# Bovine class A scavenger receptors (SR-A) exhibit specific patterns of regulation in endometrium during estrous cycle and early pregnancy

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**Abridged title**: SR-A expression in bovine endometrium

## Abstract

In mammals, tight regulation of the maternal endometrial function is critical for pregnancy success. In the bovine species, endometrial expression of members of

class A scavenger receptor (SR-A) has been listed in high-throughput analyses but very little is known about the involvement of these immune factors during implantation in mammals. To provide first insights on the contribution of SR-A to endometrial physiology, we analyzed the expression and regulation of all SR-A members during estrous cycle and early pregnancy in cattle. *SR-A1* level is increased by the pregnancy at day 20. *SR-A3* increases at day 13 of the estrous cycle and the pregnancy. *SR-A4* level reduces at day 20 of the estrous cycle but remains high in pregnant animals. *SR-A5* increases by day 13 of the estrous cycle, decreases on day 20 but remains high in pregnant animals. Interferon-tau does not affect *SR-A* gene expression whereas progesterone regulates expression of *SR-A3* and *SR-A5* transcripts. Eventually endometrial *SR-A3* appeared significantly higher in cows carrying *in vitro* produced embryos than in artificially inseminated cows. Our data suggest that members of *SR-A* family are involved in endometrial remodeling and regulation of endometrial gland physiology, both processes critical for implantation in mammals.

**Keywords:** scavenger receptors, endometrium, cattle, implantation

## Introduction

In mammals, implantation is a critical step of pregnancy, that relies on a tightly regulated and synchronized communication involving cellular and molecular interactions between the conceptus and the receptive endometrium (Guillomot, 1995; Lee and DeMayo, 2004). Since the pioneer report of Sir Medawar pinpointing the necessity of a “maternal tolerance of the allogenic foetus” graft (Billingham et al., 1953; Medawar, 1953; Colucci et al., 2014), a wealth of data has demonstrated the importance of the maternal immune system for the establishment and the progression of pregnancy (Chaouat et al., 2004, 2007; Chaouat, 2007). During implantation, regulation of the immune function in the endometrium includes modulation of secreted pro- and anti-inflammatory cytokines (Th1/Th2 balance), the regulation of the major histocompatibility complex and the recruitment of immune cells (Chaouat et al., 2004, 2007; Oliveira et al., 2010; Walker et al., 2010; Mansouri-Attia et al., 2012; Fair, 2016).

In ruminants, progesterone (P4) and interferon-tau (IFNT) regulate the endometrial function and both are required for the success of pregnancy (Bazer et al., 2008). Progesterone actions in pregnancy, involve the regulation of histotroph secretion that is critical for conceptus development (Spencer, 2002; Carter et al., 2008; Forde et al., 2011a). Progesterone was also shown to affect the expression of genes relative to the regulation of macrophage localization (Forde et al., 2011a). During elongation of the hatched embryo until implantation is completed, trophectoderm cells secrete interferon-tau (IFNT), a cytokine considered as the major signal of maternal recognition of pregnancy in ruminants (Martal et al., 1979; Bazer et al., 2008; Ealy and Yang, 2009; Forde et al., 2011b; Bazer and Thatcher, 2017). The major function of IFNT has been recognized as the inhibition of the

63 luteolytic mechanisms that leads to the maintenance of P4 secretion by the
64 functional corpus luteum (Gray et al., 2006; Spencer et al., 2007; Bazer et al.,
65 2008), thereby contributing to uterine receptivity and conceptus growth (Fair,
66 2016). In addition direct actions of IFNT on the endometrium have been
67 abundantly illustrated and include the regulation of endometrial genes implicated
68 in cellular growth, cell differentiation and apoptosis local immune system (Gray et
69 al., 2006; Spencer et al., 2007; Bazer et al., 2008; Ott and Gifford, 2010).

70 In order to decipher the highly dynamic processes that control endometrial
71 physiology of early pregnancy in cattle, numerous high-throughput analyses were
72 performed between day 5 and day 20 of estrous cycle and pregnancy, highlighting
73 a major impact of the conceptus from day 13 of pregnancy onwards (Klein et al.,
74 2006; Bauersachs et al., 2008, 2009, Mansouri-Attia et al., 2009a; b; Walker et al.,
75 2010; Forde et al., 2011b, 2012; Mamo et al., 2012; Spencer et al., 2013). These
76 molecular data also confirmed the functional differences between the two areas
77 that constitute the ruminant endometrium, namely the caruncules (CAR) and the
78 intercaruncular areas (ICAR) (Mansouri-Attia et al., 2009a; Walker et al., 2010).
79 While CAR areas are aglandular structures being part of the placentomes, ICAR
80 areas contain glandular epithelium, the major source of histotroph (Mansouri-Attia
81 et al., 2009a). Moreover, these studies also unveiled the ability of the endometrium
82 to response to embryos manipulations (Bauersachs et al., 2009; Mansouri-Attia et
83 al., 2009b). Interestingly, a member of the new class of Class A Scavenger
84 Receptors (SR-A) appeared as an endometrial implantation-regulated gene
85 (Mansouri-Attia et al., 2009a) suggesting the involvement of SR-A in the
86 endometrial physiology.

The scavenger receptors were identified in 1979 based on their ability to bind modified low-density lipoproteins (Goldstein et al., 1979). The majority of these receptors are transmembrane cell surface glycoproteins first identified in macrophages, dendritic cells and endothelial cells (Mukhopadhyay and Gordon, 2004). Based on the recent released consensus classification, SR family encompasses eight classes of protein based on their multi-domain structure (PrabhuDas et al., 2017). The class A (SR-A) is composed of five members: SR-A1, SR-A3 to SR-A6 with SR-A1 and SR-A6 being very closed structurally (PrabhuDas et al., 2017). Both are expressed in macrophages but *Sr-a1* expression is induced by the differentiation whereas *Sr-a6* is induced by infection in sub-populations of murine macrophages (Plüddemann et al., 2007). Unlike the other SR-A members, *SR-A3*, *SR-A4* and *SR-A5* do not appear to be expressed in macrophages but in various types of tissues including heart, intestine, lung, and placenta, as well as epithelial cells (PrabhuDas et al., 2017). Scavenger Receptors Class A have been considered as major components of innate immunity via their implication in recognition of various microbial pathogens as well as modified or endogenous molecules derived from the host (Mukhopadhyay and Gordon, 2004; Bowdish et al., 2007; Plüddemann et al., 2007; PrabhuDas et al., 2017). One particularity of SR-A5 is that it is unable to endocytose modified low-density lipoproteins (Plüddemann et al., 2007). Despite their established contribution in the immune function in mammals, little is known about the involvement of SR-A in the endometrial physiology. In cattle, SR-A1, -A3 and -A4 proteins were immunodetected in uterine macrophages (Naito et al., 1991). In mice, the second half of pregnancy is associated with an increase of uterine *Sr-a1* expression (Kyaw et al., 1998) and expression of *SR-A4* and *SR-A5* transcripts is regulated in

pregnant endometrium compared with cyclic tissue at day 20 post-estrous (Mansouri-Attia et al., 2009a).

In order to bring first insights about *SR-A* in the endometrium, we analyzed the expression and the regulation of all members of this class during estrous cycle and early pregnancy in cattle. Using *in vitro* and *in vivo* experimental models, the regulation of SR-A members by P4 and IFNT was investigated. Eventually, to further explore the immune component of the endometrium as a sensor of *in vitro* manipulated embryos (Sandra et al., 2011), we analyzed the impact of bovine *in vitro* fertilized embryos on endometrial *SR-A* expression of recipient cows at implantation.

## Materials and methods

*Animals*

Animal care and all experimental procedures were completed in accordance with European Community Directive 86/609/EC, the Animal Research Ethics Committee of University College Dublin and the French Ministry of Agriculture (authorization B91332). Protocols were registered by the Department of Health and Children (Ireland) or by the Regional Ethical Committee of Animal Experimentation of INRA and AgroParisTech (France, protocol 12-124). *In vitro* embryo production and embryo transfer protocols were registered by the French Veterinary Services (N°FRPB780 and FRTB910).

*Experiment 1: Endometrial SR-A expression during early pregnancy*

As previously described (Forde et al., 2011a; b) synchronised cross-bred beef heifers were artificially inseminated to generate the pregnant group or were left as non-inseminated to generate a cyclic group. Cyclic females were slaughtered at

day 5 (n=5), at day 7 (n=5), at day 13 (n=5) and at day 16 (n=5) and uteri were immediately retrieved and flushed. A similar procedure was applied to inseminated heifers (day 5: n=5, day 7: n=5, day 13: n=5 and day 16: n=5) and pregnancy was confirmed when the stage of conceptus development was consistent with the day of pregnancy as determined by stereomicroscopy (Degrelle et al., 2005). Strips of endometrium (containing CAR and ICAR areas) were collected, snap-frozen in liquid nitrogen then stored at -80°C for further analyses.

*Experiment 2: Endometrial SR-A expression during maternal recognition of pregnancy period*

As previously described (Forde et al., 2011b; Eozenou et al., 2012; Vitorino Carvalho et al., 2014, 2016) synchronised cross-bred beef heifers were artificially inseminated (AI) to generate the pregnant group or were left as noninseminated to generate a cyclic group. Cyclic females were slaughtered at day 16 (n=5) and at day 20 (n=6) and uteri were immediately retrieved and flushed. A similar procedure was applied to inseminated heifers that were considered as pregnant (day 16, n=4; day 20, n=5) when the stage of conceptus development was consistent with the day of pregnancy as determined by stereomicroscopy (Degrelle et al., 2005). Based on ovarian morphology (Arosh et al., 2002), all cyclic heifers sampled at day 20 after estrous presented a regressed corpus luteum whereas cyclic and pregnant heifers sampled at day 16 as well as pregnant heifers sampled at day 20 presented a functional corpus luteum. Endometrial CAR and ICAR areas were dissected separately from the uterine horn ipsilateral to the corpus luteum (Mansouri-Attia et al., 2009a), frozen in liquid nitrogen then stored at -80°C for further analyses.

*Experiment 3: Impact of in vivo P4 supplementation on the endometrial expression of SR-A in cyclic and pregnant heifers*

As previously described (Carter et al., 2008; Forde et al., 2011a; Eozenou et al., 2012), cyclic cross breed heifers received a P4-releasing intravaginal device containing 1.55 g of P4 (Ceva Animal Health Ltd.) on day 3 after estrous. Females were slaughtered after 2 days (day 5 after estrous, normal P4 level in cyclic heifers, n=5, high P4 level in cyclic heifers, n=4, normal P4 level in pregnant heifers, n=5 and high P4 level in pregnant heifers, n=5) and 13 days (day 16 after estrous, normal P4 level in cyclic heifers, n=5, high P4 level in cyclic heifers, n=4, normal P4 level in pregnant heifers, n=5 and high P4 level in pregnant heifers, n=5) of P4 supplementation. Strips of endometrium (containing CAR and ICAR areas) were collected, snap-frozen in liquid nitrogen then stored at -80°C for further analyses.

*Experiment 4: Impact of in vivo IFNT supplementation on the endometrial expression of SR-A in cyclic heifers*

As previously described (Eozenou et al., 2012; Vitorino Carvalho et al., 2014, 2016), cyclic Charolais cows were synchronised by the Crestar method (Mansouri-Attia et al., 2009a). At day 14 after estrous, recombinant ovine IFNT (roIFNT; 200 µg/mL, 25 mL/horn; Sandra et al., 2005) or control solution (saline buffer) was infused into the uterine lumen. Cows were slaughtered 2 h after the intra-uterine infusion and the endometrium of five IFNT-infused and five control cows was collected. Endometrial CAR and ICAR areas were dissected separately from the uterine horn ipsilateral to the corpus luteum (Mansouri-Attia et al., 2009a), frozen in liquid nitrogen then stored at -80°C for further analyses.

*Experiment 5: Endometrial SR-A in response to embryo transfer*

All females (Charolais or Holstein breeds) used in this experiment were synchronized using the Crestar method. Holstein or Charolais heifers were inseminated with semen from their respective breed (as previously described,

Vitorino Carvalho et al., 2014). Embryos from other heifers were collected at day 7 post insemination. International Embryo Transfer Society quality grades 1 and 2 blastocysts were transferred into the uterine ipsilateral horn to the corpus luteum of synchronised Charolais cows for embryo transfer (ET) pregnancies with Charolais embryos (ET-Charolais, n=5) or Prim'Holstein embryos (ET-Prim'Holstein, n=4) (2 blastocysts per recipient). Pregnant cows were slaughtered at day 20 and pregnancy was confirmed when the stage of conceptus development was consistent with the day of pregnancy as determined by stereomicroscopy (Degrelle et al., 2005) and compared to one control group of Charolais AI cows (n=6) at the same pregnancy stage. Endometrial CAR and ICAR areas were dissected separately from the uterine horn ipsilateral to the corpus luteum (Mansouri-Attia et al., 2009a), frozen in liquid nitrogen then stored at -80°C for further analyses.

*Primary cultures of endometrial cells*

Epithelial and stromal cells were isolated from bovine endometrium collected from mixed breed beef cows on day 11–17 of the estrous cycle, as previously described (Cronin et al., 2012). The cells were treated with control medium (RPMI-1640 medium (Sigma–Aldrich), 10% heat-inactivated foetal bovine serum (Sigma–Aldrich), 1% penicillin–streptomycin (Sigma–Aldrich), 1% amphotericin B (Sigma–Aldrich) or a medium containing roIFNT (100 ng/mL) for 2 h. Each experiment was carried out using isolated cells from four independent animals.

*Total RNA extraction*

Total RNA was isolated from frozen tissue by homogenisation in Trizol Reagent (Invitrogen, Cerdy-Pontoise, France) according to the manufacturer's recommendations and as previously published (Mansouri-Attia et al., 2009a; Eozenou et al., 2012). Total RNA samples were purified on Qiagen columns

according to the manufacturer's protocol (RNeasy Mini Kit, Qiagen, Courtaboeuf, France). RNA was quantified using a NanoDrop-ND1000 Spectrophotometer (Thermo Fisher Scientific Inc., Boston, MA, USA) and all samples were shown to a 260/280nm ratio greater than 1.8. RNA quality was determined using the RNA 6000 chip on the Agilent 2100 bioanalyzer (Agilent, Les Ulis, France); all samples were shown to have a RNA Integrity Number (RIN) greater than 7.8. 1 µL of RNase inhibitor (RNAsin, Promega, Charbonnières-les-Bains, France) was added to each sample before storing at -80°C.

*Quantitative real-time PCR*

As previously described (Mansouri-Attia et al., 2009a; Eozenou et al., 2012), 1 µg of total RNA was reverse-transcribed into cDNA using OligodT and SuperScript II (Invitrogen) for experiments 1 and 4 and using total RNA using the High Capacity cDNA Reverse Transcription Kit (LifeTechnologies) for experiments 2 and 3 according to the manufacturer's instructions in a 20 µL volume. Quantitative real-time PCR (qPCR) was carried out with Master Mix SYBR Green (Applied Biosystems, Saint Aubin, France) and Step One Plus system (Applied Biosystems). Primers were designed using Primer-BLAST (NCBI, http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome) or Primer Express Software (Applied Biosystems) then synthesized by Eurogentec (Angers, France). The oligonucleotide primers used for gene quantification are listed in Table 1. To assess the amplification of the correct cDNA fragments, every amplicon was sequenced and blasted on NCBI RNA bovine collection. For each gene of interest, relative expression was normalized to the expression of the most stable reference genes as determined by qBaseplus software (Biogazelle, Gent, Belgium) from the quantification of six housekeeping genes as previously

described (Vitorino Carvalho et al., 2014, 2016). Due to its very low expression level, *SR-A6* expression could not be evaluated in bovine endometrium, in our different models (Mansouri-Attia et al., 2009a; Forde et al., 2011a).

*Statistical analyses*

All statistical analyses were performed with GraphPad Prism 6 software (La Jolla, CA, USA). *SR-A* gene expression in tissues was first subjected to a two-way ANOVA followed by paired post hoc Bonferroni to analyze the effect of day, pregnancy status (cyclic or pregnant), endometrial areas (CAR and ICAR) and their interactions.

## Results

*Endometrial SR-A levels during estrous cycle and early pregnancy*

*SR-A* expression was analyzed in endometrial samples at day 5, 7, 13 and 16 of estrous cycle and pregnancy in cross bred beef heifers (Fig. 1).

During estrous cycle (Fig. 1), no significant regulation of *SR-A1* and *SR-A4* was observed whereas *SR-A3* and *SR-A5* expression increased since day 13 when compared to day 7 (respectively $P<0.0001$ and $P<0.01$). At day 16, *SR-A5* expression then appeared stable compared to day 13, *SR-A3* expression decreased at day 16 but remained higher than at day 5 and day 7 (both with $P<0.001$).

During the early pregnancy (Fig. 1), *SR-A1* levels were similar from day 5 to day 13 but higher at day 16 when compared to day 5 and day 7 (respectively, $P<0.01$ and $P<0.05$). The expression of *SR-A3* increased at day 13 and day 16 (both with $P<0.0001$) whereas *SR-A4* appeared up-regulated only at day 13 ($P<0.05$) when

compared to day 5. No regulation of *SR-A5* expression was observed during early pregnancy.

The comparison of cyclic endometrium and pregnant endometrium evidenced no significant regulation of *SR-A1*, *SR-A4* and *SR-A5* expression by the presence of a conceptus. The expression of *SR-A3* was increased by the presence of a conceptus only at day 16 ($P<0.001$).

*Endometrial SR-A expression during late estrous cycle and pre-implantation period*

*SR-A* expression was analyzed in the CAR and ICAR endometrial areas at day 16 and day 20 of the estrous cycle and pregnancy in cross bred beef heifers.

In cycle, from day 16 to day 20 post-estrous (Fig.2), no significant difference of expression was observed in CAR and ICAR areas for *SR-A1* and *SR-A3* whereas the *SR-A4* and *SR-A5* levels were significantly reduced at day 20 compared to day 16 in CAR and ICAR areas.

The comparison of the *SR-A* levels between the maternal recognition of the pregnancy (day 16) and the implantation (day 20) (Fig. 2) revealed a significant increase of *SR-A1* expression in CAR areas whereas its expression was not different in ICAR areas, in pregnant cows. On the contrary, *SR-A4* level appeared not impacted in CAR areas but its expression significantly decreased in ICAR areas. No significant regulation of *SR-A3* and *SR-A5* expression were observed during the same period.

The comparison of pregnant endometrium to cyclic endometrium (Fig. 2) evidenced two distinct patterns of regulation. Indeed, whereas *SR-A1, SR-A4 and SR-A5* expression were not impacted at day 16 in both endometrial areas, their

expression significantly increased at day 20 in CAR and ICAR areas. Moreover, *SR-A3* expression was not different in CAR areas but significantly reduced in ICAR areas only at day 20.

*Regulation of SR-A by P4-supplementation* in vivo

In order to investigate the impact of P4 on *SR-A* expression, cyclic and pregnant cross-bred beef heifers were supplemented with P4 from day 3 post-estrous onwards and the endometrium was sampled at day 5 or day 16 (Fig. 3).

2 days and 13 days of P4 treatments did not impact *SR-A1*, *SR-A4* and *SR-A5* expression in cyclic and pregnant heifers. On the other hand, no impact of P4-supplementation was observed on *SR-A3* level at day 5 of cycle and pregnancy whereas its expression was increased at day 16 of cycle ($P<0.05$) but not impacted in case of pregnancy.

*Regulation of SR-A by IFNT supplementation* in vivo *and* in vitro

No impact of IFNT supplementation was observed on *SR-A* levels in both endometrial areas (Fig. 4). In primary cultures of endometrial cells, no regulation by IFNT was observed on *SR-A* expression in stromal and epithelial cells (Fig. 5). Interestingly, *SR-A1* and *SR-A5* levels were lower and *SR-A3* and *SR-A4* levels were higher in stromal cells compared with their expression in glandular epithelial cells ($P<0.01$, $P<0.0001$, $P<0.0001$ and $P<0.0001$ respectively; Fig. 5).

*Endometrial SR-A expression in pregnant cows carrying embryos obtained by AI, ET-Charolais or ET-Holstein*

To analyze the importance of difference of genome between pregnant cow and the carrying embryo in *SR-A* levels (Fig. 6), we designed a protocol of pregnant Charolais cows carrying embryo obtained by AI or *in vivo* in Charolais or Holstein cows and transferred at day 7 after estrous. Samples were collected at day 20 of pregnancy (day of implantation).

No significant regulation by the origin of the embryo was observed for *SR-A1*, *SR-A4* and *SR-A5* levels. Nevertheless, SR-A3 expression appeared significantly higher in ET-Charolais than in AI or than in ET-Holstein in ICAR areas.

## Discussion

Implantation involves a tight regulation of the maternal immune system to accept the colonization of the uterus by conceptus cells (Billingham et al., 1953). This regulation is highly complex and includes the modulation of innate and adaptive immunity (Chaouat et al., 2004, 2007). Among the factors involved in innate immunity, SR-A have been recognized as major contributors for recognition of microbial pathogens or endogenous molecules produced by the host (Mukhopadhyay and Gordon, 2004; Bowdish et al., 2007). Nevertheless, very few information are available on the putative roles of SR-A factors in uterine function during pregnancy in mammals (Naito et al., 1991; Kyaw et al., 1998). In order to provide first insights on the implication of SR-A in endometrial physiology, expression and regulation of the five identified *SR-A* genes *(SR-A1, SR-A3 to SR-A6)* was investigated in bovine endometrium in order to (i) establish expression patterns during estrous cycle and early pregnancy (ii) define the contribution of IFNT and P4 in *SR-A* genes regulation (iii) determine if *SR-A* levels are altered at implantation upon transfer of bovine IVF-produced embryos.

Despite numerous attempts with different primers, we were unable to detect *SR-A6* transcriptional expression in our various bovine models suggesting a very low expression. This very low expression is consistent with the absence of *SR-A6* in transcriptome profiles of bovine endometrium (Mansouri-Attia et al., 2009a; Forde et al., 2011b) as well as the undetectable expression of *SR-A6* in murine uterus

(Kyaw et al., 1998) and in human endometrial cells during the menstrual cycle (Talbi et al., 2006). Altogether, these results suggest little involvement of *SR-A6* in endometrial physiology or restricted to a few specialized cells, as suggested by the expression of this factor in specific sub-populations of murine macrophages (Plüddemann et al., 2007; PrabhuDas et al., 2017). More sensitive assay – for instance, based on single cell analyses - will be necessary to conclude about the involvement of *SR-A6* in mammal endometrial physiology.

In the bovine endometrium, we detected *SR-A1*, *SR-A3, SR-A4* and *SR-A5* from day 5 to 20 of the estrous cycle, with distinct expression profiles. Whereas no regulation of *SR-A1* gene expression was observed, expression *SR-A3, SR-A4* and *SR-A5* transcripts increased during the luteal phase (day 13 or day 16) then remained high (*SR-A3*) or was greatly reduced at day 20 of the estrous cycle when P4 blood level drops (*SR-A4* and *SR-A5*). In keeping with our observations, mining human transcriptome data sets confirms that *SR-A1* expression was constant across menstrual cycle in human endometrium and *SR-A5* expression was up-regulated during the secretory phase (Talbi et al., 2006; Duncan et al., 2011; Sigurgeirsson et al., 2017). However, in contrast with our data, expression of *SR-A3* and *SR-A4* transcripts decreased during the secretory phase (Talbi et al., 2006; Kashiwagi et al., 2007; Hu et al., 2014; Sigurgeirsson et al., 2017) when endometrial cells undergo decidualization, a process that is very limited in ruminants but is critical for implantation in species with an invasive blastocyst penetration such as primates and rodents (Guillomot, 1995). Collectively, published reports and our data show that *SR-A1*, *SR-A3, SR-A4* and *SR-A5* are expressed in the endometrium of mammals, with *SR-A3* and *SR-A4* expression being variable with the type of implantation. Interestingly, previous studies report

that SR-A protein level is correlated to the mRNA level, especially for SR-A5 (Liu et al., 2013; Lee et al., 2017; You et al., 2017). Thus, even if protein regulation could be extrapolated from the mRNA expression, further analysis should be performed especially to identifying cells that express *SR-A* in the endometrium of various species.

Impact of the conceptus on the endometrial expression of *SR-A* was limited to the increase in *SR-A1* transcript level in the CAR area at day 20 of pregnancy i.e. when first permanent contacts between the elongated conceptus and endometrium take place. *SR-A1* has been described as a marker of endometrial macrophages (Chang et al., 2009; Oliveira et al., 2010) and its expression is correlated with the recruitment of immune cells, especially B lymphocytes in mouse spleen, leading to tissue reorganization (Plüddemann et al., 2007). Since expression of bovine *SR-A1* gene was higher in endometrial epithelial cells than in stromal cells, this factor could be involved in the recruitment of immune cells at the endometrium-conceptus interface particularly in CAR areas where cellular contacts with extra-embryonic tissues of the conceptus will lead to placentomes development. Epithelial localization of *SR-A1* may also reflect a potential regulatory role in endometrial protection against bacterial aggression during pregnancy as previously suggested in humans (Senn et al., 2018). Furthermore, absence of *SR-A1* leads to an increased secretion of pro-inflammatory cytokines by murine macrophages (Ohnishi et al., 2011) suggesting that *SR-A1* could have a role in the control of the Th1/Th2 balance at implantation in the bovine species (Chaouat et al., 2004; Oliveira et al., 2013). Since the recruitment of maternal immune cells (i.e. macrophages and lymphocytes) and the modulation of cytokine secretion are necessary for pregnancy success (Chaouat et al., 2007; Mansouri-

386 Attia et al., 2012; Fair, 2015, 2016), further experiments will help clarify the
387 importance of SR-A1 in the regulation of local immune system of the mother.

388 Biological functions reported for *Sr-a5* are less related to the regulation of the
389 immune system than other members of SR-A family (Jiang et al., 2006). *Sr-a5* has
390 been suggested to act as a tissue remodeler that drives cell fate of adipocytes
391 (Lee et al., 2017). *Sr-a5* overexpression regulates cell proliferation, invasion, and
392 migration and can induces apoptosis (Huang et al., 2010; Liu et al., 2013; You et
393 al., 2017). Eventually *Sr-a5* is implicated in ferritin uptake and iron traffic regulating
394 organogenesis (Li et al., 2009). Our current report highlights that *SR-A5* transcripts
395 are more abundant in epithelial cells that in stroma cells, in keeping with the high
396 expression detected in murine epithelial cells (Jiang et al., 2006) and the weak
397 expression reported in human macrophages (Senn et al., 2018). In the bovine
398 endometrium at day 20 of pregnancy (Mansouri-Attia et al., 2009a), *SR-A5*
399 transcript was localized by *in situ* hybridization in the stratum compactum layer as
400 well as in the glandular epithelium that produces histotroph critical for conceptus
401 elongation in ruminants and implantation in mammals (Spencer et al., 2013).
402 Collectively, these data suggest that *SR-A5* could be involved in endometrial
403 remodeling and histotroph secretion, two major processes in the context of
404 implantation and placental development. SR-A5 protein localization could be very
405 helpful to gain new insights of endometrial *SR-A5* function and to refine our
406 understanding of SR-A5 involvement in early pregnancy.

407 Interestingly, despite distinct expression patterns, *SR-A4* could also be involved in
408 endometrial remodeling especially in CAR areas. Indeed, *Sr-a4* is expressed in
409 vascular epithelia in mouse (Plüddemann et al., 2007) suggesting a function in
410 vascular physiology. Increased *SR-A4* expression has been described in

trophectoderm cells of implanting embryos in humans and mice (Haouzi et al., 2011; McConaha et al., 2011; Simopoulou et al., 2014). Placental development involves the reorganization of endometrial blood vascularization (Spencer et al., 2007) especially in CAR areas. Based on SR-A4 secondary structure that integrates collagen-like sequences and carbohydrate recognition domains (Haouzi et al., 2011), SR-A4 as an adhesion factor may be involved in the initial attachment of trophectoderm to the receptive endometrium in mouse and human. Considering the expression of bovine *SR-A4* in endometrial stromal cells, this scavenger receptor could take part to the endometrial remodeling as a pre-requisite for implantation in cattle.

Successful implantation process requires coordinated actions of conceptus-released factors including IFNT and maternal factors including P4. None of the analyzed *SR-A* was regulated by the IFNT *in vitro* and *in vivo*, suggesting that none of them are early immediate target gene of the IFNT signaling pathway (Vitorino Carvalho et al., 2014, 2016). Nevertheless, other factors secreted by the conceptus could be involved in the regulation of SR-A expression, such as TGFB which is expressed by the elongating conceptus in cattle (Hue et al., 2012) and regulates the SR-A5 level in cellular model (Liu et al., 2013). Further experimentations would help to identify conceptus-released factors impacting endometrial *SR-A* expression. Furthermore, in the present study, the decrease of *SR-A3* and *SR-A5* level observed during the follicular phase of estrous cycle suggest that P4 may contribute to the transcriptional regulation of these two genes. In our experimental bovine model, 2-days supplementation with P4 did not modify *SR-A3* and *SR-A5* expression in the endometrium of treated heifers whereas a 13-days treatment only increased *SR-A3* level, suggesting an

436 differential impact of P4 on SR-A3 and SR-A5 expression and the involvement of
437 other maternal factors in their regulation. In keeping with our data, endometrial
438 *SR-A5* transcripts were more abundant in heifers displaying high P4 levels (Mitko
439 et al., 2008) as well as in human endometrium collected during secretory phase
440 when P4 levels rise (Talbi et al., 2006). Further experiment will be necessary to
441 clarify the molecular mechanisms that drive regulation of *SR-A3* and *SR-A5* gene
442 expression by P4 in mammals.

443 Using embryos with distinct potencies of term development has uncovered a
444 biosensor property of the endometrium in mammalian species (Mansouri-Attia et
445 al., 2009b; Sandra et al., 2011, 2015; Macklon and Brosens, 2014). In the present
446 study, at implantation, *SR-A3* gene expression in the ICAR areas was upregulated
447 by the presence of IVF-produced conceptus compared with AI pregnancies. In the
448 mouse, biological functions identified for *Sr-a3* include tumor suppression by cell
449 death induction (Zhu et al., 2009) and sensing as well as protection against
450 oxidative stress (Brown et al., 2013; Zani et al., 2015) through *Sr-a3* expression by
451 various cell types including macrophages and fibroblasts (DeWitte-Orr et al.,
452 2010). Transferred IVF embryos are subject to a higher oxidative stress than AI
453 embryos as a consequence of culture conditions (Yang et al., 1998) and increased
454 expression of *SR-A3* as well as regulation of other endometrial factors (Mansouri-
455 Attia et al., 2009b) could represent the response of this tissue to the implanting
456 conceptus produced *in vitro*.

457 In summary, this study documents patterns of expression of all members of the
458 *SR-A* family in the endometrium of a mammalian species. Significant differences in
459 temporal expression during estrous cycle were reported for *SR-A3*, *SR-A4* and
460 *SR-A5* whereas impact of the conceptus was significant on *SR-A1* gene

expression when apposition phase initiates. Transcript levels between CAR and ICAR areas were affected by IVF-produced conceptuses. Our data including potential roles of SR-A members in the regulation of endometrial physiology are summarized in Table 2. Further investigation will be required to clarify the biological functions of SR-A family in endometrial physiology during cycle and pregnancy.

## Conflicts of Interest

The authors declare no conflicts of interest.


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## Tables

Table 1. Description of the oligonucleotide primers used for bovine gene quantification by real time RT-PCR. NTC, no-template control; ND, not detected

| Gene | Forward | Reverse | Amplicon size (pb) | Accession number | Ct of NTC | Slope | Y intercept | PCR efficiency (%) | Linear dynamic range (Ct) |
|---|---|---|---|---|---|---|---|---|---|
| *SR-A1* | TCTCTGGTTTACCTGGAGTTCGA | CCTGGTCTTTGCATGCTTCC | 120 | NM_174113 | ND | -3.296 | 35.8 | 101.07 | 26-31 |
| *SR-A3* | TAGACCTCAACGTCCGCAAC | GGGGTCTCCTTTTGGTCCTT | 188 | XM_002689483 | ND | -3.168 | 35.2 | 107.23 | 24-34 |
| *SR-A4* | CAACACCCTTGCTGCGTGTC | GCTCTGTCCACCCTTCCCAA | 120 | NM_001101843 | ND | -3.570 | 35.2 | 90.60 | 23-36 |
| *SR-A5* | GTCTTGCTTTACATCAATGAAAAACC | CCCTGAAATACAGGATCATGGCT | 120 | NM_001102499 | ND | -3.462 | 33.8 | 94.47 | 24-32 |
| *SR-A6* | AGAGGAGCTACTGGGCCAC | CGGATTATCGGCTCTAGGAA | 90 | XM_024981046 | ND | ND | ND | ND | 33-43 |
| *CNOT11* | CCTTCAAGAGCCCCCTGT | GGGTCCTTTTCCAACTCTCC | 64 | XM_002691150 | ND | -3.430 | 37 | 95.68 | 23-35 |
| *GAPDH* | GCTGACGCTCCCATGTTTGT | TCATAAGTCCCTCCACGATGC | 432 | NM_001034034 | ND | -3.382 | 27.1 | 97.55 | 17-30 |
| *RPL19* | CCCCAATGAGACCAATGAAATC | CAGCCCATCTTTGATCAGCTT | 72 | NM_001040516 | ND | -3.515 | 30.1 | 92.53 | 16-34 |
| *SLC30A6* | TGATGAGGAAACCTAGCCCTGCC | TCGGGCTGCTCCAAAAAGCGT | 142 | NM_001075766 | ND | -3.505 | 37 | 92.89 | 22-35 |
| *SUZ12* | CGTTGTGAGCAGTTTTGCCCTGT | ACCACAGTGCTTGGAGTTGGACT | 139 | NM_001205587 | 37 | -3.325 | 33.5 | 99.9 | 19-34 |

Table 2. Summary of expression data and putative contribution of *SR-A* in bovine endometrial function. ND: not detected

| | SR-A1 | SR-A3 | SR-A4 | SR-A5 | SR-A6 |
|---|---|---|---|---|---|
| **Oestrus cycle** | no variation | increased from day 13 and decreased at day 20 | no variation | increased from day 13 | ND |
| **Pregnancy** | increased in CAR | decreased in ICAR | increased | increased | ND |
| ***In vitro* cell expression** | Epithelium>Stroma | Stroma>Epithelium | Stroma>Epithelium | Epithelium>Stroma | ND |
| **Regulation by IFNT** | no variation | no variation | no variation | no variation | ND |
| **Regulation by P4** | no variation | increased | no variation | no variation | ND |
| **Putative(s) function(s)** | Immunity/Tissue remodeling | Oxidative stress | Tissue remodeling/Conceptus adhesion | Tissue remodeling/Histotroph secretion | |

## Figure captations

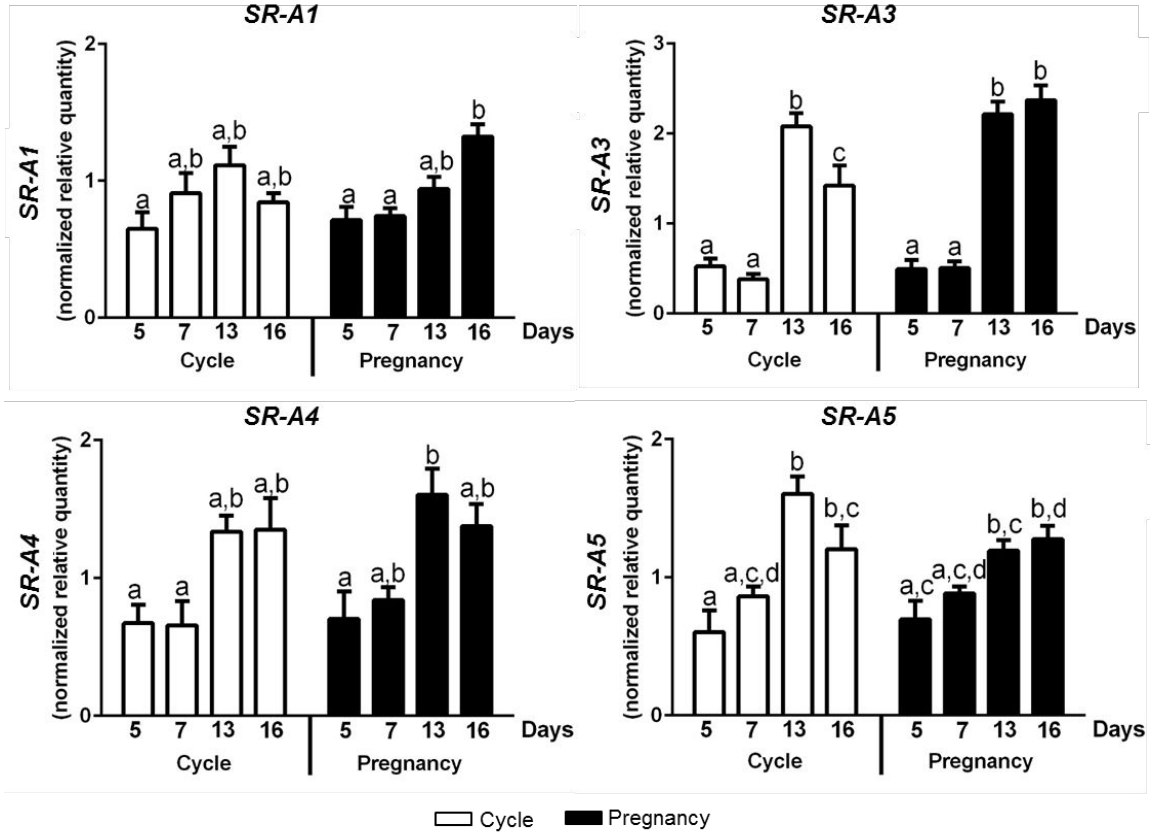


**Figure 1**

Fig. 1: Quantification of *SR-A* gene expression in cyclic and pregnant bovine endometrium on day 5 to 16 of the estrous/pregnancy. Endometrium samples were collected from cyclic (day 5: n=5; day 7: n=5, day 13: n=5 and day 16, n=5) and pregnant (day 5: n=5; day 7: n=5, day 13: n=5 and day 16, n=5) cross-bred heifers. Expression of *SR-A* was normalized to that of *GAPDH* and *RPL19* using qBasePlus. Scale Bars (mean±S.E.M.) with different lowercase letters differ significantly (P<0.05 or lower).

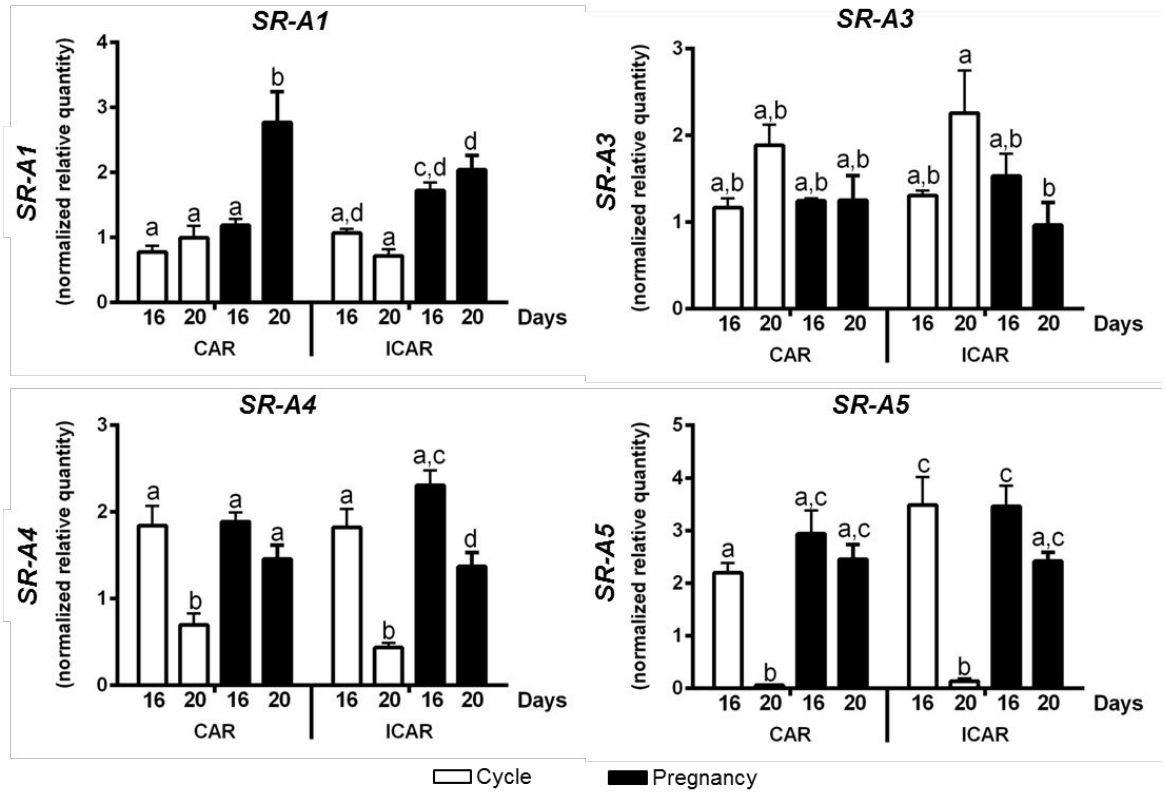


**Figure 2**

Fig. 2: Quantification of *SR-A* gene expression in bovine endometrium on day 16 and 20 of estrous cycle and pregnancy. Caruncular (CAR) and intercaruncular (ICAR) areas were collected from cyclic (day 16: n=5; day 20, n=6) and pregnant (day 16: n=4; day 20: n=5) cross-bred heifers. Expression of *SR-A* levels was normalized to that of *RPL19* and *SCL30A6* using qBasePlus. Scale Bars (mean±S.E.M.) with different lowercase letters differ significantly (P<0.05 or lower).

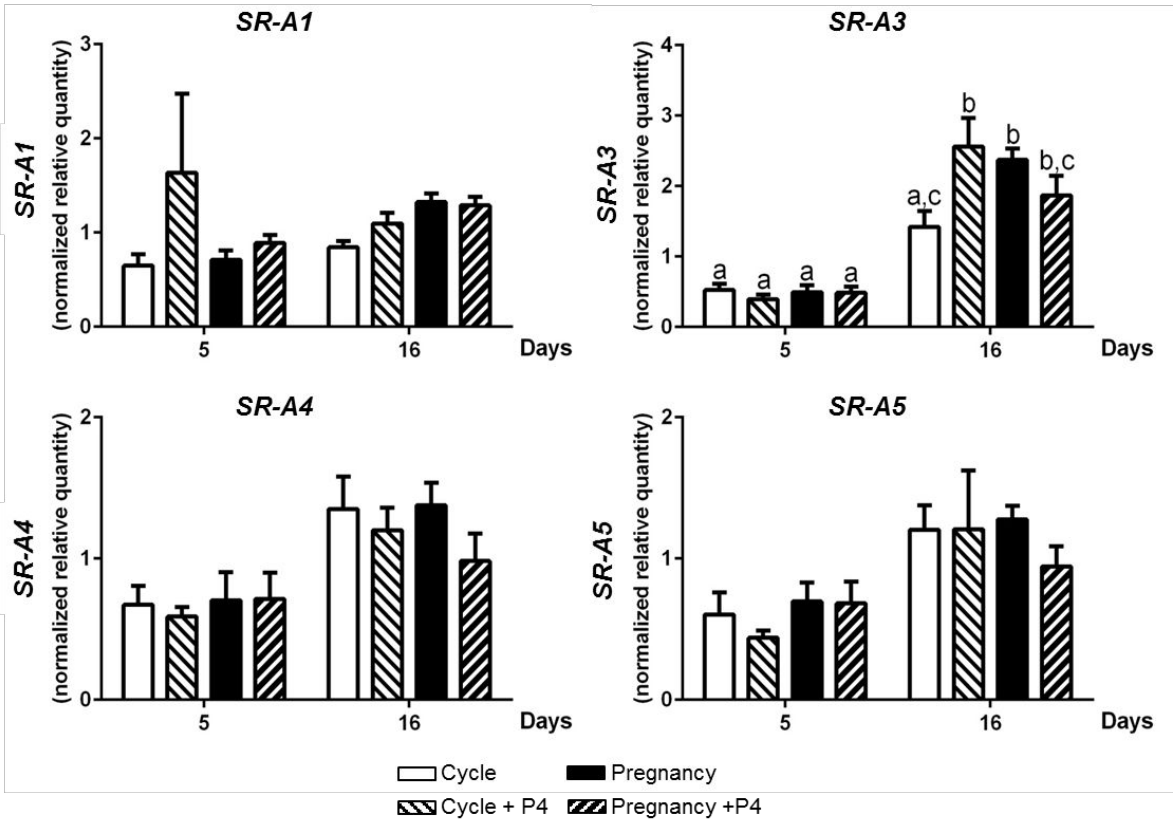


**Figure 3**

Fig. 3: *SR-A* endometrial gene expression in cyclic and pregnant cross-bred beef heifers supplemented with P4 from day 3 after estrous. The females were slaughtered after 2 days (day 5 after estrous, normal P4 level in cyclic heifers, n=5, high P4 level in cyclic heifers, n=4, normal P4 level in pregnant heifers, n=5 and high P4 level in pregnant heifers, n=5) and 13 days (day 16 after estrous, normal P4 level in cyclic heifers, n=5, high P4 level in cyclic heifers, n=4, normal P4 level in pregnant heifers, n=5 and high P4 level in pregnant heifers, n=5) of P4 supplementation. *SR-A* level was quantified by RT-qPCR and normalized to *GAPDH* and *RPL19* using qBasePlus. Scale Bars (mean±S.E.M.) represent the different conditions. Scale Bars (mean±S.E.M.) with different lowercase letters differ significantly (P<0.05 or lower).

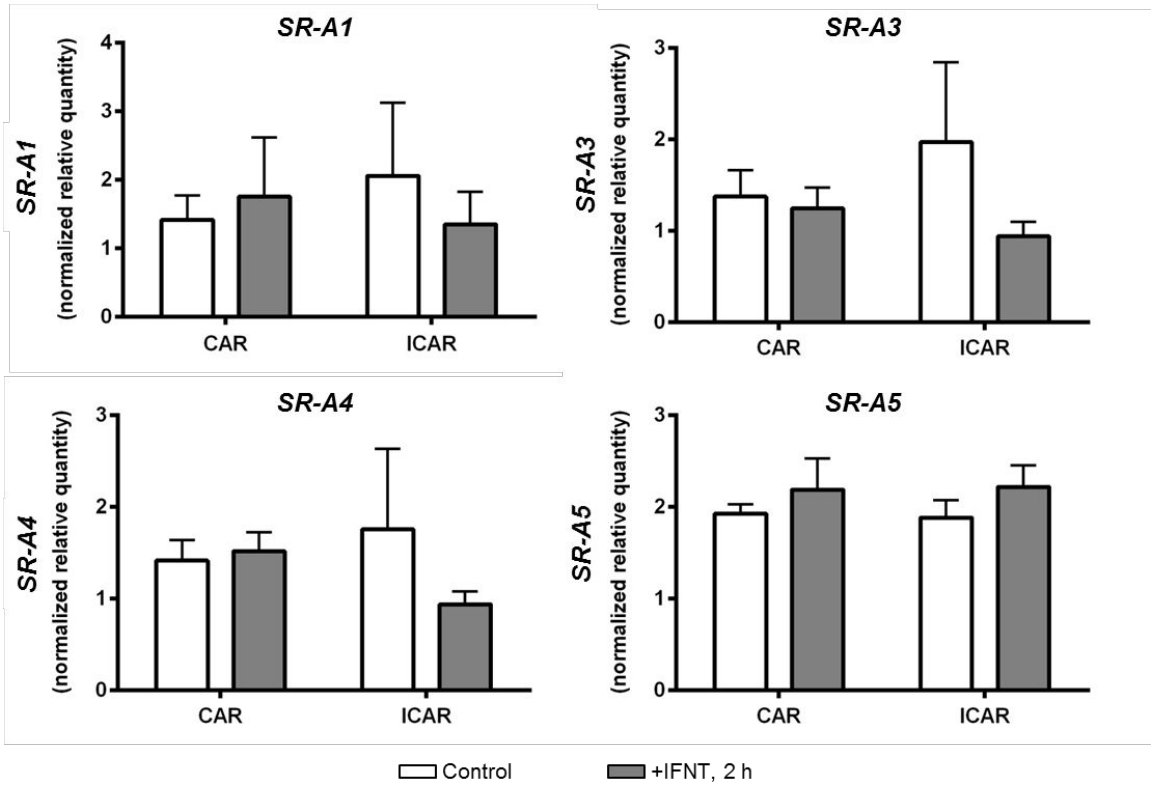


**Figure 4**

Fig. 4: Regulation of endometrial *SR-A* gene expression by IFNT *in vivo*. Caruncular (CAR) and intercaruncular (ICAR) endometrial areas were collected from Charolais cows infused with control solution (n=5) or recombinant ovine IFNT (200 µg/mL; n=5) for 2 h at day 14 of estrous cycle. *SR-A* levels were quantified by RT-qPCR and normalized to *CNOT11*, *SLC30A6* and *SUZ12* using qBasePlus. Data are the mean± S.E.M.

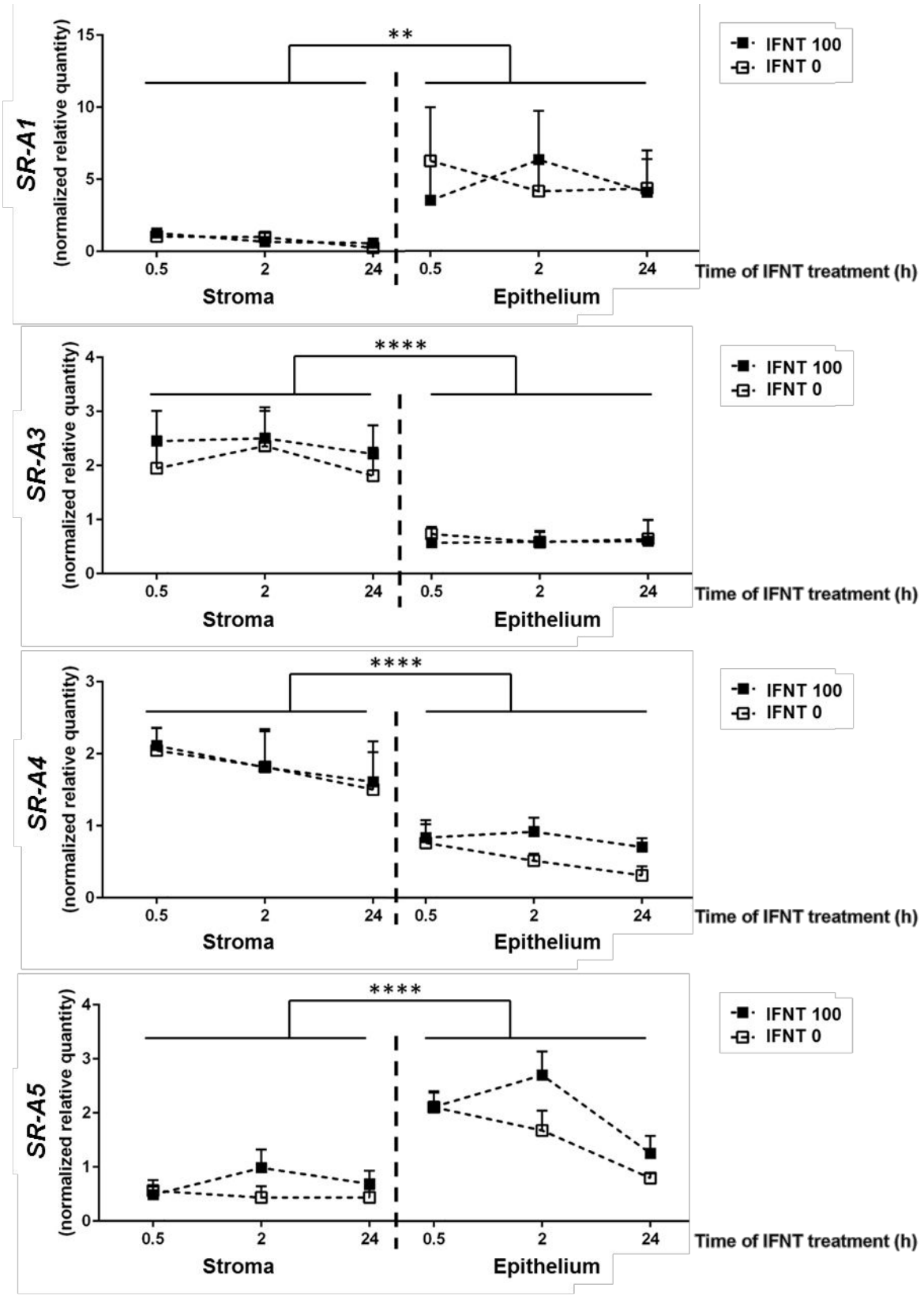
SR-A1
(normalized relative quantity)
**
IFNT 100
IFNT 0
0.5
2
24
Stroma
Epithelium
Time of IFNT treatment (h)
SR-A3
****
SR-A4
SR-A5

**Figure 5**

743

744 Fig. 5: Regulation of *SR-A* gene expression by IFNT in bovine endometrial cells. *In*
745 *vitro*, cells isolated from bovine endometrium were treated with roIFNT (100 ng/ml)
746 for 2 h. For each gene, mRNA expression was normalized to that of RPL19 and
747 ACTB using qBasePlus. Quantitative data are presented as mean +/-SEM and
748 significant differences between cell types were noted using ** : P < 0,01, **** : P <
749 0,0001.

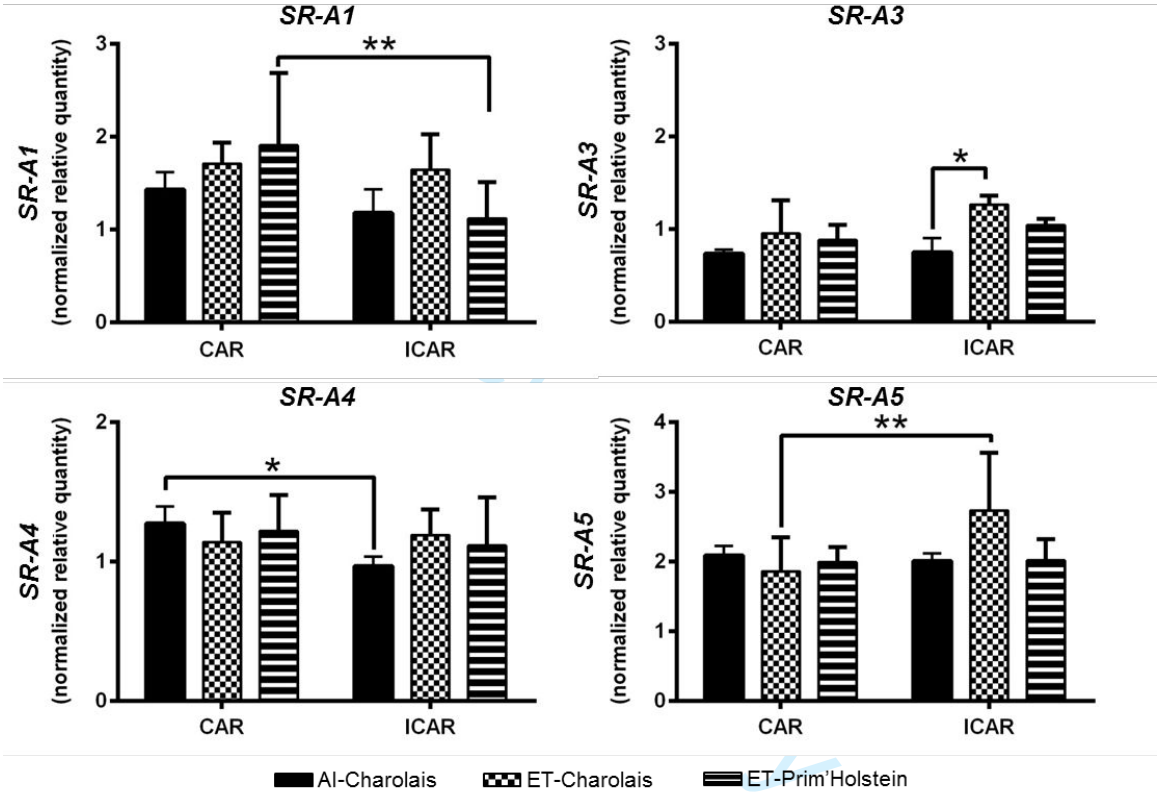


**Figure 6**

750

751 Fig. 6: Quantification of endometrial *SR-A* gene expression in pregnant Charolais
752 cows at day 20. Pregnancy was obtained by Artificial Insemination (AI, n=6) or by
753 Embryo Transfer (ET) at day 7 after estrous of two Charolais-bred embryos (ET-
754 Charolais, n=5) or two Prim'Holstein-bred embryos (ET-Prim'Holstein, n=4).
755 Caruncular (CAR) and intercaruncular (ICAR) areas were collected separatly.
756 Expression of *SR-A* was normalized to that of *RPL19* and *SCL30A6* using

757 qBasePlus. Quantitative data are presented as mean +/-SEM and significant

758 differences were noted using * : P < 0,05; ** : P < 0,01.

| Gene | Forward | Reverse |
|---|---|---|
| *SR-A1* | TCTCTGGTTTACCTGGAGTTCGA | CCTGGTCTTTGCATGCTTCC |
| *SR-A3* | TAGACCTCAACGTCCGCAAC | GGGGTCTCCTTTTGGTCCTT |
| *SR-A4* | CAACACCCTTGCTGCGTGTC | GCTCTGTCCACCCTTCCCAA |
| *SR-A5* | GTCTTGCTTTACATCAATGAAAAACC | CCCTGAAATACAGGATCATGGCT |
| *SR-A6* | AGAGGAGCTACTGGGCCAC | CGGATTATCGGCTCTAGGAA |
| *CNOT11* | CCTTCAAGAGCCCCCTGT | GGGTCCTTTTCCAACTCTCC |
| *GAPDH* | GCTGACGCTCCCATGTTTGT | TCATAAGTCCCTCCACGATGC |
| *RPL19* | CCCCAATGAGACCAATGAAATC | CAGCCCATCTTTGATCAGCTT |
| *SLC30A6* | TGATGAGGAAACCTAGCCCTGCC | TCGGGCTGCTCCAAAAAGCGT |
| *SUZ12* | CGTTGTGAGCAGTTTTGCCCTGT | ACCACAGTGCTTGGAGTTGGACT |

| Amplicon size (pb) | Accession number | Ct of NTC | Slope | Y intercept | PCR efficiency (%) |
|---|---|---|---|---|---|
| 120 | NM_174113 | ND | -3.296 | 35.8 | 101.07 |
| 188 | XM_002689483 | ND | -3.168 | 35.2 | 107.23 |
| 120 | NM_001101843 | ND | -3.570 | 35.2 | 90.60 |
| 120 | NM_001102499 | ND | -3.462 | 33.8 | 94.47 |
| 90 | XM_024981046 | ND | ND | ND | ND |
| 64 | XM_002691150 | ND | -3.430 | 37 | 95.68 |
| 432 | NM_001034034 | ND | -3.382 | 27.1 | 97.55 |
| 72 | NM_001040516 | ND | -3.515 | 30.1 | 92.53 |
| 142 | NM_001075766 | ND | -3.505 | 37 | 92.89 |
| 139 | NM_001205587 | 37 | -3.325 | 33.5 | 99.9 |

| Linear dynamic range (Ct) |
|---|
| 26-31 |
| 24-34 |
| 23-36 |
| 24-32 |
| 33-43 |
| 23-35 |
| 17-30 |
| 16-34 |
| 22-35 |
| 19-34 |

| | **SR-A1** |
|---|---|
| **Oestrus cycle** | no variation |
| **Pregnancy** | increased in CAR |
| ***In vitro* cell expression** | Epithelium>Stroma |
| **Regulation by IFNT** | no variation |
| **Regulation by P4** | no variation |
| **Putative(s) function(s)** | Immunity/Tissue remodeling |

| SR-A3 | SR-A4 |
|---|---|
| increased from day 13 and decreased at day 20 | no variation |
| decreased in ICAR | increased |
| Stroma>Epithelium | Stroma>Epithelium |
| no variation | no variation |
| increased | no variation |
| Oxidative stress | Tissue remodeling/Conceptus adhesion |

| SR-A5 | SR-A6 |
|---|---|
| increased from day 13 | ND |
| increased | ND |
| Epithelium>Stroma | ND |
| no variation | ND |
| no variation | ND |
| Tissue remodeling/Histotroph secretion | |

AI-[redacted]arolais ET[redacted]arolais ET[redacted]