

The **ReProTect** Framework Program: New Innovative Approaches for Evaluating Fertilization, Implantation and Prenatal Development

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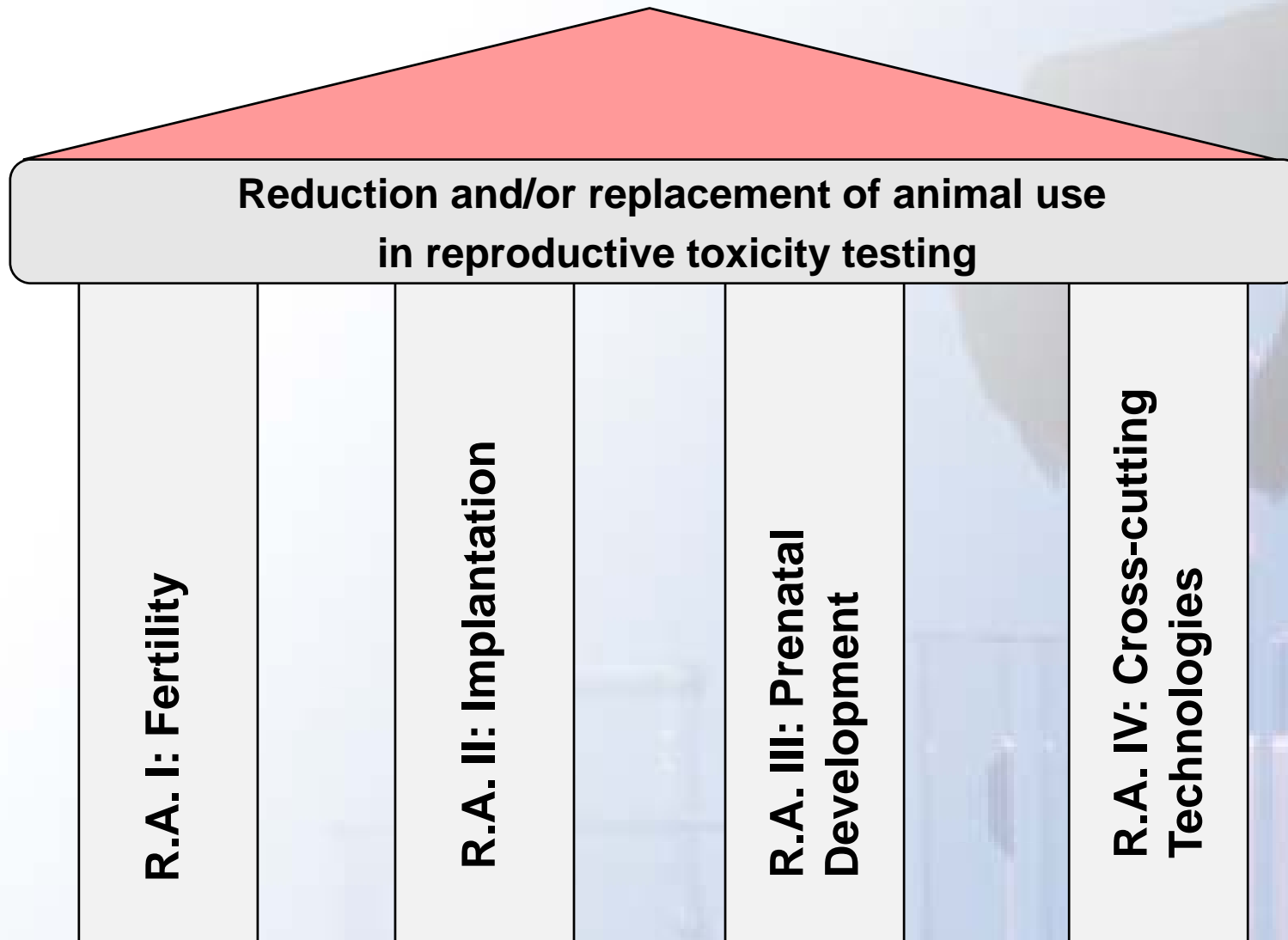
2004-2009

(www.reprotect.eu)

LSHB-CT-2004-503257

Development of a novel approach in hazard and risk assessment of reproductive toxicity by a combination and application of in vitro, tissue and sensor technologies

- **Integrated project funded through the EU FP6 program**
- **Total budget amounts: 13.2 mEUR**



Coordination:

M. Schwarz, Tübingen

Project management

(Financial):

S. Stoppel, Tübingen

(Scientific)

S. Bremer, ECVAM, Ispra

C. Pellizzer, ECVAM, Ispra

Advisory Board (chair):

Bernward Garthoff

Research Area leaders:

I.: G. Lazzari, Cremona

II: L. Dencker, Uppsala

III: H. Spielmann, Berlin

IV: A. Mantovani, Rome

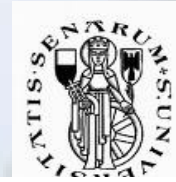


Structure of the ReProTect Project

Brussels, November 19, 2009



Imperial College



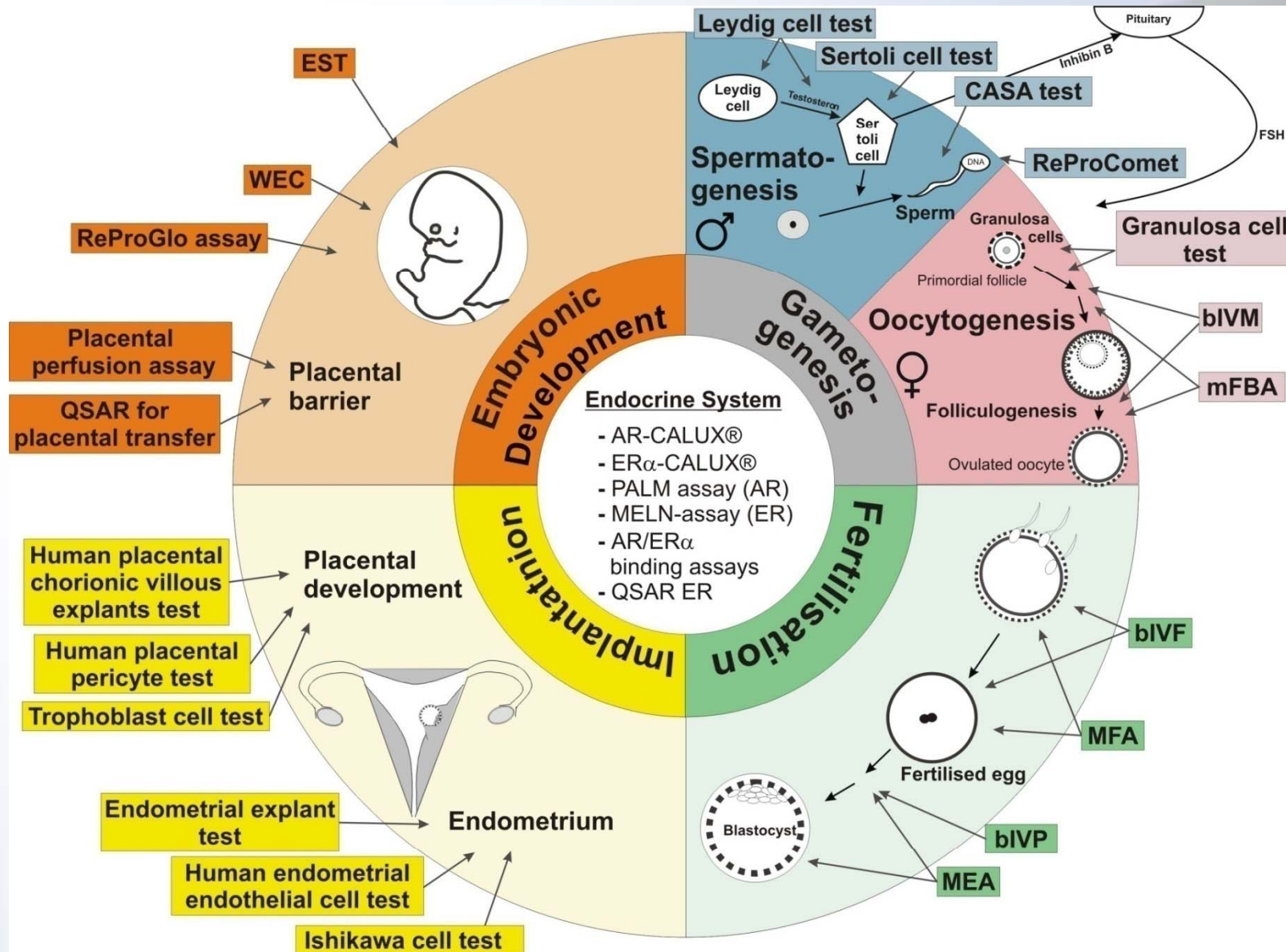
FRIEDRICH-KARLS



33 partners

from Academia, Industry, SMEs and Governmental Institutes





Bart van der Burg



Hilda Witters



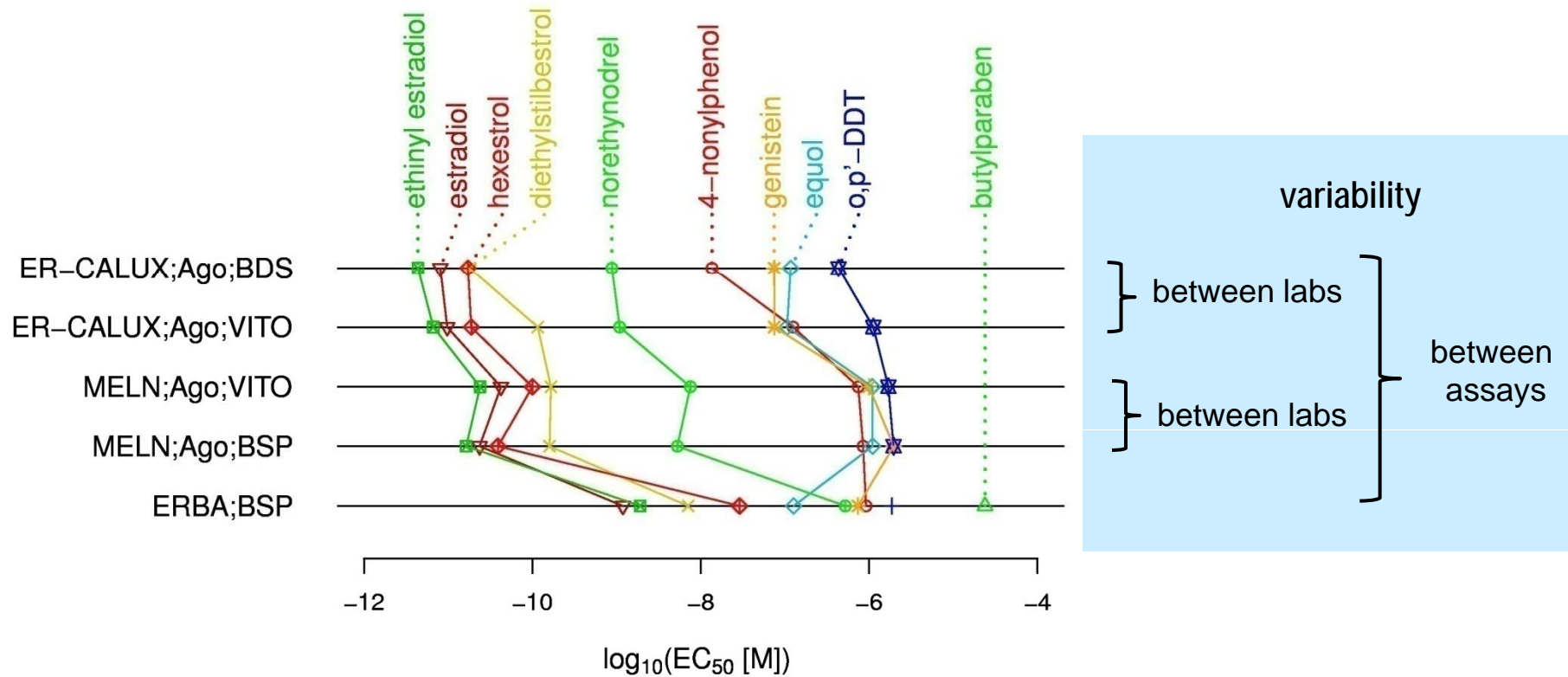
Alexius Freyberger



Endocrine System

- AR-CALUX®
- ER α -CALUX®
- PALM assay (AR)
- MELN-assay (ER)
- AR/ER α binding assays
- QSAR ER

Estrogenic agonists (in vitro)



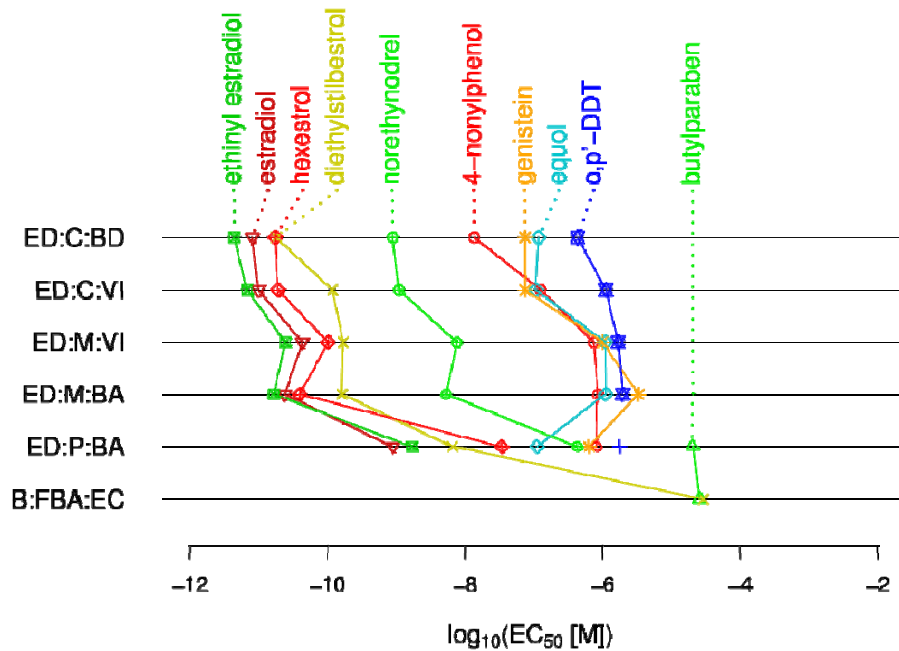
In vitro

vs.

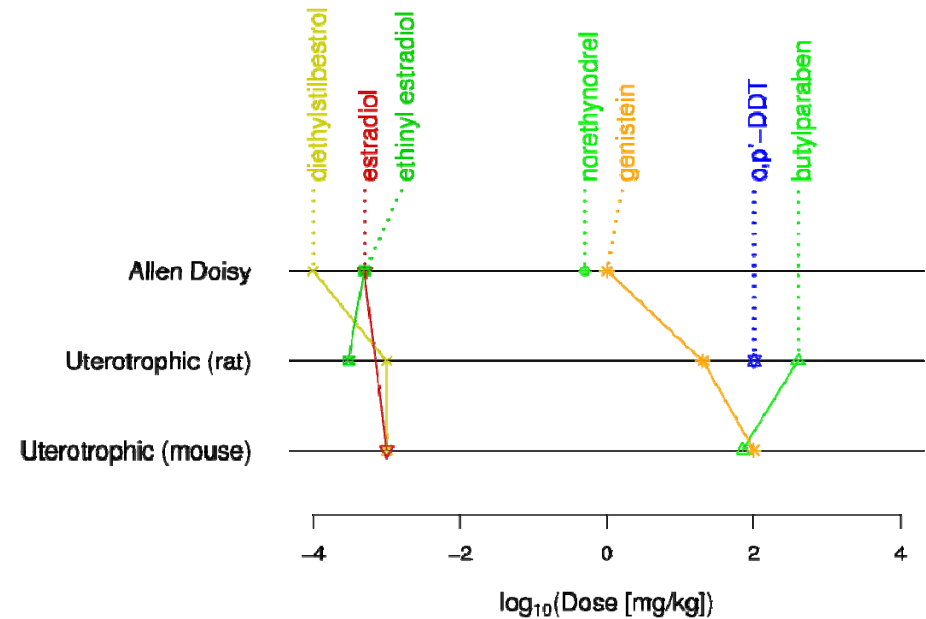
In vivo

Similar ranking

Estrogenic agonists (in vitro)



Estrogenic agonists (in vivo)



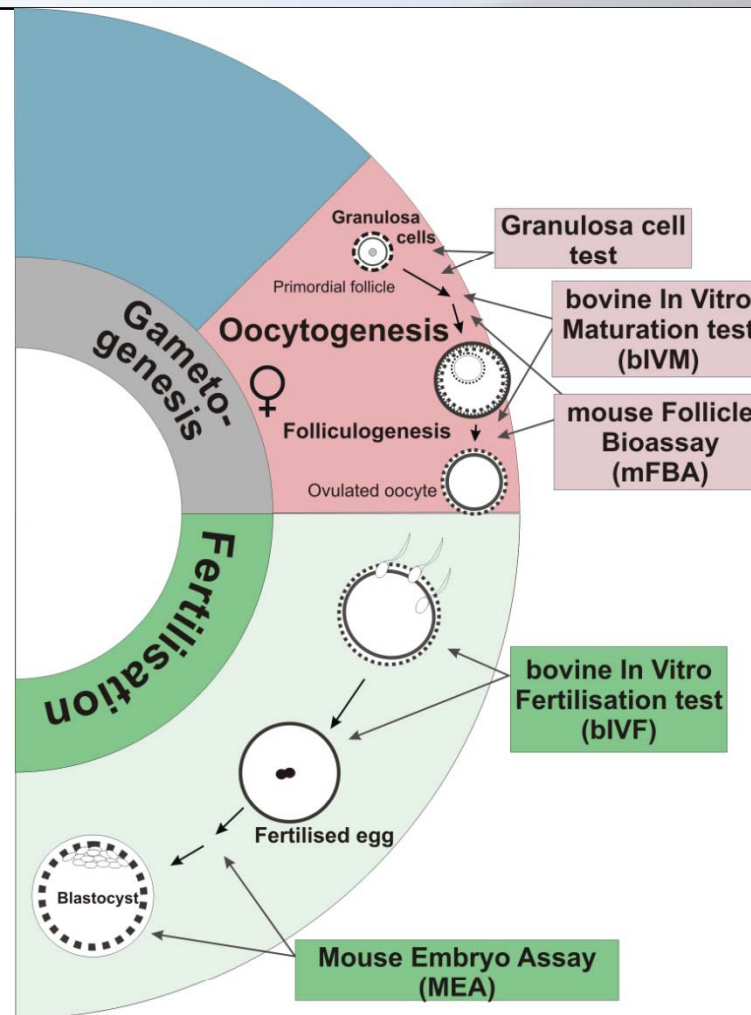
Giovanna Lazzari



Rita Cortvindt



Ilpo Huthaniemi



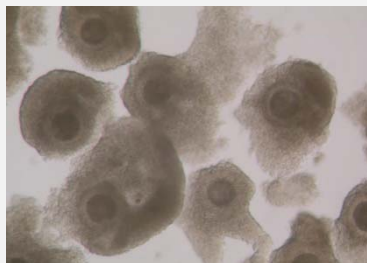
Giovanna Lazzari



In vitro **maturation** (IVM) of bovine oocytes

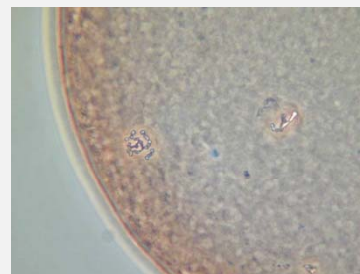
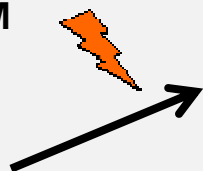


bovine ovaries



bovine oocytes

Chemical exposure (24h) during IVM



Endpoint:
% Metaphase II

In vitro **fertilisation** (IVF) of bovine oocytes

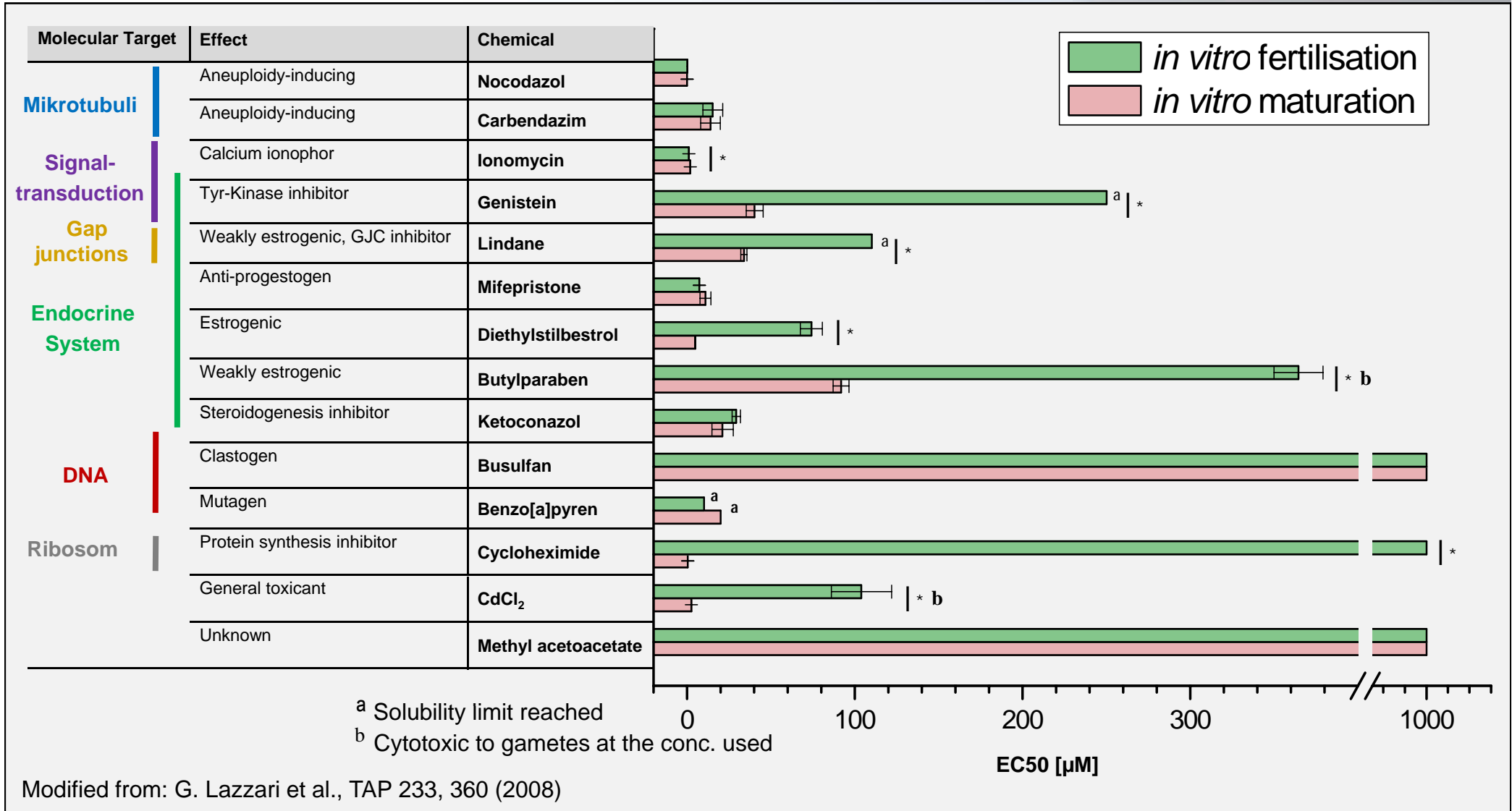


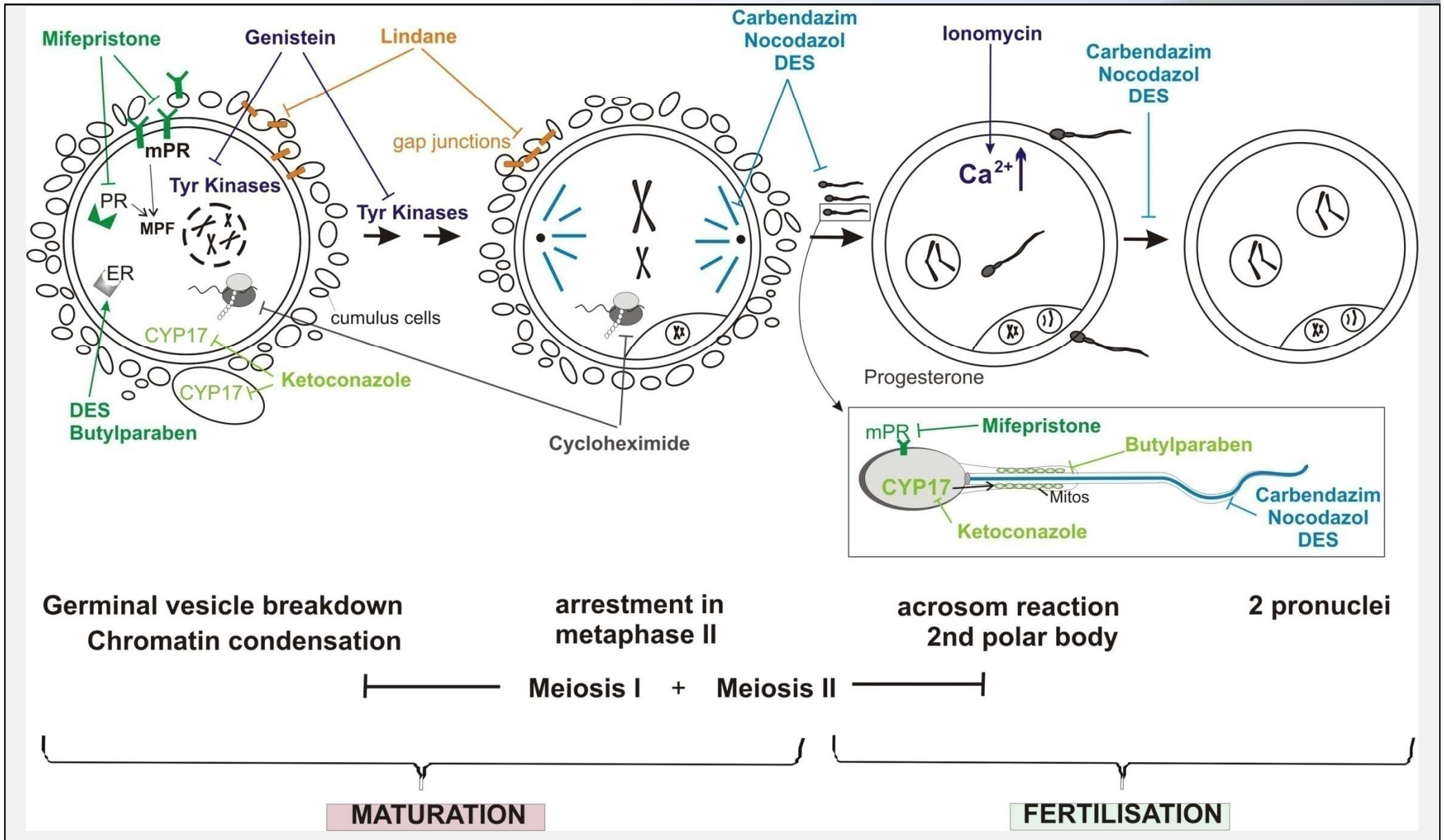
Chemical exposure (20h) during IVF



Endpoint:
% 2Pronuclei

IVM, 24 h



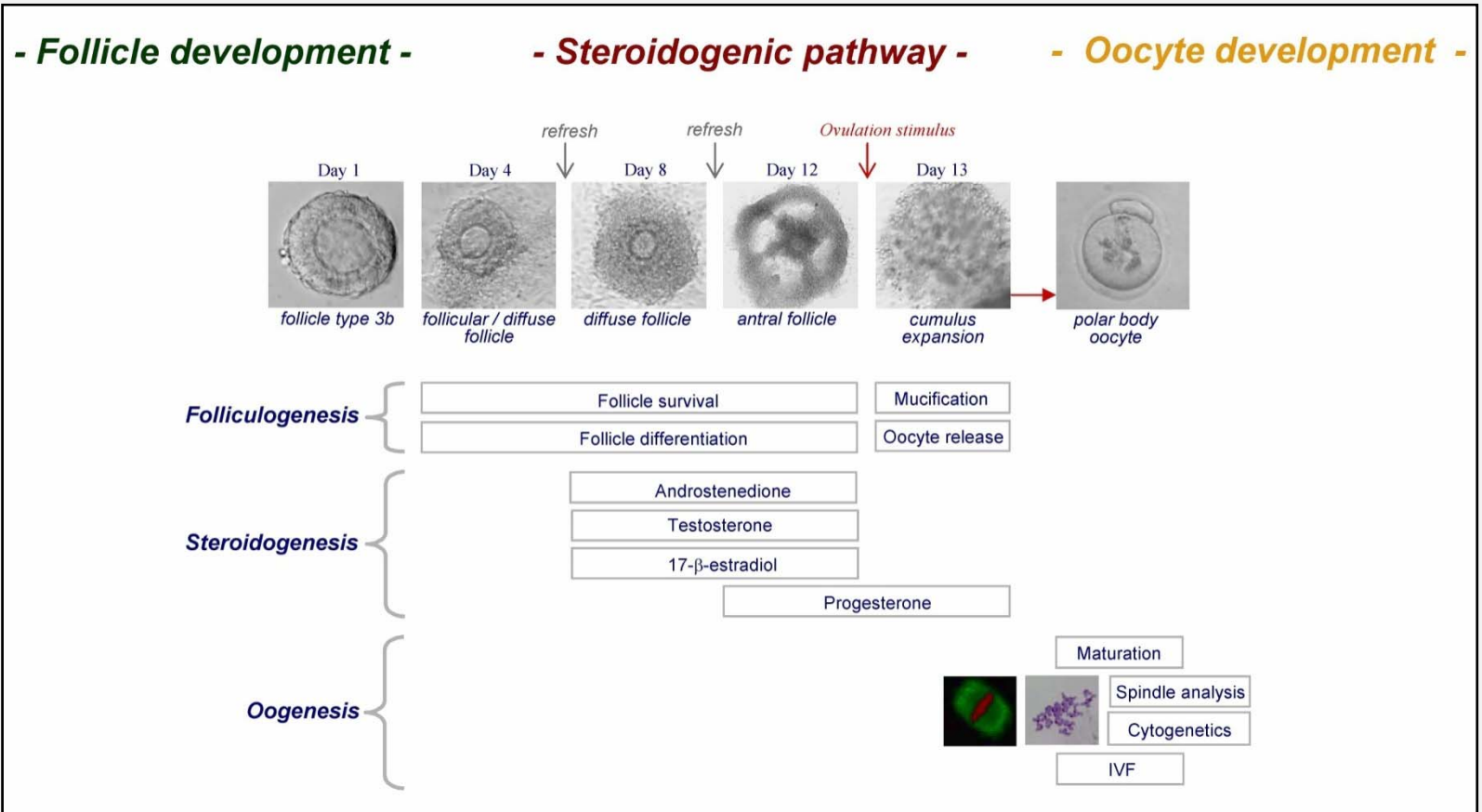


Rita Cortvindt

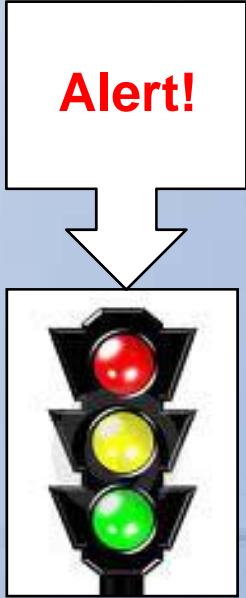


Prediction:

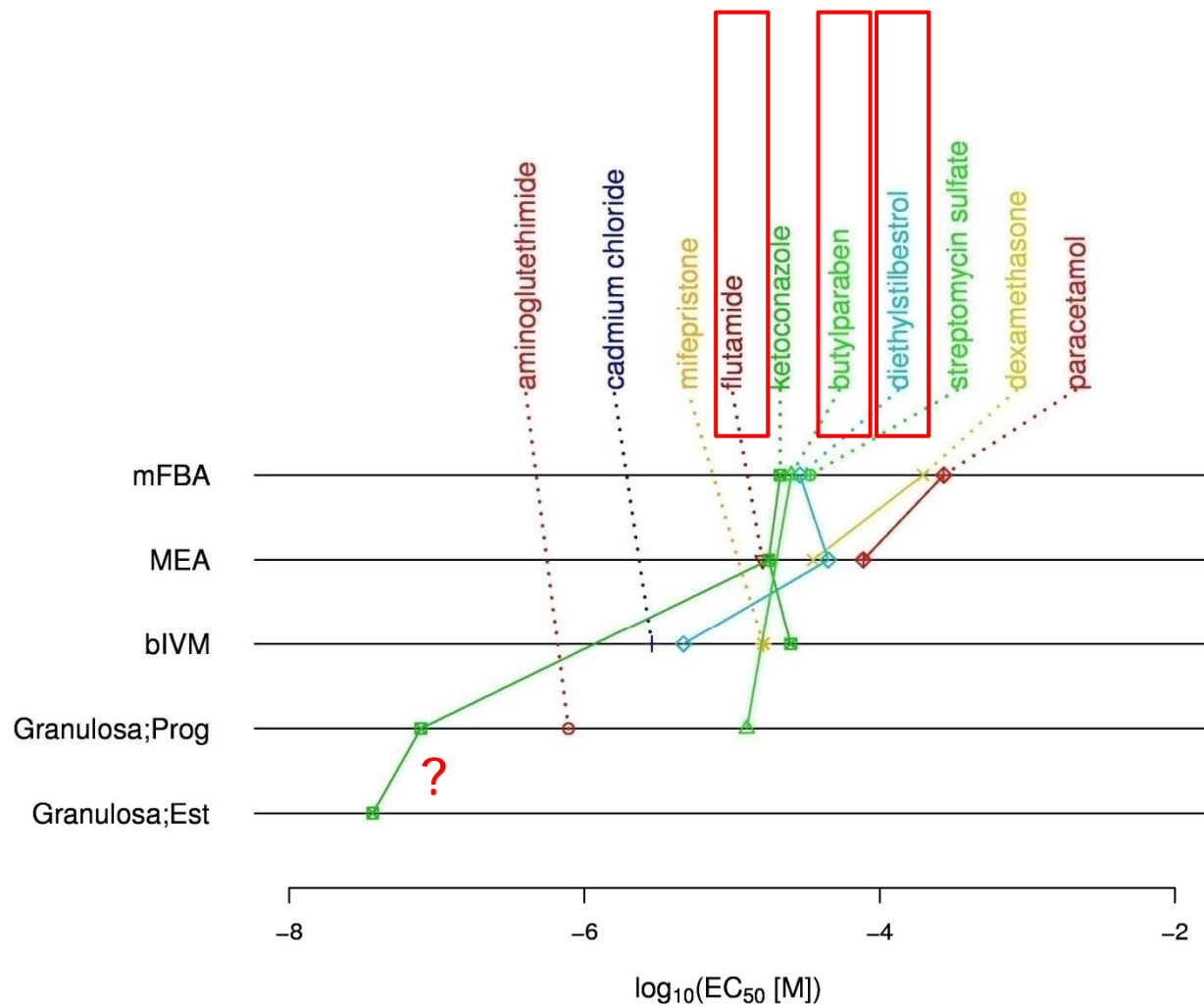
- Cycle disturbances
- Ovulation problems
- Fertilization problems
- Conception problems
- Endocrine disruption
-

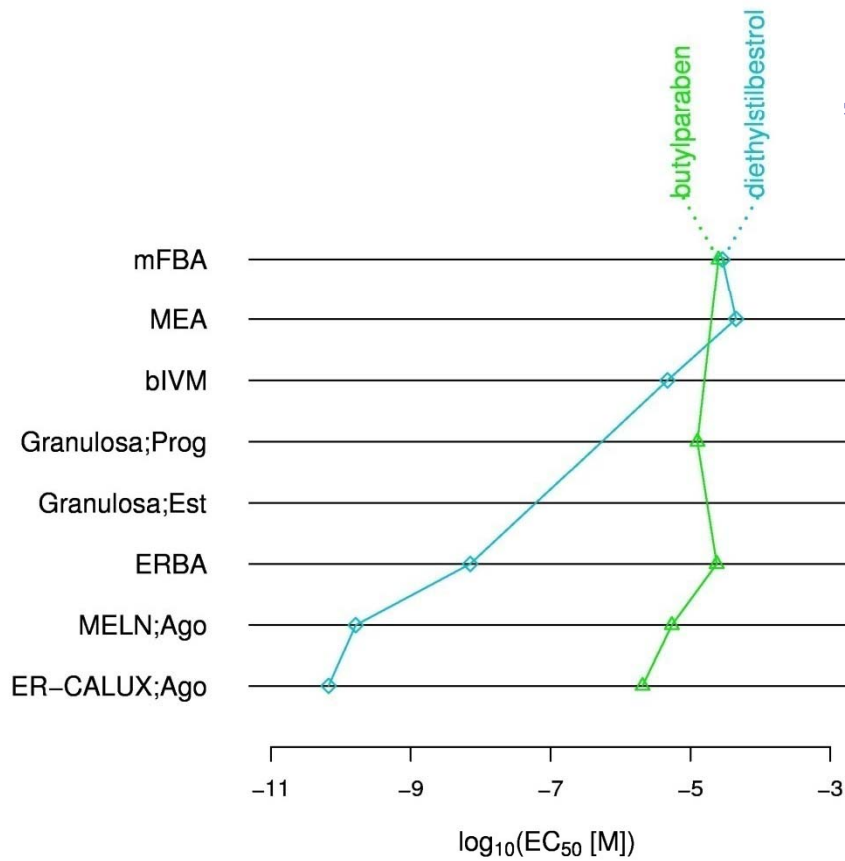


Compound	CAS	bIVM Maturation	Granulosa Estradiol			Granulosa Progesterone			MEA Total Blastula			mFBA Maturation		
aminoglutethimide	125-84-8	- - -	3	0	0	0	0	4	2	0	0	2	0	0
butylparaben	94-26-8	- - -	3	0	0	0	1	2	2	0	0	0	0	2
cadmium chloride	7790-78-5 10108-64-2	0 0 7	2	1	0	2	1	0	-	-	-	-	-	-
dexamethasone	50-02-2	- - -	-	-	-	-	-	-	0	0	2	3	1	2
diethylstilbestrol	56-53-1	0 0 7	4	0	0	3	2	0	0	2	1	0	1	1
flutamide	13311-84-7	- - -	3	0	0	1	3	0	0	0	2	-	-	-
ketoconazole	65277-42-1	0 0 6	2	2	1	2	0	1	0	0	4	0	0	2
metyrapone	54-36-4	- - -	-	-	-	-	-	-	2	0	0	2	0	0
mifepristone	84371-65-3	0 0 6	3	0	0	0	3	0	-	-	-	-	-	-
paracetamol	103-90-2	- - -	-	-	-	-	-	-	0	1	2	0	2	1
piroxicam	36322-90-4	- - -	-	-	-	-	-	-	2	0	0	2	0	0
streptomycin sulfate	3810-74-0	- - -	-	-	-	-	-	-	2	0	0	0	1	2



Numbers indicate number of independent experiments per chemical

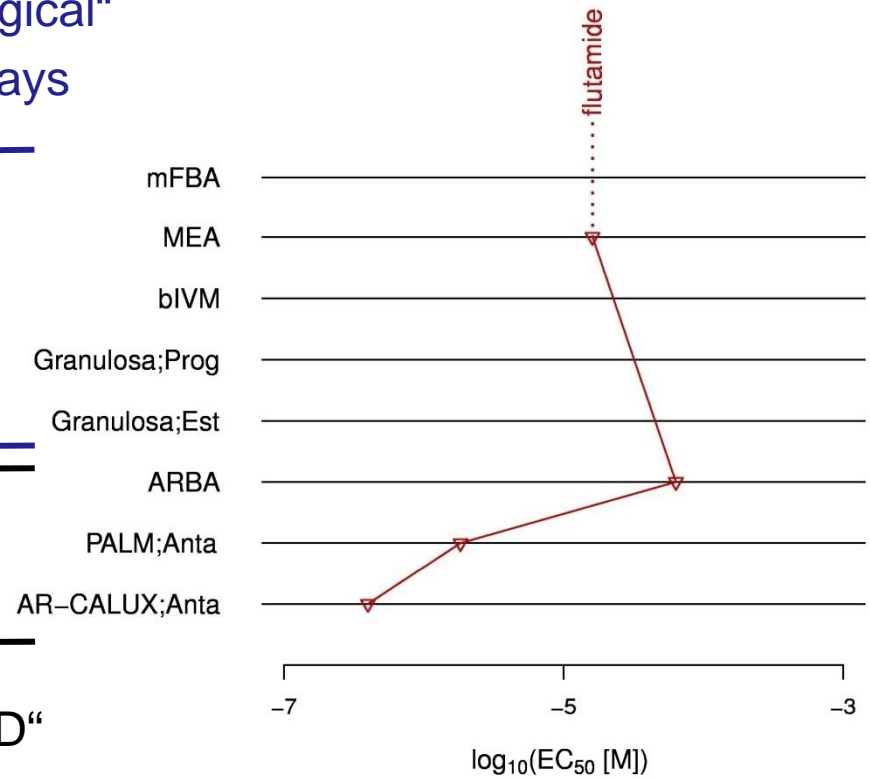




„biological“
assays



„ED“
assays





The ReProTect database

Brussels, November 19, 2009

Server: localhost | Datenbank: ReProTect

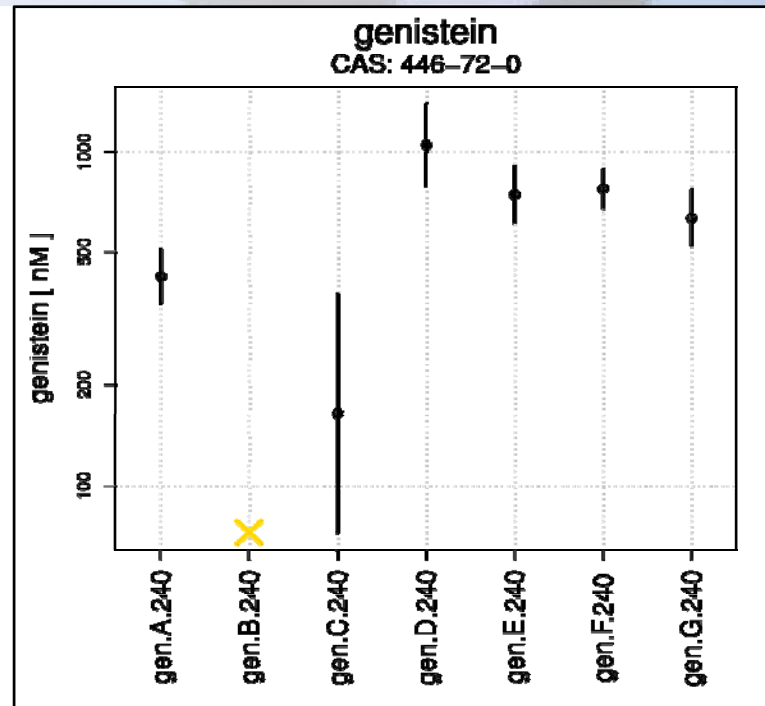
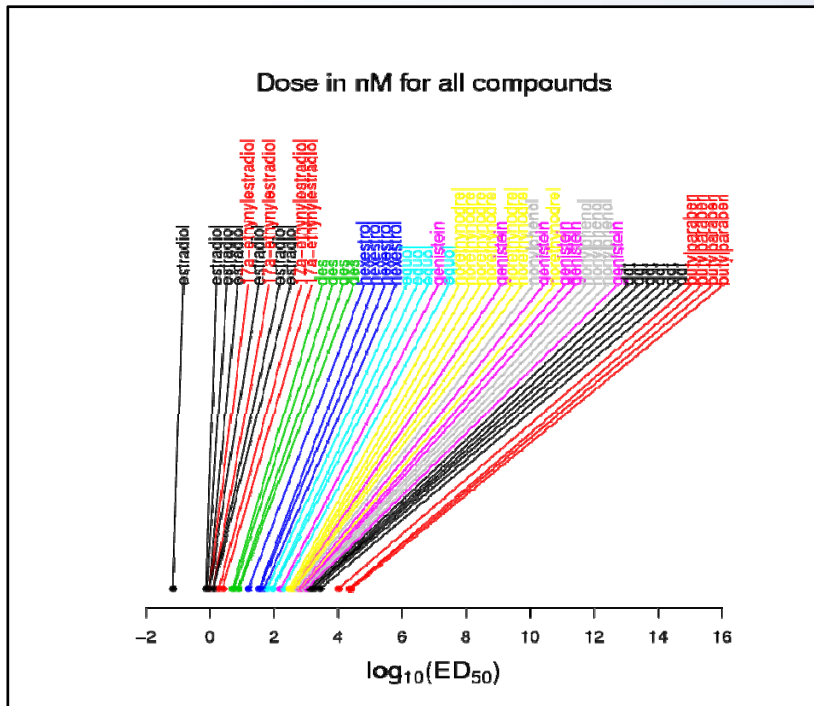
Struktur | SQL | Suche | Abfrageeditor | Exportieren | Importieren | Operationen | Rechte | Löschen

Tabelle	Aktion	Einträge	Typ	Kollation	Größe	Überhang
<input type="checkbox"/> Assay		-28	InnoDB	utf8_general_ci	48,0 KiB	-
<input checked="" type="checkbox"/> CompoundHarm		~128	InnoDB	utf8_general_ci	64,0 KiB	-
<input type="checkbox"/> CompoundInd		-155	InnoDB	utf8_general_ci	32,0 KiB	-
<input type="checkbox"/> DoseUnit		~6	InnoDB	utf8_general_ci	32,0 KiB	-
<input type="checkbox"/> ED50		-890	InnoDB	utf8_general_ci	96,0 KiB	-
<input type="checkbox"/> Endpoint		~25	InnoDB	utf8_general_ci	32,0 KiB	-
<input type="checkbox"/> Lab		-15	InnoDB	utf8_general_ci	16,0 KiB	-
<input type="checkbox"/> Run		-1,522	InnoDB	utf8_general_ci	336,0 KiB	-
<input type="checkbox"/> Target		~7	InnoDB	utf8_general_ci	16,0 KiB	-
<input type="checkbox"/> Treatment		~338	InnoDB	utf8_general_ci	96,0 KiB	-
<input type="checkbox"/> WarningsHarm		~8	InnoDB	utf8_general_ci	32,0 KiB	-
<input type="checkbox"/> WarningsInd		-632	InnoDB	utf8_general_ci	96,0 KiB	-
12 Tabellen	Gesamt	-3,754	MyISAM	latin1_swedish_ci	896,0 KiB	0 Bytes

Alle auswählen / Auswahl entfernen | markierte:

GlobalID	Assay	Target	Endpoint	Lab	Compound	CAS	Run	ED50
670			6 Standard		genistein	446-72-0	gen.A.240	422.6
672			6 Standard		genistein	446-72-0	gen.C.240	164.5
673			6 Standard		genistein	446-72-0	gen.D.240	1049.0
674			6 Standard		genistein	446-72-0	gen.E.240	744.5
675			6 Standard		genistein	446-72-0	gen.F.240	776.4
676			6 Standard		genistein	446-72-0	gen.G.240	633.1

Lower	Upper	DoseUnit	Mw	CompoundHarm	CompoundRef	RefED50	RefDoseUnit
350.2	509.9	nM	270.2369	genistein	genistein	4.226e-07	M
72.5	373.4	nM	270.2369	genistein	genistein	1.645e-07	M
788.8	1394.0	nM	270.2369	genistein	genistein	1.049e-06	M
611.3	906.6	nM	270.2369	genistein	genistein	7.445e-07	M
678.6	888.4	nM	270.2369	genistein	genistein	7.764e-07	M
520.1	770.7	nM	270.2369	genistein	genistein	6.331e-07	M



~130
compounds

Alternatives in Reproductive Tox. Testing: Where do we stand?

- ➔ **2 validated tests predictive for embryotoxicity available (mEST, WEC) But: no metabolic capacity; applicability domain unclear**
- ➔ **Several assays (Receptor binding or cell-based reporter systems) for detection of endocrine disrupters available**
- ➔ **Several assays predictive for adverse effects on female or male fertility available**

Alternatives in Reproductive Tox. testing: Potential use

- ➔ **Early drug development**
(**“in-house” use for prioritization** during lead compound optimization)
Selection of candidate compounds for further safety evaluation studies; early screen-out of compounds predicted to show undesirable reproductive toxicity properties
- ➔ **Early drug development and regulatory decision making**
Mode of action analysis for compounds that have demonstrated reproductive toxicity *in vivo*.
- ➔ Alternative tests may lead to a **Reduction** in experimental animals but presently **not to a Replacement** of the animal assay(s).

I deeply acknowledge the scientific and personal input of all partners in the ReProTect project

We acknowledge the external experts that selected the test chemicals for the feasibility study

The ReProTect Supervising Board is acknowledged for their scientific advice during the project

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I thank you for your attention

This paper was produced for a meeting organized by Health & Consumers DG and represents the views of its author on the subject. These views have not been adopted or in any way approved by the Commission and should not be relied upon as a statement of the Commission's or Health & Consumers DG's views. The European Commission does not guarantee the accuracy of the data included in this paper, nor does it accept responsibility for any use made thereof.