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

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2004-2009

(www.reprotect.eu)

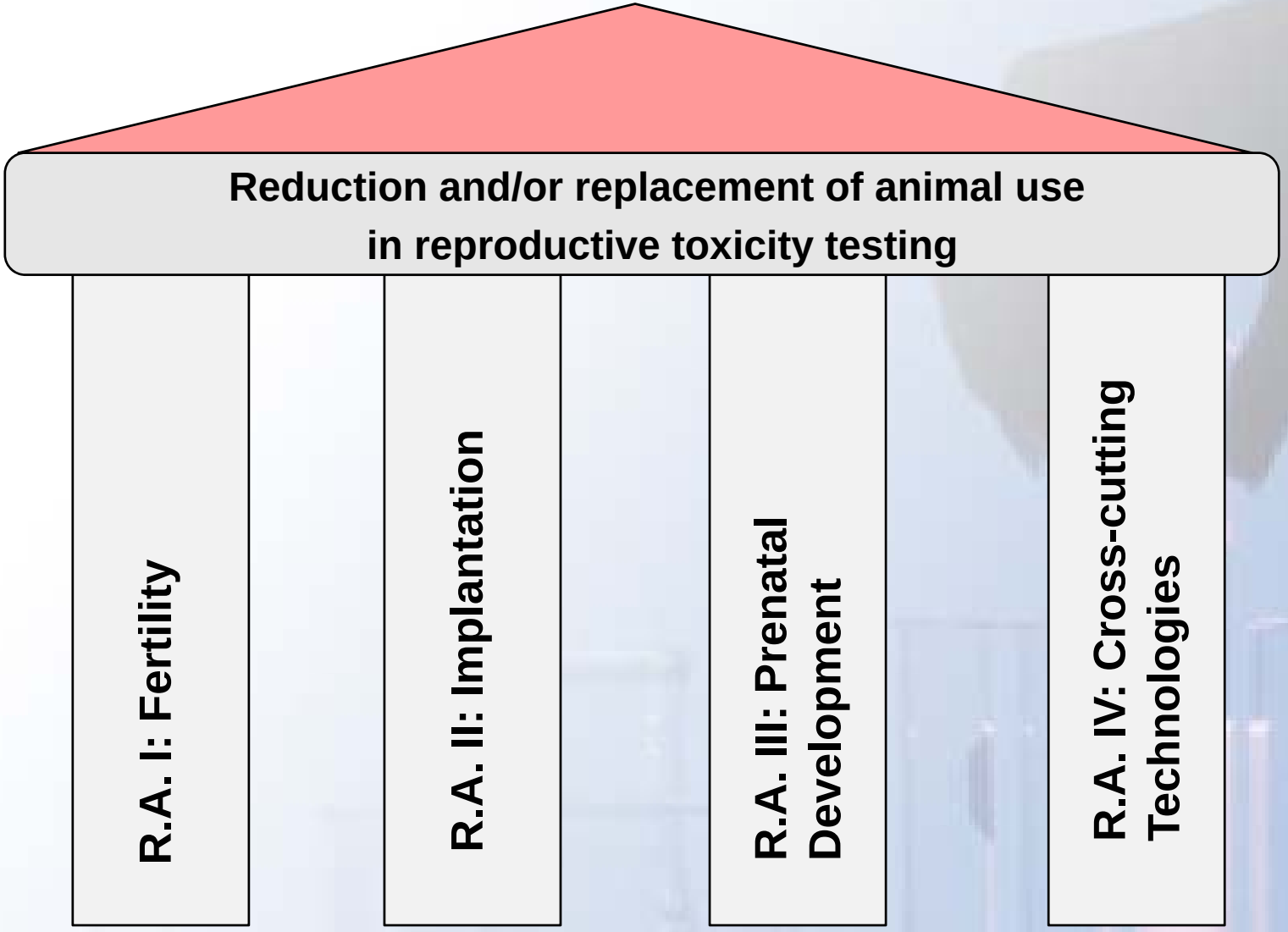
LSHB-CT-2004-503257

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**

Structure of the ReProTect Project

Brussels, November 19, 2009



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C. Pellizzer, ECVAM, Ispra

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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

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33 partners

from Academia, Industry, SMEs and Governmental Institutes

Specific endpoints in the reproductive cycle

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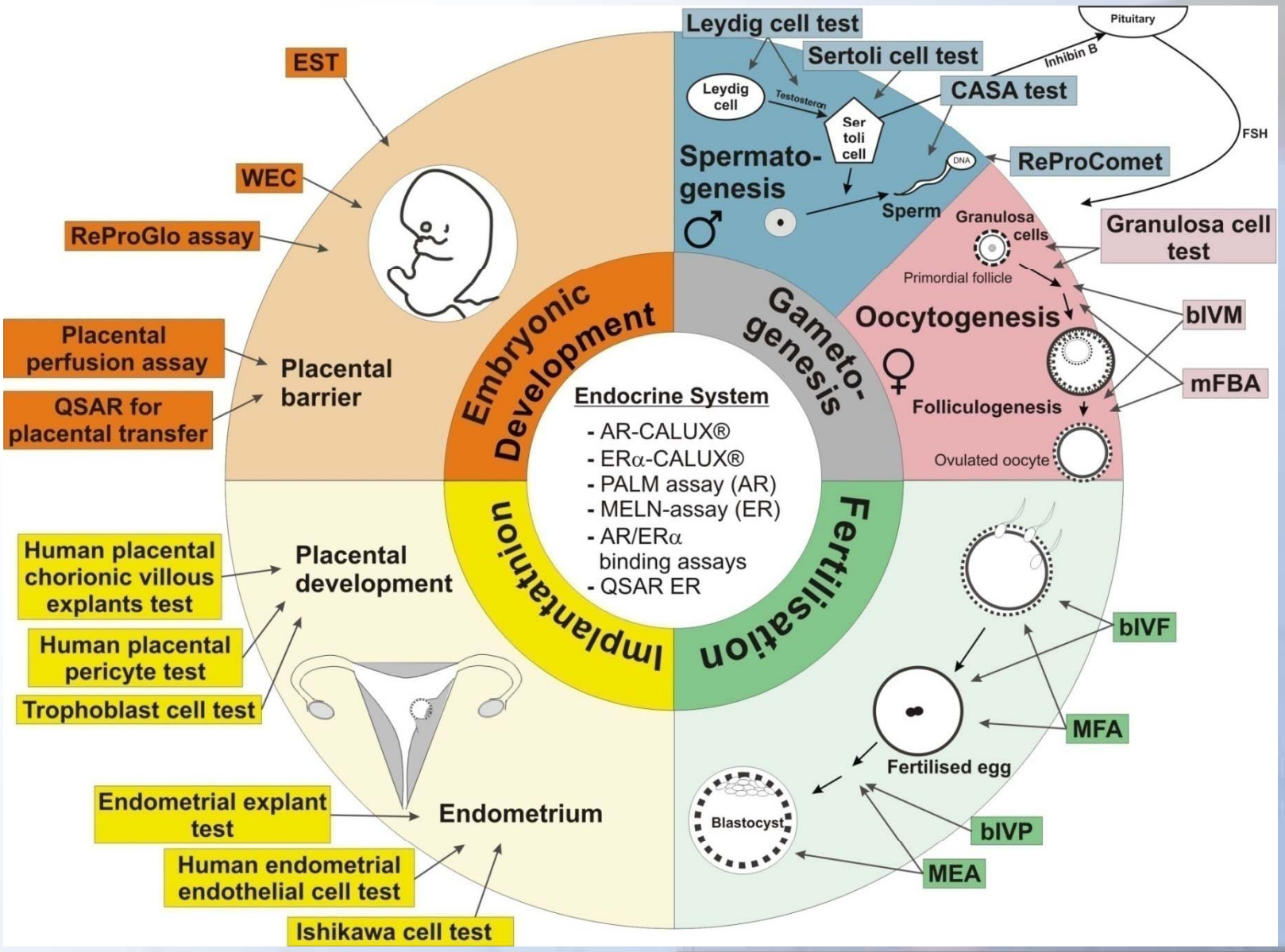

Endocrine Disruptor tests

Example I

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Bart van der Burg

Hilda Witters

Alexius Freyberger

Endocrine System

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

Endocrine Disruptor tests

Example I

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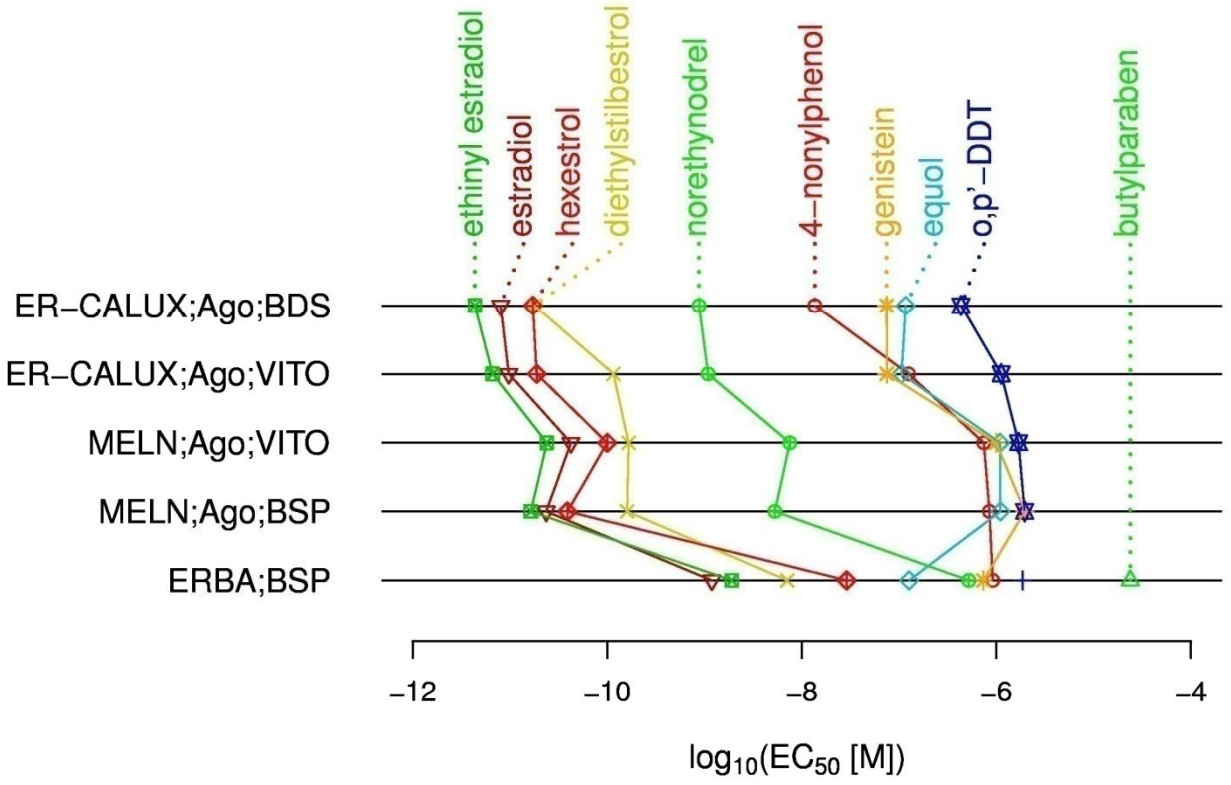
Estrogenic agonists (in vitro)



variability

- between labs
- between labs

→ between assays

Endocrine Disruptor tests

Example I

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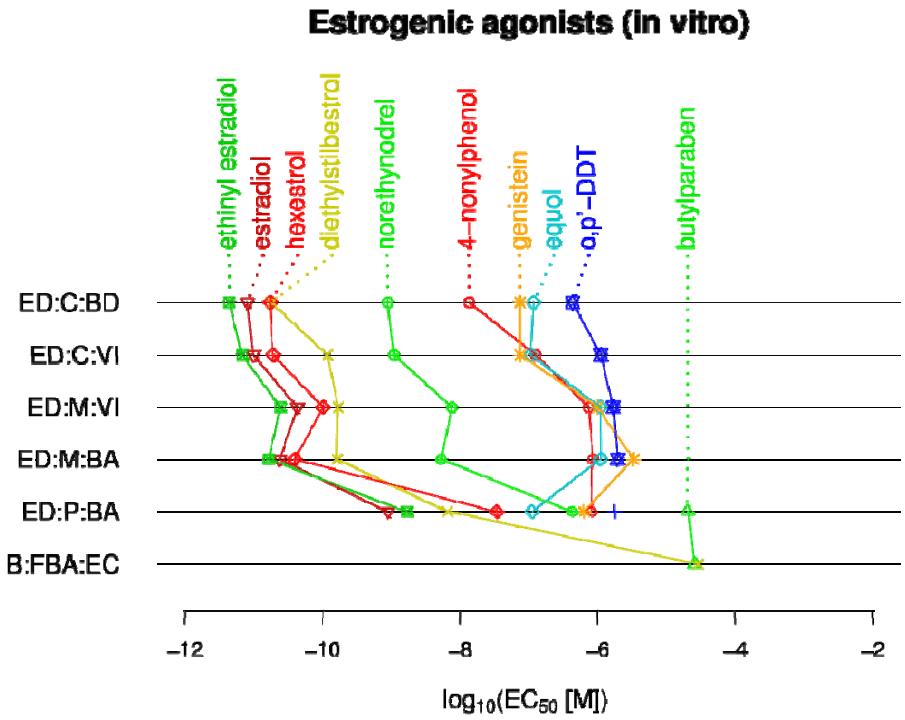
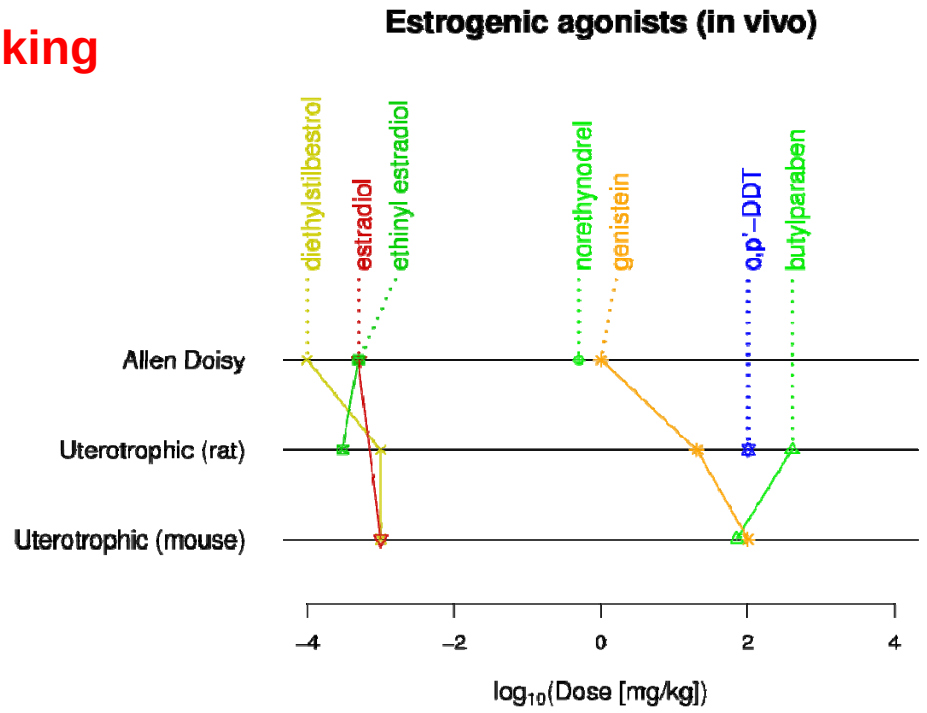
In vitro **vs.** ***In vivo***

Similar ranking





Female fertility

Example II

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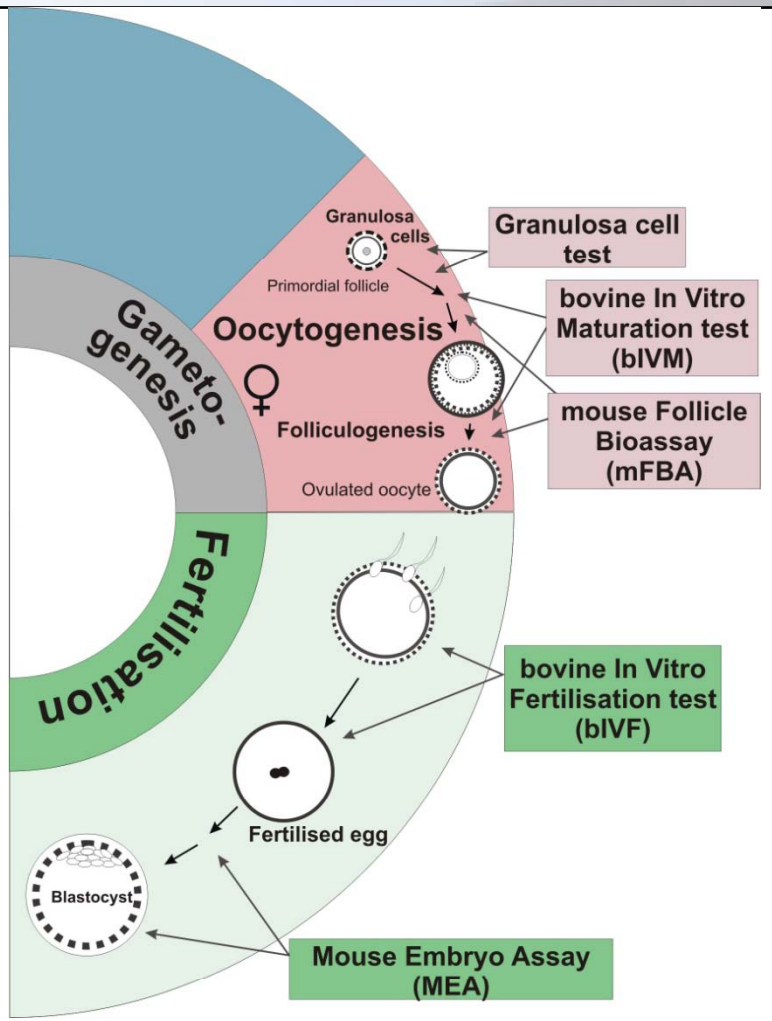
Giovanna Lazzari

Rita Cortvindt

Ilpo Huthaniemi



Use of bovine gametes for reproductive toxicity testing

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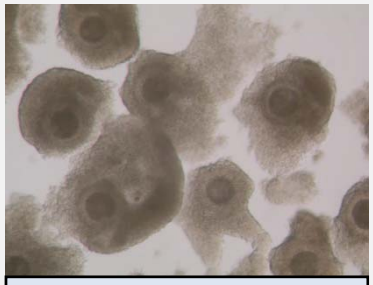
Giovanna Lazzari

AVANTEA
LABORATORIO DI TECNOLOGIE DELLA RIPRODUZIONE

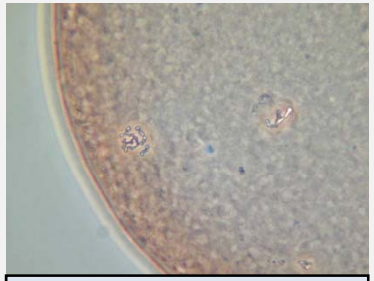
In vitro **maturation** (IVM) of bovine oocytes

Chemical exposure (24h) during IVM

bovine ovaries



bovine oocytes



Endpoint:
% Metaphase II

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

IVM, 24 h

Chemical exposure (20h) during IVF

Endpoint:
% 2Pronuclei

The bovine follicle bioassay: bIVM and bIVF

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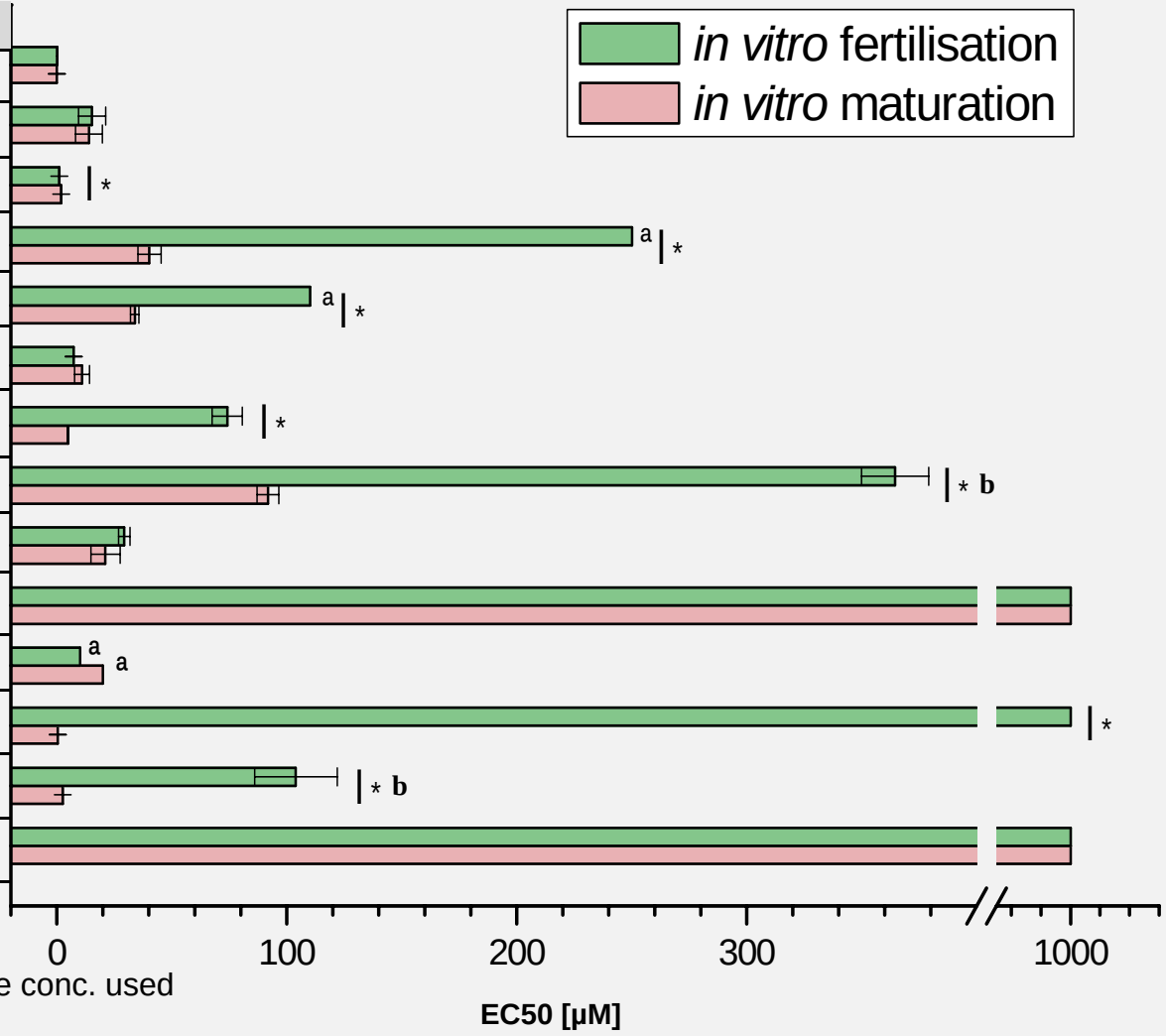
Molecular Target	Effect	Chemical
Mikrotubuli	Aneuploidy-inducing	Nocodazol
Mikrotubuli	Aneuploidy-inducing	Carbendazim
Signal-transduction	Calcium ionophor	Ionomycin
Signal-transduction	Tyr-Kinase inhibitor	Genistein
Gap junctions	Weakly estrogenic, GJC inhibitor	Lindane
Endocrine System	Anti-progestogen	Mifepristone
Endocrine System	Estrogenic	Diethylstilbestrol
Endocrine System	Weakly estrogenic	Butylparaben
Endocrine System	Steroidogenesis inhibitor	Ketoconazol
DNA	Clastogen	Busulfan
DNA	Mutagen	Benzo[a]pyren
Ribosom	Protein synthesis inhibitor	Cycloheximide
	General toxicant	$CdCl_2$
	Unknown	Methyl acetoacetate



[a] Solubility limit reached
[b] Cytotoxic to gametes at the conc. used

Modified from: G. Lazzari et al., TAP 233, 360 (2008)

Suggested mechanisms of compounds

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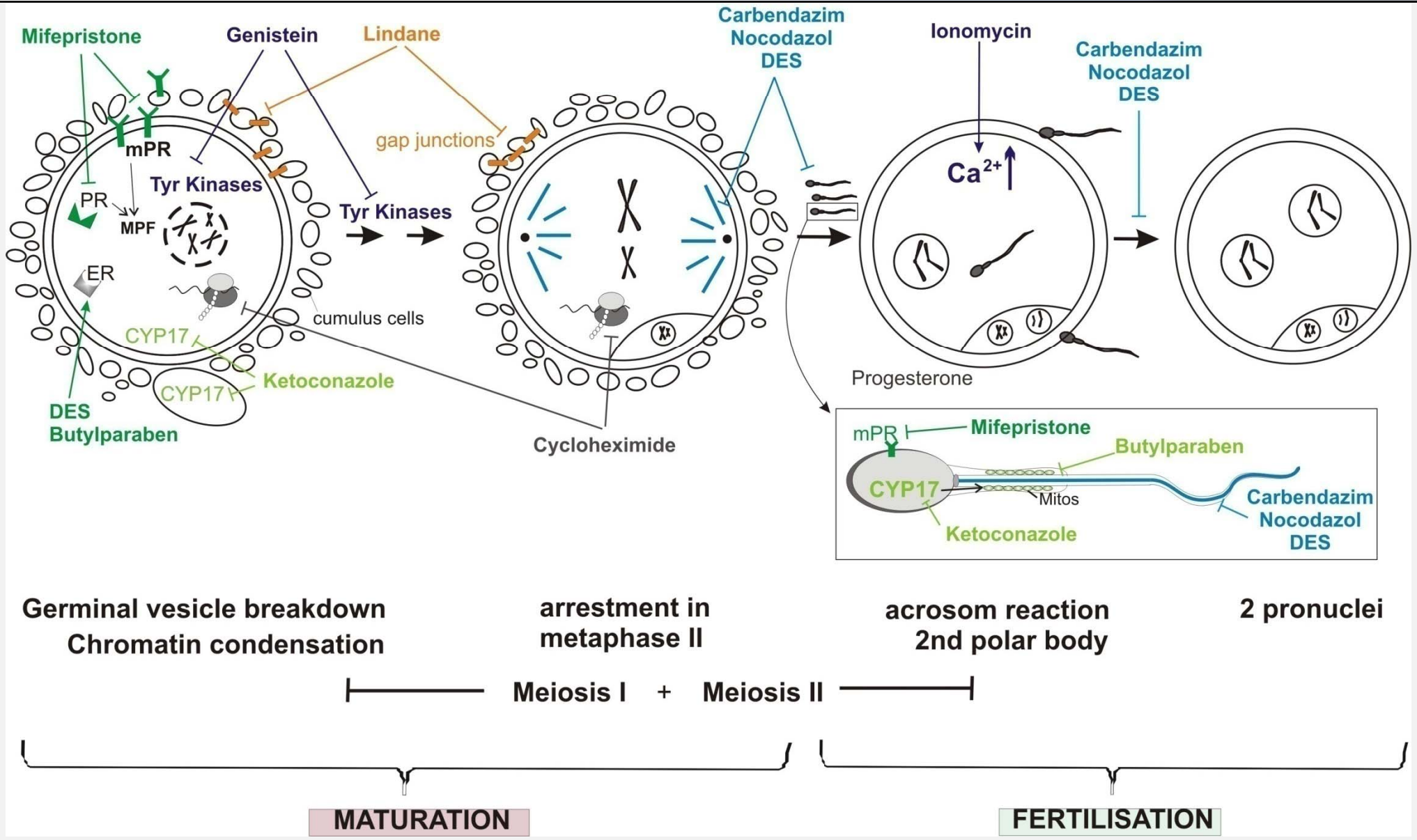

The mouse follicle bioassay

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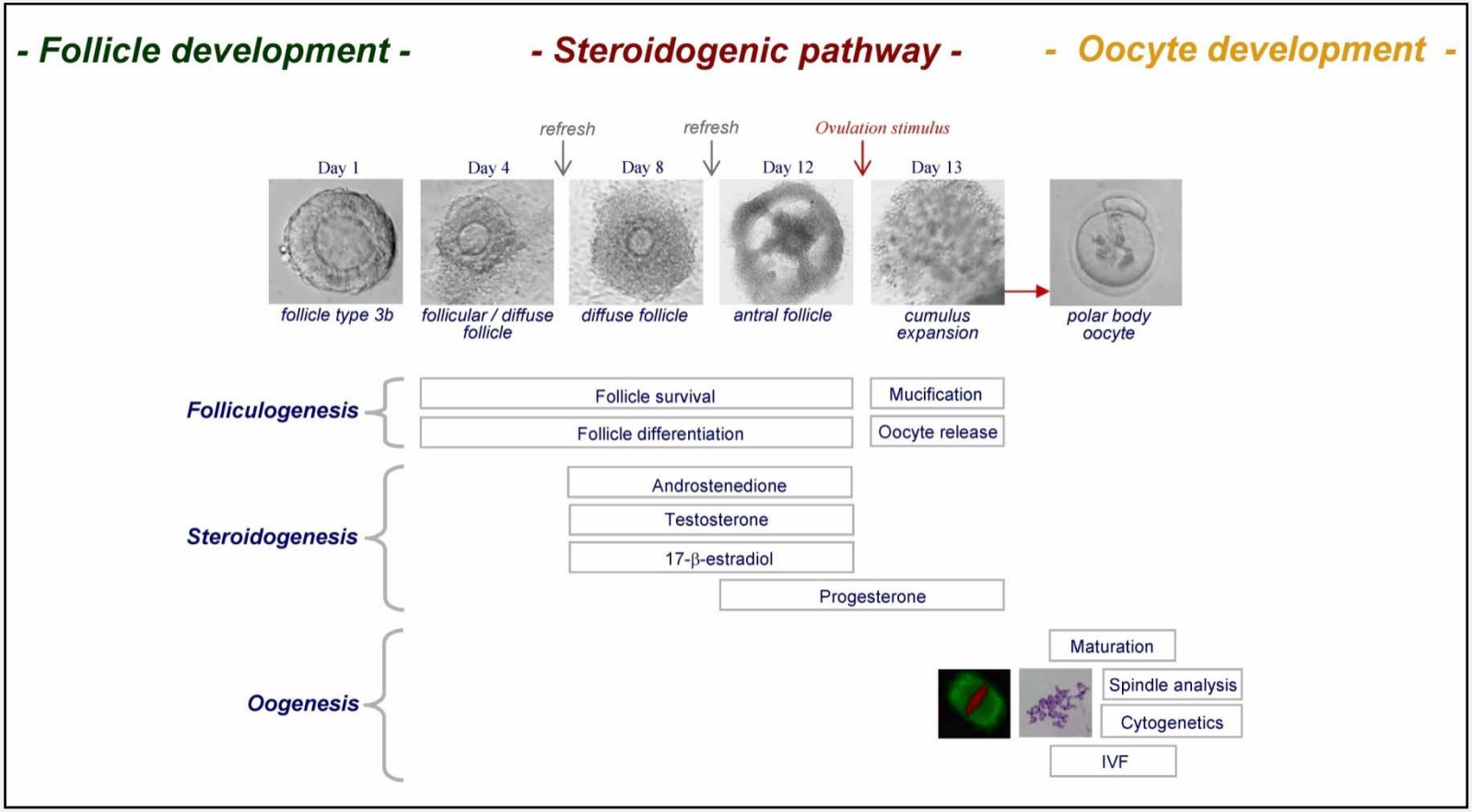
Rita Cortvindt

EggCentris
Center of Excellence for Reproductive Function Testing

Prediction:

Cycle disturbances
Ovulation problems
Fertilization problems
Conception problems
Endocrine disruption
….



Female Fertility

Example II

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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

Alert!

Numbers indicate number of independent experiments per chemical

Female Fertility

Example II

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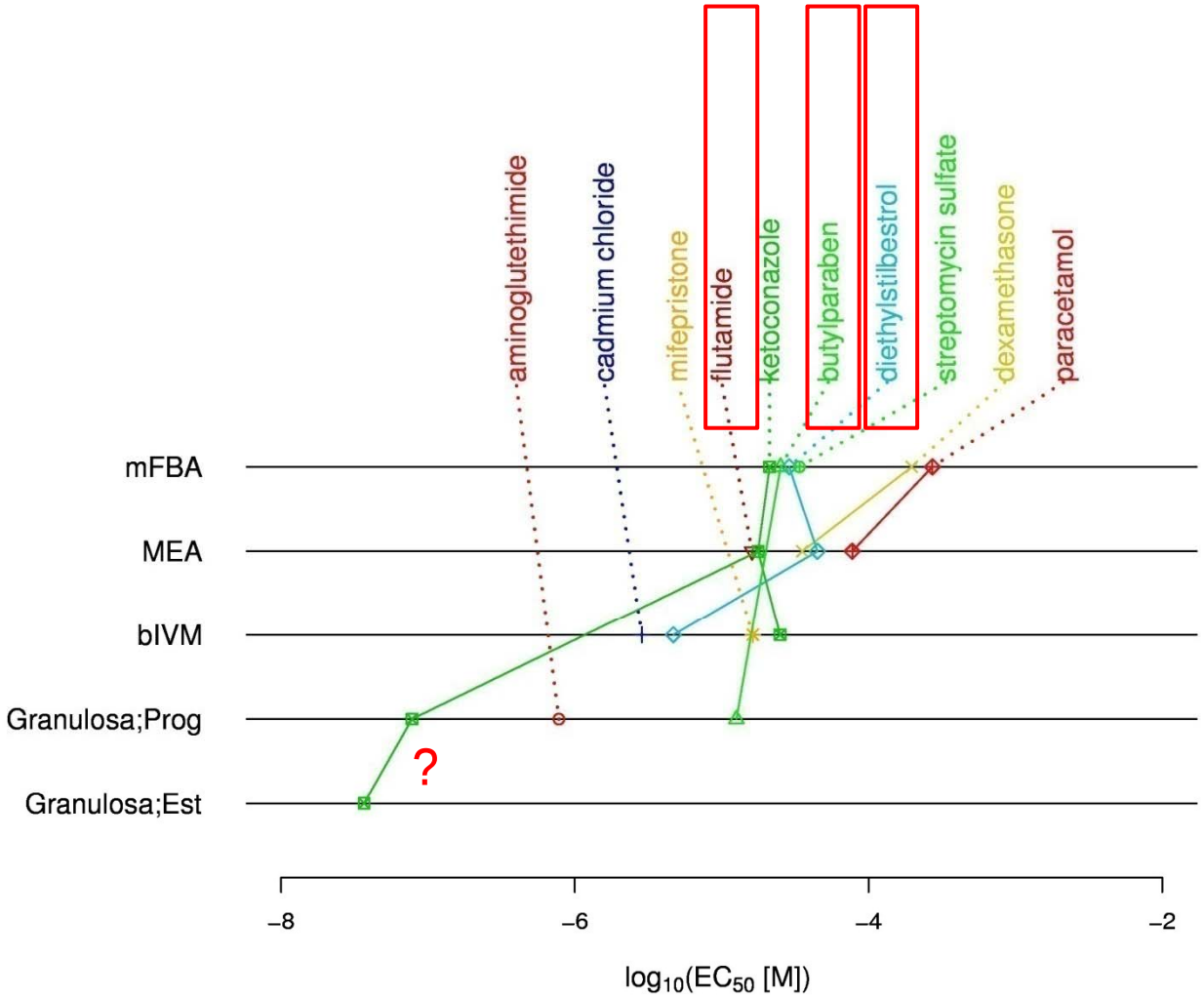

Female Fertility

Example II

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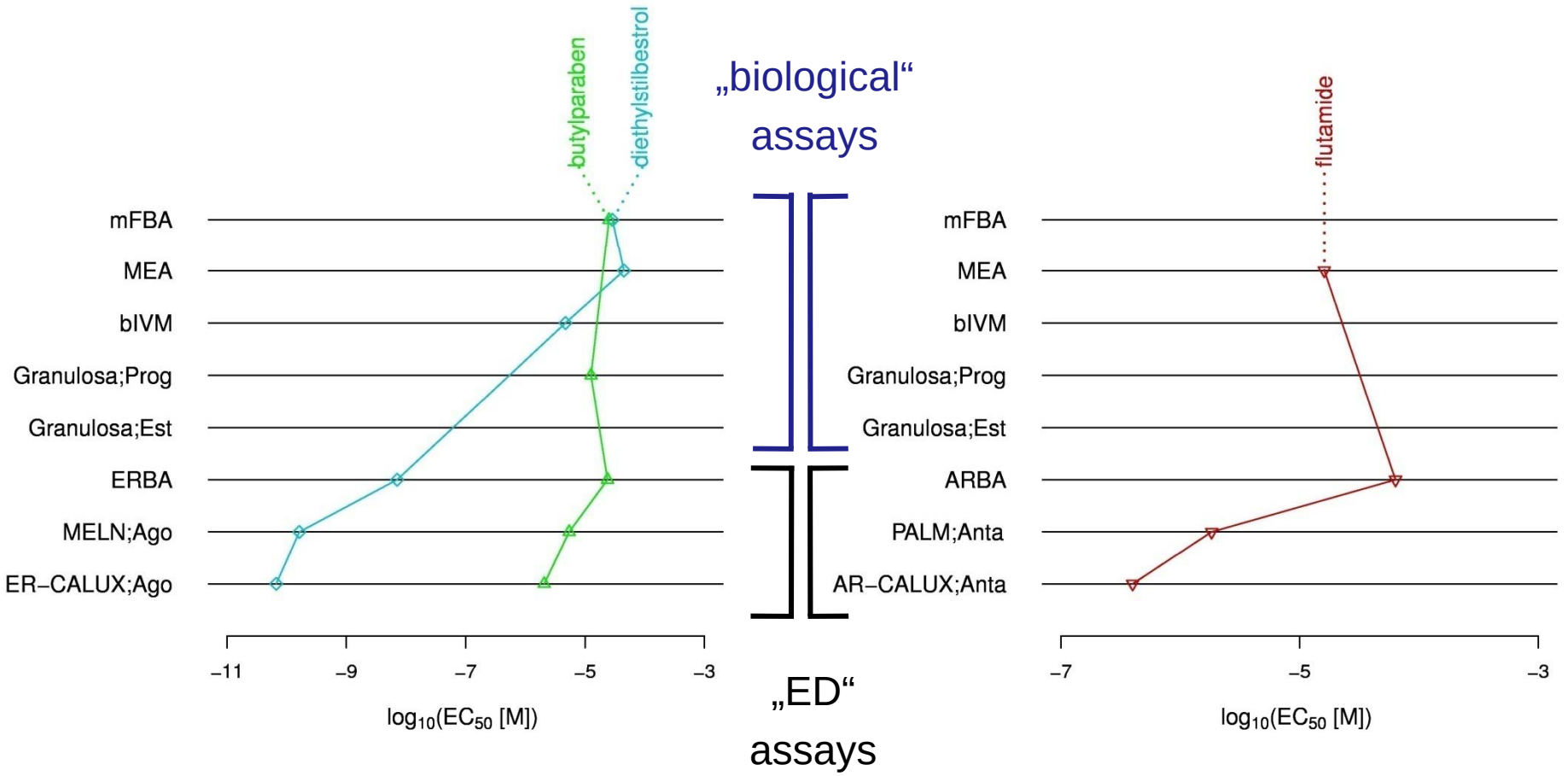

The ReProTect database

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Server: localhost ▸ Datenbank: ReProTect

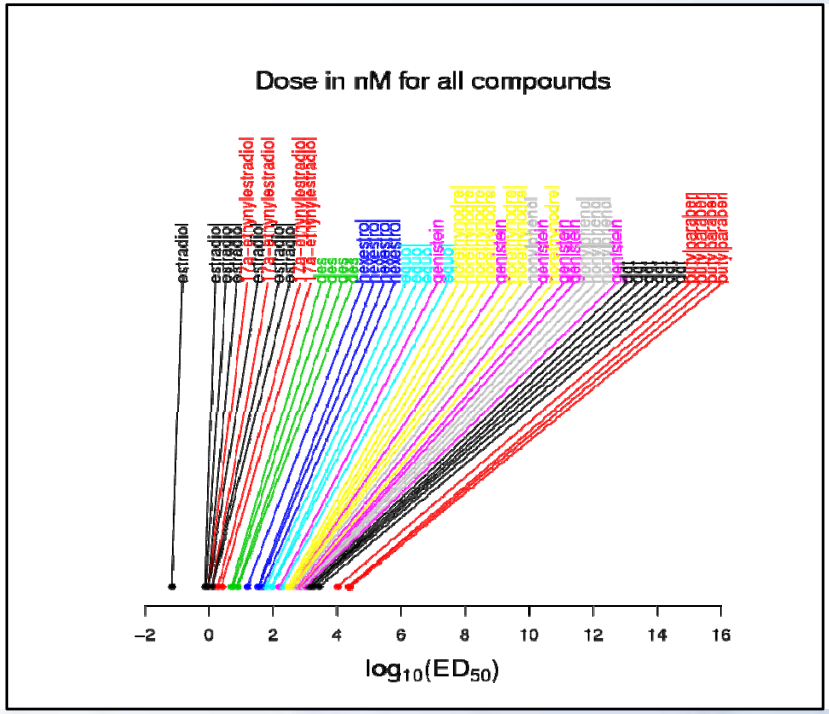
Struktur | SQL | Suche | Abfrageeditor | Exportieren | Importieren | Operationen | Rechte | Löschen

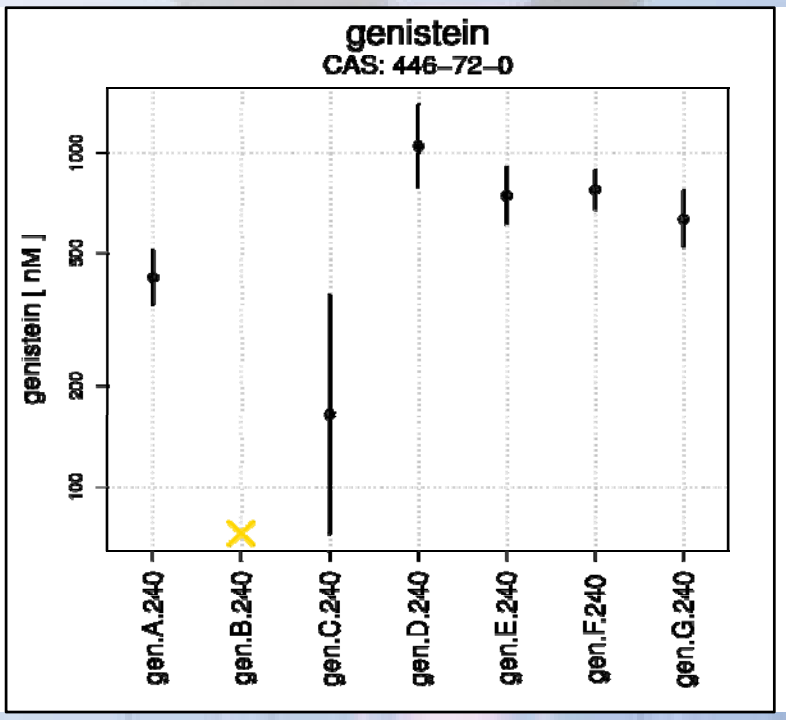
	Tabelle	Aktion	Einträge	Typ	Kollation	Größe	Überhang
☐	Assay		~28	InnoDB	utf8_general_ci	48,0 KiB	-
☐	CompoundHarm		~128	InnoDB	utf8_general_ci	64,0 KiB	-
☐	CompoundInd		~155	InnoDB	utf8_general_ci	32,0 KiB	-
☐	DoseUnit		~6	InnoDB	utf8_general_ci	32,0 KiB	-
☐	ED50		~890	InnoDB	utf8_general_ci	96,0 KiB	-
☐	Endpoint		~25	InnoDB	utf8_general_ci	32,0 KiB	-
☐	Lab		~15	InnoDB	utf8_general_ci	16,0 KiB	-
☐	Run		~1,522	InnoDB	utf8_general_ci	336,0 KiB	-
☐	Target		~7	InnoDB	utf8_general_ci	16,0 KiB	-
☐	Treatment		~338	InnoDB	utf8_general_ci	96,0 KiB	-
☐	WarningsHarm		~8	InnoDB	utf8_general_ci	32,0 KiB	-
☐	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

The ReProTect Feasibility Study

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10 <u>blinded</u> test chemicals tested in a test battery

Selection by independent experts out of the ~130 ReProTect chemicals.

Selection criteria:

- ***In vivo* effects well characterized**
- **No metabolic activation (CYP450-mediated) required**

Test #

Chemical #	1	2	3	4	5	6	7	8	9	10
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

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).

Acknowledgements

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

This project was granted by the European Commission under contract number LSHB-CT-2004-503257

I thank you for your attention