INTERFERON-STIMULATED GENES IN THE PREGNANT MOUSE UTERUS

A Senior Honors Thesis

by

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Submitted to the Office of Honors Programs
Texas A&M University
In partial fulfillment of the requirements of the

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOWS

May 2008

Major: Biomedical Science and Chemistry

ABSTRACT

Interferon-Stimulated Genes in the Pregnant Mouse Uterus

(May 2008)

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During pregnancy in the mouse, extensive communication takes place between the conceptus (embryo/fetus and associated extraembryonic membranes) and uterus. Our focus centers on the uterine response to the conceptus. In ruminants, the conceptus produces interferon tau that induces interferon stimulated genes (ISGs) which likely regulate uterine receptivity, conceptus implantation, and conceptus growth and development. Our hypothesis is that ISGs are similarly induced in uterus during pregnancy in the mouse. If ISGs have a critical role in pregnancy establishment and maintenance in mammals, it is important to identify these ISGs in order to address fertility issues in human medicine. In this research, *in situ* hybridization analysis of uteri during gestation in the mouse was conducted to understand cell specific expression of selected ISGs during pregnancy. Of the fifteen ISGs investigated, ten (*Irf1, Irf2, Irf6, Irf7, Isg15, Oas2, Plscr1, Stat1, Rsad2, Tlr4*) were found to be expressed in the uterus during pregnancy.

To my parents.

Thank you so much for your unconditional love and support. Through your patience and diligence, you have encouraged me to go beyond the status quo and pursue excellence in every aspect of my life. Any achievement I obtain is merely a reflection of your consistent support and sacrifice. For this, no magnitude of thanks could be adequate. Therefore, let me simply state: I love you.

ACKNOWLEDGEMENTS

First and foremost, thank you Dr. Kanako Hayashi for your guidance and patience! This research is the result of the time and attention that you dedicated to teaching me. Special thanks also to Drs. Tom Spencer, Fuller Bazer, David Erikson, Jo-Ann Fleming, and Gwonhwa Song for your collective encouragement and wisdom and direction! Finally, I thank The Laboratory for Uterine Biology and Pregnancy and the Honors Research Fellows Program for the contributions that made this research possible.

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I INTRODUCTION[1]

Understanding the mechanisms of reproduction is essential to the progress of modern society. By elucidating the pathways of procreation, we can ensure the efficiency of our agriculture industry. Considerable resources are required to maintain each bred cow, sow, or ewe, and, therefore, it is important that the young survive and can return a profit. Currently, U.S. beef cattle herds average a pregnancy failure rate of nearly 30%, while increasing animal production by just 3% would result in 1 million more beef cows per year [1]. Additionally, understanding the molecular pathways of pregnancy will provide insight for development of new technologies in treating infertility, a hardship that will devastate 1 in 10 American couples in their reproductive years. Conversely, this knowledge can lead to progress in contraceptive research, as the current birth control options are not free of side effects and contraindications and, therefore, prohibit our complacency in this area of research.

Therefore, this research aims to understand the cellular and molecular interactions of the conceptus, defined as the embryo/fetus and associated extraembryonic membranes, and the uterus during pregnancy. The research carried out carries ethical limitations that prevent direct human experimentation. The mouse was chosen as a model organism due to its short gestational cycle and its comparability to humans.

A Concepts of Pregnancy Establishment

The mouse has an estrous cycle of 4-6 days in which fluctuating sexual receptivity physiologic behaviors can be observed. This cycle is ultimately controlled by

[1]This thesis follows the style and format of *Reproductive Biology and Endocrinology.*

the hypothalamic-pituitary-ovarian axis. By convention, the first stage in the estrous cycle is called proestrus, and is that in which the follicles begin growing. Proestrus is followed by estrus stage in which the female is sexually receptive to the male. Pituitary luteinizing hormone (LH) induces ovulation in the mouse during estrus (spontaneous ovulation). Following ovulation, LH acts on the remaining follicular tissue, inducing the development of the corpora lutea (CL). Once developed, the CL function as endocrine glands secreting progesterone during diestrus. In the event of successful mating, progesterone prepares the uterus for implantation and allows pregnancy establishment and maintenance. If mating does not occur, the CL are quickly broken down. This final stage is known as proestrus. However, if mating does occur, the resulting cervical stimulation brings about twice daily secretions of prolactin from the pituitary. Prolactin is trophic on the CL ("luteotrophic") and prevents its breakdown ("luteolysis"). The enduring CL continues to release progesterone for pregnancy [1-5].

After mating, if fertilization is successful, the embryo undergoes its first cell division within 24 hours. Cell division continues within the confines of the zona pellucida (ZP). As a result, the cell number rapidly increases, while the total mass of the embryo does not significantly change. During this cell division the embryo leaves the ampulla, the site of fertilization, and travels through the juxtaposed loops of oviduct, aided by peristaltic contractions, entering the uterus by gestational day 3 [5]. At this point in development, the morula are typically 16-32 cells and gross differentiation begins. The first structural differentiation can be seen in which the uniformly clustered cells of the morula segregate forming the blastocyst which is characterized by a

peripheral layer of cells called the trophectoderm, and small clump of cells that aggregate on the deep surface of the trophectoderm. This group of cells is termed the inner cell mass and becomes the embryo proper [1, 4-6].

On day 3 the blastocyst hatches from the ZP [5, 7], which permits the trophectoderm to make necessary contact with the luminal epithelium (LE) during the stages of implantation. In this process, the blastocyst secretes the protease strypsin, which digests a hole in the ZP. Then the blastocyst completes its expulsion from the ZP via rhythmic contractions [8, 9]. The blastocyst will then continue to grow and make its way to its fated implantation site in the uterus.

In all Eutherian mammals, implantation is dependent on a "two-way" molecular conversation between the conceptus. Although this mechanism is highly complex, identification of certain phases in implantation can help simplify and elucidate the process. The first recognized phase of implantation is attachment, which involves the apposition of the blastocyst to the uterine LE [10]. This activity is initiated by estrogen and then progesterone, which suppresses uterine epithelial cell proliferation and induces differentiation [7, 11]. The resultant rising levels of progesterone act on the uterus to promote the proliferation of stromal cells [7, 8, 11]. On day 4 of pregnancy, a small surge of ovarian estrogen initiates implantation of the blastocyst [7, 11, 12]. To this end, it has been shown that mice, ovariectomy on day four just prior to the estrogen surge delays implantation and induces dormancy of the blastocyst, because the ovarian originated estrogen surge is absent. This state can be later reversed by injections of estrogen, and the blastocyst will implant [7, 11].

Next, extensive stromal edema acts to progressively narrow the lumen of the uterus. Progesterone furthers this process by inducing the uterine lumen to close tightly [8, 11], which ensures a juxtaposition of the conceptus trophectoderm and the uterine LE. The trophectoderm and LE then attach together using cadherins, galectins, integrins, heparin sulfate, proteoglycans, and selectins, that trigger cell-to-cell signal transduction cascades [7, 8, 11].

Following this resulting superficial attachment of the blastocyst to maternal tissue, the blastocyst trophectoderm invades the uterine tissue, completing implantation. This process is mediated by prostaglandins and histamines and results in an increase in vascular permeability in the localized endometrium. Apoptosis of the luminal epithelial cells adjacent to the trophectoderm allows for the final invasion of the conceptus [7, 8].

Concurrent with implantation, an additional process begins in which the endometrium undergoes significant changes in shape, organization, and metabolic requirements. This process, called decidualization, produces a new uterine tissue known as the decidua. Decidualization begins adjacent to the implantation site and commences with the appearance of trophoblast giant cells. The goal of these cells is to modify the vasculature of the uterus, resulting in expansive, low resistance vessels that will allow the pooling of blood as part of the placenta [13, 14]. However, this mechanism must be restrained in preservation of the mother, because complete invasion of uterine arteries will result in severe hemorrhage, hypovolemic shock, and death. Decidualization is this protective restraint. The decidua possesses dense cellular matrix that impedes trophoblast invasion and instead enables trophoblast attachment [14]. This reaction that

began juxtaposed to the conceptus progressively continues throughout the endometrium over the next several days [13]. The effect is the replacement of endometrial stroma with the decidua in a progression that radiates away from the implantation site.

B Interferon-Stimulated Genes

Interferons (IFNs) are molecules primarily responsible for the defense against viral infection. Two classes of interferons have been identified: Type I IFNs and Type II IFNs. The presence of viral component particles in a cell, especially double stranded RNA, cause the cells to produce Type I IFNs, which then induce ISGs in neighboring cells [15-18]. ISGs defend these neighboring cells from viral infection. In fact, null mice for various components of the IFN system develop severe disease when infected with certain viruses that are typically symptom-less in wild time mice [16, 18]. ISGs have a vast range of functions that aim to prevent viral infection, including inhibition of further protein synthesis, inactivating energy stores, signal transduction, etc. [19]. These functions collectively act to fight against infection perpetuation in the host.

In the 1980s, a new function was added to the repertoire of Type I IFNs with the discovery of interferon tau (IFNT). During the establishment of pregnancy in ruminants, IFNT is produced by the conceptus and acts to prolong the lifespan of the CL. In ruminants, the CL is normally triggered to break down by PGF2α [10, 15, 20-22]. This effectively inhibits luteolysis, the destruction of CL. Thus, CL can continue to secrete progesterone, which is required for establishment and maintenance of pregnancy.

IFNT, like other Type I IFNs, has the ability to induce ISG expression. As such, it has been previously shown that many ISGs are expressed in the ruminant uterus during early pregnancy [23]. It is believed that some of these ISGs mediate maternal-conceptus communication and uterine receptivity. IFNT is present only in ruminants. Although mice and humans, therefore, do not have the IFNT gene, ISGs are still expressed in the uterus and decidua during pregnancy. Most notably *ISG15* expression is present in the uterine tissues of ruminants, primates, and rodents (specifically sheep, humans, and mice, respectively) [10, 24, 25].

Many ISGs are induced by Type I IFNs via the Janus tyrosine kinase and signal transducers and activators of transcription (JAK-STAT) pathway. In this pathway, circulating Type I IFNs bind to a transmembrane receptor on the external surface of the membrane, which is composed of two subunits IFNAR1 and IFNAR2. When this receptor is triggered, it activates two kinases, Tyk1 and JAK1 on the cytoplasmic side of the membrane, resulting in their phosphyorylation. Once activated, Tyk1 acts back on the IFNAR1 subunit, which allows for the sequential recruitment and phosphorylation of STAT2 and STAT1. STAT2 and STAT1 form a complex, which then travels to the nucleus where it binds with other transcription factors, including IRF9. This new complex binds to a specific IFN-stimulated response element (ISRE) on the promoter region of ISGs to regulate their transcription [26].

II ISGs

The aim of this research is to determine if expression of various ISGs are expressed in the pregnant mouse uterus. Therefore, several ISGs have been selected for evaluation.

A Characteristics of Selected Genes

Interferon stimulated gene 15 (Isg15)

Functionally, ISG15 conjugates intracellular proteins, signaling a pathway that is thought to be similar to ubiquitin [27]. *Isg15* was one of the first ISGs discovered in the mouse [24]. *Isg15* null mice typically show no reproductive, developmental, or viability effects [28]. However, UBP43 is a protease that removes ISG15 tags from ISGylated proteins, and *Ubp43* null mice demonstrate heightened antiviral activity [27]. Additionally, *ISG15* is expressed in the endometrium during implantation in humans [24], pigs[29], cows[30-32], and mice[10, 24].

Interferon regulatory factor 1 and 2 (Irf1 & Irf2)

Irf1 is induced by Type I and II IFNs. IRF1 actions are functionally diverse, including apoptosis regulation, antiproliferative effects, natural killer cell (NK) development, signal transduction mediation, and transcription regulation of Type I IFNs [33, 34].

IRF1 and IRF2 actions are antagonistic. IRF2 suppresses the IRF1 response through competitive inhibition. Murine knockouts for *Irf1* show decreased immune

response when subjected to LCMV infection, a pathologically well-documented laboratory virus, while mice lacking the *Irf2* gene demonstrate a hypersensitivity to antigens and over production of ISGs [35, 36]. Therefore, it is believed that IRF2 acts to safeguard the body against the harmful effects of an overly vigilant immune system [35]. Interestingly, it has been recently shown that in somatic cells antiestrogens induce IRF1, while estrogens suppress it. Additionally, IRF1 increases cell sensitivity to anti-estrogens [37].

Interferon regulatory factory 6 (Irf6)

Irf6 is a transactivator and a member of the class of interferon regulatory factors. IRF6 is associated development of craniofacial, epidermal, and limb connective tissue [38]. In humans, deficiencies in *Irf6* is associated with Popliteal Pterygium Syndrome, which is symptomatically characterized by cleft palate, webbing of limb joints, as well as other less conserved manifestations. In mice, deficiencies in *Irf6* are perinatal lethal [38, 39]. IRF6 plays a role in embryo development, though the function and regulatory mechanisms are not well understood. Additionally, IRF6 is known to be absent in some carcinomas [40]. This finding, in conjunction with the pathology of Popliteal Ptergium Syndrome, lends speculation that IRF6 induces apoptotic activity essential for tissue remodeling.

Interferon regulatory factor (Irf7)

Irf7 is one of the genes that is directly responsible for the induction of Type I IFNs. During viral infection, the *Irf7* gene is phosphorylated, which allows it to interact with transcriptional coactivators, inducing IFNA and IFNB production [41]. IRF7 is responsible for transitioning a virus to from primary infection to latency [42]. Also, *Irf7* null mice have marked viral infection susceptibility, and IRF7 is necessarily required for efficient antiviral activity [42, 43]. In fact, recent studies have turned some of the spotlight of Type I IFN response initiation away from IRF1 and toward IRF7 *[42]*. A few researchers have even gone so far as to name *Irf7* as the 'master regulator' of the antiviral response [43, 44].

Phospholipid scramblase 1 (Plscr1)

A common feature of healthy cells is asymmetry across the cell's plasma membrane. In other words, the phospholipid layer has a directionality in which certain classes of phospholipids reside on each side of the membrane. However, this asymmetry is obliterated as a step in signaling apoptosis [45, 46]. This response is largely mediated by the PLSCR class of molecules. PLSCR1 effectively 'scrambles' the phospholipids in a random, two-directional manner across the membrane. Additionally, PLSCR1 can bind to genomic DNA and induce cell proliferation and differentiation. These functions are especially induced in the presence of IFN; in fact, although *Plscr1* null mice have no apparent deficiencies, PLSCR1 is strongly induced by IFNs [47, 48].

Signal transducer and activator of transcription 1 (Stat1)

STAT proteins play a vital role in cell proliferation, differentiation, and signal transduction by controlling related transcription factors [49, 50]. IFNA has a considerable influence on the production and activation of STAT1 [49, 50], and STAT1 activates transcription of ISGs [26]. Additionally, *Stat1* null mice show signaling defects when mounting an interferon response [51-53].

N-myc (and STAT) interactor (Nmi)

The *Stat* and *Myc* gene classes are known to play important roles in cell differentiation and proliferation, and whole organism development. *Nmi* has a significant influence on the activity of the *Myc* and *Stat* genes [54], and therefore, acts to regulate other transcriptional factors involved in paracrine signaling of other cytokines and hormones.

Radical S-adenosyl methionine domain containing 2 (Rsad2)

Rsad2 is a known antiviral protein that is induced by Type I interferons. Additionally, *Rsad2* is expressed in the presence of conceptus-secreted IFNT during pregnancy in sheep and cows, and it is thought to have a vital role in the maintenance of pregnancy [23].

2'-5' oligoadenylate synthetase 2 (Oas2)

Oas2 has an important role in viral RNA degradation, namely OAS2 cleaves viral mRNA from rRNA [55]. Other cellular roles include apoptosis induction and growth regulation [56]. Additionally, *Oas2* is a well-established pregnancy induced ISG in sheep [57].

Toll-like receptor4 (Tlr4)

TLR4 activity induces Type I IFNs [58]. However, unlike the molecules discuss thus far, TLR4 is specialized not for the antiviral response, but as an antibacterial defense. TLR4 recognizes lipopolysaccharide subunits, the differentiating component of gram negative bacterial walls and then initiates an immune response [59]. Interestingly, the conceptus shows restrained immunological activity while in the uterus, however, it has been recently shown that trophoblast cells not only have the ability to express *Tlr4*, but also that signaling from TLR4 could initiate the immunological interactions of implantation [60, 61].

III MATERIALS AND METHODS

All experiments and surgical procedures were approved by the Institutional Animal Care Committee of Texas A&M University. Mice were kept under light controlled conditions with free access to food and water. Females were placed with fertile males, and the morning that a vaginal plug was observed was considered gestational day 1. Pregnant mice were maintained according to normal husbandry practices. Mice were sacrificed by cervical dislocation on differing gestational days. The entire reproductive tract (uterus and ovary) was excised, and the uterus was trimmed free of the broad ligament, oviduct, and cervix. Sections from the middle of each uterine horn (approx. 0.5 cm) were fixed in 4% (w/v) paraformaldehyde in PBS (pH 7.2). After 24 h, fixed tissues were changed to 70% (v/v) ethanol and then embedded in Paraplast Plus (Oxford Labware). The remainder of the uterine horn was frozen in liquid nitrogen and stored at –80°C for RNA extraction.

A Gene Identification Procedures

RT-PCR Analysis

Expression of *Irf1, Irf2, Irf6, Irf7, Isg15, Mx1, Nmi, Oas2, Plscr1, Rsad2,* and *Stat1* studied by RT-PCR as described previously [62-65]. Primers for each component were derived from mouse genes using Primer 3[64]. A partial murine cDNA of 300–700 bp was cloned by RT-PCR using total RNA isolated from the uterus. Primer and annealing temperatures used for PCR are summarized in Table 1. The amplified PCR

products were subcloned into the pCRII cloning vector using a T/A Cloning Kit (Invitrogen Life Technologies) and sequenced in both directions using an ABI PRISM Dye Terminator Cycle Sequencing Kit and ABI PRISM automated DNA sequencer (Perkin-Elmer Applied Biosystems) to confirm identity.

In Situ Hybridization

In situ hybridization analysis of ovine uteri were conducted using methods described previously [66]. Briefly, deparaffinized, rehydrated, and deproteinated cross-

TABLE 1. Sequences for primers for RT-PCR

Primer	*GenBank Accession No.*	*Primer sequence 5' – 3' (forward and reverse)*	*Annealing temp. (C)*	*Product size (bp)*
Irf1	NM_008390	CCAGCCGAGACACTAAGAGC CTTCGGCTATCTTCCCTTCC	55.0˚	330
Irf2	NM_008391	CTCCGCTCTTCAGAAACTGG TGCGTTCTCTTCATCACTGG	55.0˚	584
Irf6	NM_016851	TGTGGAGACCGGAAAGTACC ACCGTTGATGTTCAGGAAGG	55.0˚	328
Irf7	NM_016850	CTTCTTGCTTCAGGTTCTGC TGCCTACTTCCCAGTATACC	55.0˚	470
Isg15	NM_015783	CCGTGACTAACTCCATGACG CTGGTCTTCGTGACTTGTTC	54.0˚	302
Mx1	NM_01084	CCGTATCAGAGGGAGACAGC TCTCCCAATATTCCGTCTGC	54.5˚	608
Nmi	AF019249	GAATCATGTCGTGCAGATGG TTGACCACCTCCACTTCTCC	55.0˚	516
Oas2	NM_145227	ATGCCACTTTTCGTCACTCC CCTGAGGGTGAAGTCAGACC	54.5˚	483
Plscr1	NM_011636	ACTGCTGTACCCGAAACTGC TCACAAAACCAGACCACTGC	54.5˚	353
Rsad2	BC057868	AGATGGGAACAGCACTCAGC AGATTCAGGCACCAAACAGG	55.0˚	694
Stat1	NM_009283	GAAGAGCGACCAAAAACAGG GAATGAGCTGCTGGAAGAGG	54.5˚	377

sections (5 μm) of the uteri were hybridized with radiolabeled sense or antisense cRNA probes generated from linearized plasmid DNA templates using in vitro transcription with [^{35}S-α]UTP. After hybridization, washing, and ribonuclease A digestion, slides were dipped in NTB-2 liquid photographic emulsion (Kodak), stored at 4°C for 4 to 40 days, and developed in Kodak D-19 developer. Slides were then counterstained with Gill's modified hematoxylin (Stat Lab), dehydrated through a graded series of alcohol to xylene, and protected with a coverslip.

Photomicroscopy

Relative amounts of mRNA expression from in situ hybridization were assessed visually in uterine sections from each mouse by two independent observers and scored as present or absent (no signal stronger than sense). If histologically discernable, notations were made declaring location (LE, GE, S, D, etc.) of strongest and/or most variable change over gestational days. Images of representative fields of sections hybridized with antisense or sense cRNAs were recorded under brightfield or darkfield illumination with a Nikon Eclipse 1000 photomicroscope (Nikon Instruments Inc.) fitted with a Nikon DXM1200 digital camera using constant image acquisition parameters to ensure accurate comparison.

IV RESULTS

A Cell Type Identification

One important requirement of this research is that not only should it detect up-regulation of the specified gene, but also that the *location* (cell type) of the mRNA activity can be identified according to gestational day (GD). Therefore, it is important to recognize major cell types on the histological sample. Such cell types have been identified in Figure 1.

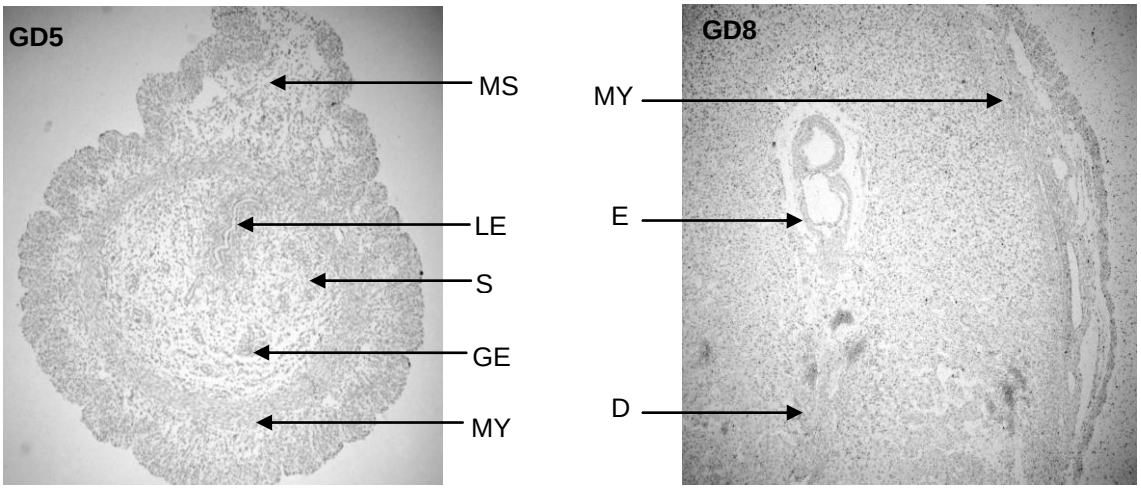


FIG. 1. Cell types labeled in tissue cross section of GD 5 and 8. MS, mesometrium; LE, luminal epithelium; S, stroma; GE, glandular epithelium; MY, myometrium; D, Decidua; E, Embryo.

B Signal Recognition

Experimental tissues subjected to *in situ* hybridization result in localized radioactive decay at the location of mRNA occurrence. Although the site of radiation emission cannot be seen by brightfield microscopy, this radiation signal is preserved by

exposure to liquid radiographic film, which deposits silver grains on the tissue. The presence of mRNA then *can* be detected in its original location by *dark* field microscopy. The results here are coupled with bright field microscopy for orientation reference. For example, see the schematic representative diagram in Figure 2.

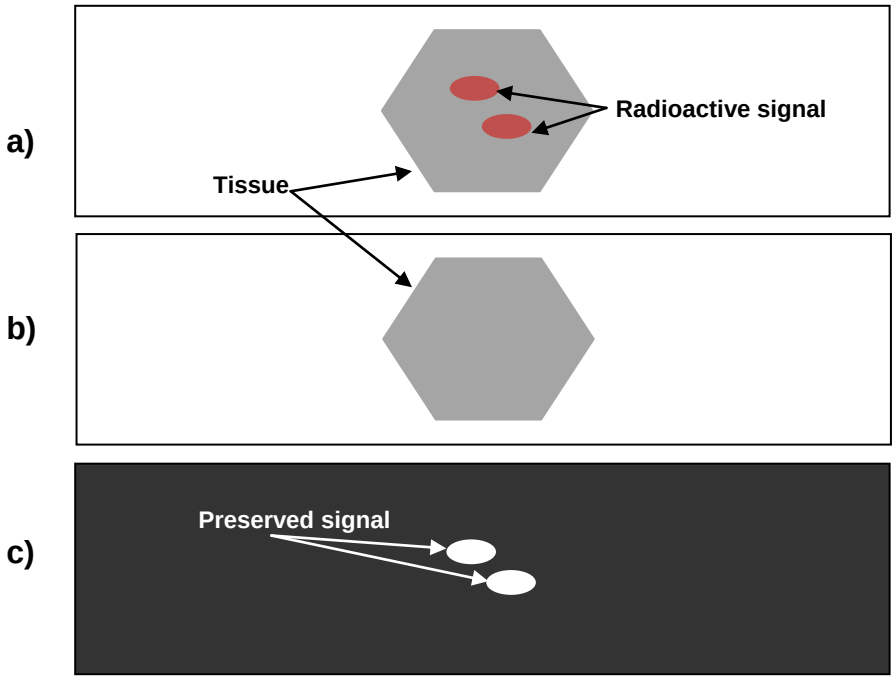


FIG. 2. – A schematic representation of post-ISH tissue slide with localized radioactivity, as seen **a)** in its theoretical state, **b)** under bright field microscopy, and **c)** under dark field microscopy

In situ hybridization studies with radiolabeled sense probe was used as negative control and was used for comparison of antisense expression.

C Summary of Results

The *in situ* hybridization studies that yielded a positive result are summarized in Figures 3, 4, and 5 under both bright field and dark field microscopy according to gene and gestational day. Individual findings will be reported subsequently.

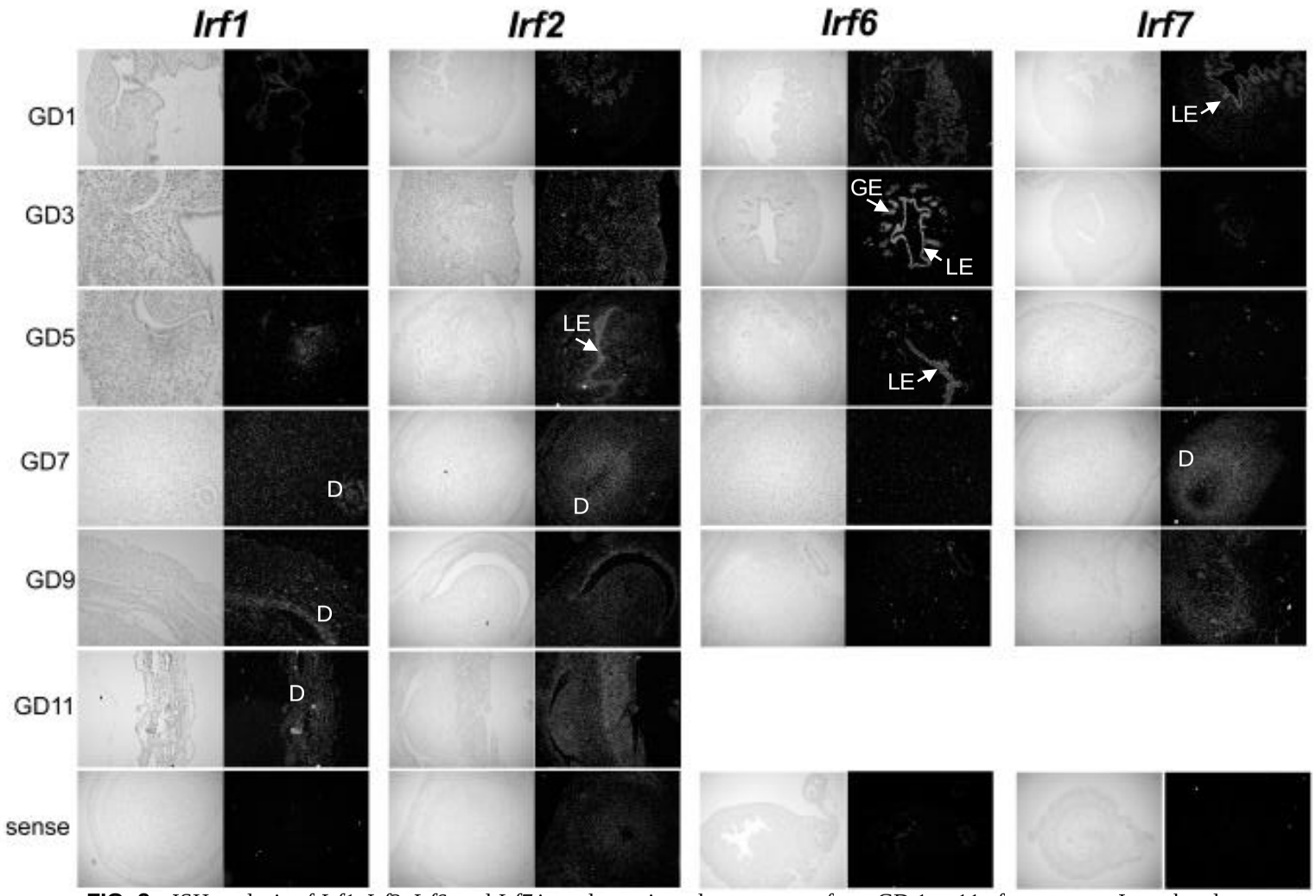


FIG. 3. ISH analysis of *Irf1*, *Irf2*, *Irf6*, and *Irf7* in endometria and conceptuses from GD 1 to 11 of pregnancy. In each column, representative photomicrographs of ISH results are presented in dark-field and bright-field illumination for each respective gene. LE, luminal epithelium; GE, glandular epithelium; D ,decidua.

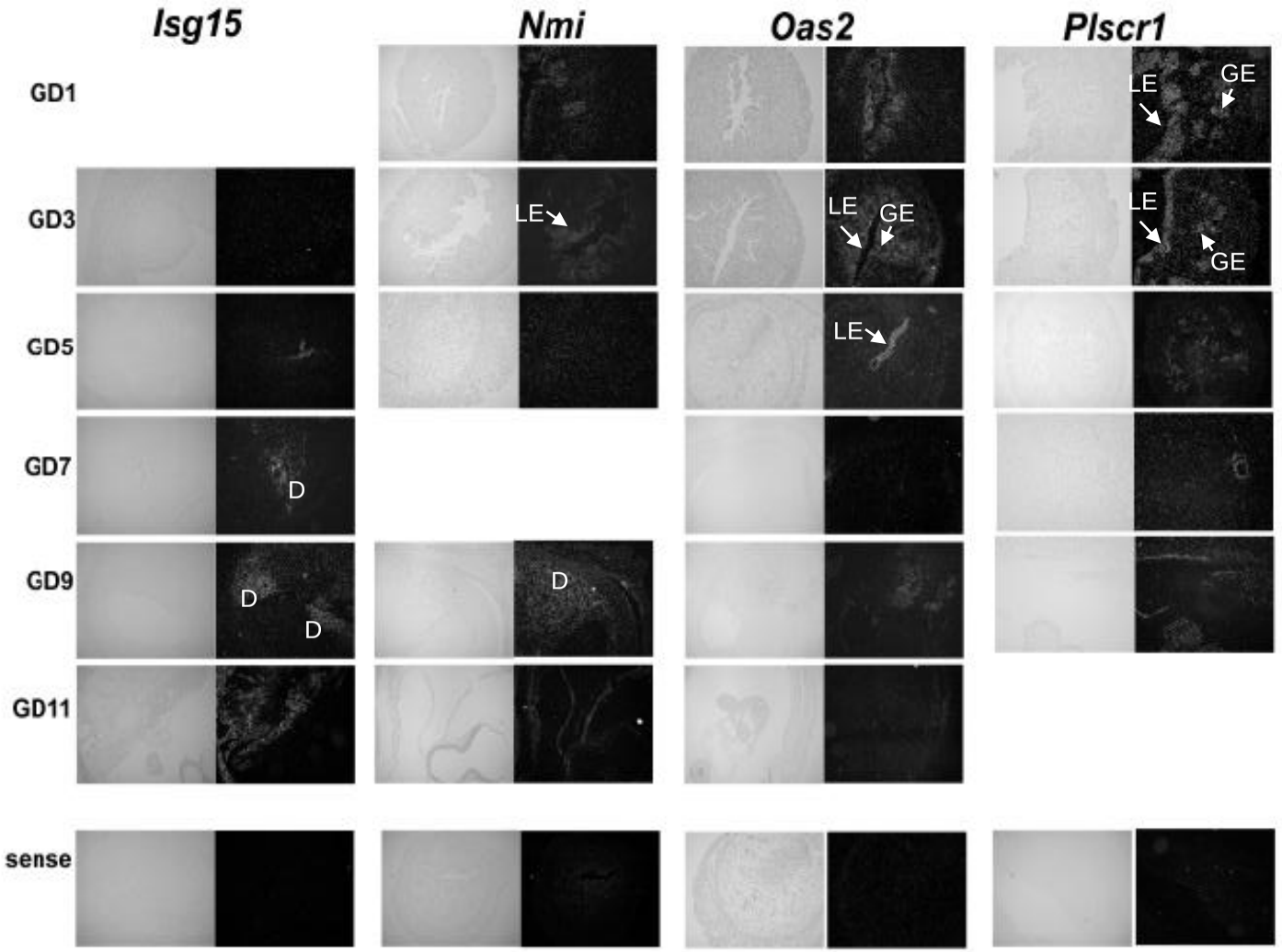


FIG. 4. ISH analysis of *Isg15*, *Nmi*, *Oas2*, and *Plscr1* in endometria and conceptuses from GD 1 to 11 of pregnancy. In each column, representative photomicrographs of ISH results are presented in dark-field and bright-field illumination for each respective gene. LE, luminal epithelium; GE, glandular epithelium; D, decidua.

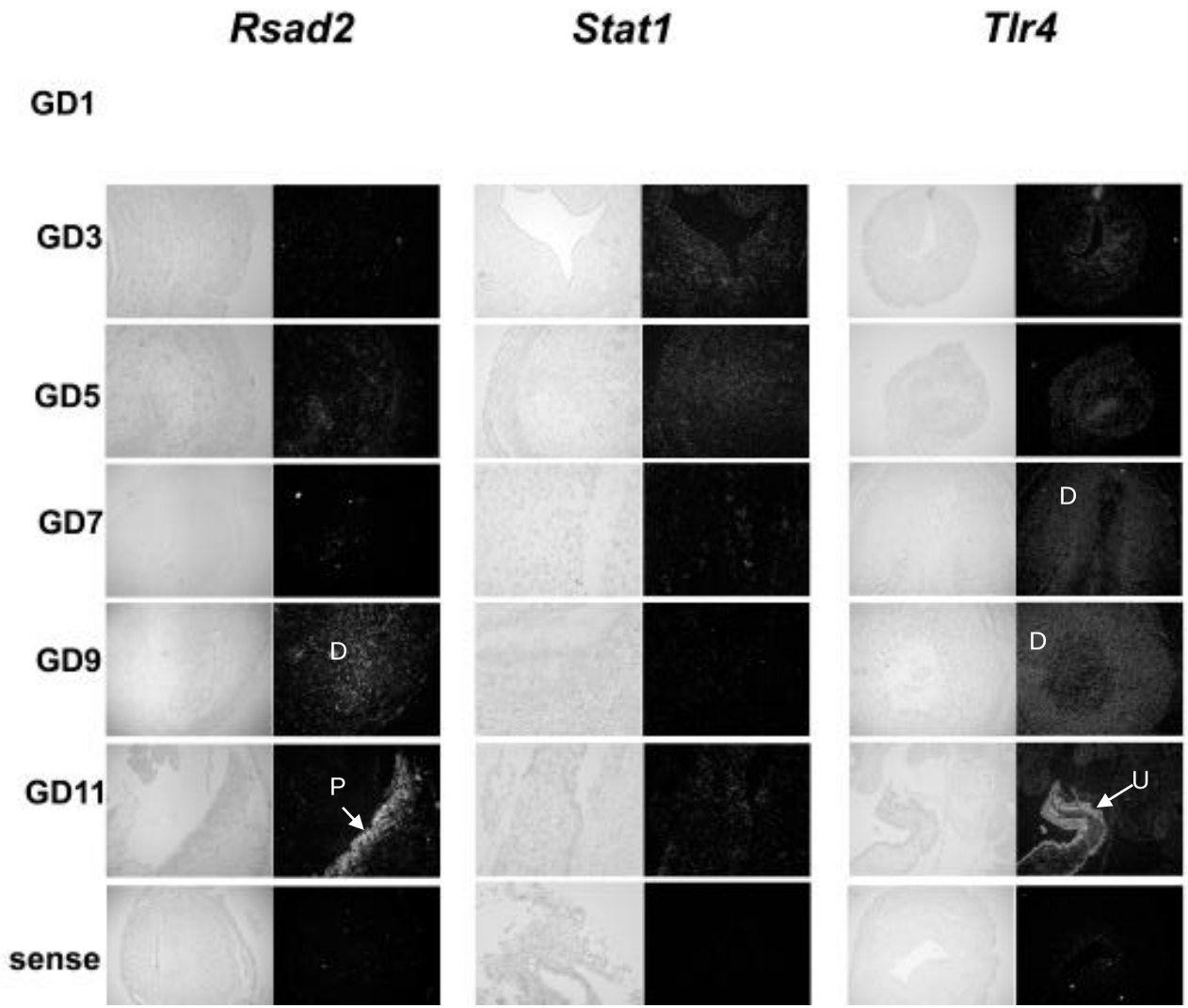


FIG. 5. ISH analysis of *Rsad2*, *Stat1*, *Tlr4* in endometria and conceptuses from GD 1 to 11 of pregnancy. In each column, representative photomicrographs of ISH results are presented in dark-field and bright-field illumination for each respective gene. LE, luminal epithelium; GE, glandular epithelium; D, decidua; P, placental tissue; U, umbilical tissue.

D Peri-implantation Signal

In situ hybridization (ISH) studies found *Ifr6* mRNA expression in uterine LE cells and GE cells. During gestational days 2-5, the *Irf6* signal is the most strong, which can be seen in Figures 3 and 6 under brightfield microscopy (BF) and darkfield microscopy (DF). This finding in addition to the role it plays in tissue development and remodeling has made it of particular interest to further studies.

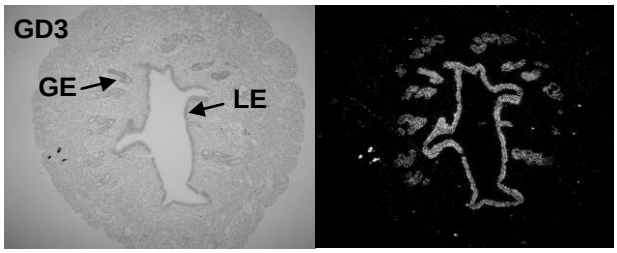


FIG. 6 ISH analysis of *Irf6* in endometria on GD3 of pregnancy in dark-field and bright-field illumination for each respective gene. LE, luminal epithelium; GE, glandular epithelium.

Additionally, ISH studies indicate that *Oas2* and *Plscr1* mRNA are also expressed in the uterine LE. Similarly, mRNA expression peaked during the peri-implantation period, namely GDs 1-5 and 2-4, respectively. These findings can be seen in Figures 4, 5, 7, and 8.

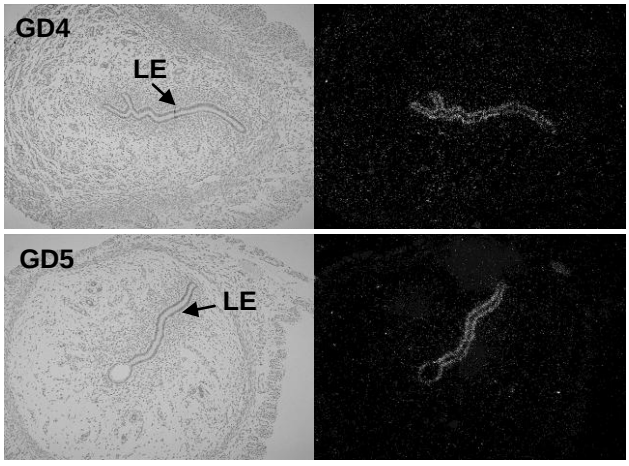


FIG. 7. ISH analysis of *Oas2* in endometria on GD4 and GD5 of pregnancy in dark-field and bright-field illumination. LE, luminal epithelium.

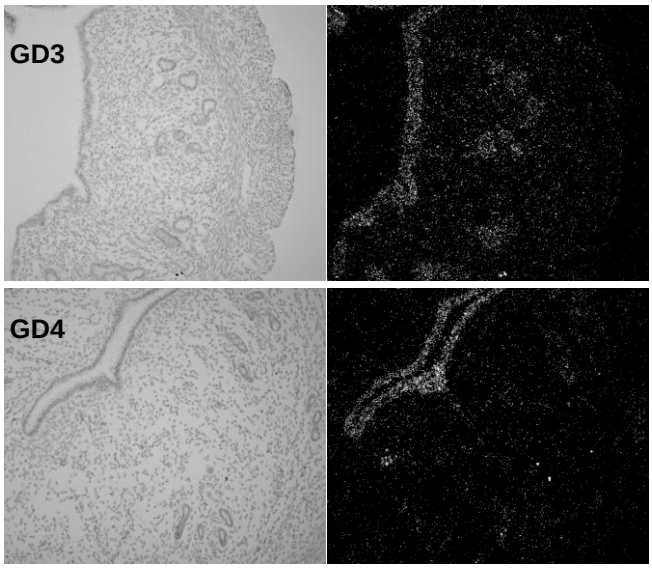


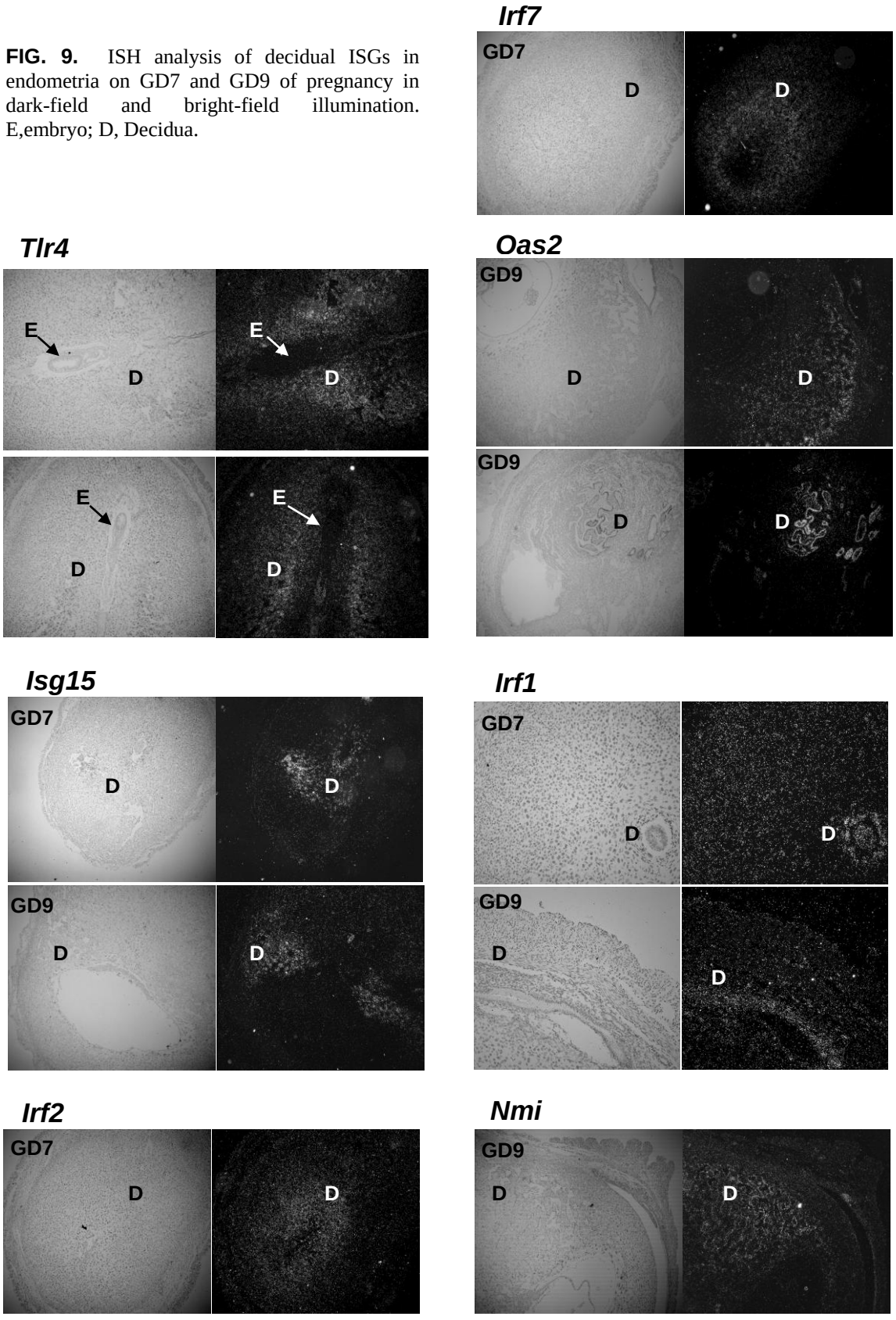
FIG. 8. ISH analysis of *Plscr1*in endometria on GD3 and GD4 of pregnancy in dark-field and bright-field illumination. LE, luminal epithelium.

E Post-implantation Decidual Signal

Many of the genes investigated (*Tlr4, Isg15, Nmi, Oas2, Irf1, Irf2, Irf7*) showed mRNA expression in the decidua that was the greatest on gestational days 7-9. As previously discussed, decidualization is a process in which a significant change in cellular composition and morphology occurs in the endometrial tissue. This process begins around the time of implantation immediately adjacent to the implantation site. Over the several days following this event, the decidualization reaction radiates to the entire breadth of the endometrium.

Because of the location of the ISH signal produced for these genes, it is speculated that these genes may have a role in the decidualization process. These results can be seen in Figure 9 under bright field microscopy (BF) and dark field microscopy (DF).

FIG. 9. ISH analysis of decidual ISGs in endometria on GD7 and GD9 of pregnancy in dark-field and bright-field illumination. E,embryo; D, Decidua.



F Other Notable Findings

In addition to the decidual mRNA expression, *Tlr4* produced interesting results in late gestation. The investigatory gene mRNA was highly expressed in the umbilical cord, while absent in the fetus (see Figure 10).

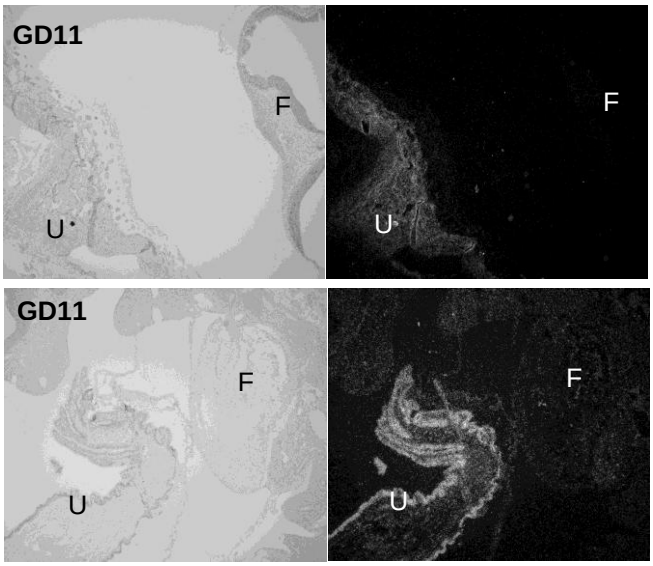


FIG. 10. *Tlr4* ISH analysis in endometria on GD11 of pregnancy in dark-field and bright-field illumination. F, fetal Tissue; U, Umbilical tissue.

Similarly, *Rsad2* was expressed in placental tissue. This signal could be seen most intensely after GD 9 and was absent in the fetal tissue. (See Figure 11.)

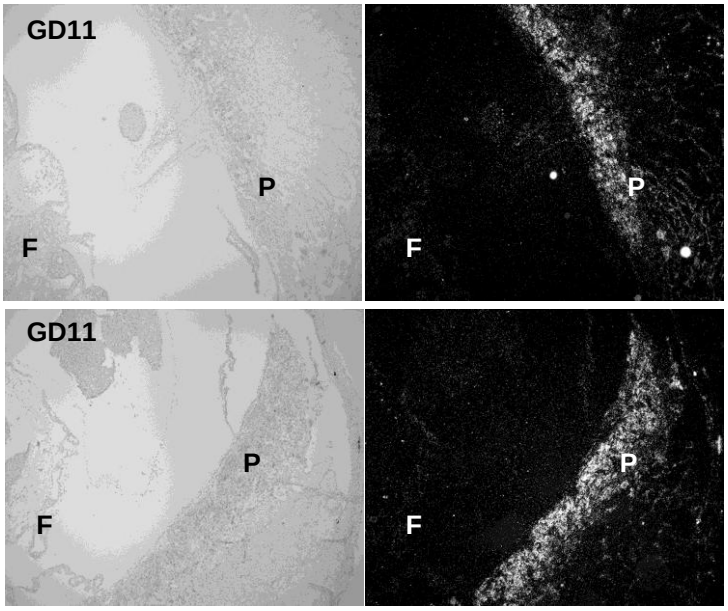


FIG. 11. ISH analysis of *Rsad2* in endometria on GD11 of pregnancy in dark-field and bright-field illumination. F, fetal Tissue; P, placental tissue.

G No Result

Finally, *Mx1* and *Stat1* showed no result. In the case of *Mx1*, RT-PCR yielded no product, and *in situ* hybridization studies were not attempted. This was to be expected as it is known that highly inbred mice, such as those routinely used in laboratory studies, carry deletions and/or mutations in the *Mx* genes [67]. *Stat1*, however, had a successful RT-PCR study, however mRNA expression was below detectable limits in our ISH studies.

V DISCUSSION

As discussed, several genes are specifically expressed around the time of implantation: *Irf6, Oas2,* and *Plscr1*. In order for implantation to be successful, the conceptus must approach the uterine wall with a specific orientation, uterine tissue must undergo conformational modifications, the conceptus must hatch from the ZP, the microvilli between the conceptus and uterine epithelium must interdigitate, the lumen mist close around the conceptus, the conceptus must invade the uterine endometrium, and, finally, in tandem, the uterine endometrium must permit invasion by the conceptus [13].

Although the mechanisms for many of these processes are unknown, it is clear that the entire process is continuous and depends on constant combative input from both the maternal and fetal signals. It is interesting that the genes found to be expressed in this study around the time of implantation are those that are known to induce tissue remodeling, growth and development, and cell communication. Now that these genes have been identified, further study is needed to investigate the specific gestational functions of these genes.

Accompanying implantation, decidualization occurs in the stromal tissue immediately surrounding the implantation site. Over time, this reaction initiation radiates through the uterine stroma [14]. Interestingly, many of the genes studied that have positive ISH signals after gestational day 5, display a signal that encircles the conceptus from a distance (refer to Figure 9). In addition, many of these genes are known to function as signal transducers, transcriptional modifiers, and proliferation and

differentiation regulators. It is possible that the genes identified are active participants in decidualization. As previously stated, these genes will also need further study to investigate these claims.

Finally, *Tlr4* and *Rsad2* may have a role in placental and umbilical development and maintenance. This is likewise speculated based on the extent of mRNA expression of these genes in their respective histological locations. As addressed, these functional speculations demand further study.

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II. Academic Background

Institution	Degree	Year	Major
Texas A&M University	B.S.	2009 (est.)	Biomedical Science
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III. Current Titles

2007 – Present: University Honors Research Fellow, Center for Animal Biotechnology and Genomics, Texas A&M University.

2007 – Present: Teaching Assistant, Department of Veterinary Physiology and Pharmacology, Texas A&M University

2008 – Present: International Humanitarian Volunteer. Development and Education Programme for Daughters and Communities, Mai Sai, Thailand.

2004 – Present: Officer, Aggies for Life. Student organization, Texas A&M University
Current Office: President. Past Offices: Vice President, Publicity Chairman.

IV. Past Titles

2007: Short term international medical volunteer (Spring Break). International Student Learning. Puntarenas, Costa Rica

2007: Physician Shadow, Dr. Tracy Papa, Fort Worth Perinatal Associates. Fort Worth, Texas.

2005-2007: Chris Pregnancy Counselor Volunteer. Coalition for Life, Bryan, Texas.

2005-2006: Student Researcher. Laboratory for Cardiovascular Chemistry, Texas A&M University.

2004: Physician's office receptionist, Fort Worth Perinatal Associates. Fort Worth, Texas.

V. Honors and Awards

- Dean's List. College of Veterinary Medicine and Biomedical Sciences.
- Academic Excellence Award. Association of Former Students.
- Who's Who Among Students in American Universities and Colleges.
- 2nd place, Oral Presentation. 2008 Student Research Week competition.
- 2nd place, Poster Presentation. 2006 Student Research Week competition.
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