

# Rôle prédictif des cytokines maternelles dans l'implantation

CPMA/FABER

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# The NIDUS study

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# Background

- IVF rate of success: only 25%
- Rate-limiting step: embryo implantation
- A lot of factors involved:
  - Integrins
  - Metalloproteinases
  - VEGF
  - Interleukins...
- Historically: Th1 (IFN $\gamma$ , IL-2, TNF $\beta$ )  $\rightarrow$  Th2 (IL-4, IL-5, IL-6, IL-10)  
Nowadays: Th3 (TGF $\beta$ ), Tr1 (IL-10),...  $\Rightarrow$  more complex

# Maternal biochemical analyses

- Aim: to measure cytokines in peripheral circulation to evaluate patient chances of pregnancy, on the day of embryo transfer opposite to up to now, where post-transfer markers, with only poor prognostic value

# Study design

- Prospective study (160 patients)
- Fresh transfers after stimulated cycles and transfers of cryopreserved zygotes in natural cycles
- Blood sample on the day of embryo transfer
- Four outcome groups:
  - Pregnancy failures (N=119)
  - Biochemical pregnancies (N=8) 5%
  - First-trimester miscarriages (N=4) 2.5%
  - Normal term deliveries (N=29) 18%

# Maternal characteristics

| N=160                                |   | Pregnancy failure<br>N=119   | Biochemical pregnancy<br>N=8 | First-trimester miscarriage<br>N=4 | Normal term delivery<br>N=29 |
|--------------------------------------|---|------------------------------|------------------------------|------------------------------------|------------------------------|
| Age (years)                          |   | 34±4 (25-44)                 | 37±4 <sup>†</sup> (31-42)    | 33±5 (28-39)                       | 34±4 (26-42)                 |
| BMI (kg/m <sup>2</sup> )             |   | 22.9±3.9                     | 26.4±9.2                     | 23.3±2.5                           | 23.9±4.3                     |
| Gravidity (%)                        | 0<br>1<br>≥2  | 56<br>24<br>20               | 63<br>12<br>25               | 50<br>0<br>50                      | 38<br>24<br>38               |
| Parity (%)                           | 0<br>1<br>≥2  | 70<br>20<br>10               | 75<br>25<br>0                | 50<br>50<br>0                      | 52<br>31<br>17               |
| Infertility origin (%)               | paternal<br>maternal<br>both conjugated<br>idiopathic/unknown | 52<br>24<br>17<br>7          | 38<br>38<br>24<br>0          | 75 <sup>‡</sup><br>0<br>0<br>25    | 52<br>34<br>14<br>0          |
| Infertility type (%)                 | primary<br>secondary  | 82<br>18                     | 75<br>25                     | 100<br>0                           | 66<br>34                     |
| Previous transfers (fresh or frozen) |   | 3.4±2.8                      | 6.3±5.7                      | 2.8±1.7                            | 3.7±3.1                      |
| Transfer type (%)                    | fresh<br>frozen   | 44.5<br>55.5                 | 87.5<br>12.5                 | 50<br>50                           | 62.1<br>37.9                 |
| Oocytes (%)                          | collected<br>ripe<br>ripe/collected                           | 12.5±6.5<br>10.3±5.7<br>0.83 | 11.0±6.0<br>8.9±5.6<br>0.79  | 12.0±5.8<br>8.5±2.6<br>0.78        | 13.0±7.3<br>11.0±6.0<br>0.86 |

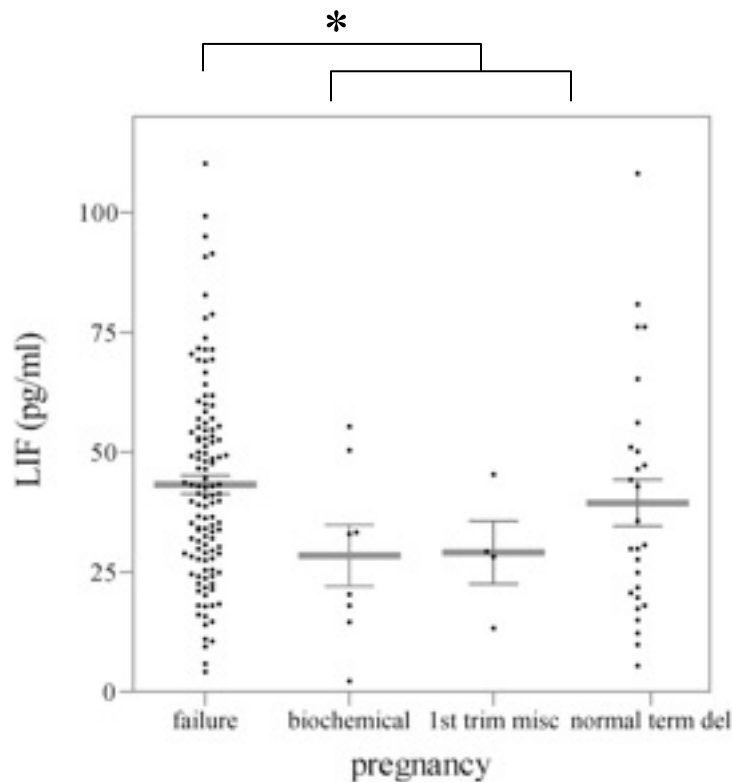
# Maternal biochemical analyses

- Cytokines measured in maternal plasma:
  - interleukin-6 (IL-6)
  - soluble interleukin-2 receptor (sIL-2R)
  - interferon a (IFNa) (above detection limit)
  - interferon b (IFNb) (above detection limit)
  - interleukin-1b (IL-1b) (above detection limit)
  - leukemia inhibitory factor (LIF)
  - matrix metalloproteinase 2 (MMP2)

# Leukemia inhibitory factor (LIF)

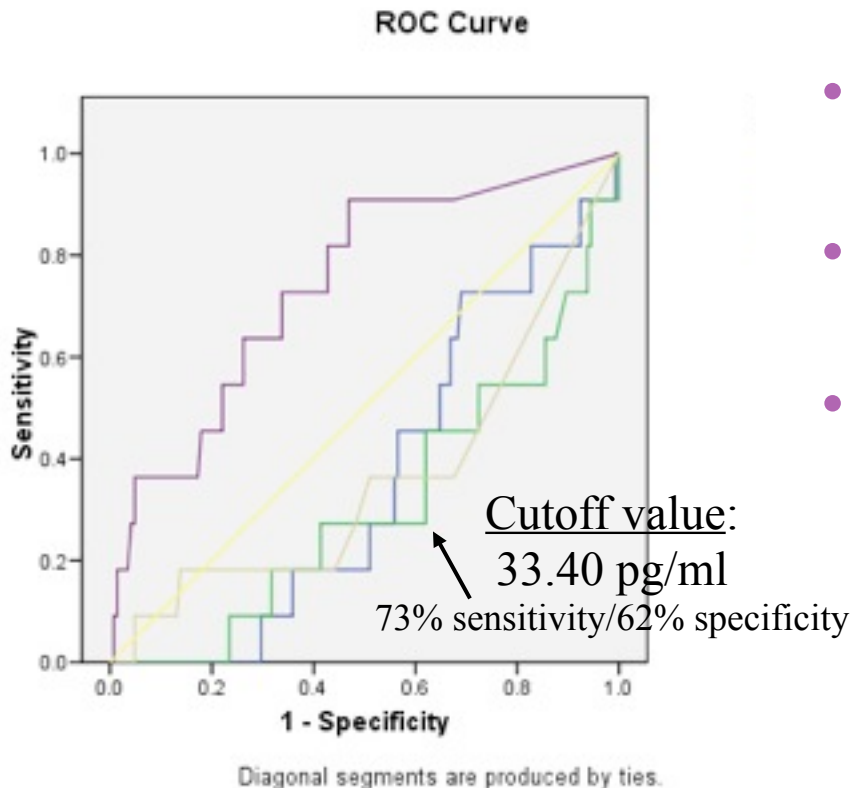
- **Role:**
  - cytokine of the IL-6 family
  - necessary for early embryo implantation:  
no blastocyst implantation in LIF(-/-) mice
- **Previously shown:**
  - lower LIF in intrauterine flushings of women with unexplained infertility
  - less LIF produced by endometrial tissue explants of infertile women

# LIF concentrations in maternal plasma on the day of embryo transfer



- LIF concentrations were 1/3 lower in biochemical pregnancies/first-trimester miscarriages than in pregnancy failures.

# LIF ROC curve analysis

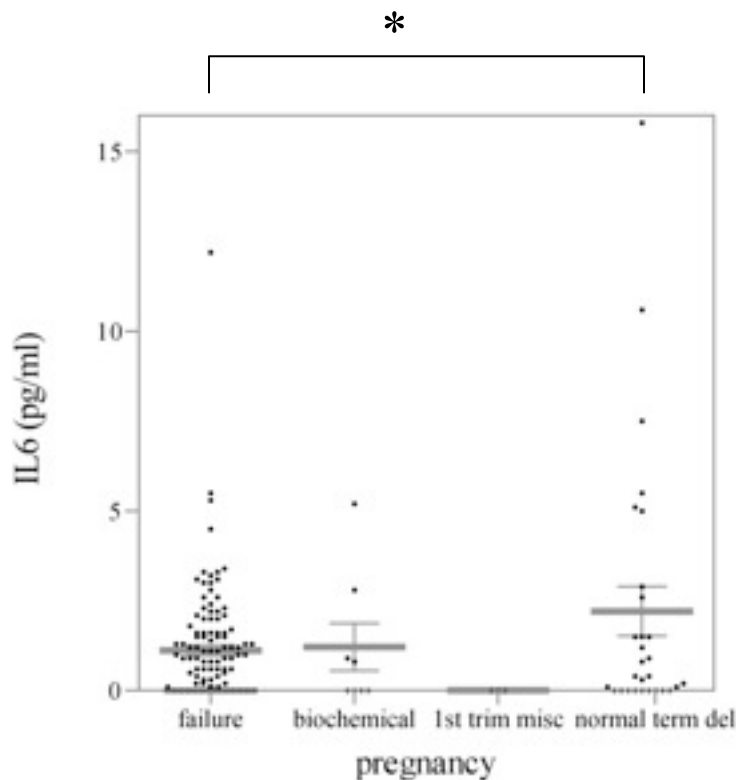


- ROC curve:  
fair predictive value
- $AUC=0.7$ ,  $95\%CI=0.5-0.8$ ,  
 $P=0.039$
- Risks of biochemical  
pregnancy/first-trimester  
miscarriage modified as such:  
 $OR=5.6$ ,  $95\%CI=1.4-22.0$ ,  
 $P=0.010$ .

# Interleukin 6 (IL-6)

- **Role:**
  - induced by IL-1
  - mediates some of the pro-implantation effects of IL-1
- **Previously shown:**
  - endometrial IL-6 mRNA suppressed in the mid-secretory phase of patients with recurrent abortion

# IL-6 concentrations in maternal plasma on the day of embryo transfer

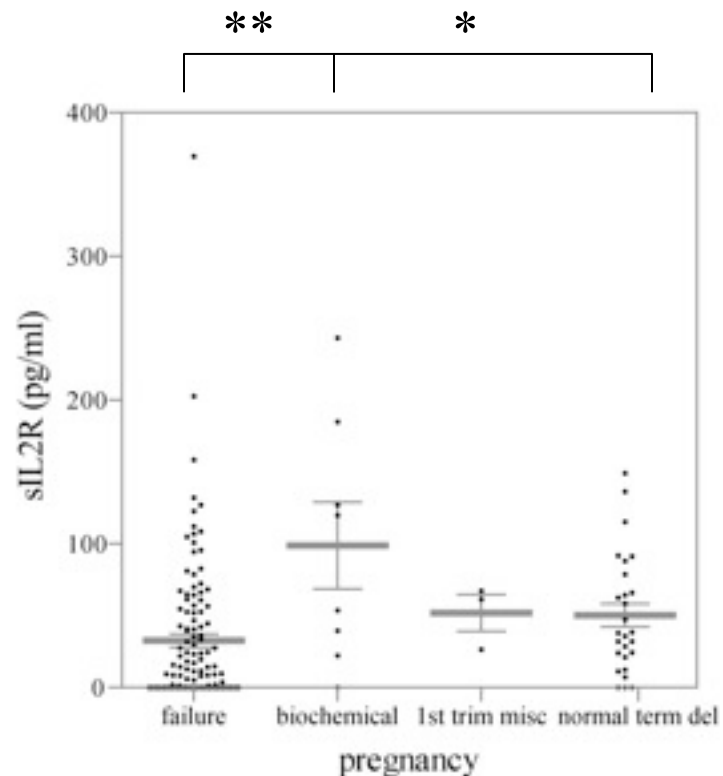


- IL6 concentrations 2-fold higher in normal term deliveries than in pregnancy failures
- ROC curve analysis: no predictive value

# Soluble interleukin 2 receptor (sIL-2R)

- **Role:**
  - part of the membrane IL-2R
  - marker of T cell activation
  - only expressed at the surface of T cells after stimulation or in disorders like organ allograft rejection, T cell mediated autoimmune disease and certain hematological malignancies
- **Previously shown:**
  - sIL-2R maternal serum concentration higher in biochemical pregnancies/SAB than in term deliveries at ET+11 in an IVF population

# sIL2R concentrations in maternal plasma on the day of embryo transfer



- sIL2R concentrations 3-fold higher in biochemical pregnancies than in pregnancy failures

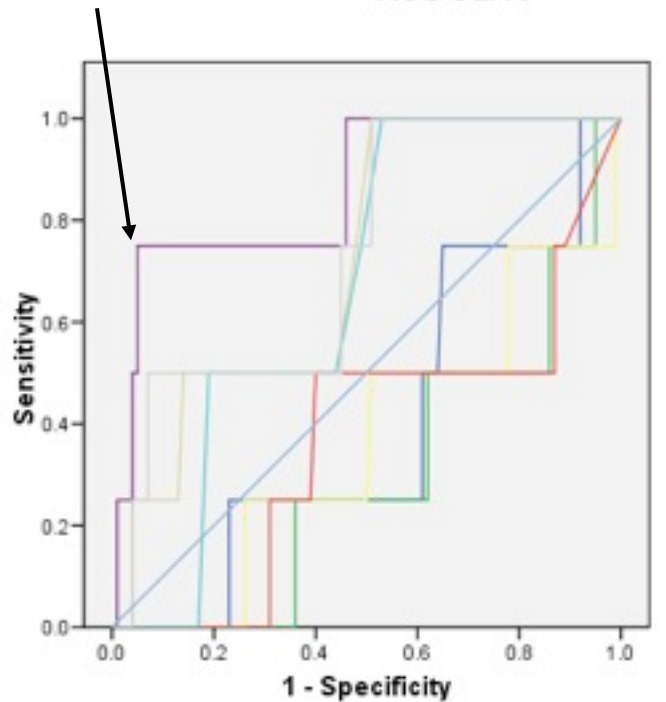
# sIL-2R ROC curve analysis

Cutoff value:

115.95 pg/ml

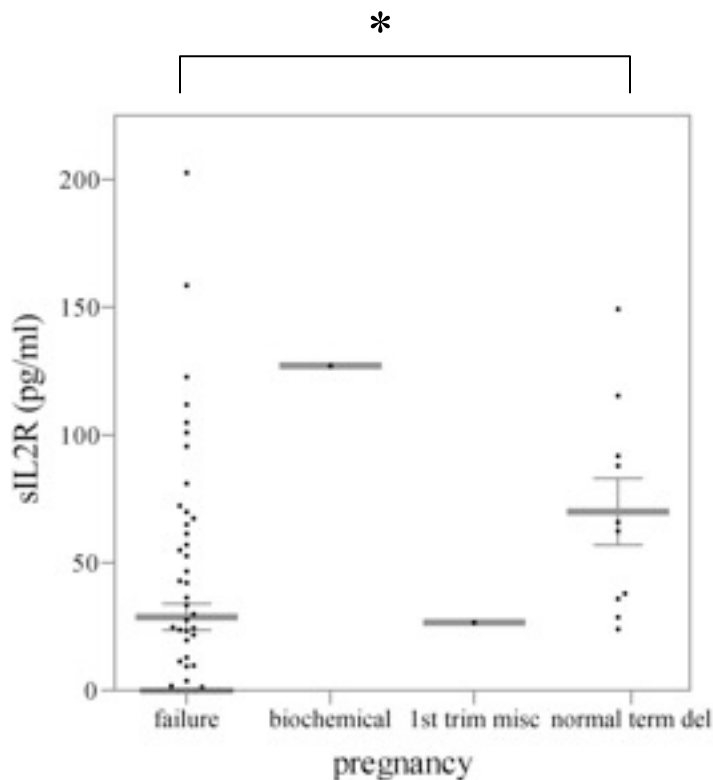
75% sensitivity/95% specificity

ROC Curve



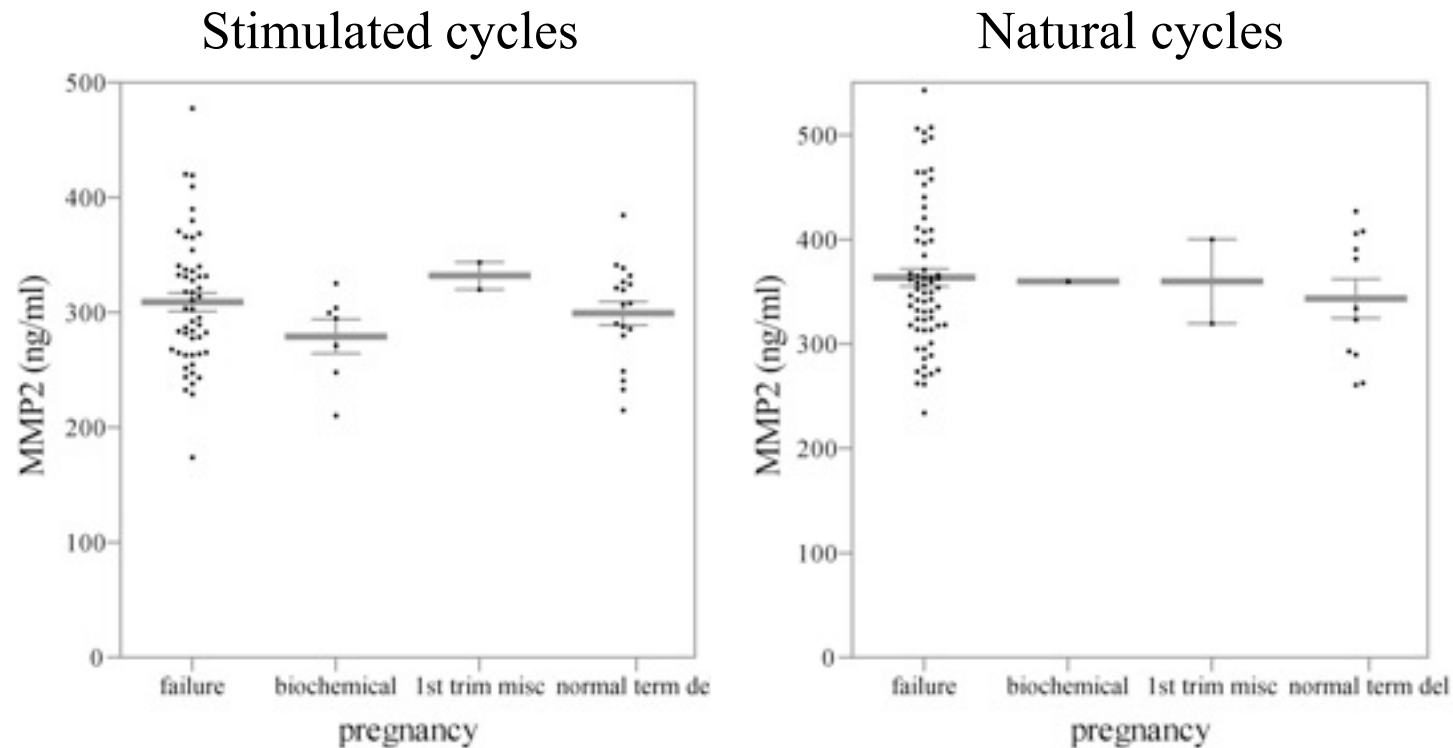
- ▶ ROC curve:  
excellent predictive value
- ▶  $AUC=0.9$ ,  $95\%CI=0.7-1.0$ ,  
 $P=0.015$
- ▶ Risks of biochemical pregnancy  
modified as such:  
 $OR=17.6$ ,  $95\%CI=3.7-83.7$ ,  
 $P=0.001$ .

# sIL2R concentrations in maternal plasma in natural cycles only



- sIL2R concentrations 2.5-fold higher in normal term deliveries than in pregnancy failures
- ROC curve analysis:  
good predictive value  
AUC=0.8, 95% CI=0.7-0.9,  $P=0.003$
- Cut-off value of 34.75 pg/ml (88% sensitivity and 72% specificity)
- Chances of normal term delivery modified as follows:  
OR=8.5, 95%CI=1.7-43.6,  $P=0.005$ .

# MMP2 concentrations in maternal plasma on the day of embryo transfer



- MMP2 concentrations similar in all four IVF outcome groups

# Conclusion I

- low maternal plasma concentration of LIF associated with biochemical pregnancy/first-trimester miscarriage and high maternal concentration of sIL2R with biochemical pregnancy
- moderately high sIL2R values predictive of normal term delivery in natural cycles.
- an excessive inflammatory reaction likely result in embryo rejection, while a poor inflammatory reaction probably incompatible with successful pregnancy establishment.

# Conclusions II

- two biochemical markers present in the maternal peripheral circulation can score IVF success already when measured on the day of embryo transfer (ETo), which could help guide IVF patient management between embryo transfer (favorable cytokine profile identification) or embryo transfer delay (unfavorable cytokine profile identification), in a particular IVF cycle, moreover in an easily measurable way during the normal follow-up of an IVF procedure or in stimulated cycles prior to any oocyte pick-up (OPU).

# Maternal genetic analyses

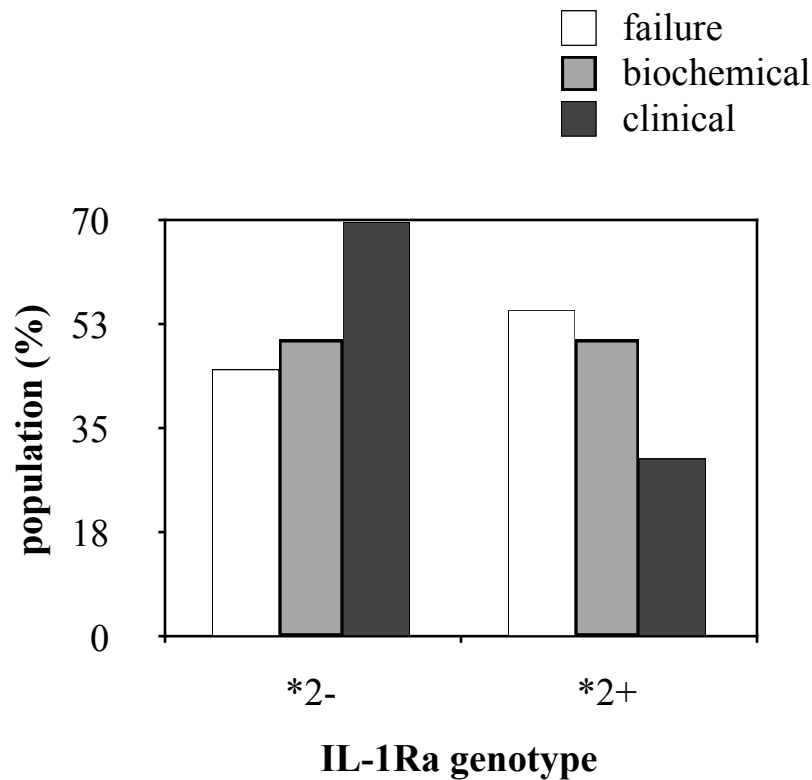
- Polymorphisms tested:

- |   |               |
|---|---------------|
| □ matrix metalloproteinase 2 (MMP2)             | MMP2(C-1306T) |
| □ matrix metalloproteinase 9 (MMP9)             | MMP9(C-1562T) |
| □ interleukin-1b (IL-1b)                        | IL-1B+3953    |
| □ interleukin-1 receptor<br>antagonist (IL-1Ra) | IL-1RN VNTR   |

# Interleukin 1 receptor antagonist (IL-1Ra)

- **Role:**
  - antagonizes pro-inflammatory role and then pro-implantation role of IL-1
- **Previously shown:**
  - 3-fold increase of IL-1RN\*2 frequency in women with recurrent pregnancy loss
  - recipient IL-1RN\*2 allele carriage associated with chronic graft-vs-host disease in graft acceptance

# IL-1Ra allele 2 analysis



- 2-fold less IL-1RN\*2 carriers in clinical pregnancy than in pregnancy failure group

# Genetic analyses conclusion

- IL-1RN\*2 allele carriers have 3-fold less chances to get pregnant than non-carriers in IVF programs (P=0.017)