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Aurora Kinase mRNA expression is reduced with increasing gestational age and in severe early onset fetal growth restriction

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Abstract

Introduction: Oxidative damage and biochemical ageing are implicated in placental dysfunction and potentially fetal death. Cellular senescence may play a role in the pathophysiology of fetal growth restriction (FGR) and preeclampsia (PE). Aurora kinases (*AURKA*, *B* and *C*) are important regulators of cellular division in mitosis and meiosis with implications in cellular senescence. We aimed to investigate whether aurora kinase expression is altered with placental dysfunction or placental ageing.

Methods: Placenta and blood was obtained across gestation from pathological pregnancies complicated by PE, FGR or both PE and FGR, as well as gestation-matched control samples from women who delivered for other maternal or fetal reasons. Expression of *AURKA*, *B* and *C* mRNA was examined using real time qPCR in both the placenta and maternal circulation.

Results: Placental aurora kinase expression decreased as gestation progressed: *AURKA* and *AURKB* were significantly reduced at 37–40 weeks, whereas *AURKC* was significantly reduced at 34–37 weeks, when compared to <34 weeks. In the maternal circulation, mRNA levels of *AURKB* was significantly reduced at >40 weeks compared to <34 weeks gestation. A significant reduction in *AURKC* was seen in FGR pregnancies <34 weeks compared to gestation-matched controls.

Discussion: Placental *AURK* expression is reduced with increased gestation. Circulating *AURKB* mRNA reduces at >40 weeks gestation, when compared to <34 weeks. *AURKC* is significantly reduced in placentas from pregnancies complicated by severe early onset (<34 weeks) FGR compared with gestation-matched controls. The functional role of aurora kinase in the placenta and in gestational age warrants further investigation.

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Keywords

Placenta; preeclampsia; fetal growth restriction; aurora kinase

INTRODUCTION:

Preeclampsia is a life-threatening complication of pregnancy, characteristically causing hypertension and proteinuria, which can lead to multi-organ maternal injury in the kidneys, liver and brain, and in some cases, fetal growth restriction (FGR) (1). Isolated FGR is caused by significant placental dysfunction leading to impaired intrauterine growth which, in severe cases, can lead to stillbirth (2). Both placental disorders can necessitate pre-term delivery, that may lead to death or significant long-term disability in the neonate especially if they occur at extreme pre-term gestations (3).

Oxidative damage and biochemical ageing have been implicated as a cause of fetal death (4), and cellular senescence may play a role in the pathophysiology of preeclampsia, FGR and even spontaneous pre-term birth (5). Cellular senescence can be induced by a variety of factors, such as oxidative stress, strong mitotic signals and dysregulation or activation of tumour suppressor genes (6). Aurora kinases A (AURKA), B (AURKB) and C (AURKC) are members of a group of serine/threonine protein kinases with important regulatory roles in mitosis and meiosis, including M-phase entry, spindle formation and regulation, and cytokinesis (7). *AURKB* expression is decreased in a p53-dependent manner in human primary cells (human dermal fibroblasts and human umbilical vein endothelial cells) undergoing senescence (7) and oocyte-specific *Aurkb* knockout female mice undergo premature age-related infertility (8). Collectively, these data indicate an important role of *AURKB* in cellular ageing. Although AURKA and AURKB are found in all cell types throughout the body, significant levels of AURKC are found only in cells that undergo meiosis (9). AURKC is also essential for healthy early pregnancy and knockout mice are subfertile with meiotic abnormalities and compromised embryonic development (10).

As well as a potential role in placental dysfunction, cellular senescence is also implicated in the pathophysiology of cancer (6). Aurora kinases are overexpressed in highly invasive human tumours, leading to their consideration as a potential therapeutic target (11–15). Activation of AURKB through the lysine methylation of Heat Shock Protein 70 (HSP70) may promote cancer cell proliferation (16). HSP70 is also elevated in preeclampsia and is thought to originate from syncytiotrophoblasts and villous endothelial cells (17).

The link between cellular senescence and the aurora kinases, and the link between impaired early pregnancy development and AURKC, led us to first assess the possibility of a role by evaluating changes in the expression of aurora kinases in pregnancy in women. We aimed to investigate whether *AURKA*, *AURKB* and *AURKC* are altered with either disorders of placental dysfunction, or with placental ageing.

METHODS:

Study subjects

Control placental and blood samples were obtained from women who delivered at various gestations with an appropriately grown fetus, in the absence of hypertensive diseases of pregnancy and with no clinical or histopathological evidence of chorioamnionitis. Placental tissue was obtained from across gestations and were divided into early pre-term (<34 weeks; n=17), late pre-term (34–37 weeks; n=15), term (37–40 weeks; n=21) and post-term (>40 weeks; n=9). Those delivering pre-term were delivered on maternal or fetal grounds excluding reasons mentioned above. Term and post-term pregnancies were delivered with no known obstetric, medical or surgical complications in pregnancy. All deliveries occurred via caesarean section, thus not confounded by labour (Table 4). Blood samples were also obtained across gestation at early pre-term, (n=17), term (n=7) and post-term (n=5) in controls that all eventually went on to deliver a term (>37 weeks), normally grown fetus without any known pregnancy complications (Table 5).

Placental (Table 1–3) and blood (Table 6) samples were also obtained from women with pregnancies complicated by preeclampsia (with or without coexistent FGR), or FGR in isolation. Preeclampsia was defined according to ACOG guidelines, with persistently elevated blood pressure of >140mmHg systolic or >90mmHg diastolic, and >0.3g/day of proteinuria, after 20 weeks gestation (18). FGR was defined as a fetus born less than the 10^{th} centile for gestation (using sex specific charts) according to contemporary Australian birthweight centiles (19).

Ethical approval was obtained from the Mercy Health Human Research Ethics Committee (R11/34). All women donating tissue or blood samples at the Mercy Hospital for Women in Melbourne gave informed written consent.

Tissue and blood collection

Placental tissue was obtained within 30 minutes of a caesarean section delivery. Maternal and fetal surfaces were removed, and samples were taken from multiple sites according to CoLab recommendations (20). Samples were washed in sterile phosphate-buffered saline (PBS). Samples were stabilised in RNAlater and snap frozen with liquid nitrogen and stored at −80°C until processing for RNA extraction. Peripheral whole blood samples (2.5ml) were collected in PAXgene blood RNA tubes (PreAnalytix, Hombrechtikon, Switzerland). They were stored at room temperature for 24 −72 hours as per manufacturer's instructions before being transferred to −20°C for 24 h, and finally stored at −80°C until processing for RNA extraction.

RNA extraction

Total RNA was extracted from placental tissue using the RNeasy mini kit (Qiagen, Valencia, CA). Total RNA was extracted from peripheral whole maternal blood using the PAXgene Blood Kit (PreAnalytix, Hombrechtikon, Switzerland), as per manufacturer's instructions as previously described (21). Genomic DNA was removed using DNAse treatment, and total RNA eluted and stored at −80°C.

Real Time Quantitative RT-PCR

RNA quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and quantified using a Nanodrop ND 1000 spectrophotometer (NanoDrop technologies Inc, Wilmington, DE). RNA was converted to cDNA using the Applied Biosystems high capacity cDNA reverse transcriptase kit (Applied Biosystems; Thermofisher Life technologies, Mulgrave, Australia) as per manufacturer guidelines. Expression of AURKA, AURKB and AURKC mRNA was assessed by qPCR using Taqman Gene Expression Assays: Hs01582072_m1, Hs00177782_m1 and Hs00152930_m1 respectively and TaqMan Universal PCR Master Mix on a CFX 384 (BioRad, Foster CA) with the following cycling conditions: 50°C for 2 mins, 95°C for 10 mins, and 40 cycles of 95°C for 15 sec, 60°C for 1 min. Fold changes in gene expression were determined by the comparative CT method normalized against the mean expression of housekeeping reference genes (TOP1/CYC1 for placental tissue and GUSB/B2M for circulating mRNA). Data was analysed using the ΔΔCT method of analysis.

Statistical Analysis:

All qPCR reactions were performed in triplicate. Statistical analysis was performed using GraphPad Prism. Data were tested for normality; non-parametric data were analysed with a Mann-Whitney U or Kruskal-Wallis test and parametric data with an unpaired t-test or One-way ANOVA. $P<0.05$ was considered significant.

RESULTS:

Placental expression of *AURK* across gestation

To determine whether the aurora kinases are altered in disorders of placental dysfunction, or with placental ageing, we first evaluated their mRNA expression levels by quantitative real-time PCR in placentas during the later stages of normal gestation. The change in placental expression of all three aurora kinases decreased as gestation progressed (Figure 1). Notably, expression of both *AURKA* and *AURKB* mRNA was significantly reduced at 37–40 weeks compared to earlier in gestation, at <34 weeks ($p<0.05$ and $p<0.01$ respectively). *AURKC* expression was significantly reduced at earlier gestations than the other two *AURKs* at 34–37 weeks when compared to <34 weeks ($p<0.05$). These data suggest that placental expression of the *AURK*s reduce with gestational age.

Placental expression of *AURK*s in preeclamptic and fetal growth restricted pregnancies

Next, we evaluated *AURK* expression in dysfunctional placentas obtained at less than 34 weeks of gestation. Compared to control pregnancies the mRNA expression of *AURKA* and *AURKB* was unchanged in all subgroups of diseases tested (Figure 2A–B). For *AURKC* there were no differences observed in placental expression in pregnancies complicated by preeclampsia, with or without FGR, at <34 weeks. However, *AURKC* was significantly reduced in placentas from pregnancies complicated by FGR compared to gestation matched controls at <34 weeks gestation ($p<0.01$) (Figure 2C).

In late pre-term gestation (34–37 weeks) or term (37–40 weeks) gestation, there were no differences in placental expression of *AURKA*, *AURKB* or *AURKC* in preeclampsia or

FGR compared with gestation matched controls (Figures 3 and 4). Therefore, the data suggest that a decrease in placental *AURKC* expression is associated with FGR that results in pre-term birth <34 weeks, but not late pre-term FGR, nor term FGR.

Circulating *AURK* mRNA expression across gestation

This is the first study to consider whether aurora kinases are released into the maternal circulation during pregnancy. We assessed the mRNA expression of these kinases at three gestational time points, early pre-term, term and post-term. The expression levels of *AURKA* and *AURKC* were unchanged over time. Similar to the aged placenta however, circulating *AURKB* mRNA was significantly reduced as pregnancy progressed. We observed a reduction of its expression in post-term pregnancies (>40 weeks) compared to pre-term pregnancies (<34 weeks) ($p<0.05$) (Figure 5B).

Circulating *AURK* mRNA expression in preeclamptic and fetal growth restricted pregnancies

Finally, we assessed the mRNA expression levels of the aurora kinases in circulating maternal blood from pregnancies complicated by preeclampsia, FGR, or both placental diseases at <34 weeks gestation. In contrast to the changes in placental *AURKC* expression (Figure 3), there was no difference in the relative expression of circulating *AURKA*, *AURKB* or *AURKC* mRNA in pregnancies complicated by either preeclampsia or FGR compared to gestation-matched controls (Figure 6).

DISCUSSION:

Placental ageing and cellular senescence are implicated in multiple aspects of placental dysfunction in pregnancy, including pre-term birth, fetal growth restriction, preeclampsia, and ultimately fetal death. The aurora kinases play an important role in cellular ageing and are known regulators of multiple cell-cycle stages. Because of this connection, we assessed aurora kinase mRNA levels in the placenta and maternal circulation, in both normal physiologically ageing placentas, and in the pathological states of preeclampsia and fetal growth restriction.

We identified that the mRNA levels of *AURKA*, *AURKB* and *AURKC* were all reduced with increasing gestation in placentas from normotensive pregnancies with a baby delivered at appropriate size for gestational age. Expression of both *AURKA* and *AURKB* at early pre-term gestation was higher than those at term gestation. During late pregnancy, it is hypothesized that the fetal nutritional requirements exceed the placental ability to meet demands, stimulating the generation of reactive oxygen species (ROS). Therefore, these demands place the placenta in a state of oxidative stress. AURKA becomes hyperphosphorylated under oxidative stress, disturbing its function in mitotic spindle formation and impeding further mitotic progression (22). Furthermore, therapeutic inhibitors of AURKB have been shown to increase amounts of ROS (23).

We also identified that circulating *AURKB* mRNA levels were significantly reduced in post-term pregnancies, although we did not observe the same change for circulating *AURKA* or *AURKC* mRNA expression. This reduction in circulating *AURKB* corresponds with

the decreasing expression of *AURKB* in placental tissue as gestation progresses and the placenta ages. Messenger RNA, as well as surface and cytoplasmic proteins and microRNA are known to leak or be released from the placenta throughout gestation (24), and thus potentially explains the change in maternal circulation of *AURKB* observed here.

We found a significant reduction in placental *AURKC* expression in pregnancies complicated by severe early onset fetal growth restriction compared to gestation matched controls delivered <34 weeks for other indications. Interestingly, when fetal growth restriction occurred at later gestations, there was no change in placental *AURKC* expression. There is a suggestion that early onset and late onset fetal growth restriction are different pathophysiological conditions (25). Potentially, *AURKC* may be important earlier in gestation while the placental cells are proliferating, and aberrant expression contributes to the development of early onset fetal growth restriction. Further studies exploring *AURKC* in early gestation could help improve our understanding.

In conclusion, here we demonstrate there is a significant reduction in placental expression of *AURKA*, *AURKB* and *AURKC* across gestation, which is consistent with aurora kinase expression in other ageing human and mouse tissues. We also found that the circulating mRNA levels of *AURKB* were reduced with advancing gestation, as in the placental tissue. We found a significant reduction in *AURKC* placental expression in placentas from pregnancies complicated by severe early onset FGR compared to gestation-matched controls, however this was not reflected in FGR delivered at later gestations, nor in placentas from preeclamptic pregnancies. The significance of these findings remains uncertain, and the roles of aurora kinase in the placenta warrants further investigation.

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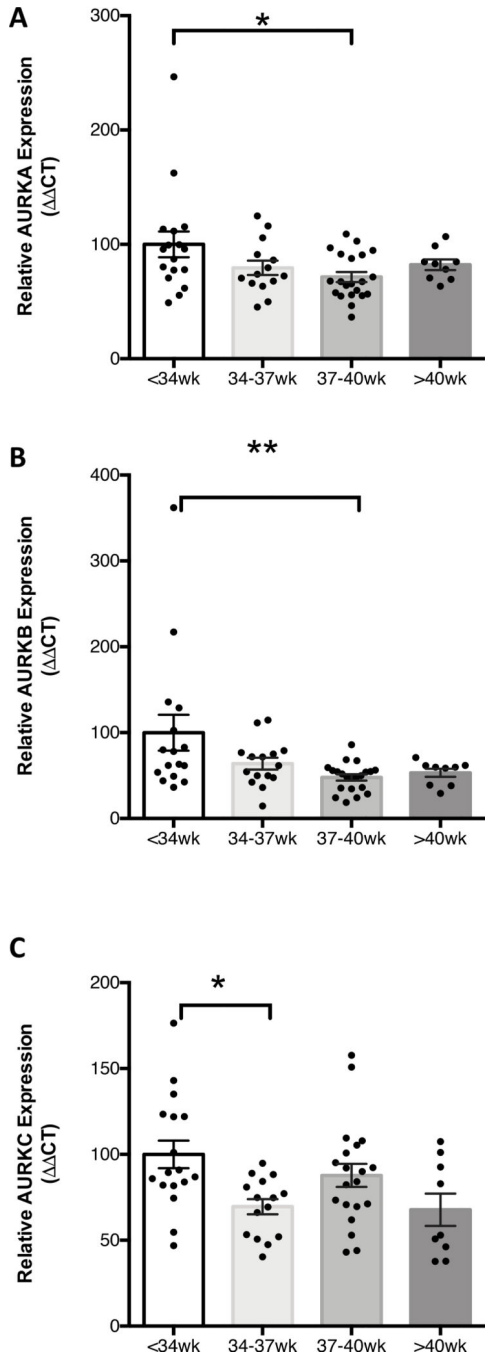

Figure 1: Placental *AURK* expression decreases across gestation.

A-C): Relative expression of *AURKA* (A), *AURKB* (B), and *AURKC* (C) in placentas obtained from pregnancies at the indicated gestation time. Data expressed as mean ± SEM. *p<0.05, **p<0.01.

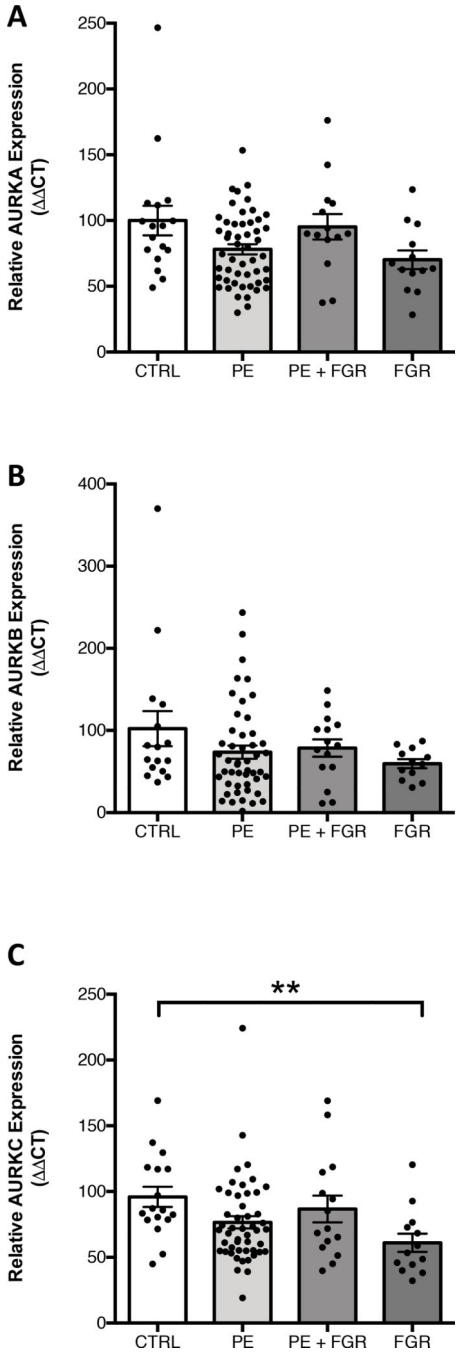

Figure 2: Placental *AURKC* expression is significantly decreased in pregnancies complicated by fetal growth restriction at <34 weeks gestation.

A-C): Relative expression of *AURKA* (A), *AURKB* (B), and *AURKC* (C) in placentas obtained from pregnancies prior to 34 weeks from the indicated pathology. CTRL = gestation-matched control; PE = preeclampsia (without fetal growth restriction); PE + FGR = preeclampsia (with fetal growth restriction); FGR = fetal growth restriction. Data expressed as mean ± SEM. **p<0.01.

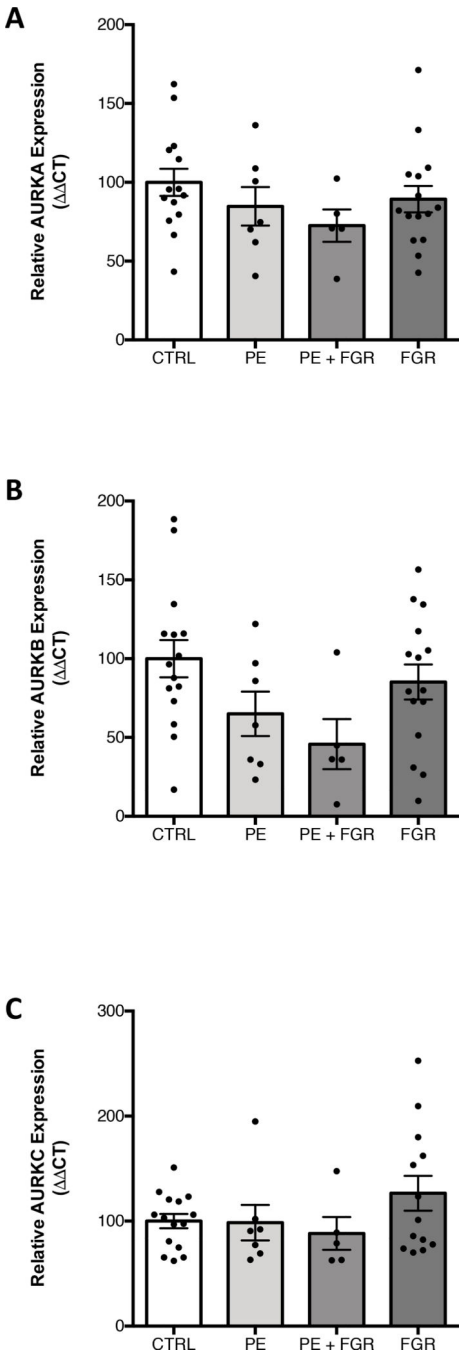

Figure 3: Placental *AURK* expression is not altered in pathological pregnancies at 34–37 weeks gestation.

A-C): Relative expression of *AURKA* (A), *AURKB* (B), and *AURKC* (C) in placentas obtained from pregnancies delivering between 34–37 weeks from the indicated pathology. CTRL = gestation-matched control; PE = preeclampsia (without fetal growth restriction); PE + FGR = preeclampsia (with fetal growth restriction); FGR = fetal growth restriction. Data expressed as mean ± SEM.

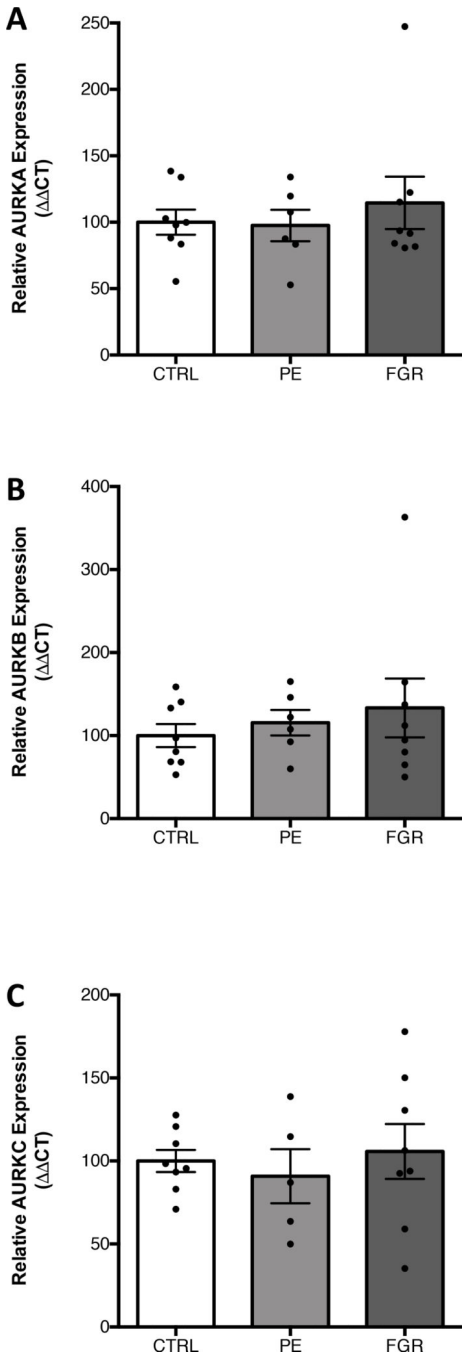

Figure 4: Placental *AURK* expression is not altered in pathological pregnancies at 37–40 weeks gestation.

A-C): Relative expression of *AURKA* (A), *AURKB* (B), and *AURKC* (C) in placentas obtained from pregnancies delivering between 37–40 weeks from the indicated pathology. CTRL = gestation-matched control; PE = preeclampsia; FGR = fetal growth restriction. Data expressed as mean ± SEM.

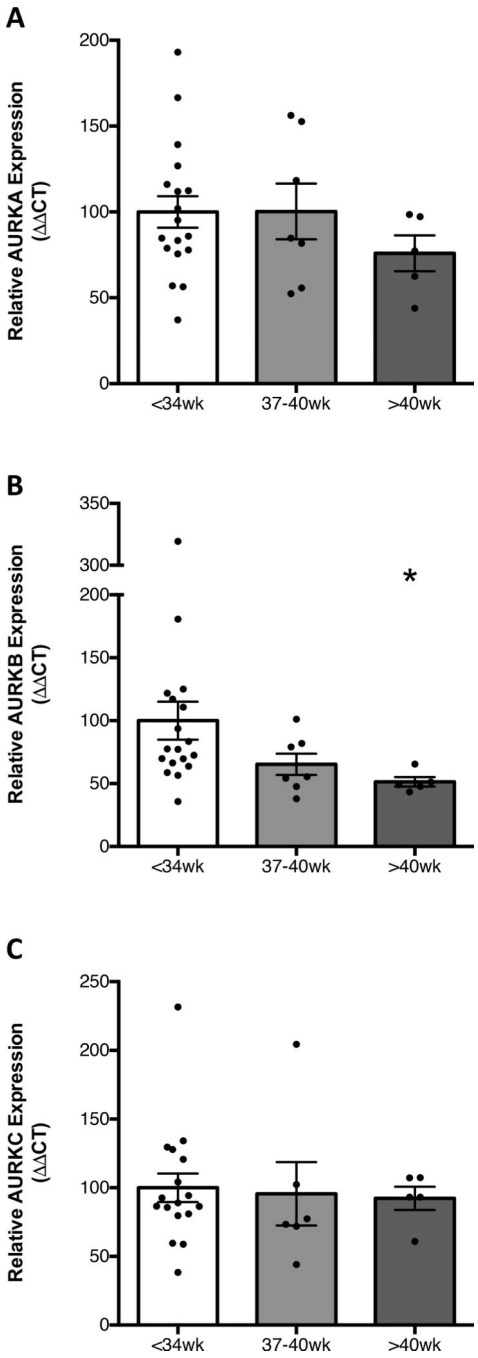

Figure 5: Circulating *AURKB* mRNA levels significantly decrease with gestation greater than 40 weeks.

A-C): Relative expression of *AURKA* (A), *AURKB* (B), and *AURKC* (C) in maternal circulating blood obtained from pregnancies at the indicated gestation time. Data expressed as mean ± SEM. *p<0.05.

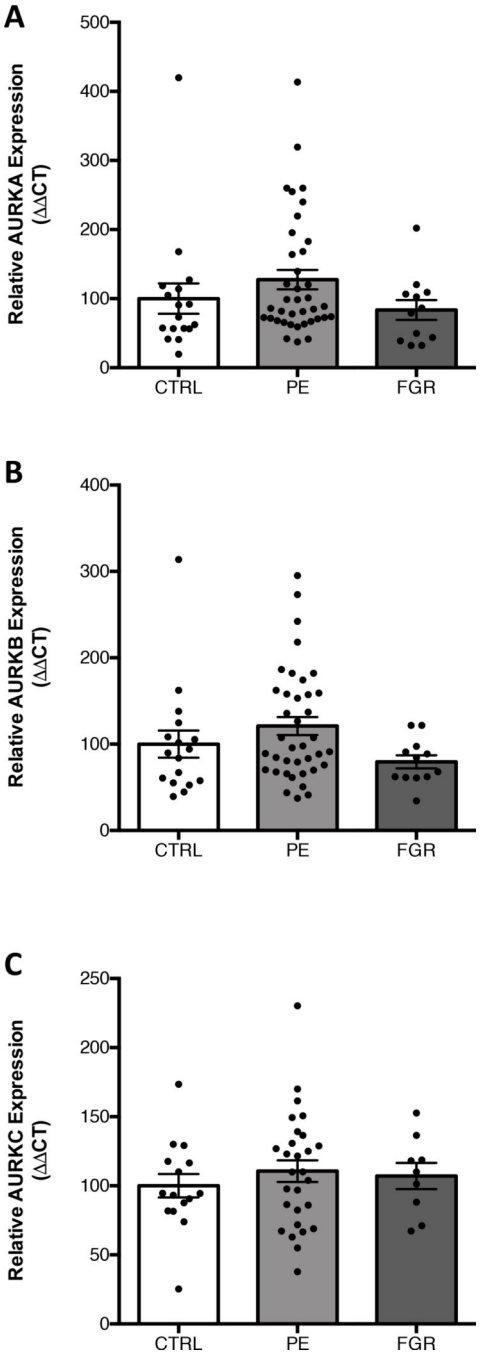

Figure 6: Circulating AURK mRNA is not altered in pathological pregnancies at <34 weeks gestation.

A-C): Relative expression of *AURKA* (A), *AURKB* (B), and *AURKC* (C) in maternal circulating blood obtained from pregnancies prior to 34 weeks with the indicated placental pathology. CTRL = gestation-matched control; PE = preeclampsia; FGR = fetal growth restriction. Data expressed as mean ± SEM.

Table 1:

Patient characteristics for placental tissue samples collected at <34 weeks

	Control (n = 17)	**Preeclampsia (without FGR) (n=51)**	**Preeclampsia (with FGR) (n=14)**	**FGR (n=13)**
Maternal Age Median (IQR)	32 (23–36)	31 (28–35)	31 (24.5–32.3)	29 (21–35)
Gestation at Delivery Median (IQR)	30.7 (28.9 – 31.9)	30.4 (28.4 – 31.9)	30.1 (27.3 – 30.9)	31.4 (30.2 – 32.7)
BMI (kg/m^2) Median (IQR)	27.3 (24–35.2)	27 (25–37)	27.1 (25–32)	23 (18.8–30)
Parity no. (%)				
0	5 (29.4)	36 (70.6)	13 (92.9)	10 (76.9)
1	7 (41.2)	10 (19.6)	1 (7.1)	1 (7.7)
≥2	5 (29.4)	5 (9.8)	0 (0)	2 (15.4)
Highest SBP prior to delivery (mmHg) Median (IQR)	125 (110–130)	175 (165–182) ****	165 (155–176) ***	120 (116–132)
Highest DBP prior to delivery (mmHg) Median (IQR)	70 (68–80)	100 (100–110) ****	100 (94–110) ****	80 (70–84)
Birth weight (g) Median (IQR)	1589 (1278–1943)	1329 (996–1470)	770 (581–1214) ***	999 (864–1214) *

BMI data unavailable for n=5 control, n=8 preeclampsia (without FGR), and n=3 preeclampsia (with FGR).

* P<0.05

*** p<0.001

**** p<0.0001.

Table 2:

Patient characteristics for placental tissue samples collected between 34–37 weeks

	Control (n = 15)	**Preeclampsia (without FGR) (n=7)**	**Preeclampsia (with FGR) (n=5)**	**FGR (n=15)**
Maternal Age Median (IQR)	31 (28 – 36)	34 (27 – 34)	36 (27 – 38.5)	32 (29 – 35)
Gestation at Delivery Median (IQR)	34.9 (34.4 – 35.3)	35.6 (35.1 – 36.3)	34.9 (34.3 – 36.1)	34.9 (34 – 36)
BMI (kg/m^2) Median (IQR)	24.2 (20.9 – 26.8)	25.3 (24 – 32)	22 (20.6 – 34.8)	28.7 (25.4 – 33.7)
Parity no. (%)				
0	6 (40)	5 (71)	3 (60)	8 (53.3)
1	6 (40)	1 (14.5)	1 (20)	3 (20)
≥2	3 (20)	1 (14.5)	1 (20)	4 (26.7)
Highest SBP prior to delivery (mmHg) Median (IQR)	120 (110 –125)	180 (170 – 180) ****	160 (150 – 195) ***	130 (120 – 140)
Highest DBP prior to delivery (mmHg) Median (IQR)	80 (70 – 80)	100 (90 – 112) ***	110 (95 – 112) ***	80 (75 – 85)
Birth weight (g) Median (IQR)	2695 (2395 – 2830)	2226 (2053 – 2514)	1833 (1688 – 2015) **	1700 (1655 – 1950) ****

BMI data unavailable for n=1 control and n=1 FGR.

** p<0.01

*** p<0.001

**** p<0.0001.

Table 3:

Patient characteristics for placental tissue samples collected between 37–40 weeks

	Control (n = 8)	**Preeclampsia (n=6)**	**FGR (n=8)**
Maternal Age Median (IQR)	32 (29.5 – 35)	34 (30.8 – 37)	33 (31 – 36.5)
Gestation at Delivery Median (IQR)	38.4 (37.4 – 38.7)	37.3 (37.1 – 37.5)	37.4 (37.2 – 38.1)
BMI (kg/m^2) Median (IQR)	25.3 (23.1 – 28.1)	29 (23.5 – 38.7)	23.8 (22 – 25)
Parity no. (%)			
0	5 (62.5)	2 (33.3)	4 (50)
1	1 (12.5)	3 (50)	4 (50)
≥2	2 (25)	1 (16.7)	0 (0)
Highest SBP prior to delivery (mmHg) Median (IQR)	120 (120 – 125)	160 (144 – 174) **	116 (111 – 131)
Highest DBP prior to delivery (mmHg) Median (IQR)	71 (70 – 75)	98 (93 – 101) **	73 (61 – 83)
Birth weight (g) Median (IQR)	2940 (2833 – 3683)	2985 (2558 – 3182)	2229 (2115 – 2308) ***

BMI data unavailable for n=1 FGR.

** p<0.01

*** p<0.001.

Table 4:

Patient characteristics for placental tissue samples collected across gestation

Gestation	**<34 weeks (n = 17)**	**34–37 weeks (n=15)**	**37–40 weeks (n=21)**	**>40 weeks (n=9)**
Maternal Age Median (IQR)	32 (23 – 36)	31 (28 – 35)	32.5 (31 – 37.3)	31 (27.5 – 35.5)
Gestation at Delivery Median (IQR)	30.7 (28.9 – 31.9)	34.9 (34.4 – 35.3)	39 (38.6 – 39.2)	40.7 (40.5 – 41)
BMI (kg/m^2) Median (IQR)	27.3 (24 – 35.2)	21.2 (20.9 – 26.8)	25.1 (22.6 – 27.4)	23.7 (22.3 –28.3)
Parity no. (%)				
0	5 (29.4)	6 (40)	9 (42.9)	1 (11.1)
1	7 (41.2))	6 (40)	9 (42.9)	8 (88.9)
≥2	5 (29.4)	3 (20)	3 (14.3)	0 (0)
Highest SBP prior to delivery (mmHg) Median (IQR)	125 (110 – 130)	120 (110 – 125)	120 (113 – 125)	120 (106 – 138)
Highest DBP prior to delivery (mmHg) Median (IQR)	70 (68 – 80)	80 (70 – 80)	75 (70 – 80)	75 (70 – 84)
Birth weight (g) Median (IQR)	1589 (1278 – 1943)	2695 (2395 – 2830) *	3410 (3025 – 3740) ****	3640 (3330 – 4165) ****

BMI data unavailable for n=5 <34 weeks and n=1 34–37 weeks.

* $p<0.05$

**** $p<0.0001$.

Table 5:

Patient characteristics for maternal blood samples collected across gestation

	<34 weeks (n=17)	**37–40 weeks (n=7)**	**>40 weeks (n=5)**
Maternal Age Median (IQR)	30 (29 – 33)	35 (28.5 – 36.5)	31 (29 – 34)
Gestation at Blood Collection Median (IQR)	27.4 (21.8 – 28.1)	38.3 (38.0 – 39.1) **	40.4 (40.2 – 40.6) ****
Gestation at Delivery Median (IQR)	39.4 (38.9 – 40)	39.1 (38.9 – 39.5)	40.4 (40.4 – 40.7) *
BMI (kg/m^2) Median (IQR)	22 (21 – 25.2)	29.4 (22.9 – 36.2)	27.9 (22.5 – 29.3)
Parity no. (%)			
0	9 (52.9)	1 (14.3)	1 (20)
1	6 (35.3)	4 (57.1)	4 (80)
≥2	2 (11.8)	2 (28.6)	0 (0)
Highest SBP prior to delivery (mmHg) Median (IQR)	125 (120 – 130)	115 (110 –115)	125 (115 – 130)
Highest DBP prior to delivery (mmHg) Median (IQR)	75 (70 – 80)	70 (70 – 73)	78 (70 – 85)
Birth weight (g) Median (IQR)	3620 (3320 – 3870)	3290 (3190 – 3875)	3640 (3508 – 3730)

* $p<0.05$

** $p<0.01$

**** $p<0.0001$.

Table 6:

Patient characteristics for maternal blood samples collected from pregnancies complicated by preeclampsia or FGR and gestational matched controls

	Control (n=17)	Preeclampsia (n=38)	FGR (n=12)
Maternal Age Median (IQR)	30 (29 – 32)	32 (27.3 – 34.8)	32.5 (28.3 – 37)
Gestation at Blood Collection Median (IQR)	29 (28 – 30.3)	29.4 (27.9 – 30.9)	31.7 (30.1 – 32.5)
Gestation at Delivery Median (IQR)	39.7 (39 – 40.4)	29.9 (27.9 – 31.7) ****	31.8 (30.3 – 32.5) ***
BMI (kg/m^2) Median (IQR)	23.0 (21.0 – 27.0)	27.5 (24.8 – 35.2) *	26.2 (21.3 – 29.5)
Parity no. (%)			
0	8 (47.1)	28 (73.7)	9 (75)
1	8 (47.1)	6 (15.8)	1 (8.3)
≥2	1 (5.9)	4 (10.5)	2 (16.7)
Highest SBP prior to delivery (mmHg) Median (IQR)	130 (125 – 130)	170 (161 – 180) ****	120 (118–131)
Highest DBP prior to delivery (mmHg) Median (IQR)	75 (70 – 80)	100 (95 – 105) ****	76 (70 – 80)
Birth weight (g) Median (IQR)	3610 (3290 – 3760)	1099 (844 – 1453) ****	1031 (973 – 1370) ****

BMI data unavailable for n=5 preeclampsia.

* p<0.05

*** p<0.001

**** p<0.0001.