometrium. An overall stimulation of the SOCS transcripts was described in the pregnant ewes with two specific patterns. Unilaterally pregnant ewes confirmed the conceptus-produced factors as regulators of the SOCSs 1–3 expression at day 16 of pregnancy. Intrauterine injection of recombinant ovine interferon τ (IFNτ) in cyclic ewes stimulated the expression of the SOCS mRNA with various potencies, therefore suggesting that the SOCS could take part in the negative regulation of the IFNτ signalling pathway.

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Introduction

A tightly regulated communication between the conceptus (the embryo or foetus and associated membranes) and the endometrium is necessary for the establishment and maintenance of pregnancy (Paria *et al.* 2002). To be successful, implantation requires both uterine and conceptus secretions (Bowen & Burghardt 2000). In the endometrium of ruminants, the intercaruncular zones synthesize and secrete numerous substances including cytokines, growth factors and hormones – collectively termed histotroph – whereas the aglandular caruncles represent the sites of superficial placentation. These regions are both essential for conceptus survival, development and implantation (Gray *et al.* 2001).

Among the conceptus-synthesized factors, interferon τ (IFNτ) is considered as the major antiluteolytic pregnancy recognition signal in ruminants (Roberts *et al.* 1992). IFNτ is secreted exclusively by the ovine trophectoderm from days 10 to 22 after mating. It is not detected in the maternal serum and acts in a paracrine fashion on the endometrium to prevent the increase of oxytocin and oestrogen receptors, thereby abrogating the luteolytic mechanism (Martal *et al.* 1998). IFNτ is a member of the type I IFN family, binds to type I IFN receptors (IFNARs), signals through the Janus kinase/signal transduction and activators of transcription (JAK/STAT) pathway and regulates a set of IFN-responsive genes including STATs 1 and 2, β2-macroglobulin, IFN regulatory factors (IRFs) 1 and 9, ISG17 Mx protein and 2′,5′-oligoadenylate synthetase (Spencer & Bazer 2002). Unravelling the molecular mechanisms of IFNτ transduction pathway and identifying new IFNτ target genes may help to elucidate the molecular mechanisms sustaining the implantation in ruminants.

Currently eight molecules have been identified as members of the suppressor of cytokine signalling (SOCS) protein family: the cytokine-inducible SH2-domain-containing protein CIS and SOCSs 1–7 (Yoshimura *et al.* 1995, Endo *et al.* 1997, Naka *et al.* 1997, Starr *et al.* 1997). They are generally present at low levels in unstimulated cells but are rapidly and transiently induced by various cytokines, interferons and hormones signalling through the JAK/STAT pathway (Fujimoto & Naka 2003). SOCS proteins contain a central SH2 domain, which is flanked by a variable N-terminal domain and a C-terminal SOCS box (Krebs & Hilton 2000). SOCS proteins have been shown to attenuate signal transduction by binding and inhibiting the JAK tyrosine kinases activity, by competing with STATs for phosphorylated tyrosine residues on cytokine receptors or by targeting bound signalling proteins for proteasomal degradation (Kile *et al.* 2002). Therefore SOCS proteins are inducible inhibitors of cytokine signalling that control the intensity and the duration of cytokine responses via a classical negative-feedback loop but which are also involved in crosstalk between different signalling pathways.

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Because the crucial functions of SOCS in the regulation of cytokine signalling pathway are well established and numerous cytokines, IFNs, growth factors and hormones are required for a successful implantation (Martal *et al.* 1997), we have hypothesized that the cascade of conceptus and maternal intercellular mediators controlling the endometrium receptivity affects the expression of SOCS genes during the peri-implantation period. The aim of this study was to determine the expression of CIS and SOCSs 1–3 as well as to analyze their regulation by the conceptus signals, especially IFNτ, in caruncular and intercaruncular endometrium compartments during early pregnancy in the ewe.

Materials and methods

Animals and tissue samples

Procedures for handling animals were conducted in compliance with the guidelines for Care and Use of Agricultural Animals in Agricultural Research and Teaching (authorization no. 78–34). Primiparous ewes of the Prealpes-du-Sud breed were synchronized using a 14-day treatment of intravaginal sponges containing 40 mg fluorogestone acetate (Intervet, Angers, France). The ewes then were given 400 IU equine chorionic gonadotrophin (Intervet) and were mated with fertile rams. For every experiment, uteri were collected after slaughter and exsanguination. Intercaruncular (INTERCAR in the figures) and caruncular (CAR in the figures) endometrium zones were dissected free of the myometrium, snap-frozen immediately in liquid nitrogen and stored at –80 °C until use. In experiment 1, cyclic ewes were sampled at days 12 and 16 of the oestrous cycle and pregnant ewes at days 12, 16, 20, 26 and 46 of pregnancy ($n=3$ ewes/status for each day). The stages of pregnancy were confirmed by the presence and the morphology of the conceptus in uterine flushings. In experiment 2, mono-ovulated pregnant animals ($n=4$) were prepared surgically to limit pregnancy to one uterine horn as described previously, with slight modifications (Bazer *et al.* 1979). Briefly, at day 5 of pregnancy a uterine ligature was placed at the base of the horn contralateral to the corpus luteum. The pregnant uteri were recovered at day 16. In experiment 3, non-pregnant ewes (day 10 post-oestrus) received a single intra-uterine injection (2 ml) of either recombinant ovine IFNτ (roIFNτ) produced in yeast (Rogez *et al.* 2003; 300 μg; 1×10^8 anti-viral units/mg; $n=8$) or 0·9% NaCl solution containing 300 μg BSA (vehicle; $n=8$) in the horn ipsilateral to the corpus luteum. Injections were performed by injecting the ovine IFNτ or the vehicle solution in the oviduct upon careful insertion of the needle in the unfundibulum. Four animals from each group were slaughtered 2 or 24 h later and caruncles as well as intercaruncular zones sampled from the injected horn.

Reverse transcriptase PCR cloning of partial cDNA for ovine CIS and SOCSs 1–3

Total cellular RNA was extracted from a mix of ovine endometrium and corpus luteum using the TRIzol LS reagent (Invitrogen Life Technologies, Cergy-Pontoise, France) according to the manufacturer's instructions. An aliquot of of total RNA (2 μg) was reverse-transcribed for 60 min at 37 °C in the presence of 0·5 μg oligo(dT)12–18 primer and 200 U Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies) in a total volume of 20 μl. Partial cDNAs for ovine CIS (314 bp), SOCS1 (344 bp), SOCS2 (325 bp) and SOCS3 (313 bp) were amplified by PCR using 1 μl of the reverse transcriptase reaction and specific primers for CIS (forward, 5′-GGAGGATC TGCTGTGCATAG-3′; reverse, 5′-CAGYRCAGGA GGCCACATAG-3′), SOCS1 (forward, 5′-GCCGAT TACCGGCGCATCAC-3′; reverse, 5′-GCTCC TGCAGCGGCCGCACG-3′), SOCS2 (forward, 5′-CGCATTCAGACTACCTACTAAC-3′; reverse, 5′-GGTAAAGGCAGTCCCCAG-3′) or SOCS3 (forward, 5′-TGCGCCTCAAGACCTTCAGC-3′; reverse, 5′-ACCAGCTTGAGCACGCAGTC-3′). The PCR were performed with *Taq* DNA polymerase (Q.BIOgene, Illkirch, France) for 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s followed by a final extension step at 72 °C for 10 min. The PCR products of the appropriate size were cloned in the pGEM-T Easy Vector System (Promega, Charbonnières, France) and then sequenced in both directions.

Northern-blot and dot-blot analysis

The quantity of RNA was estimated spectrophotometrically and the integrity of RNA examined by gel electrophoresis in a 1% agarose gel. Northern blotting was performed with 15 μg total RNA by hot agarose electrophoresis on a 1·2% TAE gel according to Almeida *et al.* (2000) and then capillary-transferred overnight in 10 × SSC (where 1 × SSC is 0·15 M NaCl/0·015 M sodium citrate) to nylon membrane (Hybond-N; Amersham Biosciences, Orsay, France). Dot blotting was carried out with 10 μg total RNA loaded on to nylon membrane using a Bio-Dot device (Touzart & Matignon, Courtaboeuf, France). The membranes were fixed by UV irradiation.

Loading and samples integrity in Northern blot membranes were confirmed by staining RNA with Methylene Blue. Specific radioactive probes for each SOCS were generated with the RediprimII random primer-labelling kit (Amersham Biosciences) using the PCR-generated cDNAs and [α-^{32}P]dCTP, followed

by cleanup with MicroSpin S-200 HR columns (Amersham Biosciences). Hybridizations of the membranes were carried out at 65 °C in an aliquot of 1% BSA/1 mM EDTA/0·5 M phosphate buffer/1% SDS (Church & Gilbert 1984) containing 1×10^6 c.p.m./ml labeled probe. After washing under high-stringency conditions (Sandra *et al.* 2001), Northern blots were exposed to autoradiography using Kodak MS X-ray films (Eastman Kodak, Paris, France). After exposure to a sensitive screen, radioactive signals of the dot blots were revealed with a FLA-3000 phosphorimager (FujiFilm, Düsseldorf, Germany) and intensity was quantified using the Advanced Image Data Analyzer software (Fujifilm). 18 S mRNA intensities were used for normalization before comparison. Stripping and reprobing were performed using the same membranes. Mean values of relative expression intensities were used for final data presentation.

Statistical analysis

All data are presented as the mean values ± S.E.M. arbitrary units (AI). Statistical differences were assessed using two-way ANOVA followed by Tukey–Kramer multiple-comparisons test when appropriate (GraphPad Prism, GraphPad Prism software; San Diego, CA, USA). Differences were considered significant when $P<0·05$.

Results

Expression of SOCS transcripts in the endometrium during early pregnancy

In the intercaruncular (Fig. 1A) and caruncular (data not shown) zones of the endometrium, a single transcript with the specific size of each SOCS was detected at various days of the oestrous cycle and pregnancy.

Using slot-blot hybridization analysis, steady-state levels of SOCS mRNA were determined in the caruncular and intercaruncular zones collected at day 12 and 16 of the cycle as well as at day 12, 16, 20, 26 and 46 of pregnancy (Fig. 1B). Between days 12 and 16 of the cycle, the expression of CIS, SOCS1, SOCS2 and SOCS3 mRNA was stable and identical between endometrium zones. The level of all SOCS transcripts was also unaffected by pregnancy status at day 12.

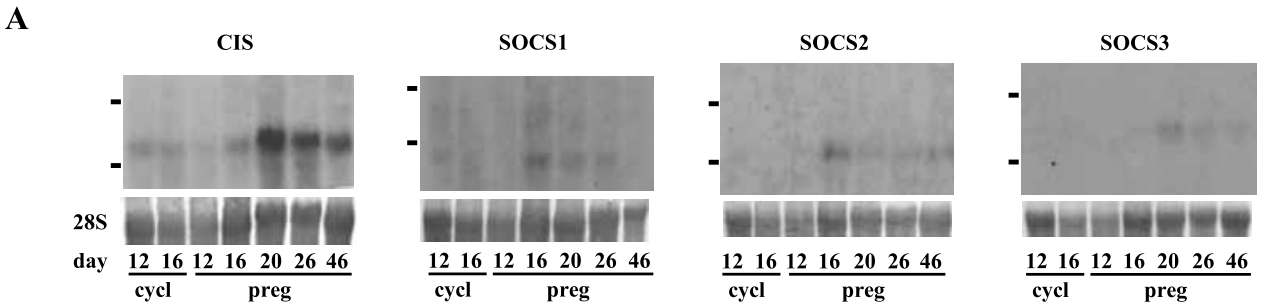
During the first 46 days of pregnancy, the expression of CIS mRNA was stimulated by pregnancy in the caruncular zone at days 16 and 20 compared with day 12 ($P<0·01$ and $P<0·001$ respectively). The levels of CIS mRNA expression clearly increased in the intercaruncular zone at days 20, 26 ($P<0·01$) and 46 ($P<0·05$). In addition the intercaruncular zone displayed an approx. 2-fold increase in CIS and SOCS3 mRNA expression compared with the caruncular zone from day 20 to day 46 of pregnancy ($P<0·05$). During pregnancy, the expression of the SOCS1 transcript was altered with a peak of expression occurring at day 16 in both endometrium tissues when compared with day 12 ($P<0·001$). No significant change in SOCS2 was detected during early pregnancy. However both caruncular and intercaruncular zones expressed higher SOCS2 mRNA levels (2·8- and 3·7-fold respectively; $P<0·05$) at day 16 of pregnancy as compared with day 16 of the oestrous cycle. For both SOCS1 and SOCS2 mRNAs, analysis indicated a comparable level of expression in both endometrium tissues. In the caruncular zone, the level of SOCS3 transcript was greater at days 16, 20 ($P<0·001$), 26 ($P<0·01$) and 46 ($P<0·05$) of pregnancy as compared with day 12. In the intercaruncular zone, a similar increase was observed at days 20 ($P<0·001$), 26 ($P<0·01$) and 46 ($P<0·005$). At days 20, 26 and 46, the SOCS3 transcript was approximately twice more expressed in the intercaruncular than in the caruncular zone ($P<0·05$).

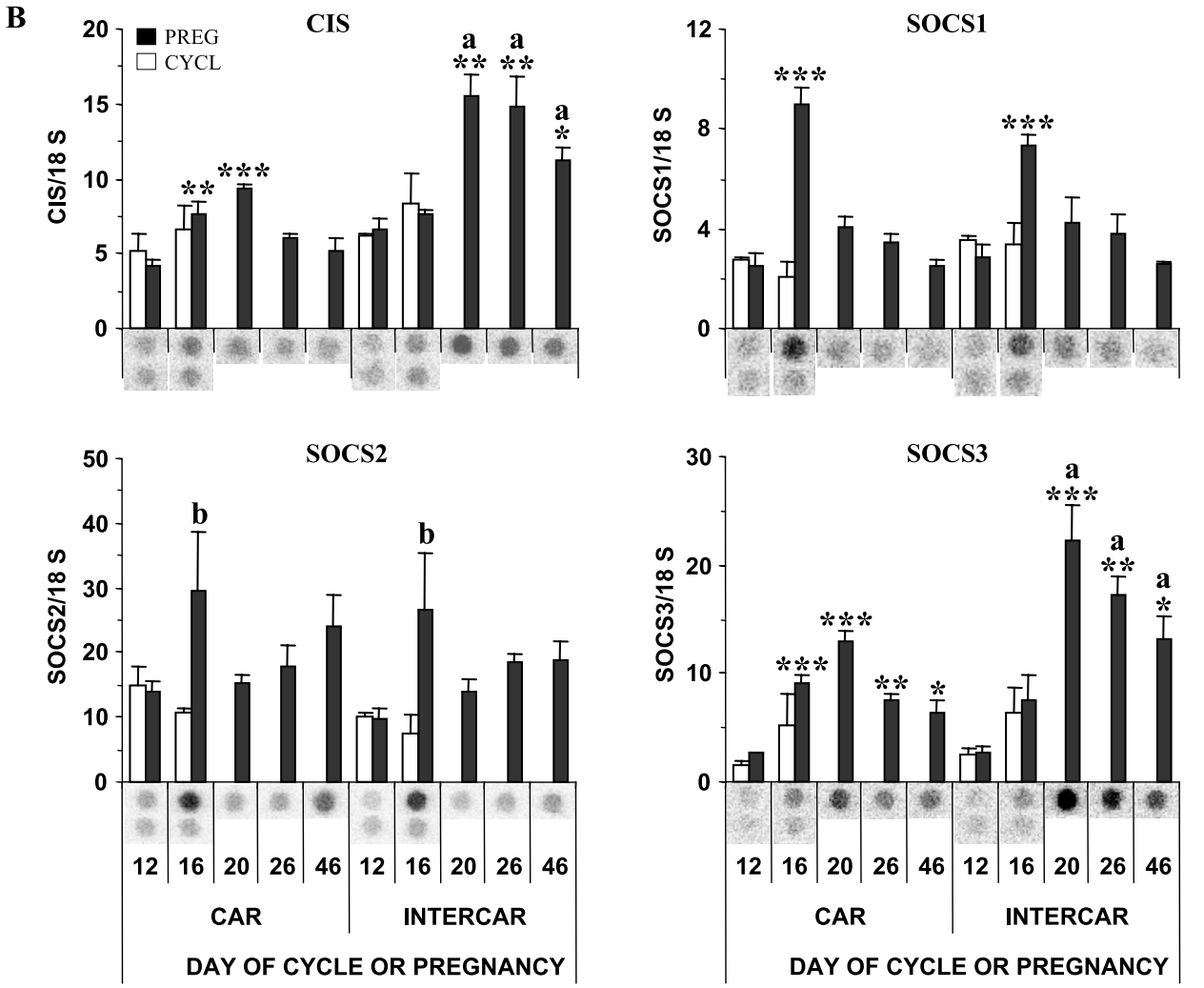
Regulation of SOCS transcripts in the endometrium by conceptus-secreted factors

To investigate the importance of the local factors secreted by the conceptus in regulating the expression of the SOCS genes, a ligature was placed at day 5 of pregnancy around the uterine horn contralateral to the corpus luteum. The conceptus was therefore blocked in the ipsilateral horn and uteri collected on day 16 of pregnancy (Fig. 2). No variation of CIS mRNA expression was detected in the caruncular zone from the non-pregnant horn compared with those collected from the pregnant horn. In the intercaruncular zone the presence of the conceptus led to a significant decrease in the levels of CIS mRNA (0·84-fold; $P<0·05$). In contrast, a significant increase was detected in the expression of SOCS2 and SOCS3 transcripts (respectively 1·7- and 1·56-fold; $P<0·05$) in the caruncular zone from the pregnant horn compared with the non-pregnant horn. With regard to SOCS1 in the caruncular zone, one of the ewes displayed a level of SOCS1 transcript higher in the non-pregnant horn than in the pregnant horn (10·23 versus 8·39). Although there was still a trend towards an increase of SOCS1 expression in the caruncular zone, the difference was not statistically different ($P=0·237$). An up-regulation was also reported for both SOCS1 (2·0-fold; $P<0·05$) and SOCS2 mRNA (2·7-fold; $P<0·005$), but not for SOCS3 mRNA, in the intercaruncular zone of the pregnant horn as compared with the same region of the non-pregnant horn.

IFNτ induces the transient expression of SOCS transcripts in the cyclic endometrium

To assess whether IFNτ affects the levels of SOCS transcripts, cyclic ewes received a single intrauterine injection of either roIFNτ or a control solution.





The uterus was collected at 2 or 24 h (Fig. 3). After 2 h of roIFNτ administration, the CIS mRNA expression was not affected in the intercaruncular zone and induced less than 2-fold in the caruncular zone ($P<0·05$). The transcripts encoding SOCSs 1, 2 or 3 were up-regulated with various intensities in both the caruncular (respectively 6-, 2- and 4·5-fold induction compared with the control; $P<0·001$, $P<0·01$ and $P<0·01$) and intercaruncular (respectively 2·6-, 2-, and 2·9-fold induction compared with the control; $P<0·01$, $P<0·05$ and $P<0·01$) zones. No induction of any SOCS transcript was detectable at 24 h after roIFNτ administration regardless of the endometrium zone.

Discussion

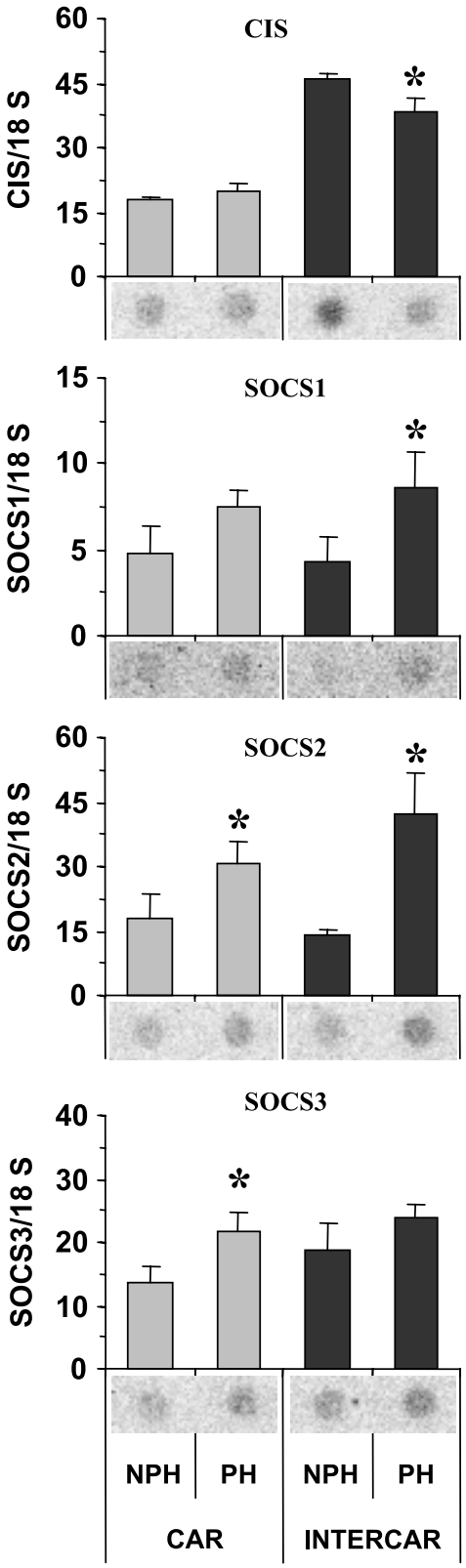
To the best of our knowledge, this is the first report that the peri-implantation period is associated with a clear regulation of SOCS-gene expression in the endometrium of a mammalian species. A major feature is the overall stimulation of the ovine SOCS-gene expression according to two distinct patterns, namely a peak centred on day 16 for SOCS1 and SOCS2 in both the caruncular and intercaruncular zones but a marked up-regulation for CIS and SOCS3 starting at day 16 in the caruncles and day 20 in the intercaruncular zones and lasting until day 46 in both endometrium zones. These results support our hypothesis that the conceptus contributes to the regulation of endometrial SOCS during early pregnancy. Furthermore, although a mechanical effect of the uterine stretch affecting the expression of SOCS cannot be totally ruled out in the pregnant horn, the use of the unilaterally pregnant ewe model confirmed that the conceptus induced the expression of SOCSs 1, 2 and 3 transcripts at day 16.

The striking correlation between the temporal changes in SOCS1 and SOCS2 genes expression (present study) and the known secretion profile of IFNτ (Roberts *et al.* 1992, Martal *et al.* 1998) led us to investigate if this locally acting signal could actually modulate the expression of the SOCS genes. The expression of the SOCSs 1, 2 and 3 mRNA was stimulated by a single intrauterine injection of roIFNτ in cyclic ewes after 2 h. This stimulation is rapid and transient as expected from what was reported in other biological systems where SOCS are known as cytokine-inducible immediate early genes (Yoshimura *et al.* 1995, Endo *et al.* 1997, Krebs & Hilton 2000). The SOCSs 1, 2 and 3 mRNA levels were induced in the caruncles, consistent with the expression of both ovine type I IFNAR subunits in these endometrium zones (Rosenfeld *et al.* 2002) as well as the expression of IRF-1 and various STAT proteins (Choi *et al.* 2001, Stewart *et al.* 2001), all required for the induction of SOCS genes (Krebs & Hilton 2000, Fujimoto & Naka 2003). Taken together, these data suggest that SOCSs 1, 2 and 3 are IFNτ target genes as formerly reported in human T-cells for IFNα, another member of the type I IFN family (Brender *et al.* 2001).

The SOCS1 and SOCS3 proteins, but not SOCS2, inhibit the IFNα-mediated antiviral and antiproliferative activities (Song & Shuai 1998). Pregnancy as well as conceptus-induced SOCS2 and SOCS1 display a similar regulation, thereby suggesting that SOCS2 can also take part to the negative regulation of IFNτ signal transduction. Given the various biological properties of IFNτ (Roberts *et al.* 1992, Martal *et al.* 1998), further studies about SOCS 1, 2 and 3 are definitely required to establish their respective contribution in the negative regulation of the IFNτ signalling pathway.

Interestingly, whereas no pregnancy-induced regulation of CIS was detected before day 20 in the intercaruncular zone (Fig. 1), the ligature experiment shows a moderate but significant down-regulation of the CIS transcript in the pregnant horn at day 16 (Fig. 2). This result reflects a negative local effect of the conceptus secretions on the expression of CIS and does not support CIS as a major member of the feedback-regulatory loop of the IFNτ signalling pathway. Whether this decrease is linked to the expression of a CIS-downregulating factor acting on the endometrial cells and/or to the disappearance of a CIS-producing cell population in the intercaruncular zones is currently unknown. Since pregnancy profoundly modifies the morphological structure of the uterus, including the accumulation of

Figure 1 Expression of SOCS mRNAs in cyclic and pregnant endometrium of the ewe. Total RNA was extracted from the caruncular (CAR) or the intercaruncular (INTERCAR) zones at various days of the oestrous cycle (CYCL) or pregnancy (PREG). (A) Northern-blot analysis of CIS, SOCS1, SOCS2 and SOCS3 transcripts in intercaruncular zone of cyclic and pregnant ewes. Each lane represents 15 µg total RNA pooled from three ewes. Dashes indicate the positions of the 28 and 18 S RNA. 28 S rRNAs were visualized by Methylene Blue staining of the membrane. (B) Quantification of SOCS mRNA: 10 µg total RNA were analyzed using dot-blotting and a [^{32}P]dCTP-labelled cDNA probe specific for CIS, SOCS1, SOCS2 or SOCS3. A representative dot blot is presented in each histogram. Over the two stages of the oestrous cycle (day 12 or 16), no change of CIS or SOCS 1, 2 or 3 expression was detected and levels were identical in the caruncular and intercaruncular zones. Data are means±S.E.M. (n=3). Asterisks indicate a significant difference between day 16, 20, 26 or 46 and day 12 of pregnancy; *$P<0·05$, **$P<0·01$, ***$P<0·001$ versus day 12 of pregnancy. Letters above bars: a indicates a significant difference between the caruncular and intercaruncular zones on the same day of pregnancy ($P<0·05$); b indicates a significant difference between day 16 of pregnancy and day 16 of the oestrous cycle ($P<0·05$).

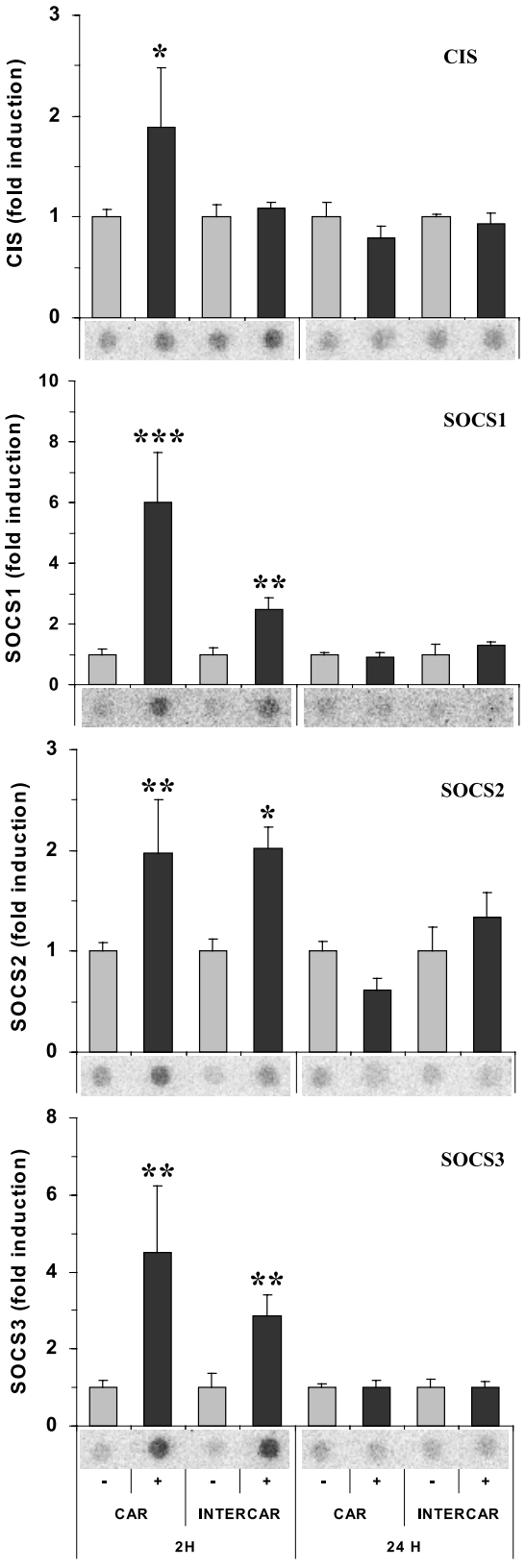


immune cells (Hansen 1998), the accurate identification of the CIS-expressing cells should give insights into explaining the specific regulation of CIS at day 16 of pregnancy.

In addition to IFNτ, the increase of ovine Leukemia Inhibitory Factor (LIF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and bovine macrophage (M)-CSF expression has been reported in the ruminant endometrium and conceptus during the peri-implantation period (Schäfer-Somi 2003). To the best of our knowledge, the expression of their receptors has not been described in ruminant uterus but, in both mice and humans, endometrial effects of these factors are essential for implantation (Saito 2001). These cytokines are known to induce the SOCS genes and SOCS1 or SOCS3 are clearly part of the negative regulatory loop of the M-CSF, GM-CSF and LIF signalling pathways (Fujimoto & Naka 2003). Therefore the regulation of these ovine SOCS transcripts between day 12 and 20 may include the effects of these cytokines and could be a marker for the successful implantation process.

During the first trimester of pregnancy, a clear increase in CIS and SOCS3 was detected from day 20 onwards, lasting until day 46 in the intercaruncular zone. For these SOCS genes, the regulation beyond day 20 of pregnancy cannot result from IFNτ, whose secretion ends at days 20–21 of pregnancy (Roberts *et al.* 1992, Martal *et al.* 1998). In addition to the persisting endometrial production of cytokines such as LIF, CSF or interleukins 2, 4 or 6 beyond the implantation stage (Martal *et al.* 1997, Rahman *et al.* 2004), ligands and receptors of the ovine placental lactogen (PL)/growth factor (GH) and insulin-like growth factor (IGF) systems are also expressed when the placentation starts. Ovine PL begins to be secreted by the trophectoderm at days 15–16 and ovine GH at day 27. Ovine PL binds to a prolactin (PRL) receptor homodimer or a PRL/GH receptor heterodimer whereas GH binds to a GH receptor homodimer, both subunits being expressed in the pregnant ovine endometrium (Spencer *et al.* 2004). Ovine uterus is a rich source of IGF-I and IGF-I receptor is clearly present throughout the first trimester of pregnancy (Wathes *et al.* 1998). These three factors contribute to regulating the development and the functions of the endometrial gland epithelium during pregnancy (Wathes *et al.* 1998, Spencer *et al.* 2004).

Figure 2 Expression of SOCS transcripts in the pregnant (PH) and non-pregnant (NPH) uterine horns in unilaterally pregnant ewes at day 16. Total RNA was extracted from the caruncles (CAR) or intercaruncular (INTERCAR) zones. 10 µg total RNA were analyzed using dot-blotting and a [^{32}P]dCTP-labelled cDNA probe specific for CIS, SOCS1, SOCS2 or SOCS3. A representative dot blot is presented in each histogram. Data are means±S.E.M. (n=4). *$P<0{\cdot}05$ versus NPH.



Since the signalling pathways of PRL, GH and IGF-I receptors are known to be down-regulated by SOCS proteins (Fujimoto & Naka 2003, Krebs & Hilton 2003), ovine PL, GH and IGF-I represent relevant candidates to account for the regulation of CIS and SOCS3 occurring during early pregnancy.

Another interesting finding is the higher expression of CIS and SOCS3 transcripts reported in the intercaruncular zones. One of the striking differences between the caruncles and the intercaruncular zones is the presence of the uterine glands in the latter region. In the ewe, a strong expression of GH and IGF-I receptors as well as an exclusive localization of the PRL receptor has been described in these endometrial glands (Wathes *et al.* 1998, Spencer *et al.* 2004). Therefore the correlation between the expression of these receptors and our results may explain the higher expression of CIS and SOCS3 detected in the intercaruncular zones. On the other hand, numerous immune cells are also distributed within the intercaruncular zones (Hansen 1998, Meeusen *et al.* 2001). In the murine and human species, the regulation of immune-cell networks is clearly controlled by SOCS proteins and, for example, SOCS3 skews helper T-cell differentiation toward Th2 cells, which contribute to the maintenance of pregnancy (Kubo *et al.* 2003). A detailed analysis of the SOCS-producing cells will be required to sort out the respective contribution of the immune versus non-immune cells of the endometrium to the expression of SOCS.

In conclusion, the SOCS transcripts are expressed and differentially regulated by the conceptus in the ovine endometrium during early pregnancy. Our data suggest that SOCS1 and SOCS2 would be involved in the regulation of the endometrium receptivity and the attachment of the blastocyst whereas CIS and SOCS3 would take part in the endometrial-gland morphogenesis, uterine secretory activity and placental differentiation. Further investigations are definitely necessary to substantiate these hypotheses and to delineate the specific functions of each SOCS protein during implantation in mammals.

Figure 3 Regulation of SOCS genes expression by roIFNτ in the endometrium of cyclic ewes. Cyclic ewes received an intrauterine injection of recombinant oIFNτ (+) or vehicle (–) at day 10 of the oestrous cycle; then, caruncular (CAR) or intercaruncular (INTERCAR) zones were sampled from the injected horn 2 or 24 h later. 10 µg total RNA were analyzed using dot-blotting and a [^{32}P]dCTP-labelled cDNA probe specific for CIS, SOCS1, SOCS2 or SOCS3. A representative dot blot is presented in each histogram. Caruncular or intercaruncular data from control ewes injected with vehicle were adjusted to a value of 1. No variation in SOCS-gene expression was detected in any endometrium zone at 24 h. Data are means±S.E.M. (n=4); *$P<0{\cdot}05$, **$P<0{\cdot}01$, ***$P<0{\cdot}001$ versus vehicle.

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References

Almeida TA, Perez JA & Pinto FM 2000 Size-fractionation of RNA by hot agarose electrophoresis. *Biotechniques* **28** 414–416.

Bazer FW, Roberts RM, Basha SM, Zavy MT, Caton D & Barron DH 1979 Method for obtaining ovine uterine secretions from unilaterally pregnant ewes. *Journal of Animal Science* **49** 1522–1527.

Bowen JA & Burghardt RC 2000 Cellular mechanisms of implantation in domestic farm animals. *Seminars in Cell and Developmental Biology* **11** 93–104.

Brender C, Nielsen M, Röpke C, Nissen MH, Svejgaard A, Billestrup N, Geisler C & Ødum N 2001 Interferon-alpha induces transient suppressors of cytokine signalling expression in human T cells. *Experimental and Clinical Immunogenetics* **18** 80–85.

Choi Y, Johnson GA, Burghardt RC, Berghman LR, Joyce MM, Taylor KM, Stewart MD, Bazer FW & Spencer TE 2001 Interferon regulatory factor-two restricts expression of interferon-stimulated genes to the endometrial stroma and glandular epithelium of the ovine uterus. *Biology of Reproduction* **65** 1038–1049.

Church GM & Gilbert W 1984 Genomic sequencing. *PNAS* **81** 1991–1995.

Endo TA, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H *et al.* 1997 A new protein containing an SH2 domain that inhibits JAK kinases. *Nature* **387** 921–924.

Fujimoto M & Naka T 2003 Regulation of cytokine signaling by SOCS family molecules. *Trends in Immunology* **24** 659–666.

Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW & Spencer TE 2001 Developmental biology of uterine glands. *Biology of Reproduction* **65** 1311–1323.

Hansen PJ 1998 Regulation of uterine function by progesterone - lessons from sheep *Journal of Reproductive Immunology* **40** 63–79.

Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HM & Hilton DJ 2002 The SOCS box: a tale of destruction and degradation. *Trends in Biochemical Sciences* **27** 235–241.

Krebs DL & Hilton DJ 2000 SOCS: physiological suppressors of cytokine signaling. *Journal of Cell Science* **113** 2813–2819.

Krebs DL & Hilton DJ 2003 A new role for SOCS in insulin action. *Science STKE* **pe6** 1–3.

Kubo M, Hanada T & Yoshimura A 2003 Suppressors of cytokine signaling and immunity. *Nature Immunology* **4** 1169–1176.

Martal J, Chene N, Camous S, Huynh L, Lantier F, Hermier P, L'Haridon R, Charpigny G, Charlier M & Chaouat G 1997 Recent developments and potentialities for reducing embryo mortality in ruminants: the role of IFN-tau and other cytokines in early pregnancy. *Reproduction, Fertility and Development* **9** 355–380.

Martal JL, Chene NM, Huynh LP, L'Haridon RM, Reinaud PB, Guillomot MW, Charlier MA & Charpigny G 1998 IFN-tau: a novel subtype I IFN1. Structural characteristics, non-ubiquitous expression, structure-function relationships, a pregnancy hormonal embryonic signal and cross-species therapeutic potentialities. *Biochimie* **80** 755–777.

Meeusen EN, Bischof RJ & Lee CS 2001 Comparative T-cell responses during pregnancy in large animals and humans. *American Journal of Reproductive Immunology* **46** 169–179.

Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K, Akira S & Kishimoto T 1997 Structure and function of a new STAT-induced STAT inhibitor. *Nature* **387** 924–929.

Paria BC, Reese J, Das SK & Dey SK 2002 Deciphering the cross-talk of implantation: advances and challenges. *Science* **296** 2185–2188.

Rahman AN, Snibson KJ, Lee CS & Meeusen EN 2004 Effects of implantation and early pregnancy on the expression of cytokines and vascular surface molecules in the sheep endometrium. *Journal of Reproductive Immunology* **64** 45–58.

Roberts RM, Cross JC & Leaman DW 1992 Interferons as hormones of pregnancy. *Endocrine Reviews* **13** 432–452.

Rogez C, Martin M, Dereuddre-Bosquet N, Martal J, Dormont D & Clayette P 2003 Anti-human immunodeficiency virus activity of tau interferon in human macrophages: involvement of cellular factors and beta-chemokines. *Journal of Virology* **77** 12914–12920.

Rosenfeld CS, Han CS, Alexenko AP, Spencer TE & Roberts RM 2002 Expression of interferon receptor subunits, IFNAR1 and IFNAR2, in the ovine uterus. *Biology of Reproduction* **67** 847–853.

Saito S 2001 Cytokine cross-talk between mother and the embryo/placenta. *Journal of Reproductive Immunology* **52** 15–33.

Sandra O, Le Rouzic P, Rentier-Delrue F & Prunet P 2001 Transfer of tilapia (*Oreochromis niloticus*) to a hyperosmotic environment is associated with sustained expression of prolactin receptor in intestine, gill, and kidney. *General and Comparative Endocrinology* **123** 295–307.

Schäfer-Somi S 2003 Cytokines during early pregnancy of mammals: a review. *Animal Reproduction Sciences* **75** 73–94.

Song MM & Shuai K 1998 The suppressor of cytokine signaling SOCS1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities. *Journal of Biological Chemistry* **273** 35056–35062.

Spencer TE & Bazer FW 2002 Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Frontiers in Bioscience* **7** d1879–d1898.

Spencer TE, Johnson GA, Burghardt RC & Bazer FW 2004 Progesterone and placental hormone actions on the uterus: insights from domestic animals. *Biology of Reproduction* **71** 2–10.

Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA & Hilton DJ 1997 A family of cytokine-inducible inhibitors of signalling. *Nature* **387** 917–921.

Stewart DM, Johnson GA, Vyhlidal CA, Burghardt RC, Safe SH, Yu-Lee LY, Bazer FW & Spencer TE 2001 Interferon-tau activates multiple signal transducer and activator of transcription proteins and has complex effects on interferon-responsive gene transcription in ovine endometrial epithelial cells. *Endocrinology* **142** 98–107.

Wathes DC, Reynolds TS, Robinson RS & Stevenson KR 1998 Role of the insulin-like growth factor system in uterine function and placental development in ruminants. *Journal of Dairy Science* **81** 1778–1789.

Yoshimura A, Ohkubo T, Kiguchi T, Jenkins NA, Gilbert DJ, Copeland NG, Hara T & Miyajima A 1995 A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. *EMBO Journal* **14** 2816–2826.

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