Interleukin (IL)-1 in rat parturition: IL-1 receptors 1 and 2 and accessory proteins abundance in pregnant rat uterus at term - regulation by progesterone

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Abstract

Although numerous studies have investigated the involvement of interleukin-1 (IL-1), a pro-inflammatory cytokine, in parturition, none have evaluated the expression of IL-1 receptor (IL-1R) 1, IL-1R2, IL-1R accessory protein (IL-1RAcP), and its predominantly brain isoform, IL-1RAcPb, during late gestation in the uterus in the Long-Evans rat. We therefore assessed changes in their mRNA and protein relative abundance in the uterus and compared IL-1RAcP and IL-1RAcPb mRNA abundance in uterus, cervix, ovaries, placenta and whole blood of Long-Evans rats during late gestation or in RU486 and progesterone-treated dams using quantitative real-time PCR and western immunoblotting. IL-1R1, IL-1RAcP, and IL-1RAcPb mRNA abundance significantly increased in the uterus at delivery whereas IL-1R2 mRNA abundance significantly decreased. IL-1R1 protein increased at term and IL-1R2 protein decreased at term compared to non-pregnant uteri. IL1-RAcPb mRNA abundance was less than IL-1RAcP, but in the lower uterine segment it was the highest of all tissues examined. RU486 stimulated preterm delivery and an increase in IL-1R1 mRNA abundance whereas progesterone administration extended pregnancy and suppressed the increase in IL-1R1. These data suggest that changes in uterine sensitivity to IL-1 occur during late gestation and suggest another level of regulation for the control of delivery. The roles for IL-1RAcP and IL-1RAcPb need to be determined, but may relate to

different intracellular signaling pathways.

Introduction

It is widely accepted that the process of parturition utilizes inflammatory mediators (6, 15, 26). Interleukin-1 (IL-1α and β forms) (30) and IL-6, (9, 25) for instance, are essential for on-time delivery since IL-6 gene deletion (25) or receptor inhibition by the specific antibody, tocilizumab, (43) or administration of the naturally-occurring IL-1 receptor antagonist (IL-1RA) (29) or 101.10, a specific small peptide allosteric receptor antagonist to IL-1R accessory protein (IL-1R AcP) (19), delay term or preterm delivery in mouse models (26). Mouse models are exquisitely sensitive to lipopolysaccharide (LPS), a gram-negative, toll-like receptor (TLR) 4 agonist, lipoteichoic acid (LTA) a gram-positive TLR2 agonist or IL-1β induction of preterm delivery (11, 19). In primate models, intra-amniotic administration of IL-1β or LPS leads to a robust inflammatory response, increases in myometrial contractions and cervical dilatation, and preterm delivery (2, 31). Elevations in the concentrations of cytokines have been reported in both term and preterm labor with or without infection in gestational tissues, amniotic fluid, fetal membranes, myometrium and cervix in several species (5, 20, 26, 28). Rats, however, are anomalies since administration of IL-1β or LPS generally has no effect upon the timing of delivery, and there are no reports of increased cytokines in rat uterine tissues or fluids at normal term delivery (10, 18).

The IL-1 family has 11 members of which IL-1α and IL-1β are the most studied in relation to parturition (38). Interleukin-1β expression is inducible and not constitutive, unlike IL-1α. Interleukin-1β is produced by hematopoietic cells such as dendritic cells, blood monocytes, T cells and tissue macrophages (13, 24, 32), but can also be synthesized by uterine tissues and cells including myometrial smooth muscle (21, 19, 44, 46). The induction of its transcription is triggered by microbial stimuli, damage associated molecular patterns (DAMPs) from maternal tissues under progressively increasing physical stress as gestation advances, or lung surfactants released from the maturing fetus, via toll-like receptor 4 (TLR4) or pro-inflammatory cytokines, including itself (24). Receptors for IL-1 are composed of extracellular immunoglobulin domains and the IL-1 receptor domain in the cytoplasmic portion of cells (38). Interleukin-1α, IL-1β, and IL-1RA have two receptors: IL-1R1, which is biologically active, and IL-1R2, which lacks a signalling-competent cytoplasmic tail and acts as a decoy. Interleukin-1R1 is activated once the IL-1RAcP binds IL-1 and IL-1R1 to form a complex that activates intracellular adapter molecules (3). Interleukin-1RAcP has an alternatively spliced isoform that varies in the C-terminal region termed IL-1RAcPb because it is primarily expressed in the central nervous system (CNS) (39).

Given the apparently divergent responses between mice and rats to IL-1β and its complex relationship with its receptors and accessory proteins, we hypothesized that another level of regulation of IL-1β action at the receptor level exists in relation to its role in parturition, *i.e.* regulation by progesterone. We therefore explored the relative abundance of IL-1 receptors and accessory proteins in Long-Evans rats and reproductive tissues in late gestation and compared the roles of IL-1β, progesterone and its receptor antagonist, RU486, on their expression as well as the timing of delivery.

Materials and Methods

Animals

The University of Alberta Health Sciences Animal Policy and Welfare Committee approved all animal experiments. Female virgin Long-Evans Rats (Charles River, Portage, MI, U.S.A.) were housed with male Long-Evans rats for 3h (from 14:00 to 17:00) to impregnate rats. The pregnant rats weighing 250-350 g were transferred from Charles River to our facility at gestational day (GD) 14. They were individually housed under standard environmental conditions: in a 12h light: 12h dark cycle in the University of Alberta Health Sciences Laboratory Animal Services and were fed food and water *ad libitum*. Start of GD 1 was defined at 15:30, corresponding to the midpoint of the mating time. Under these conditions, the average time for labor to start in normal pregnant rats (non-treated) was early GD 22 (mean time 528.1h). In this study, parturition was defined as rats having at least one pup.

Experimental Design

IL-1β treated group. To evaluate whether the administration of IL-1β might induce preterm labor in rats, pregnant rats were randomly treated with increasing amounts of IL-1β (5, 10, 20, and 100 μg, intraperitoneal injection, at 10:00, in 0.3 ml sterile saline; Prospec-Tany TechnoGene Ltd., Ness Ziona, Israel) or vehicle on GD 17. The rats in each

group were euthanized on GD 20, 21 and during labor, and the tissue samples (upper uterus, lower uterus and plasma) were collected for mRNA analysis or protein analysis.

Non-treated group. Uterine horns were divided into three equal-length segments: upper, middle, and lower, and the upper and lower segments were examined. All tissue samples (upper uterus, lower uterus, cervix, ovaries, placenta, and blood) were collected at 9:00 on GD 17, 20, and 21 or during labor immediately following the delivery of the first pup (n = 3-7 animals/group).

RU486-treated group. The pregnant rats were randomly treated with either the progesterone receptor antagonist, RU486 (mifepristone, 11β-[4-(dimethylamino)phenyl]-17β-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one; Cayman Chemical Company, MI, U.S.A.) at 10 mg/kg, subcutaneous injection, at 10:00, in 0.4 ml corn oil containing 10% ethanol, or vehicle on GD 17 (17, 33-37). The rats in both groups (each n = 4-5) were euthanized at 12h and 24h after drug or vehicle administration, and the uterine tissue samples were collected for mRNA analysis.

Progesterone-treated group. Pregnant rats were treated daily beginning on GD19 with P4 (medroxyprogesterone acetate, 16 mg/kg/d, subcoutaneous injection, at 10:00 in 0.4 ml corn oil containing 10% ethanol; Sigma-Aldrich) (17, 33-37). The P4-treated dams were

euthanized on GD 21 and 23 (n = 4-5 at each time point), and the tissue samples (upper uterus and lower uterus) were collected.

Tissue and Plasma Collection

The animals were euthanized according to the approved local policy with a lethal dose of isoflurane (Halocarbon Products Corporation, U.S.A.) by inhalation in a large beaker. Tissue samples were washed in phosphate buffered saline buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4), flash-frozen in liquid nitrogen and stored at −80ºC. For each gestational day, tissue samples were collected, blotted, immediately frozen in liquid nitrogen and stored at -80°C.

Quantitative Real-Time PCR

Total RNA was isolated from the frozen tissue samples using Qiagen RNeasy kits (Qiagen, Valencia, CA, USA) with TRIzol Reagent (Life Technologies). Complementary DNA (cDNA) was synthesized from 500 ng total RNA by reverse transcription using the qScript™ cDNA SuperMix (Quanta BioSciences, Gaithersburg, MD, USA). The resultant cDNA was used as a template to perform real-time polymerase chain reaction (RT-PCR) using gene-specific primers summarized in Table 1. Quantitative RT-PCR was conducted using the iCycler (Bio-Rad, Hercules, CA, USA) with the SYBR Green FastMix (Quanta BioScience) and specific sets of primers (Table 1) (22, 40) as previously described (8).

Reaction mixtures contained 1 μL cDNA (50 ng/μL), 10 μL SYBR Green FastMix, 0.5 μL forward primer (10 μM), 0.5 μL reverse primer (10 μM), and sterile water in a total reaction volume of 20 μL. Reactions were incubated at 95ºC for 10 min and then for 40 cycles of 95ºC for 15s, and each sample was annealed (Table 1) for 1 min. Melt curve analysis was performed following amplification to verify that amplification of nonspecific products was not present. There was no amplification of non-specific products with each set of primers. All protocols were performed according to the manufacturer's instructions. The expression of target genes was normalized to that of *Cyclophilin a* as the housekeeping gene.

Enzyme-linked immunosorbent assay

The plasma concentrations of progesterone were measured directly by Enzyme-linked immunosorbent assay (ELISA) using a Progesterone Enzyme Immunoassay Kit (EIA-1561; Both intra- and inter-assay coefficient < 10%; DRG International Inc., New Jersey, U.S.A.), according to manufacture's instruction. To adjust the concentrations of every sample to the range of the assay (between 0 to 40 ng/mL), each sample was diluted 25-fold with the buffer provided in the kit.

Statistics

Data are presented as the mean ± standard error of the mean, and analyses were performed on homogenously distributed data. Comparisons were made using the Student's

t-test or one-way analysis of variance (ANOVA) with Dunn's multiple comparison test or Tukey's multiple comparison test or two-way ANOVA with Sidak's multiple comparison test. All statistics were performed with GraphPad Prism Version 6.0a software (GraphPad Software, San Diego, CA, USA). $p < 0.05$ was considered statistically significant.

Results

The effect of IL-1β administration on timing of delivery, uterine mRNA and protein expression of Il1a, Il1b, Ptgs2 and Ptgfr

In mice we observed that intrauterine administration of IL-1β to pregnant dams once on GD 16.5 (term = 19.5d) stimulated preterm delivery starting 12h later and increased uterine myometrial mRNA abundance of *Il1a, Il1b, Ptgs2 and Ptgfr* (19). We therefore assessed whether the Long-Evans rat responded similarly. We administered IL-1β (5-100 μg/dam, intraperitoneally) based upon published work (23) on GD17 at 10:00. We were unable to stimulate preterm delivery in rats (Fig. 1A). While we observed gestational age-dependent increases in abundance of *Ptgs2* mRNA in upper ($p = 0.0038$) and lower uterus ($p = 0.0018$) and of *Ptgfr* in upper ($p = 0.022$) and lower uterus ($p < 0.0001$), we were unable to stimulate mRNA expression of *Il1a, Il1b* or *Ptgfr* in the upper or lower uterine segments (Fig. 1B,C). Only at delivery did our IL-1β administration lead to an increase in *Ptgs2* in upper uterus (Fig. 1C, $p < 0.05$). The late gestation fall in plasma progesterone was not affected by IL-1β administration (Fig. 1D).

Since the rat was relatively unresponsive to IL-1β in ways the mouse was responsive, we studied the expression of IL-1 receptors during late gestation.

The abundance of Il1r1, IL1r2, Il1rap, and Il1rapb in the uteri of parturient rats

Figures 2A and B demonstrate that *Il1r1* relative mRNA abundance in both upper and lower uterine segments increased from GD17 to parturition. There was a significant difference in the mRNA abundance of *Il1r1* in both uterine segments on GD21 (upper uterus; $p < 0.05$, lower uterus; $p < 0.001$) and during labor (upper uterus; $p < 0.0001$, lower uterus; $p < 0.0001$). We observed a significant increase in protein relative abundance in uteri from term animals compared to non-pregnant uteri ($p < 0.05$).

Il1r2 mRNA expression (Fig. 2C and D) in the upper uterus significantly decreased before parturition (GD17 vs. GD20 and GD21; $p < 0.05$). The protein abundance of IL-1R2 significantly decreased in lower uterus between non-pregnant and laboring dams.

The mRNA relative abundance of *Il1rap* in both upper and lower uterine segments (Fig. 2E, F) gradually but significantly increased from GD17 to parturition ($p < 0.05$). Figure 2H describes the 4-fold increase in the mRNA abundance of *Il1rapb* in the lower uterine segment from GD17 to parturition ($p < 0.001$). Based upon PCR cycles, it is estimated that the relative abundance of *Il1rap* mRNA is about 10-fold more than that of *Il1rapb*. There was no difference in uterine protein mass in IL-1RAcP or IL-1RAcPb between non-pregnant and delivering dams (data not shown).

Effects of RU486 and progesterone on IL-1RI mRNA relative abundance in the uterus of pregnant rats

Figure 3 shows the mRNA expression of *Il1r1* in the uterus at 12 h and 24 h after the subcutaneous injection of vehicle only or RU486 on GD17. Delivery occurred on GD18, 24.04 ± 0.69h (n = 6) after RU486 administration. None of the vehicle-administered dams were in labor. There was a significant increase of *Il1r1* mRNA abundance in the upper uterine segment at delivery ($p < 0.05$). There was no difference in the mRNA abundance of *Il1r2*, *Il1rap* or *Il1rapb* due to RU486 treatment.

Figure 4 illustrates that progesterone prevented the delivery-associated increase in *Il1r1* mRNA abundance in both upper and lower uterine segments on GD23 compared with that observed in vehicle-treated controls dams in conjunction with prolonged gestation (upper uterus; $p < 0.001$, lower uterus; $p < 0.01$). Progesterone treatment had no significant effect upon *Il1r2*, *Il1rap*, and *Il1rapb* mRNA abundance. These data suggest that the relative abundance of *Il1r1* mRNA is suppressed by progesterone.

Il1rap and Il1rapb relative abundance in reproductive and blood tissues of rats during late gestation

All tissue samples expressed rather similar levels of *Il1rap* mRNA (Fig. 5A). The relative abundance of *Il1rapb* mRNA in the lower uterine segment was considerably higher

than that in the other tissues examined, and it increased significantly in late gestation only in the uterine tissues (Fig. 5B).

Discussion

The inability of IL-1β to stimulate preterm delivery or expression of *Il1a*, *Il1b* or *Ptgfr* plus inconsistent stimulation of *Ptgs2* mRNA expression until late in gestation suggests a relative insensitivity to IL-1β until very late in gestation. Hence our examination of the timing of expression for IL-1 receptors in late gestation is justified and reveals an unrecognized and hitherto unknown role for these receptors in pregnancy maintenance and termination in pregnant Long-Evans rats. We showed that the mRNA and protein abundance of IL-1R1 increases whereas IL-1R2, the decoy receptor, abundance decreases in late gestation at term. Both *Il1rap* and *Il1rapb* increased near parturition. Further, changes in *Il1r1* mRNA abundance were induced by RU486 and inhibited by P4, and these changes were correspondingly associated with decreased or extended pregnancy length.

Our results suggest that the reciprocal nature of changes in IL-1R1 and IL-1R2 in the uterus toward parturition is related to the initiation of labor. Presumably the uterine myometrium becomes more sensitive to IL-1β and there is less decoy receptor present to bind and thereby neutralize IL-1β. One regulator of this change is progesterone, which decreases in late gestation (Fig. 1D). This can explain our observation of the insensitivity of the rat to administration of IL-1β prior to the very end of gestation. The effects of RU486 or P4 on rat uterine IL-1R1 are similar to their effects on the expression of monocyte chemoattractant

protein-1 (MCP-1/CCL-2) (37), oxytocin receptor, and prostaglandin F2α (PGF2α) (7). This result is compatible with the hypothesis that progesterone inhibits the inflammatory response to maintain uterine quiescence (14).

Other regulators of *Il1r1* are IL-1β and PGF2α. IL-1α and IL-1β induce the expression of oxytocin receptors (41), *Ptgs2* (4), and NFκB in human myometrium (41), which have a crucial role in parturition. Recently we demonstrated that IL-1β and PGF2α induce *Il1r1* in cultured human myometrial smooth muscle cells (42), and IL-1β induces the expression of *Il1r1* in mouse uterus (19). IL-1β administration predictably induces preterm birth in mice (27) and nonhuman primates (31).

IL-1β (and IL-1α) bind to IL-1R1 which then complexes with IL-1RAcP (38). The third ligand that is bound by IL-1R1 is IL-1 receptor antagonist (IL-1RA). When IL-1RA is administered to mice, it prevents the induction of preterm delivery by IL-1β (29), suggesting that IL-1RA competes with IL-1β for binding to IL-1RI. In addition to IL-1RA, the other naturally occurring inhibitor of IL-1β and IL-1R1 binding is IL-1R2, a decoy receptor. It can exist in a membrane-bound form or a soluble form that binds both IL-1β and IL-1RAcP but does not signal. Our observation that IL-1R2 decreases relative abundance near term while the expression of IL-1R1 and IL-1RAcP increases suggesting an intriguing mechanism for

effecting parturition.

We reported that uterine mRNA abundance of IL-1RAcPb increases near parturition particularly in the lower segment. Previously, Smith *et al*. reported that IL-1RAcPb was nearly exclusively expressed in the CNS of humans (39). Not only did we find it in the uterus, we also found its expression in the cervix, ovaries, placenta, and blood but in much lower abundance levels than that in the uterus. The role of IL-1RAcPb in parturition is unknown. They suggested that IL-1RAcPb modulates the responses to IL-1 in the CNS (39). In other work, this group indicates that IL-1β complexed with IL-1R1 and IL-1RAcPb does not activate NFκB but rather affects SRC phosphorylation and MAPK activity (12). This is consistent with the work of McIntyre *et al*. who reported that NFκB activation is not required for inflammation (LPS)-driven preterm birth but that AP-1/JNK activation is sufficient for delivery (16). Perhaps IL-1RAcPb may be the more important accessory protein in the uterus for IL-1β-driven parturition events. Alternatively, it may serve as a cell adhesion molecule as suggested by Yoshida *et al*. in the brain (45). Further investigation will be required to define its role.

These data suggest that additional levels of regulation involving interleukin receptors and accessory proteins are required for the initiation of parturition, especially in the rat.

Importantly, with an improved understanding of physiological and cellular mechanisms leading to birth, more opportunities for identifying biomarkers that predict women at risk of preterm birth and more targets for therapeutic intervention to delay preterm delivery and to prolong and improve pregnancy outcomes may present themselves. In the long-term, these data may lead to better diagnostics and therapeutics.

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Figure Captions

Figure 1. Effect of increasing amounts (5–100 μg) of IL-1β or vehicle administration on gestational length (*n* = 2–7 dams at each concentration). There is no effect of IL-1β on the length of gestation.

Figure 2. Gestational age-dependent changes in *Ptgs2* (A, B) and *Ptgfr* (C, D) mRNA abundance in late gestation in rat uterus and effect of IL- 1β administration (20 μg, i.p.) or vehicle in upper (A, C) and lower (B, D) uterine segments (*n* = 3–7 dams). There were gestational age- dependent increases in mRNA abundance of *Ptgs2* in upper (**P* < 0.05) and lower uterus (***P* < 0.01) and of *Ptgfr* in upper (***P* < 0.01) and lower uterus (***P* < 0.01). IL-1β administration stimulated a late increase in laboring dams in *Ptgs2* mRNA abundance in upper uterus (**P* < 0.05).

Figure 3. Circulating plasma progesterone (P4) concentrations in late gestation and effect of IL-1β administration (20 μg, i.p.) or vehicle in rats (*n* = 4–7). Progesterone decreased in late gestation (***P* < 0.01) and IL-1β had no effect on these concentrations.

Figure 4. Relative abundance of IL-1R1 in late gestation rat uterus. A, C *Il1r1* mRNA abundance increases at labor versus GD17 and GD20 in upper uterus and lower uterus (**P* < 0.05). B, D IL1-R1 protein mass increases at labor compared to nonpregnant in upper uterus and lower uterus (*P* < 0.05); (mRNA, *n* = 5 dams at each time point; protein, *n* = 3–6 dams). GD, gestational day.

Figure 5. Relative abundance of IL-1R2 in late gestation rat uterus. A, C *Il1r2* mRNA abundance decreases on GD20 and GD21 versus GD 17 in upper uterus (**P* < 0.05) but there is no change in lower uterus. B, D median IL-1R2 protein abundance decreases in laboring versus nonpregnant upper uterus (NS) and lower uterus (**P* < 0.05); (mRNA, *n* = 5 dams at each time point; protein, *n* = 3–6 dams).GD, gestational day.

Figure 6. Relative abundancw of IL-1RAcP in late gestation rat uterus. (A, C) *Il1rap* mRNA abundance increases at labor versus GD17 in upper uterus (**P* < 0.05) and there is nonsignificant trend to increase lower uterus. (B, D) median IL-1RAcP protein abundance is not different in laboring versus nonpregnant dams; (mRNA, *n* = 5 dams at each time point; protein, *n* = 3-6 dams).

Figure 7. Relative abundance of IL-1RAcPb in late gestation rat uterus. (A, B) median *Il1rapb* mRNA abundance increases in upper (NS) and lower uterus at labor versus GD17 (*$P < 0.05$); ($n = 5$ dams at each timepoint). GD, gestational day.

Figure 8. The effect of RU486 (A upper uterus, B lower uterus) on *Il1r1* relative mRNA abundance. Dams were treated with vehicle ($n = 5$ at each time point) or RU486 ($n = 4$ at each time point). RU486 increases *Il1ra* mRNA abundance in upper uterus 24 h after administration (*$P < 0.05$). GD, gestational day.

Figure 9. The effect of progesterone (P4) (A upper uterus, B lower uterus) on *Il1r1* relative mRNA abundance in the myometrium. Dams were treated with vehicle ($n = 5$ at each time point) or P4 ($n = 4$ at each time point). P4 significantly decreased *Il1r1* mRNA abundance in upper and lower uterus on GD23 (**$P < 0.01$, ***$P < 0.001$). GD, gestational day.

Figure 10. The relative abundance of *Il1rap* and *Il1rapb* in reproductive tissues and blood. The relative mRNA abundance of *Il1rap* in upper and lower uterine segments, cervix, ovaries, placenta, and whole blood of rats during late gestation ($n = 5$ at each time point, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). The relative mRNA abundance of *Il1rapb* in upper and lower uterine segments, cervix, ovaries, placenta, and whole blood of rats during late gestation. There was a significant increase in lower uterus with approaching delivery ($n = 5$ at each time point, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Figure 1

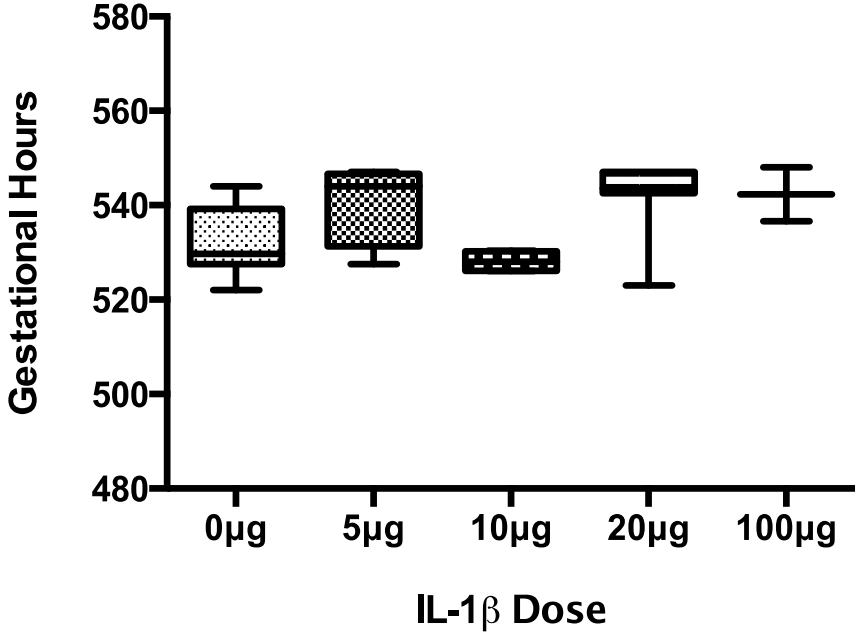

580
560
540
520
500
480
Gestational Hours
0µg
5µg
10µg
20µg
100µg
IL-1β Dose

Figure 2

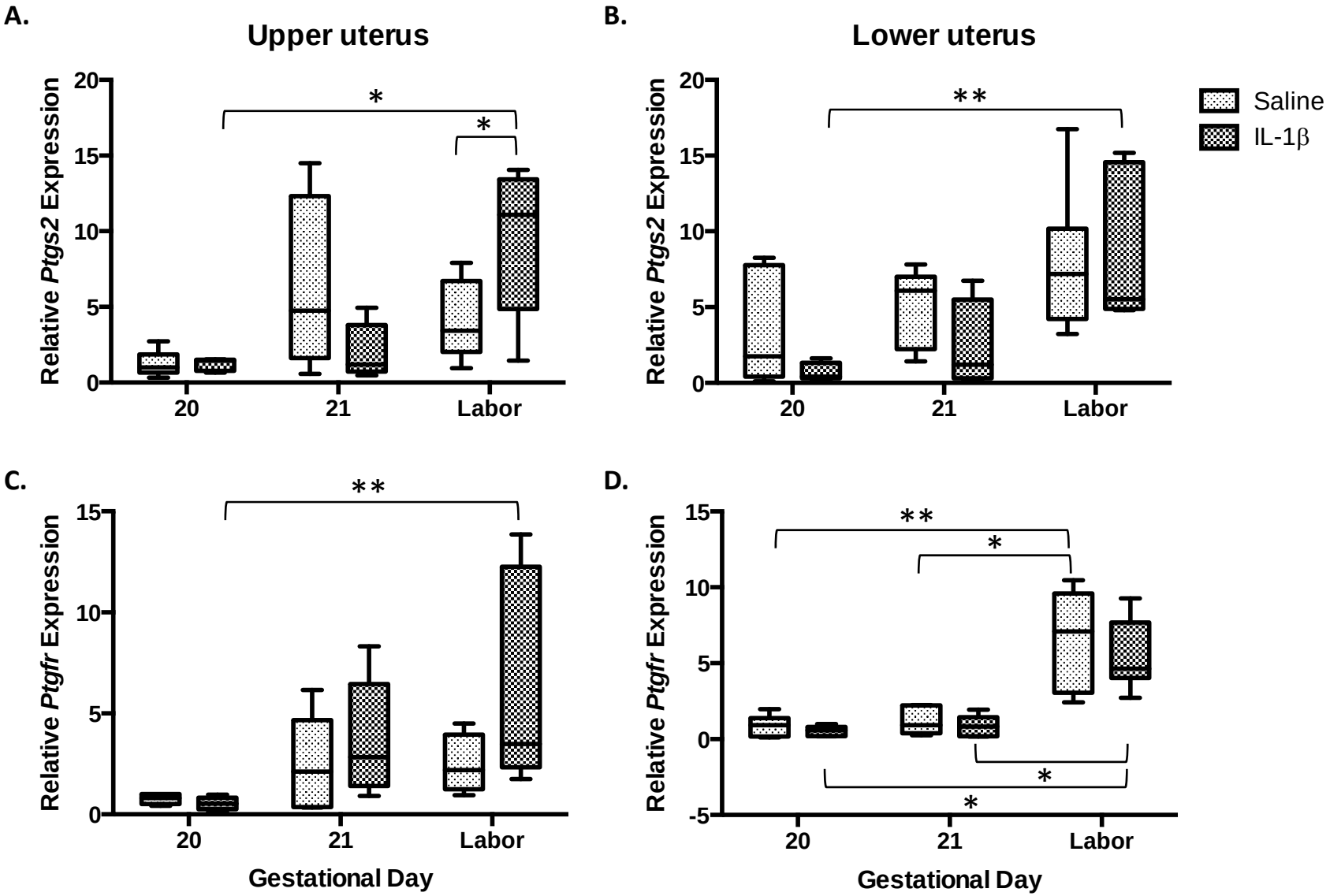

A.
Upper uterus
Relative Ptgs2 Expression
20
15
10
5
0
*
*
20
21
Labor
B.
Lower uterus
Relative Ptgs2 Expression
20
15
10
5
0
**
20
21
Labor
Saline
IL-1β
C.
Relative Ptgfr Expression
15
10
5
0
**
20
21
Labor
Gestational Day
D.
Relative Ptgfr Expression
15
10
5
0
-5
**
*
*
*
20
21
Labor
Gestational Day

Figure 3

Saline
IL-1β
120
80
40
0
Serum P4 level (μg/ml)
**
*
**
20
21
Labor
Gestational Day

Figure 4

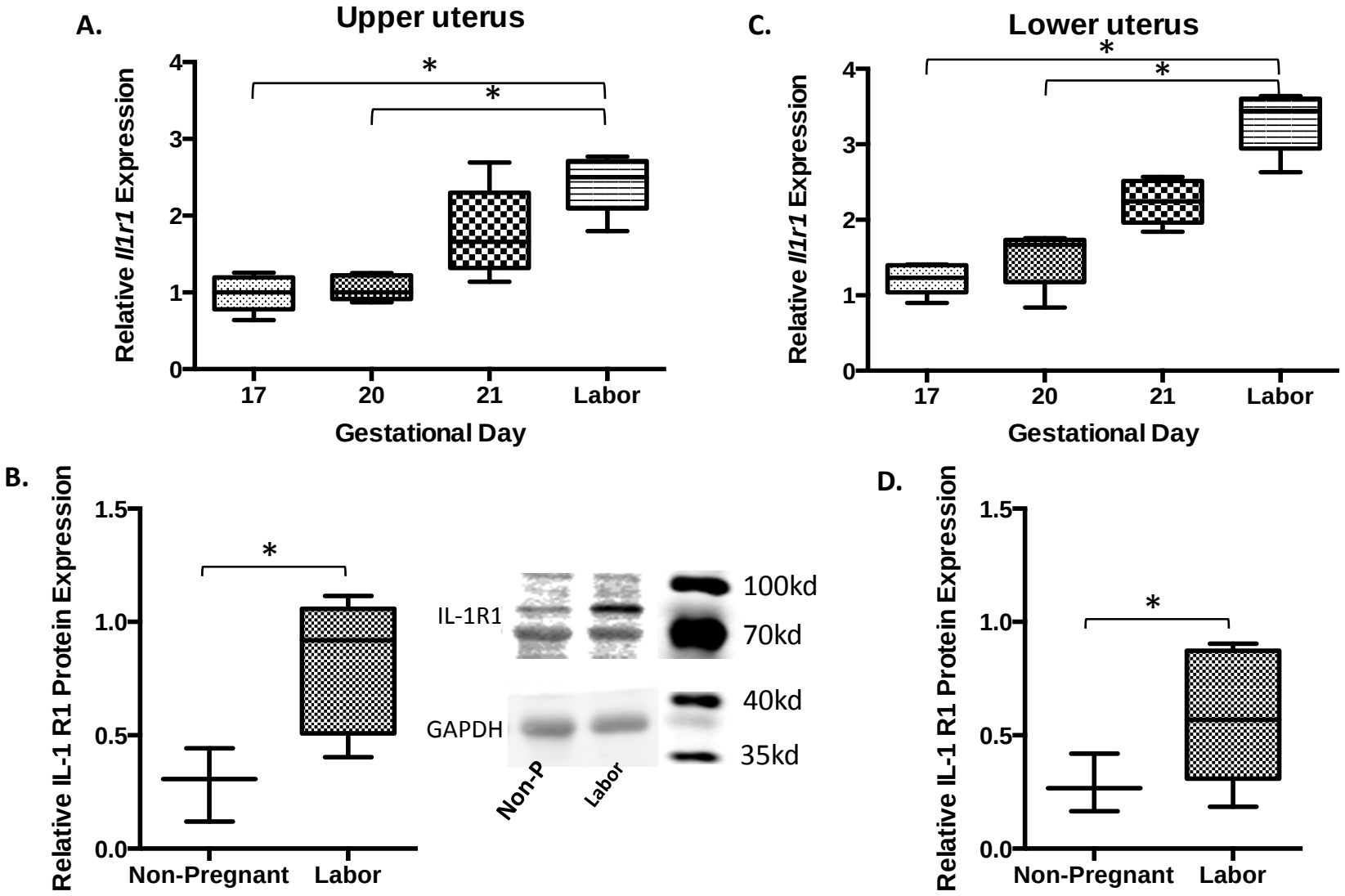

A.
Upper uterus
Relative Il1r1 Expression
0
1
2
3
4
*
*
17
20
21
Labor
Gestational Day
C.
Lower uterus
Relative Il1r1 Expression
0
1
2
3
4
*
*
17
20
21
Labor
Gestational Day
B.
Relative IL-1 R1 Protein Expression
0.0
0.5
1.0
1.5
*
Non-Pregnant
Labor
IL-1R1
GAPDH
Non-P
Labor
100kd
70kd
40kd
35kd
D.
Relative IL-1 R1 Protein Expression
0.0
0.5
1.0
1.5
*
Non-Pregnant
Labor

Figure 5

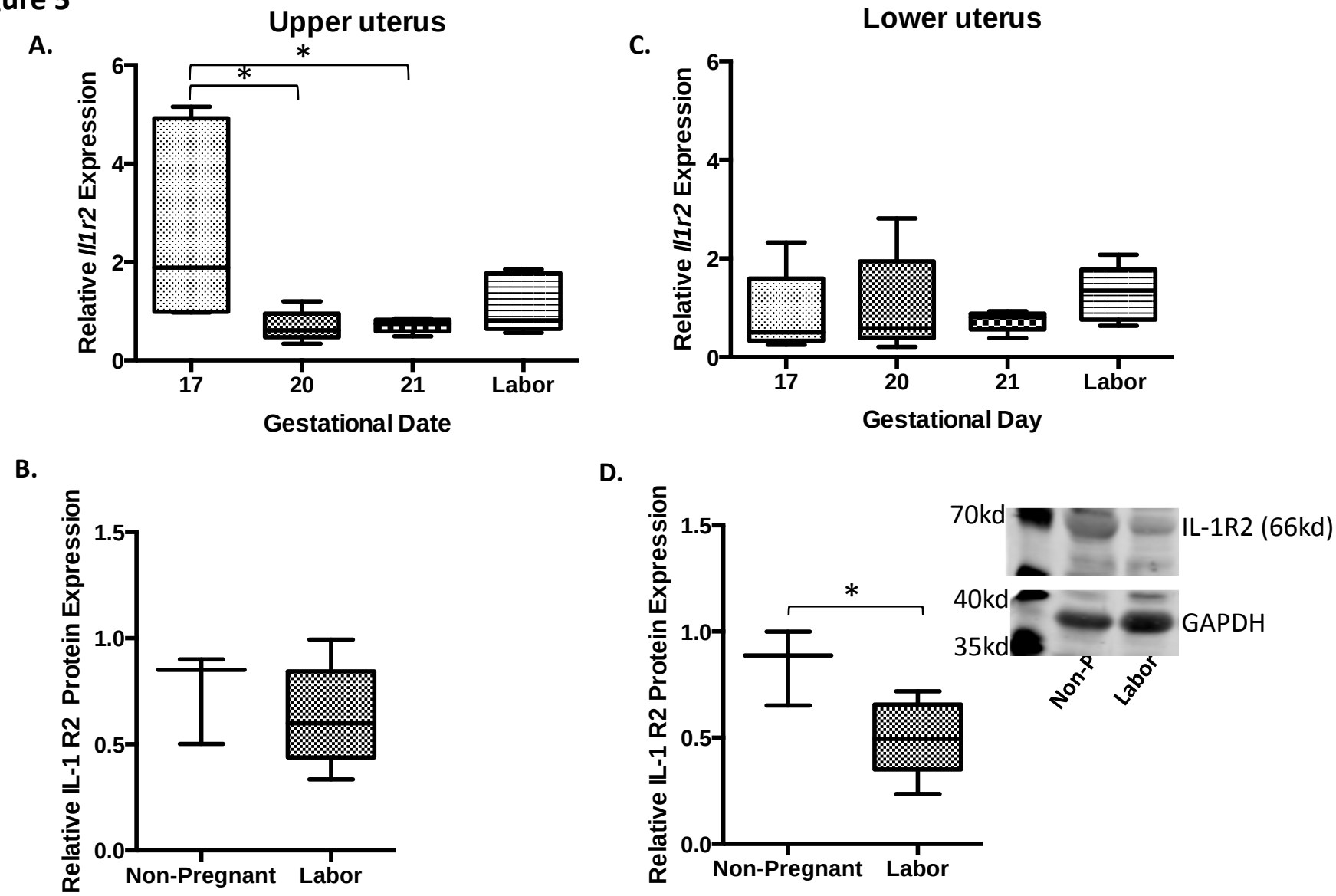

A.
Upper uterus
Relative Il1r2 Expression
0
2
4
6
*
*
17
20
21
Labor
Gestational Date
C.
Lower uterus
Relative Il1r2 Expression
0
2
4
6
17
20
21
Labor
Gestational Day
B.
Relative IL-1 R2 Protein Expression
0.0
0.5
1.0
1.5
Non-Pregnant
Labor
D.
Relative IL-1 R2 Protein Expression
0.0
0.5
1.0
1.5
*
Non-Pregnant
Labor
70kd
IL-1R2 (66kd)
40kd
35kd
GAPDH
Non-P
Labor

Figure 6

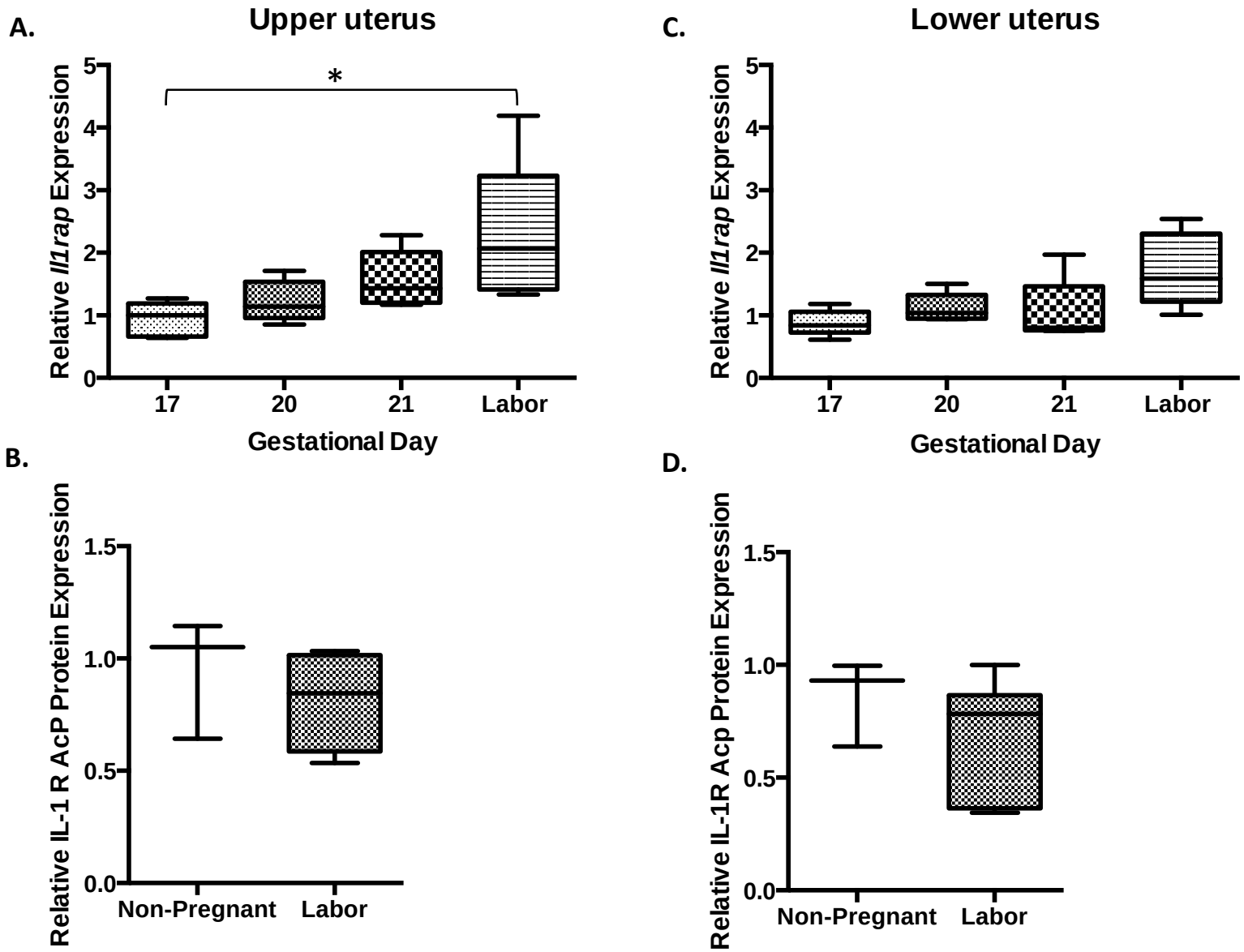
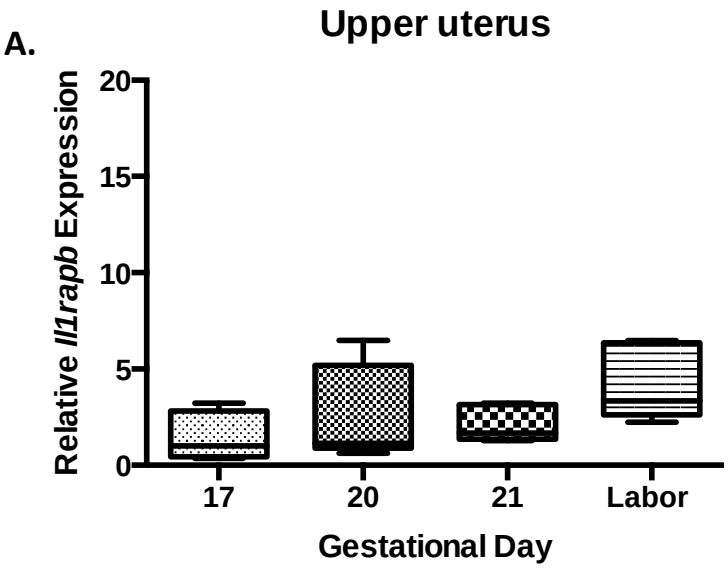

A.
Upper uterus
*
Relative Il1rap Expression
5
4
3
2
1
0
17
20
21
Labor
Gestational Day
C.
Lower uterus
Relative Il1rap Expression
5
4
3
2
1
0
17
20
21
Labor
Gestational Day
B.
Relative IL-1 R AcP Protein Expression
1.5
1.0
0.5
0.0
Non-Pregnant
Labor
D.
Relative IL-1R Acp Protein Expression
1.5
1.0
0.5
0.0
Non-Pregnant
Labor

Figure 7


A.
Upper uterus
Relative Il1rapb Expression
20
15
10
5
0
17
20
21
Labor
Gestational Day

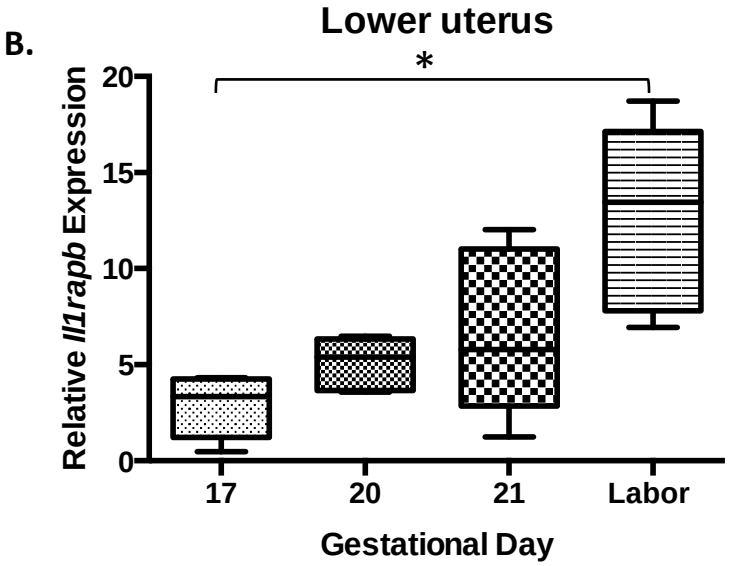

B.
Lower uterus
*
Relative Il1rapb Expression
20
15
10
5
0
17
20
21
Labor
Gestational Day

Figure 8

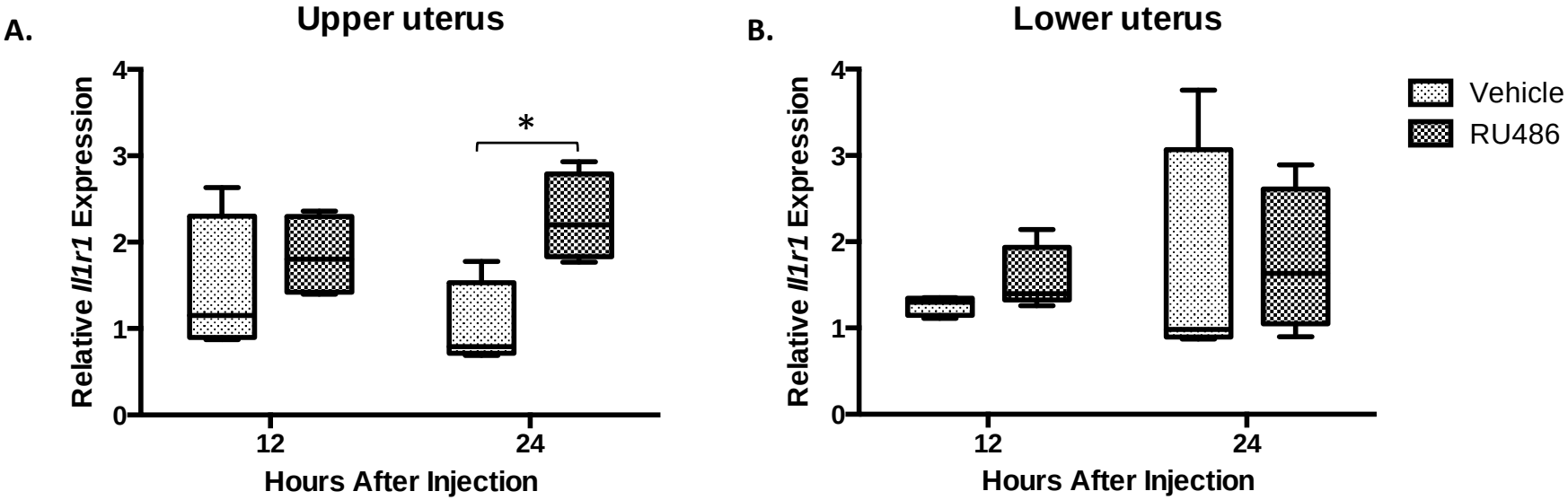

A.
Upper uterus
Relative Il1r1 Expression
0
1
2
3
4
*
12
24
Hours After Injection
B.
Lower uterus
Relative Il1r1 Expression
0
1
2
3
4
12
24
Hours After Injection
Vehicle
RU486

Figure 9

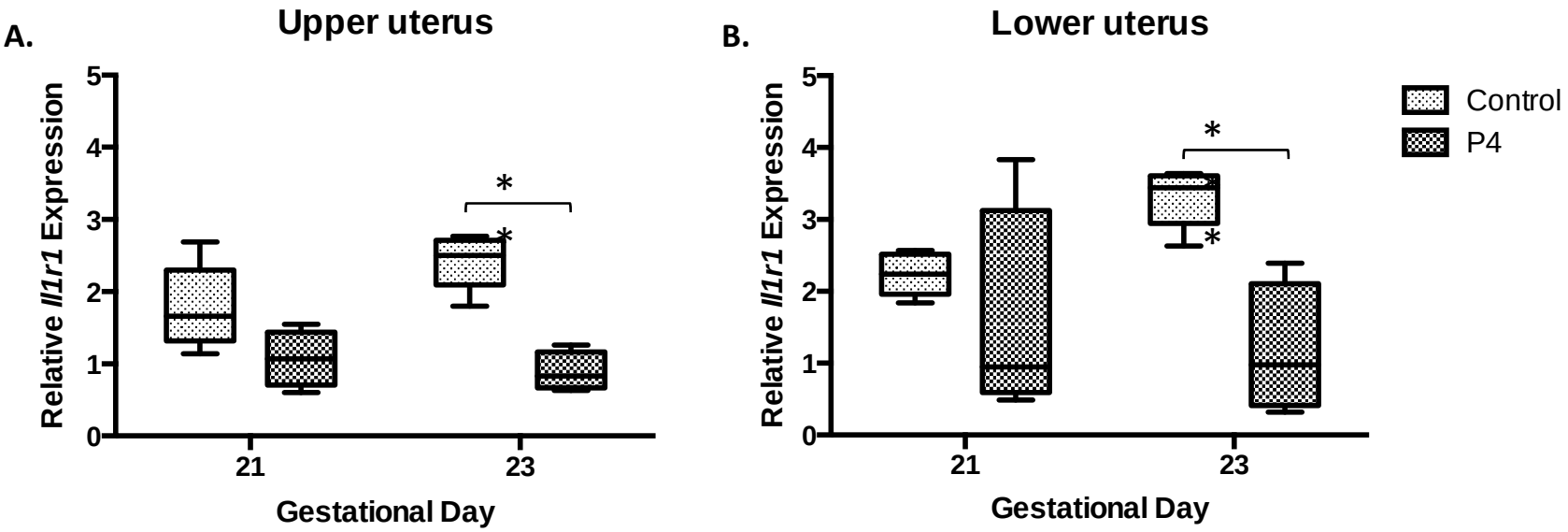
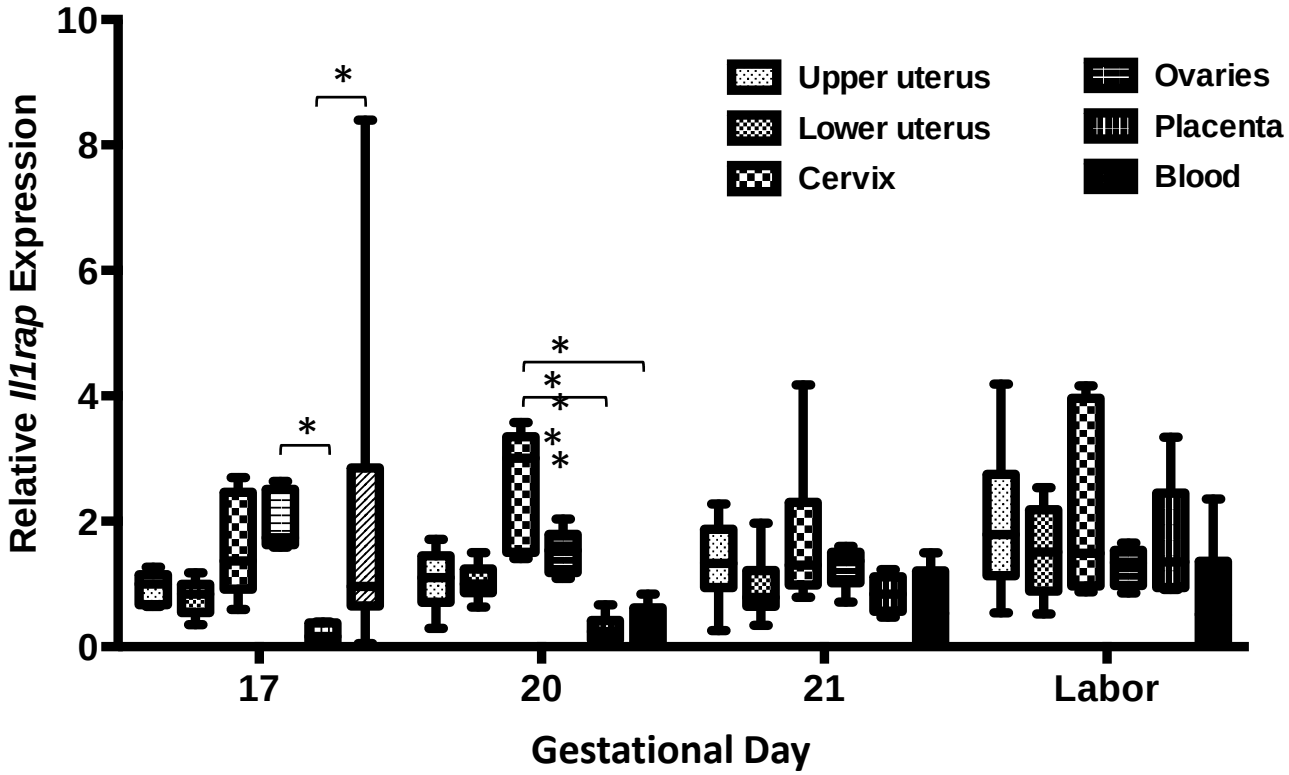

A.
Upper uterus
Relative Il1r1 Expression
0
1
2
3
4
5
*
*
21
23
Gestational Day
B.
Lower uterus
Relative Il1r1 Expression
0
1
2
3
4
5
*
*
21
23
Gestational Day
Control
P4

Figure 10

A.


Relative Il1rap Expression
10
8
6
4
2
0
Upper uterus
Lower uterus
Cervix
Ovaries
Placenta
Blood
*
*
*
*
*
*
*
17
20
21
Labor
Gestational Day

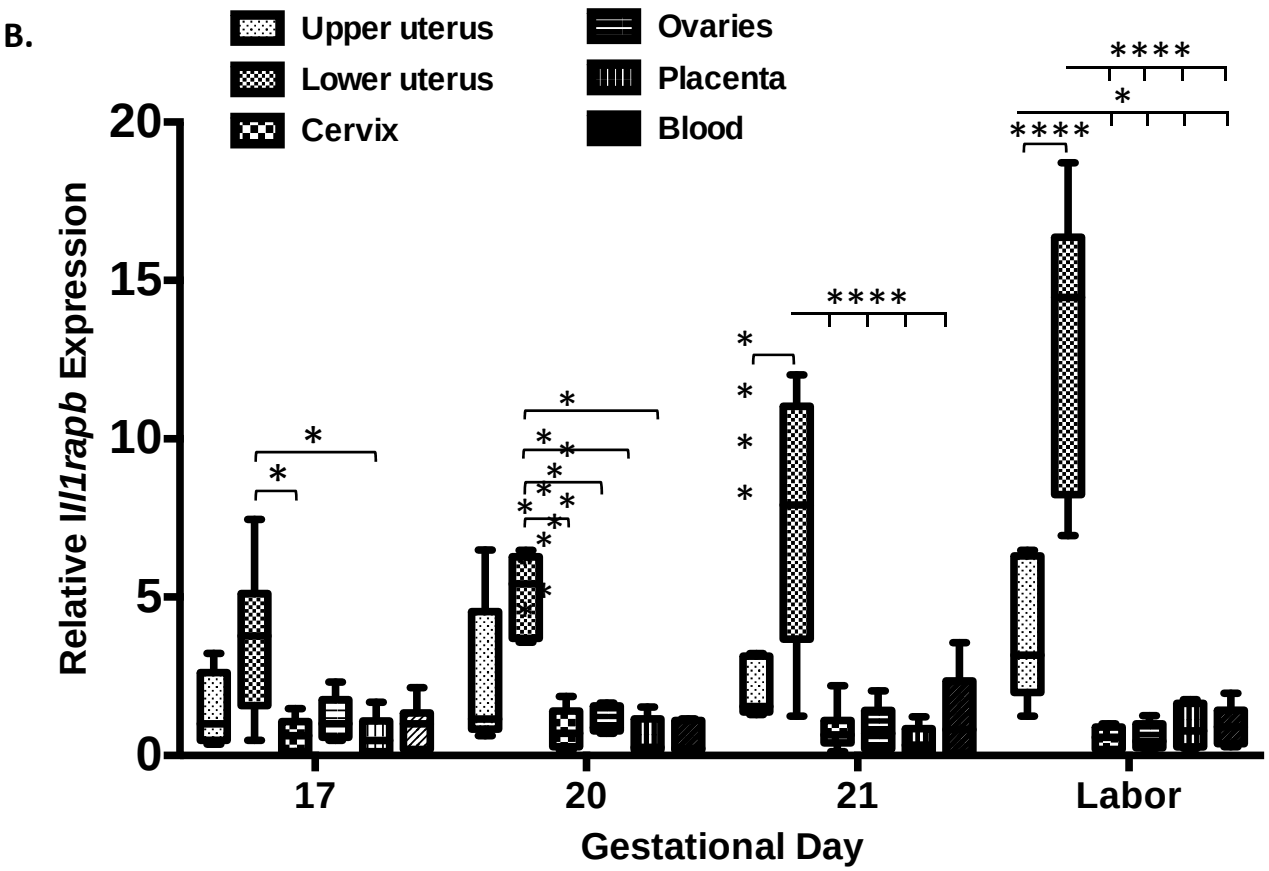

B.
Upper uterus
Lower uterus
Cervix
Ovaries
Placenta
Blood
Relative Il1rapb Expression
20
15
10
5
0
17
20
21
Labor
Gestational Day
*
****

Table 1. Primers used in real-time polymerase chain reaction (RT-PCR)

Gene		Primer sequences	Annealing temperature (°C)	NCBI reference sequences*
	Forward	5’- GGC TAA GTT TCA ATC AGC CCT TT -3’		
Il1a			60	NM_017019.1
	Reverse	5’- AGG TGC TGA TCT GGG TTG GAT -3’		
	Forward	5’- CTC AAT GGA CAG AAC ATA AGC C -3’		
Il1b			51	NM_031512.2
	Reverse	5’- GGT GTG CCG TCT TTC ATC A -3’		
	Forward	5’- CCT TGA ACA CGG ACT TGC TCA C -3’		
Ptgs2			60	S67722.1
	Reverse	5’- TCT CTC TGC TCT GGT CAA TGG A -3’		
	Forward	5’- CTG GCC ATA ATG TGC GTC TC -3’		
Ptgfr			60	NM_013115.1
	Reverse	5’- TGT CGT TTC ACA GGT CAC TGG -3’		
	Forward	5’- CCT GTG ATT ATG AGC CCA CG -3’		
Il1r1			58	NM_013123.3
	Reverse	5’- CGT GTG CAG TCT CCA GAA TAT G -3’		

	Forward	5’- AGA TGA GCC AAG GAT GTG GG -3’		
Il1r2			62	NM_053953.1
	Reverse	5’- ATC AAT AGG CGT GTG GGG TCT ATA CCA CTG TAT CTT TCC A -3’		
	Forward	5’- GGG CAA CAT CAA CGT CAT TTT AG -3’		
Il1rap			68	NM_012968.1
	Reverse	5’- CAG CTC TTT CAC CTT CAA GTC CTT -3’		
	Forward	5’- GGA GTT TAA GCT GGG TGT CAT GT -3’		
Il1rapb			68	NM_001167840.1
	Reverse	5’- TGC TCA AGC GGA CGG TAC T -3’		
	Forward	5’- CAC CGT GTT CTT CGA CAT CAC -3’		
Cyclophilin a			60	NM_017101.1
	Reverse	5’- CCA GTG CTC AGA GCA CGA AAG -3’		

* NCBI, National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).