

# ROLE OF microRNAS AND CD4+ T CELL SUBSETS IN THE REGULATION OF THE IMMUNE SYSTEM DURING PREGNANCY

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# miRNAs

- Micro RNAs are single-stranded non-coding RNAs that regulate expression of complementary RNAs
- They may regulate aprox. 50% of protein coding genes (post-transcriptional regulation) by promoting degradation of target mRNA or repression of protein translation.
- Trophoblasts secrete exosomes which contain placenta-specific miRNAs
- miRNAs have been linked to physiological and pathological processes during pregnancy

# miRNAome of the placenta

TABLE 1. Target genes and their functions of miRNAs in the trophoblasts or placenta.

miRNA	Target genes	Trophoblast	Regulatory role in trophoblast
miR-17, miR-20a, miR-20b	VEGFA, EFNB2, EPHB4, MMP2, HIF1A	HUVEC, BeWo	Trophoblast differentiation (?), angiogenesis (?)
miR-34a	PAI-1 (possible)	JAR	Inhibit trophoblast invasion
miR-34a	Notch1, Jagged1	BeWo, JAR	Inhibit trophoblast invasion
miR-152	HLA-G	JEG-3	Increase NK cell-mediated cytotoxicity
miR-155	Cyclin D1	HTR8/SVneo	Inhibit cell proliferation and invasion, and increased cell number at the G1 stage
miR-195	Acvr2a (ActRIIA)	HTR8/SVneo	Promote cell invasion
miR-210	Iron-sulfur cluster scaffold homologue (ISCU)	Swan 71	Inhibit cell invasion
miR-210	EFNA3, homeobox-A9	Primary CTB, JAR	Inhibit cell migration and invasion
miR-205	MED1	Primary CTB, HTR8/SVneo	?
miR-376c	ALK5 ALK7	HTR8/SVneo, placental explant	Induce cell proliferation, migration, and invasion and promote explant outgrowth
miR-378a-5p	Nodal	HTR8/SVneo	Enhance cell survival, proliferation, migration, and invasion
miR-517b, miR-519a	?	Placental villi	Inhibit cell proliferation

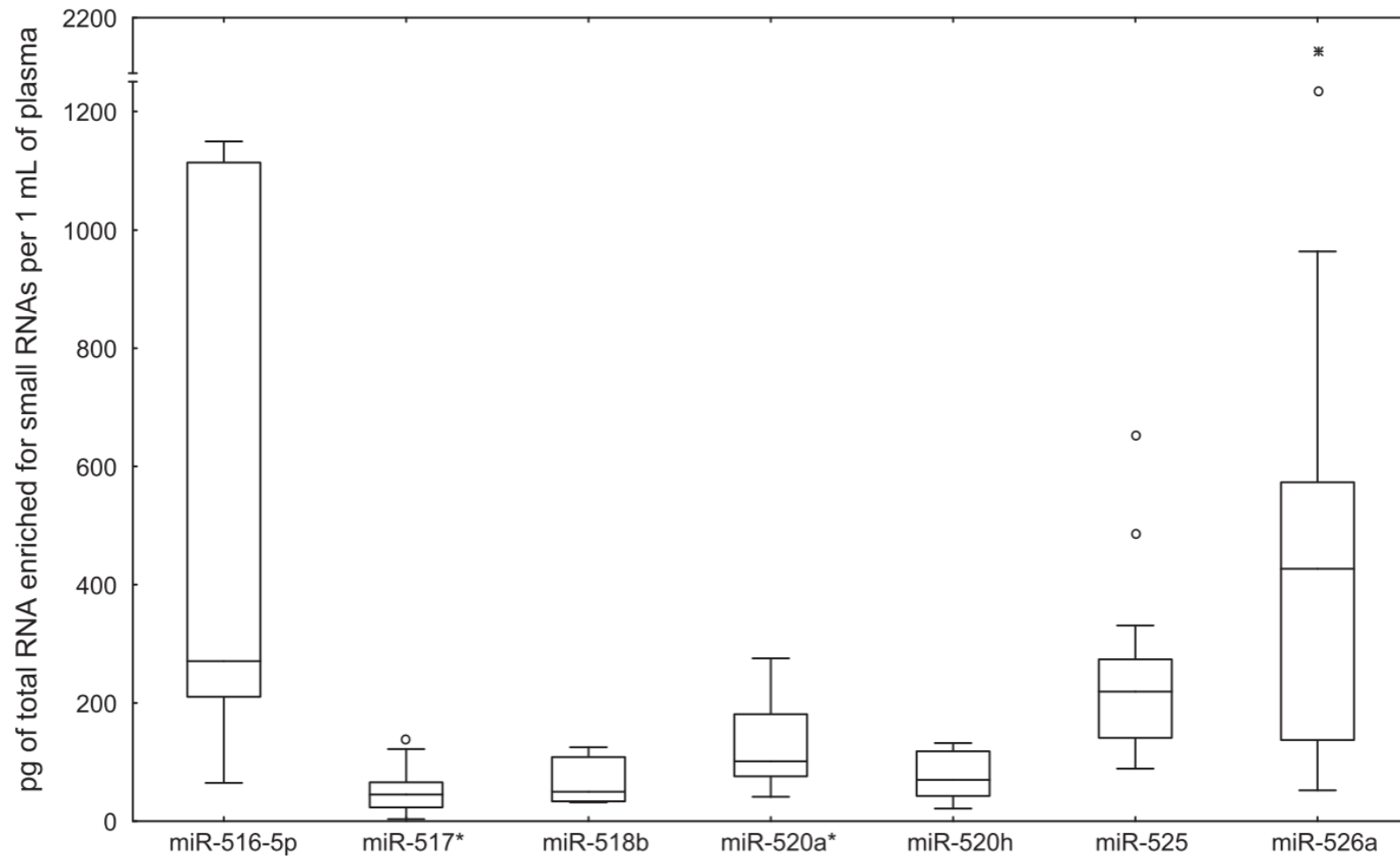
# Placental microRNAs

- The majority of placenta-derived exosomes contain miRNAs of the C19MC cluster.
- Some placental miRNA are highly abundant and rise as pregnancy progresses
- Gilad et al. propose miR-520, miR-526 and miR-527 as microRNAs that can help distinguish between pregnant and non-pregnant women
- Biological role of many of these miRNAs is not fully understood

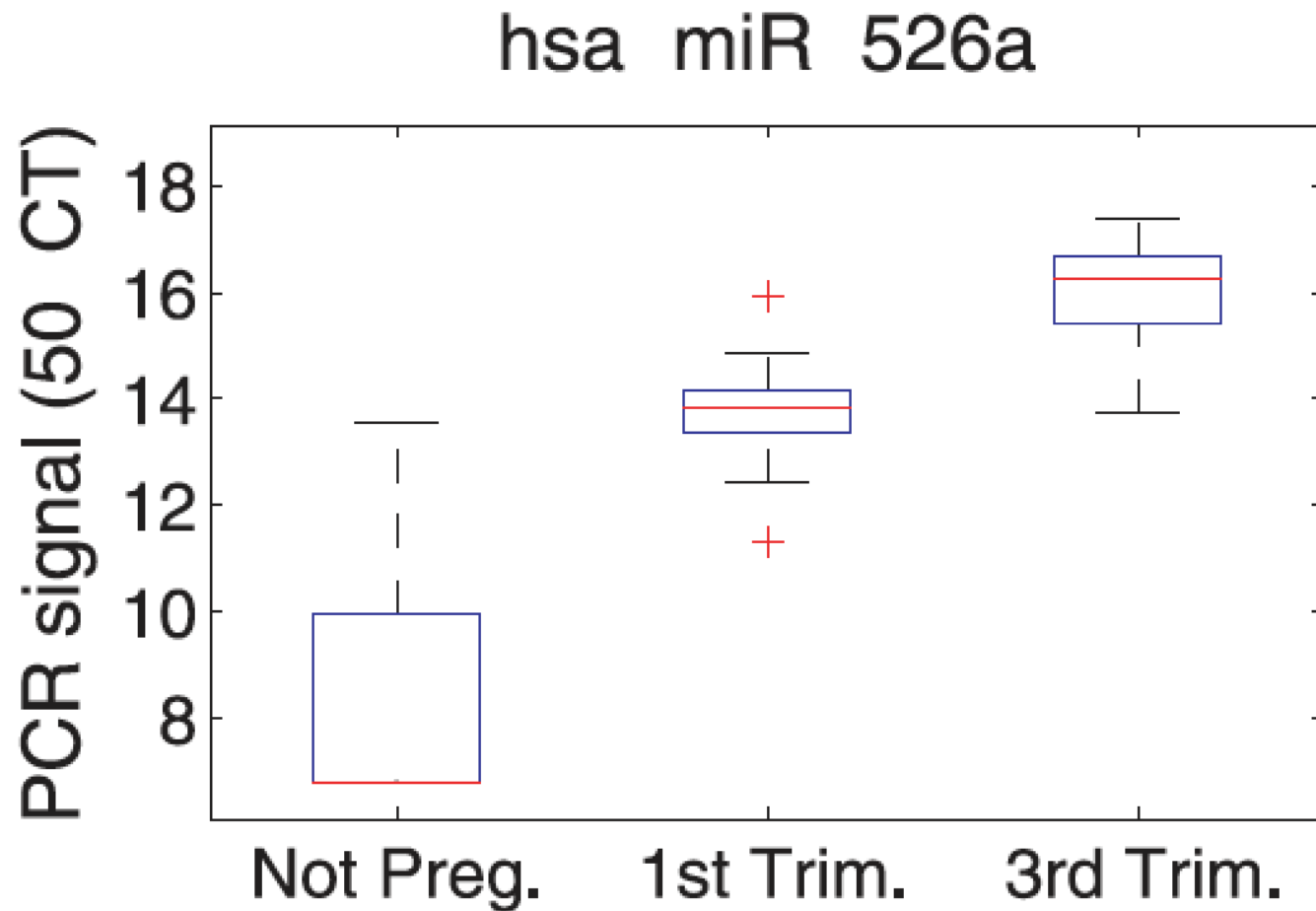
Bullerdiek and Flor *Molecular Cytogenetics* 2012, **5**:27

**Citation:** Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, et al. (2008) Serum MicroRNAs Are Promising Novel Biomarkers. *PLoS ONE* 3(9): e3148. doi:10.1371/journal.pone.0003148

# Pregnancy-associated microRNAs at 36w

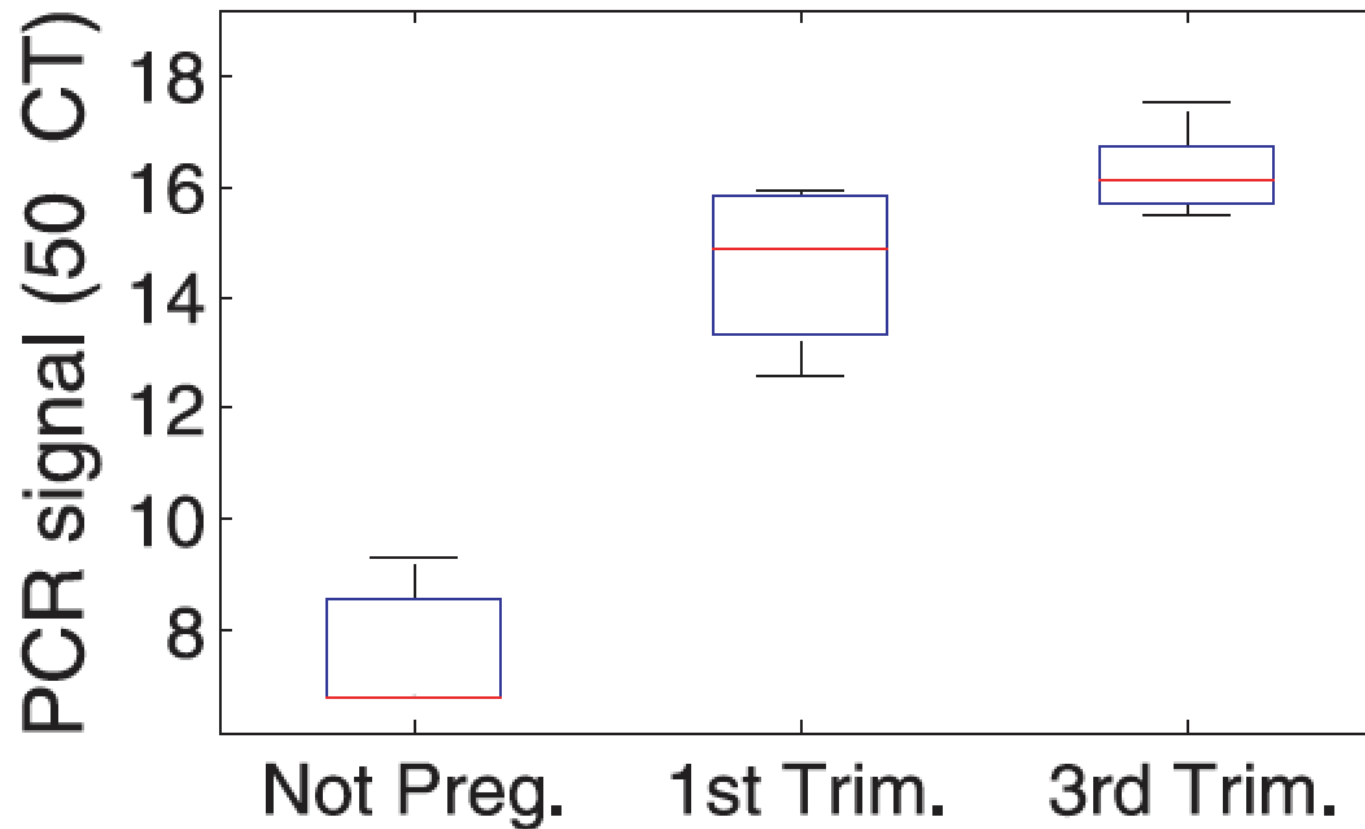


**Citation:** Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, et al. (2008) Serum MicroRNAs Are Promising Novel Biomarkers. PLoS ONE 3(9): e3148. doi:10.1371/journal.pone.0003148

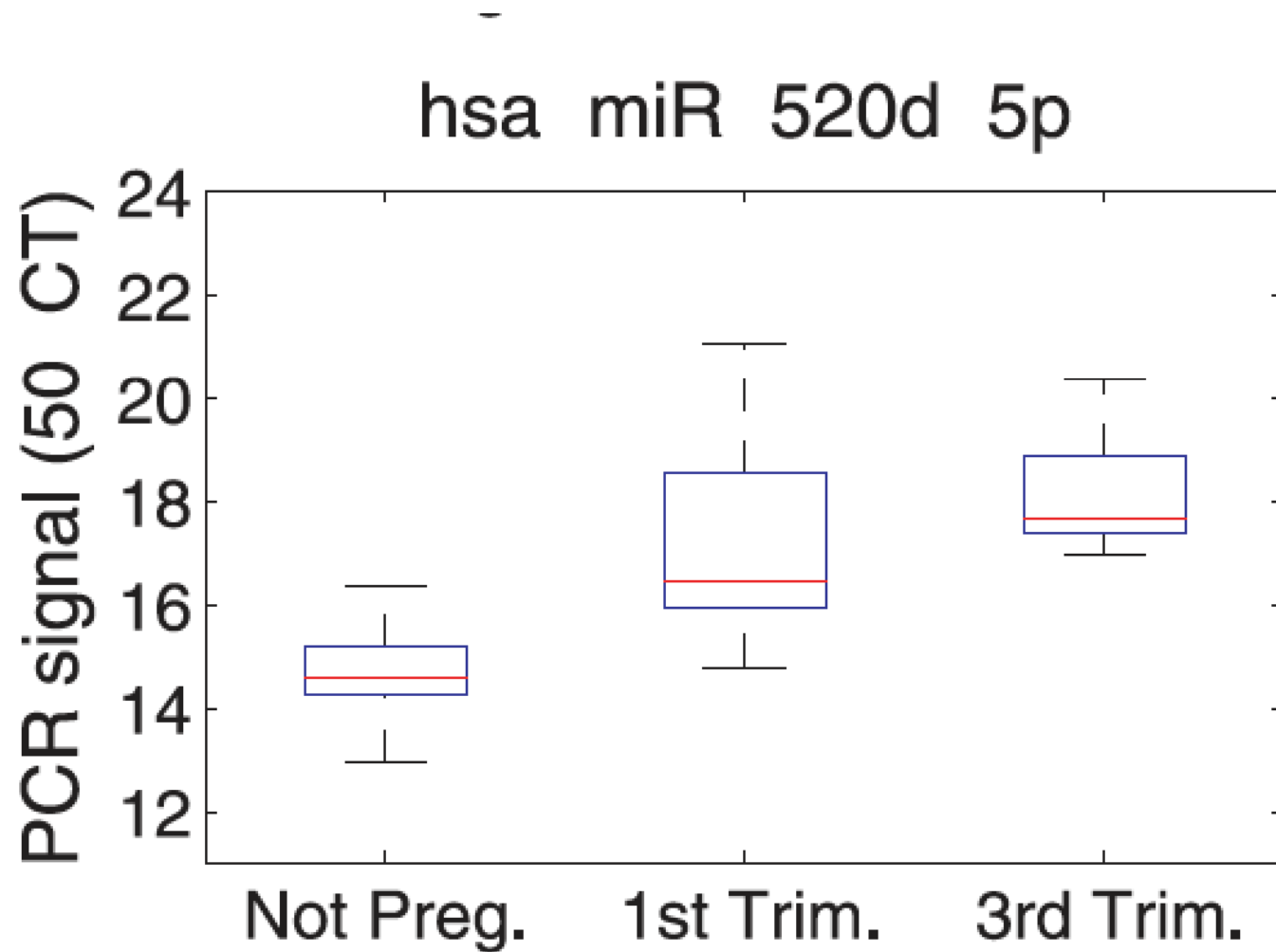


**Citation:** Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, et al. (2008) Serum MicroRNAs Are Promising Novel Biomarkers. PLoS ONE 3(9): e3148. doi:10.1371/journal.pone.0003148

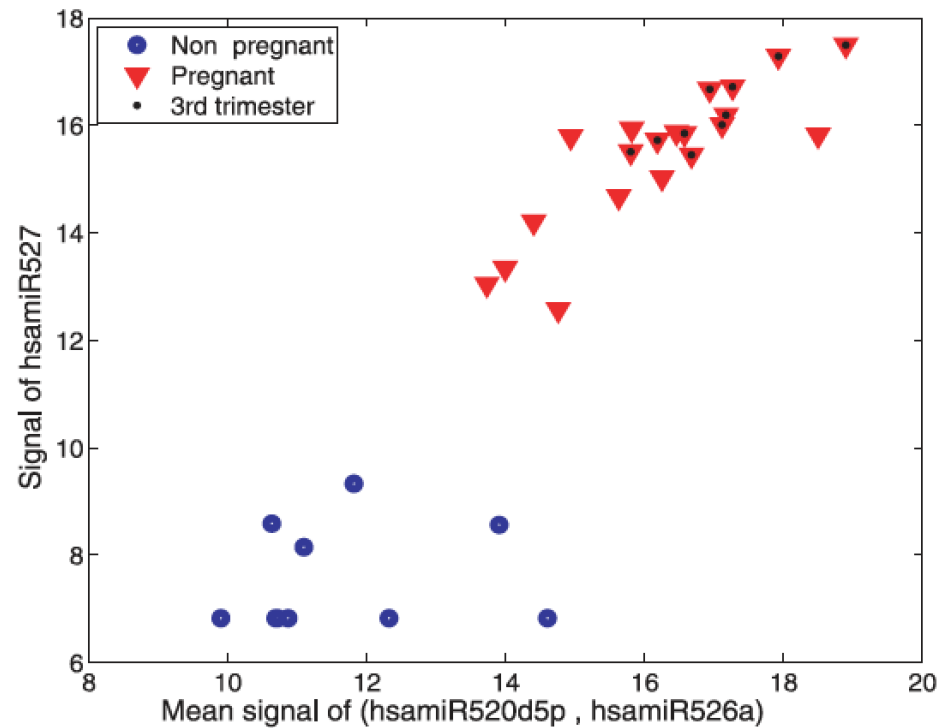
## hsa miR 527



**Citation:** Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, et al. (2008) Serum MicroRNAs Are Promising Novel Biomarkers. PLoS ONE 3(9): e3148. doi:10.1371/journal.pone.0003148








**Figure 5. "Pregnancy classification" according to the levels of three microRNAs in the sera of pregnant vs. non pregnant women.** Discrimination of pregnant women from non pregnant women based on microRNA levels in their sera. Blue circles represent non pregnant women and red triangles represent pregnant women. The location of each symbol in the plot represents the collective expression of all three microRNAs in a given serum. The y axis indicates the amount of mir-527, and the x axis indicates the average level of miR-520d-5p and miR-526a.  
doi:10.1371/journal.pone.0003148.g005

# C19MC miRNAs as immunomodulators in pregnancy

- Chromosome 19 miRNA cluster C19MC is usually silent except for embryonic stem cells and the placenta
- These are derived from placenta-derived exosomes
- May play a role in placental/maternal communication and possibly in immune system regulation during pregnancy, preventing the embryo from being attacked

Bullerdiek and Flor *Molecular Cytogenetics* 2012, **5**:27


**Cite this article:** Takahashi H, Ohkuchi A, Usui R, Takizawa T, Matsubara S, et al. (2014) Importance of Chromosome 19 miRNA Cluster in Pregnancy. *Med J Obstet Gynecol* 2(2): 1032.



C19MC microRNAs participate in normal pregnancy but may also be involved in obstetrical/placental diseases.

miRNA	Obstetrical disease	Reference
miR-520d-5p, miR-526a	NA	Gilad (2008) [65]
miR-517, miR-518	NA	Luo (2009) [9]
miR-515-3p, miR-517a, miR-517c, miR-518b, miR-526b	NA	Miura (2010) [11]
miR-516-5p, miR-517*, miR-518b, miR-520a*, miR-520h, miR-525, miR-526a	NA	Kotlabova (2011) [66]
miR-517a, miR-518b, miR-518e, miR-519d, miR-525-5p	FGR	Donker (2012) [67]
miR-518c	Preeclampsia	Ishibashi (2012) [24]
miR-516-5p, miR-517*, miR-520a*, miR-525, miR-526a	Preeclampsia	Hromadnikova (2013) [21]
miR-518b, miR-1323, miR-520h, miR-519d	FGR	Higashijima (2013) [22]
miR-520f, miR-520b, miR-520c-3p	Complete hydatidiform mole	Hasegawa (2013) [23]


FGR: Fetal growth restriction; NA: Not applicable



Embryo/baby is considered  
as a semi-allograft!

Trophoblast must promote maternal  
immune tolerance in order to survive.

microRNAs seem to modulate the  
immune system very early in the  
gestational period



Placenta-derived exosomes can share membrane components and target immune cells locally at the maternal/fetal interphase or systemically by binding to immune system cells' membranes

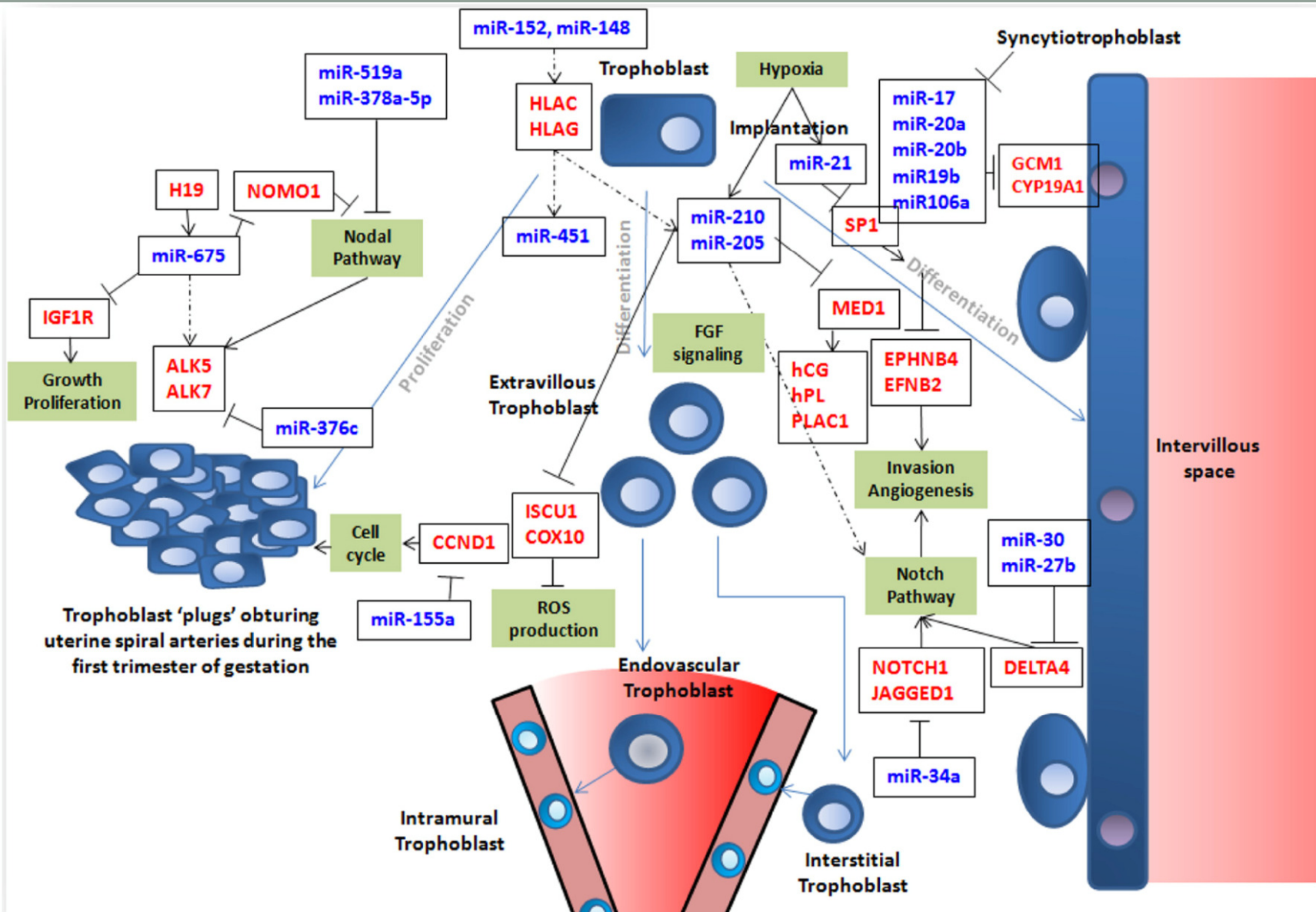
However, activated lymphocytes may also release miRNA containing exosomes...

miRNA	Targets	Biological role	Reference
miR-152	HLA-G	Materno–fetal immune dialog	Tan et al. (2007), Kulkarni et al. (2011), Manaster et al. (2012)
miR-148	HLA-G, HLA-C	Syncytialization	Kumar et al. (2013)
miR-106a	hCYP191A1, hCGB, GCM1		
miR-19b	hCYP191A1, hCGB, GCM1		
miR-17, miR-20a, miR-20b	EPHB4, EFNB2	Spiral arteries remodeling, vasculogenesis, TGF $\beta$ signaling	Wang et al. (2012), Chen and Wang (2013)
miR-34a	Notch1, Jagged1		Pang et al. (2010)
miR-210	HOXA9, EPHA3		Pineles et al. (2007), Zhang et al. (2012)
miR-27b	Delta4, Spry2		Biyashev et al. (2012)
miR-126	SPRED1		Yan et al. (2013)
miR-29	MCL1, MMP2, VEGFA, ITGB1		Li et al. (2013b)
miR-378a-5p	Nodal		Luo et al. (2012)
miR-376c	ALK5/ALK7		Fu et al. (2013b)
miR-21		Oxygen sensing	Cindrova-Davies et al. (2013)
miR-548c			Ishibashi et al. (2012)
miR-205	MED1		Mouillet et al. (2010)
miR-424	FGFR1		Mouillet et al. (2013)
miR-21	SP1		Cindrova-Davies et al. (2013)
miR-210	ISCU1/2, COX10, HSD17B1		Pineles et al. (2007), Chen et al. (2010), Enquobahrie et al. (2011), Mayor-Lynn et al. (2011)
miR-518c	HSD17B1		Ishibashi et al. (2012)
cluster C19M	General pathways of development, differentiation and cell cycle	Imprinting	Flor et al. (2012)
miR-379	<i>DIO3</i>		
miR-431, miR-127, miR-136	<i>RTL1</i>		Girardot et al. (2012)
miR-675	Igf1R, NOMO1		Munir et al. (2004), Gao et al. (2012), Keniry et al. (2012)

# miRNAs as immunomodulators during pregnancy

- **miR152:** Overexpression suppresses HLA-G expression, thus promoting NK cell cytotoxicity
  - Important, considering that 70% of leukocytes in early decidua are uterine NK cells
- **miR-148:** Targets HLA-G expression in EVT and specifically HLA-C expression
- **miR-210:** Is up-regulated in CD4+ T cells by sHLA-G, affecting their function





# miRNAs as immunomodulators during pregnancy

- **miR-155:** Positively regulates Treg and Th17 differentiation and IL-17 production by targeting SOCS1 (supressors of cytokine signaling). miR-155 deficient mice have decreased numbers of Tregs.

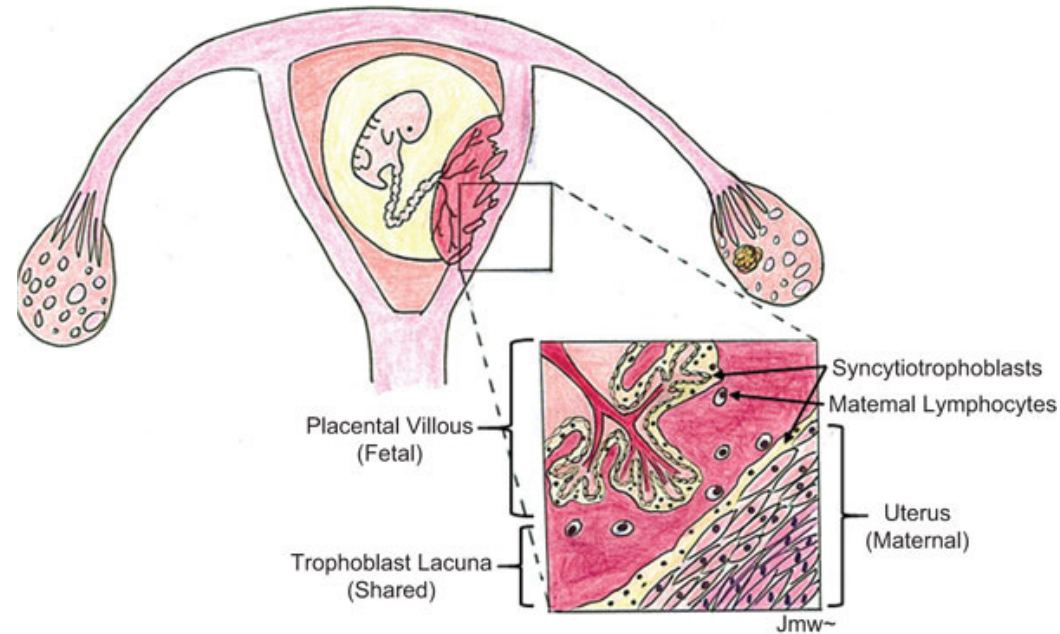
**Citation:** Yao R, Ma Y-L, Liang W, Li H-H, Ma Z-J, et al. (2012) MicroRNA-155 Modulates Treg and Th17 Cells Differentiation and Th17 Cell Function by Targeting SOCS1. PLoS ONE 7(10): e46082. doi:10.1371/journal.pone.0046082

- **miR-326:** Promotes Th17 differentiation.

*Nature Immunology* **10**, 1252 - 1259 (2009)

# MicroRNAs, immune cells and pregnancy

Mallikarjun Bidarimath<sup>1,4</sup>, Kasra Khalaj<sup>1,2,4</sup>, Jocelyn M Wessels<sup>2,3</sup> and Chandrakant Tayade<sup>1,2</sup>



<p><u>Uterine miRNAs</u></p> <ul style="list-style-type: none"> <li>• miR-10a, -27a, -29c, -323, -331-5p, -339-3p, -374b-5p, and -935<sup>49</sup></li> <li>• miR-7a, -24, -99b, -155, -181b, -451, and -429<sup>55</sup></li> <li>• miR-17-5P, 18a, -20b<sup>(Unpublished Data)</sup></li> </ul>	<p><u>miRNAs at the maternal-fetal interface</u></p> <ul style="list-style-type: none"> <li>• miR-148<sup>71</sup> and -152<sup>67,68,69</sup></li> <li>• miR-210 and -451<sup>70</sup></li> <li>• Let-7a and miR-320<sup>72,73</sup></li> <li>• miR-222, 221, 181b, 27b, 29b, 507, 143, 101, 30d, 30c, and 23a<sup>74</sup></li> <li>• miR-150, -17-5p, -18a, -19a and -296-5P<sup>(Unpublished Data)</sup></li> </ul>
<p><u>Angiogenic miRNAs</u></p> <ul style="list-style-type: none"> <li>• miR-17, -20a, -20b<sup>127</sup>, -20<sup>133</sup>, miR-29b<sup>106</sup>, and miR-182<sup>128</sup></li> <li>• miR-126<sup>129</sup></li> <li>• miR-125b<sup>130</sup></li> <li>• miR-10a<sup>131</sup></li> <li>• miR-92b and -125b<sup>133</sup></li> </ul>	<p><u>Placental miRNAs</u></p> <ul style="list-style-type: none"> <li>• miR-517a-3p, 519a-3p, and -520c-3p<sup>9</sup></li> <li>• miR-29b<sup>107</sup>, -210, -93, -205, -335, -224, -424, -451, and -491<sup>104</sup></li> <li>• miR-15b-5P, -18a and -222<sup>(unpublished Data)</sup></li> </ul>

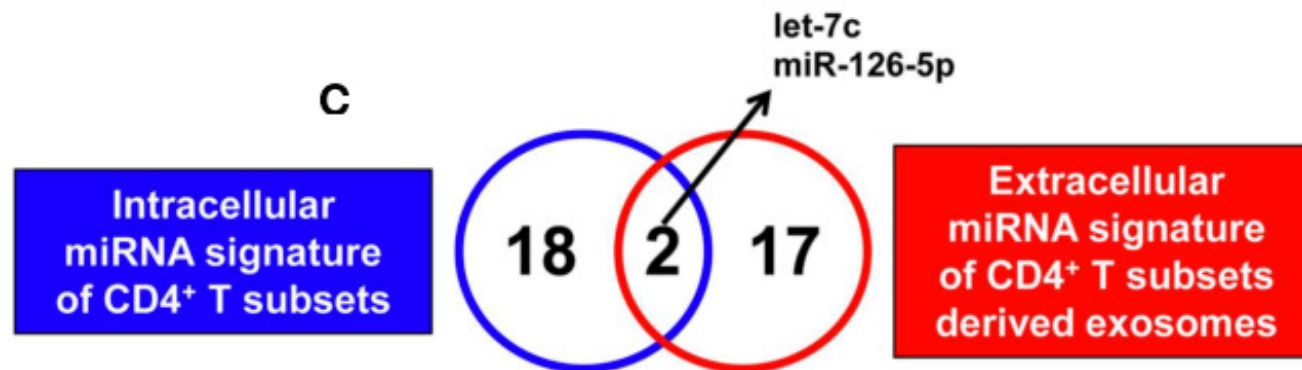
# miRNAs as immunomodulators during pregnancy

- **miR- 126:** Expressed by plamacytoid dendritic cells. Is a regulator of innate immunity, regulating TLR7,9 and VEGFR2. Considered to be proangiogenic

*Nature Immunology* **15**, 54–62 (2014) | doi:10.1038/ni.2767

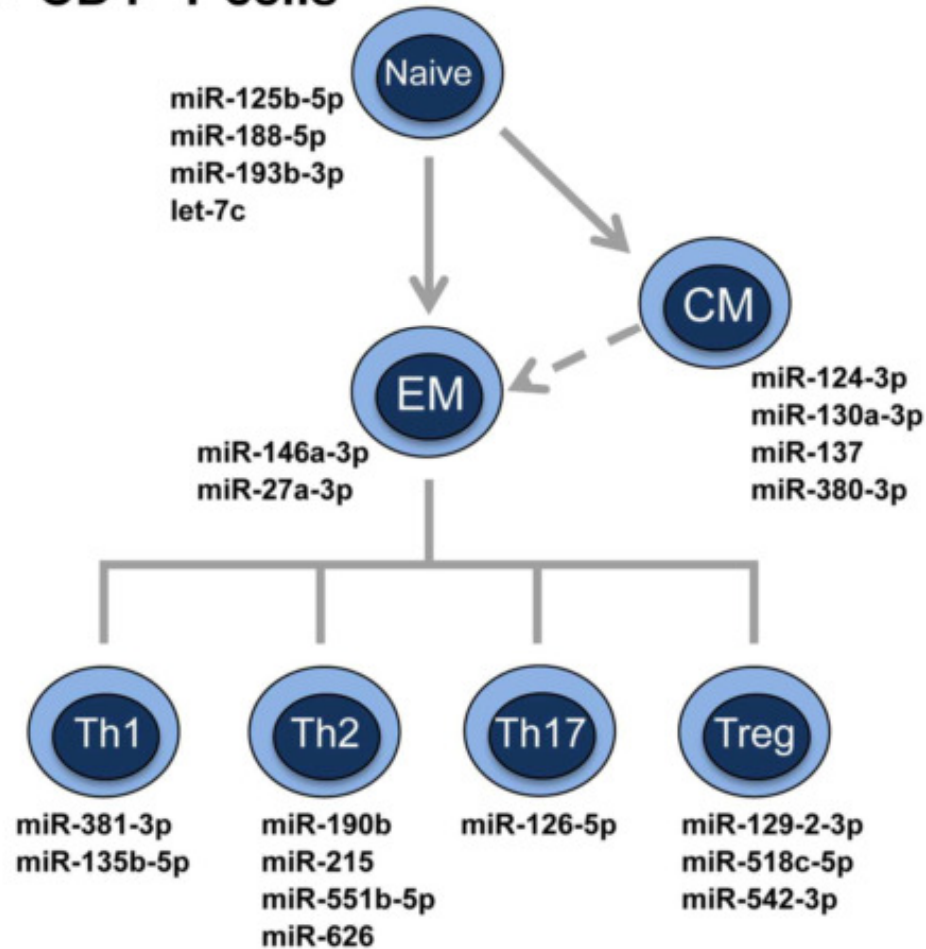
# miRNAs released by the immune system

- miRNAs of CD4<sup>+</sup> T cell exosomes are different from the intracellular versions of the same cells



# Intracellular miRNAs relevant for the development of CD4<sup>+</sup> T cell subsets

## B CD4<sup>+</sup> T cells



# Th1, Th2, Th17, Treg paradigm in pregnancy

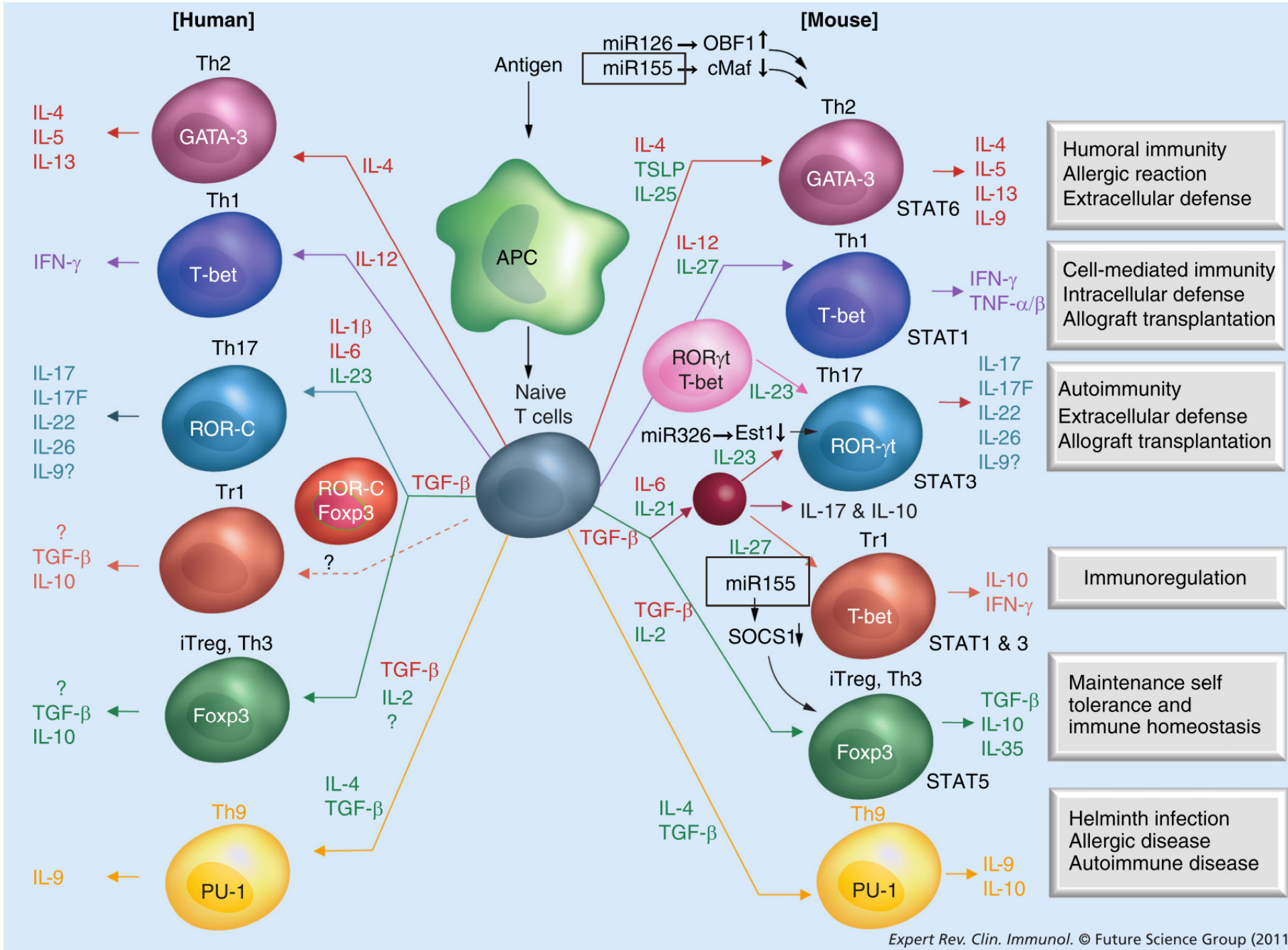
**Table I** Th1/Th2/Th17 and Treg Cells in Normal Pregnancy and Abortion

	Normal pregnancy		Abortion		Depletion of Th1, Th2, Th17 or Treg cells
	Peripheral blood	Uterus	Peripheral blood	Uterus	
Th1 cells	↘	↓	↗ →	↑ →	Abortion is not observed.
Th2 cells	↗	↑	→	↓ → ↑ (conflict data)	Abortion is not observed.
Th17 cells	→ ↘	↗	→ ↗	→ (missed abortion) ↑ (inevitable abortion) ↑ (recurrent abortion: inevitable abortion)	There is no data, but IL-17 null mice are fertile.
Treg cells	↑	↑↑	→	→	Abortion and implantation failure are observed in allogeneic pregnancy.

→ : no change, ↗ : slightly elevate, ↑ : elevate, ↑↑ : markedly elevate, ↘ : slightly decrease, ↓ : decrease.

Saito S, Nakashima A, Shima T, Ito M.  
Th1/Th2/Th17 and regulatory T-cell paradigm  
in pregnancy. Am J Reprod Immunol 2010; 63:  
601–610

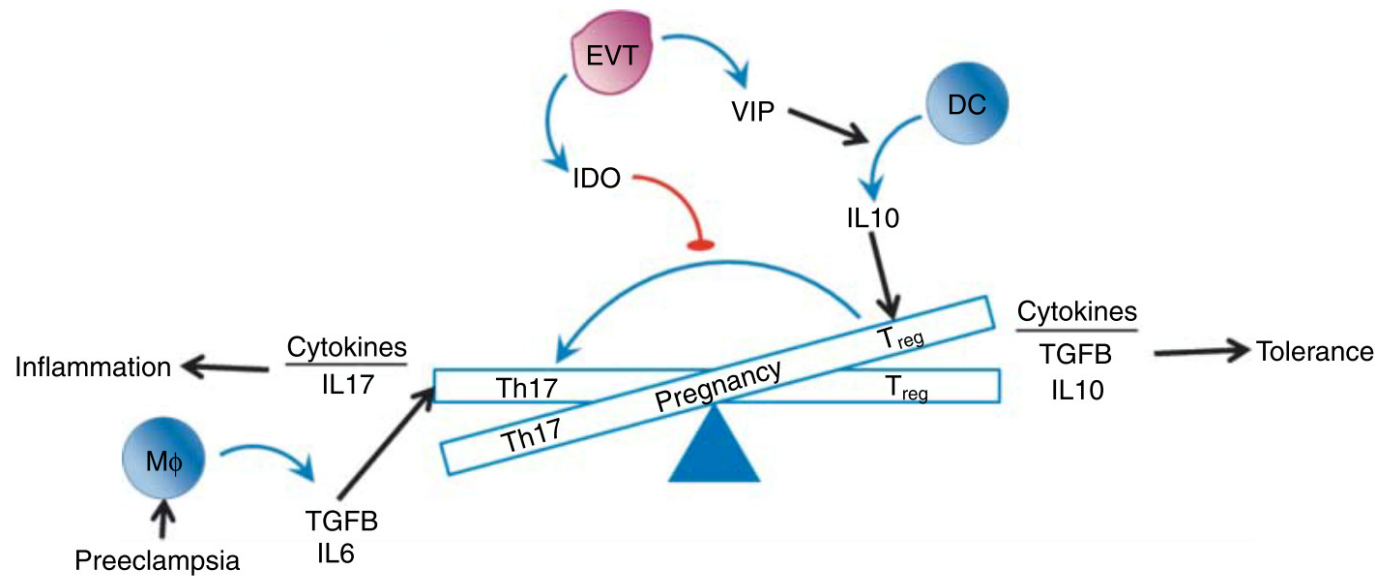






# Immune regulation during pregnancy

*Fetal mechanisms prevent rejection by maternal immune system*



# Conclusions

- miRNAs may play an important role in the regulation of the immune system during the gestational period
- The role of placenta-derived microRNAs is not fully understood
- microRNAs are produced by trophoblast, immune system cells and other cells (exosomes and intracellular)
- They not only participate in immune system regulation, they may regulate placental functions and angiogenesis...and they may be involved in pregnancy complications as well.



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# THANK YOU!

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