# Female immunsystem and pregnancy

Interventional tolerance induction by Omega-3-fatty acids and *Vitamin-D* 

Sebastian Pfeiffer M.D. Labor Benrath, Duesseldorf, Germany

# Types of Transplantations

### **Transplantation**

The grafting of tissue, usually from one individual to another.



**<u>Isograft</u>** (iso/syngeneic)

Allograft

Xenograft

(xenogeneic)

(allogeneic)

- to a genetically identical individual

- to a genetically disparate member of the same species

- to a different species

# Transplanted tissues

#### Cornea

From cadaver Immunosuppression not required 40,000 transplants per year

#### Lung

From brain-dead donor Procedure recently developed; little data available 845 transplants in 1998 Often heart/lung transplant (45 in 1998)

#### Heart

From brain-dead donor HLA matching useful but often impossible Risk of coronary artery damage, perhaps mediated by host antibody 2,340 transplants in 1998

#### Liver

From cadaver Surgical implantation complex Resistant to hyperacute rejection Risk of GVHD 4,450 transplants in 1998

#### Skin

Mostly autologous (burn victims) Temporary grafts of nonviable tissue Allogeneic grafts rare, require immunosoppression

#### Blood

Transfused from living donor ABO and Rh matching required Complications extremely rare An estimated 14 million units used each year

#### Pancreas

From cadaver Islet cells from organ sufficient 253 transplants in 1998 Increasingly, panreas/kidney transplant for advanced diabetes (965 in 1998)

#### Kidney

From live donor or cadaver ABO and HLA matching useful Immunosuppression usually required Risk of GVHD very low 11,900 transplants in 1998

#### Bone marrow

Needle aspiration from living donor Implanted by IV injection ABO and HLA matching required Rejection rare but GVHD a risk



In solid tissue transplantation, there are three major types of graft rejection:

#### hyperacute rejection:

preformed antibodies minutes – days

<u>acute</u> rejection:

T-cells days – weeks, also late

chronic rejection

T-cells, antibodies macrophages, cytokines (weeks -) months - years

## **Reception of Signals**



# Immunogenity of cells, tissues and organs

Why, and how will allogenic (under certain conditions also autologous) cells, tissues and organs be rejected

# Immunogenity of cells, tissues and organs

#### Conventional conception:

The immune system can only discriminate between "non-self" and "self"

#### Actual conception:

The immune system can discriminate between "self", "non-self AND dangerous" and "non-self and NOT dangerous = tolerance"

















"Classical" therapeutical prevention of "dangerous signals"

Corticosteroid therapy		Steroids inhibit	
Effect on	Physiological effects	NFKB activation	
IL-1, TNF-α, GM-CSF   IL-3, IL-4, IL-5, CXCL8	Inflammation   caused by cytokines	IL-2R-expression	
‡ NOS	ŧио	CD4+-T- Cellproliferation	
↓ Phospholipase A <sub>2</sub> ↓ Cyclooxygenase type 2 † Annexin-1	↓ Prostaglandins ↓ Leukotrienes		
‡ Adhesion molecules	Reduced emigration of leukocytes from vessels		
† Endonucleases	Induction of apoptosis in lymphocytes and eosinophils		
Figure 15-2 Immunobiology, 7ed. (# Garland Science 200	8)		

The antiinflammatoric potential of Omega-3-fatty acids from fish oil

 $_{\Omega}$  Synthesis of Lipid mediators (Eicosanoids, Lipoxines, Resolvines, Protektines) with an modulating effect on:

- $\boldsymbol{\Omega}$  Blood clotting
- $\Omega$  Blood pressure
- $\Omega$  Immune response
- $\Omega$  Inflammation
- $\Omega$  Integral part of biologic membranes
  - $\Omega$  Effect on membran properties like viscosity
  - $\Omega$  Especially for DHA: in brain and nerve tissue  $\rightarrow$  Development of intelligence in the retina  $\rightarrow$  Visual functions
  - $\Omega$  Stabilization of heart rhythm

# Different biologic effects of the Eicosanoids derived from Arachidonic acid (AA) and EPA

Thrombocytes	Endothelial cells	Leukocytes	
vaso-constrictive	vasodilatory	<b>†</b> Inflammation	
procoagulatory	anticoagulatory	<pre>fImmune reactions fMonocytary adhesion</pre>	
Thromboxan A <sub>2</sub>	Prostaglandin I <sub>2</sub>	Leukotrien B <sub>4</sub>	
	Arachidonic acid Omeg	<b>9a-6</b>	
	Eicosapentaenacid Omeg	a-3	
Thromboxan A <sub>3</sub>	↓ Prostaglandin I <sub>3</sub>	Leukotrien B <sub>5</sub>	
minor effect	vasodilatory	↓Inflammation ↓Immune reactions ↓Monocytary adhesion	
Source: Trautwein, A. (1999): Fette und F dobler, H. F., Meyer, A. H. (Hrsg.)	anticoagulatory ettbegleitstoffe. Omega-3-Fettsäur : Praxishandbuch Functional Food,	en. In: Erbers- Behrs Verlag Hamburg	

Effects of Alimentation with less Arachidonic acid (AA) and more EPA

 Alimentation with less Arachadonic acids and more EPA leads to a reduced inflammatory potential by

→ Displacement of Arachidonic acid (AA) from cell membranes by EPA: reduced synthesis of proinflammatoric Eicosanoids and increased release of "healthy"

Eiconsanoids from EPA

 $\rightarrow$  reduced synthesis of the high proinflammatoric Leukotrien B<sub>4</sub> (LTB<sub>4</sub>) from Arachidonic acid

# Positive Effects of Eicosanoids derived from EPA



# The supply with Omega-3-fatty acids is insufficient



Source:

Ernährungsbericht der DGE (2004), Nationale Verzehrsstudie II (2008), Leipziger Lipid Studie (Richter; V. et al. (2007): Cardiovascular risk factor profile on a population basis: Results from the Lipid Study Leipzig. Exp Clin Cardiol; 12: 51-53)

## Regulatory T-cells under Omega-3-supplementation targets for optimizing the conditions in pregnancy?

10.00

a range (0,9-2,2 cells / nl) under oral  $\omega_3$ -imake (r < 0,001), impanied by a significant (p < 0,001) increase in the CD4+T ce

position with the established reference range of 0.5-1,2 cellsh. We further found a not significant recease in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in an increase in the relative mount of the CD4\*CD25\*Te<sub>pin</sub> cells represented in an increase in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in an increase in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in an increase in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in an increase in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in an increase in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in an increase in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in a significant relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in an increase in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in an increase in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in an increase in the relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in a significant relative frequency of CD4\*CD25\*Te<sub>mic</sub> cells represented in a significant relative frequency of CD4\*CD25\*Temic cells represented in a significant relative frequency of CD4\*CD25\*Temic cells represented relative frequency of CD4\*CD25\*Temic cells represented relative frequency of CD4\*CD25\*Temic cells represented relative frequency of CD4\*CD25\*Temic cells relative frequency of CD4\*CD25\*Tem ring values before and under oral intake of ω3-FAs after atleast four weeks a more we found changes in the median value of granulocytes, B-lymphocytes a

Concession The flow cytometry analysis of parameters belonging to the cellular immun system, especially the CDC+T-imprinciples and the the regulatory T-cells, might be a used. I loot for a laboratory monitoring the cellular the cellular cellular cellular cellular cellular cellular cellular cellular cellular established reference range which might partly be due to slight expansion of the CD4+CD25-TRAP cellular. This results, site cellular cellular cellular cellular cellular cellular cellular cellular cellular population. This results is cellular c

Evaluation of prognostic parameters for interventional tolerance induction by oral omega-3 fatty BENRATH acid substitution during in vitro fertilization (IVF) therapy Wolff, C.: Pfeiffer, S.: Neulen, J.: 1: Labor-Berrath, Düsseldorf, Germany 2: University hospital Aachen, clinic for gynaecological endocrinology and reproductive medicine, Aachen, Germany

Objectiv: Aim of this prospective study was the evaluation of prognostic parameters for oral u3-FA substitution in women undergoing in vitro-fertilization (IVF) therapy by flow cytometry. The thesis of this study can be seen in the assumption that oral u3-substitution increases the frequency of succesful IVF treatments by optimizing the immunological conditions for implantation.

The pictures below are examples of our T<sub>Re</sub> assay result screen. The picture on the left side (pictur shows a typical isotype control staining while the picture on the right (picture 2) shows a typical T<sub>Reg</sub> staining.

<u>Introduction</u> <u>Interpretation</u> has been reported that a large part of spontaneous abortions has no genetical origin. The data about the frequency of periodially motivated abortions vay (1), (2). Some publications report has the source 50% of the abortions have no genetical origin. The cases heartening the source of the interpretation of the source of the source of the source cases heartening the interpretation of the source of the source of the source of the collater immunological origin on the periodial origin. The registration's collection of the collater immunological the source of source of the source of the source of the source of the source of assumption that use the postellation for induction of the source of the source of the source of origin. The clientities of our survey were flexible or the source of the source of the clientity and the thereined is a clientity client as well as the Labor-cemany and the herbertenist is can be needed to the 2008 and source of the source of the Max Ruber-Institute, in Germany which pointed out that all age groups show a loo low us Staty and consumption. Whit networks boar one should be of inferentiate that normal computing for du-da consumption. Whit networks boar one should it is of inferentiat that means computing for du-da consumption. The source of the source one should it is of inferentiate that normal computing for du-da consumption. tion. With reference to our own study it is of interest that normal cor ws no severe side effects as the national consumption survey pointe

Methodology: 79 females with a history of 2 or more miscarriages were included in this study. The follow-up included 23 patients undergoing IVF therapy. Blood was collected for analysis before and about 4 weeks after oral substitution of 800 mg/d salmon oil (Queisser Pharma, Germanye), which book = weeks alter char substatuto fr do wing samon on (Usesser Finatine, Germany) explored equal to 480 mg/du du-344tg adds : The following parameters were analyzed by flow cytown sing specific antibodies from Beckin Dickinsone: Granuboytes, tymphocytes, CD4-T-Heper co eds(-T-suppressor eds), CD5-41K, cells and CD19-Pecells. Tage-gother, measured us thittodies against CD4, CD22 and CD127 from BeckmanCoutlerey were added to the pamel lat PS-Siva was def tratilistical analysis. For Median analysis the Wiccon-Het was used. n the course of this study the diagnostics shown in table 1 are carried out

cary o			day 28	
Anamnesia				
Taking of the blood sample			Taking of the blood sampl	•
Blood count	Heemoglobin		Blood count	Haemoglobin
	Heematocrit			Haematocrit
	MCV, MCH, I	NCHC		MCV, MCH, MCHC
	Thrombocyte	•		Thrombocytes
	Leukocytes			Laukocytes
	Lymphocylea			Lymphocytea
	Granufocytes			Granulocytes
	Basophil gran	worytes		Basophil granulocytes
Neutrophil granulo		anulocytes		Neutrophil granulocytes
	Ecsinophil gr	anulocytes		Ecsinophil granulocytes
	Monocytes			Monocyles
Cellular immun status	T cells (absol	ute)	Cellular immun status	T cella (absolute)
	B cells (abso	lube)		B cells (absolute)
	NK cells (abs	clute)		NK cells (absolute)
	CD4" T cells	(absolute)		CD4* T cells (absolute)
CD8" T		(absolute)		CD8* T cells (absolute)
	CD41/CD81-F	batio		CD41/CD81-Ratio
Councils and CD4'CD25'-TZellen			CD4'CD25'-Tax-Zellen	
For immun status cells and NK cell The T <sub>Reg</sub> cell stai	s measurem s. ning carried	ent the Becton E out according to	tickinson® Multiset IM	Kit was used to stain T ce n table 2.
Table 2:				
	CD3 CD4	IgG1 IgG2a		
Control (Micronic 1)				

substitution therapy as shown in table :

hang, XX, et al., Genetic analysis of first trimester miscarniages with a combination of cytopenetic karyopyping, microstatellite genopping and amy CGH. Clin Genet. 2 of a S. et al., A reflection protocol for the detection of chromosonia abornamilies in sportaneous microinarges or fortal detects. Eur J Obstet Gynecol Reprod Bio. 2 mak-Kim, J. K.M. Yang, and A. Gilman-Sacha, Recurrent pregnancy loss: a disease of inflammation and coagulation. J Obstet Gynecol Res, 2009. 35(4): p. 609-22. Nite. E. Detects yield socia. Am Ear Migratian. 2009. 8(4): p. 545-00. Genet, 2009. 75(2): p. 133-40. d Biol, 2009. 147(2): p. 144-50 munology of pregnancy: cellula mechanisms allowing fetal survival within the maternal uterus. Expert Rev Mol Med, 2007. 9(10): p. 1-

We thank Beckman Coulter GmbH for the friendly support

Immunmodulation with Omega-3-fatty acids leads to reduced activation signs and an increase in the population of regulatory Tcells, optimizing the conditions for tolerating the fertilized egg

The intake of Omega-3-fatty acids will improve the conditions for tolerating the fertilized egg by reducing "dangerous signals" Beside this immunologic effect, Omega-3-fatty acids have a positive effect in brain developement of the fetus

## Vitammin-D and fertility issues

Interactive Map

for Vitamin-D-

children/young

adults and adults

for each country

http://www.iofbonehealth.org/fa cts-and-statistics/vitamin-d-

publications

regarding

worldwide

studies-map



Worldwide supply with Vitamin-D, published 2009





# Vitamin-D and fertility issues





- Pregnancy fulfills the same criteria like a allotransplantation
- A specific cellular immune system with a good capability for inducing tolerance is one condition for a positive outcome in

pregnancy

 Diseases accompanied by dangerous signals like proinflammation, autoimmune diseases, atopic disposition and infections will reduce consequently the ability for the induction of tolerance

Screening for a lack of Vitamin-D and consequently substitution can increase the percentage of regulatory T-cells Base line laboratory tests, including Tcell-subsets, regulatory T-cells, pro- and antiinflammatoric cytokines, could be a helpful tool in detecting immunologic disorders prior fertility treatment Monitoring regulatory T-cells during pregnancy might be a helpful prognostic tool

- Immunomodulation with Omega-3-fatty acids can optimize the conditions for nidation and pregnancy without significant side effects
- A additional therapy with steroids demands a strong indication, depending on the base line clinical and laboratory findings
- Due to the side effects of steroids, short term blood controls are strongly recommended

# Thank you for your attention