Reproductive toxicology in preclinical safety

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Outline

- Normal reproduction: why do we need special testing?
- How does this relate to clinical testing?
- Animal models
- How do we do preclinical testing?
- What is the outcome of our work?

Why is preclinical repro testing important?

- Function of reproductive system is not tested in standard pre-clinical studies
- Micropathology may point towards adverse effects upon testes/ovaries and reproductive accessory organs
- Individuals can live without reproducing
- Populations cannot survive without individuals reproducing

Human repro testing

- There are no follow-up tests in man to check on reproductive function
- Effects seen in man are likely to be retrospective and may not be detected until considerable damage has been done - **Thalidomide**
- Human reproductive processes are considerably less efficient than in animal models

Pre-clinical testing– what are we looking for?

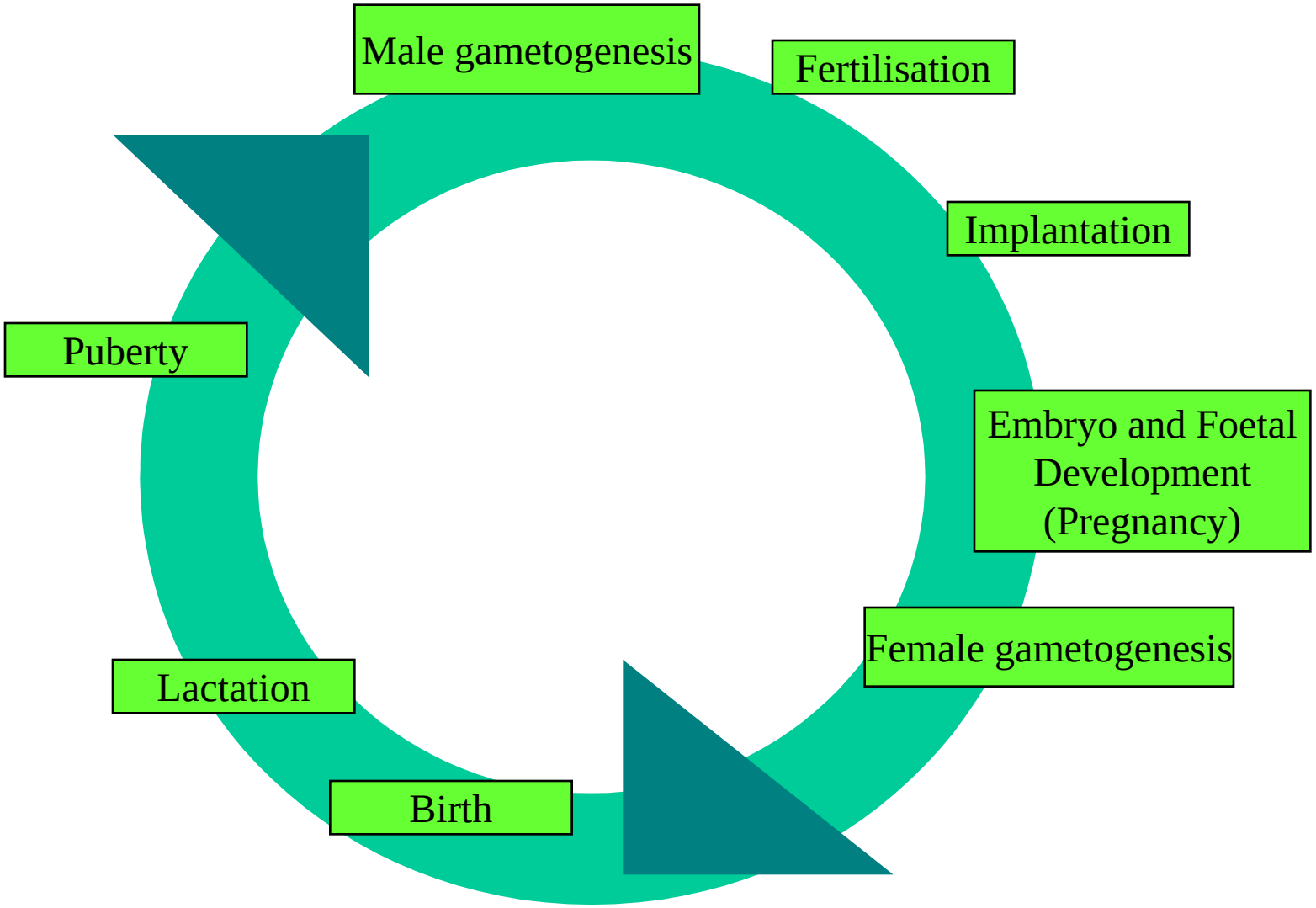
- Adverse effects of treatment
- Dose relationships
- Evidence of different sensitivity of reproductive organ systems compared to other systems
- Differential effects in mother and babies
- Safe “NOAEL” values

Critical study data outcome 6

Probably treatment related	Probably adverse
Dose response or effect in high dose only #	General function affected
Homogenous data	Primary effect
Precise data	Persistant
Biological and statistical concordance	Above concern levels
Values outside historical ranges	Multiple parameters affected
Consistent with expected effects	Not adaptive
	Seen in other models

- may have threshold effects, not all dose relationships are continuous, max effect may not be at max dose

The reproductive cycle



History of reproductive testing

- Short- not considered as a separate area until the 60's when it became clear that test materials could cross the placenta and damage the fetus - **Thalidomide**

How to test?

- Chemicals/agrochemicals tend to test to cover all phases at once – multigeneration study
- But – pharmaceuticals divide the cycle into small areas so that effects can be related to projected duration of treatment and outcome on specific areas of fertility can be assessed

Hitting the target

- General toxicity studies are unlikely to detect damage to reproductive function, but may identify damage to the testes and ovaries
- Functional testing requires specific study designs to focus on critical areas of reproduction

Perspective on fetal abnormality

- 3% human newborns detected as having a congenital abnormality
- one-third of these potentially lethal
- examination as get older suggest 6% of humans have abnormalities
- only 10% of the abnormalities believed to be related to teratogenic agents
- of 1200 compounds listed as teratogenic in animals only 40 proven in man (Shepherd 1995)

A reminder: why is preclinial repro testing special?

- Unlike testing in most preclinical areas there are NO follow-up tests in man to assess effects upon reproduction
- Reproduction is a moving target and requires special studies to assess effects at specific points in the reproductive cycle.

Pharmaceutical clinical trials

Phase I	Human pharmacology studies - very limited numbers, often in volunteers, may be in patients
Phase II	Therapeutic exploratory studies - limited numbers of patients - proof of concept
Phase III	Therapeutic confirmatory studies - larger numbers of patients to confirm safety and efficacy

Timing of repro studies for pharmaceuticals

Study type	Sex/status	EU	USA	Japan
Before Phase I/II	F - childbearing age	**Embryo-fetal** studies- 2 species	Can be included in trials if use contraception	Embryo-fetal studies- 2 species Female fertility study (women to use contraception)
	F - non childbearing potential	Can be included in trials	Can be included in trials	Can be included in trials
	M	No specific repro testing - histo data from tox studies	No specific repro testing - histo data from tox studies	Fertility often performed or histo data from tox studies
Before Phase III	F - childbearing age	**Female fertility** study	Embryo fetal - 2 species Female fertility study (women to use contraception)	
	M	**Fertility study**	Fertility study	Fertility study
Before marketing approval	F - childbearing age	**Pre and post natal study**	Pre and post natal study	Pre and post natal study

ICH = International Conference on Harmonisation - for medicinal products

- The European Community
- Japan
- The United States
- Meetings started 1991 and implemented in all three regions by 1995
- Studies performed to ICH guidelines should be acceptable in all regions

If we don't spot it – what chance have you?

- Pre-clinical testing
  - Well established animal models
  - Background control data
  - Targeted treatment periods
  - Specific end points
  - Animal tests use high doses
- Epidemiology
  - collects what it can find – sifts through the data and tries to establish links

Available animal models

- **Rat**
- **Rabbit**
- **Mouse**
- Minipig, ferret, sheep, primate, dog

Compare - Man/rat/rabbit

	Man	Rat	Rabbit
Size	70 kg	0.4kg	4 kg
Nutrition/ enzymes	Omnivore	Omnivore	Herbivore + coprophagy
Life span	70 years	2 years	7 years

Compare - Man/rat/rabbit

	Man	Rat	Rabbit
Cycle/ ovulation	Menstrual Sex at any time	Oestrous Sex at oestrus	Reflex ovulator sex at any time
Babies	One at a time	Lots at a time	Lots at a time
Fertility	Low	High	High

ICH guidance on dose level selection states:

- Dosage levels should be selected to provide information on a dose-response relationship, including a toxic dose and a no-observed-adverse-effect-level (NOAEL)

Regulatory requirements JMAFF

‘ the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering. In case of mortality this should not be more than approximately 10% in the parental animals’.

Regulatory requirements EPA/OECD

The middle dose should produce minimal but observable toxicity and the lowest dose level will be a no effect level

‘In a study which demonstrates an absence of toxic effects, further investigations to establish absorption and bioavailability of the test material should be considered’.

Tests for medicines

- 6 areas of repro recognised

  A - Pre-mating to conception - gametes, reproductive behaviour, fertilisation

  B - Conception to implantation

  C - Implantation to closure of hard palate - organogenesis

  D - Closure of hard palate to birth - organ maturation

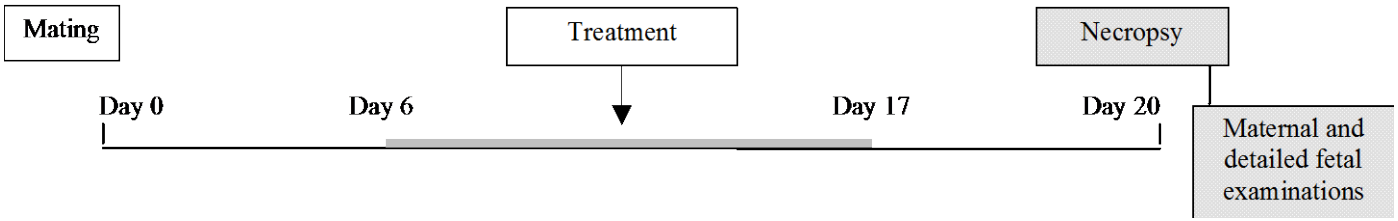
  E - Birth to weaning - function maturation

  F - Weaning to sexual maturity

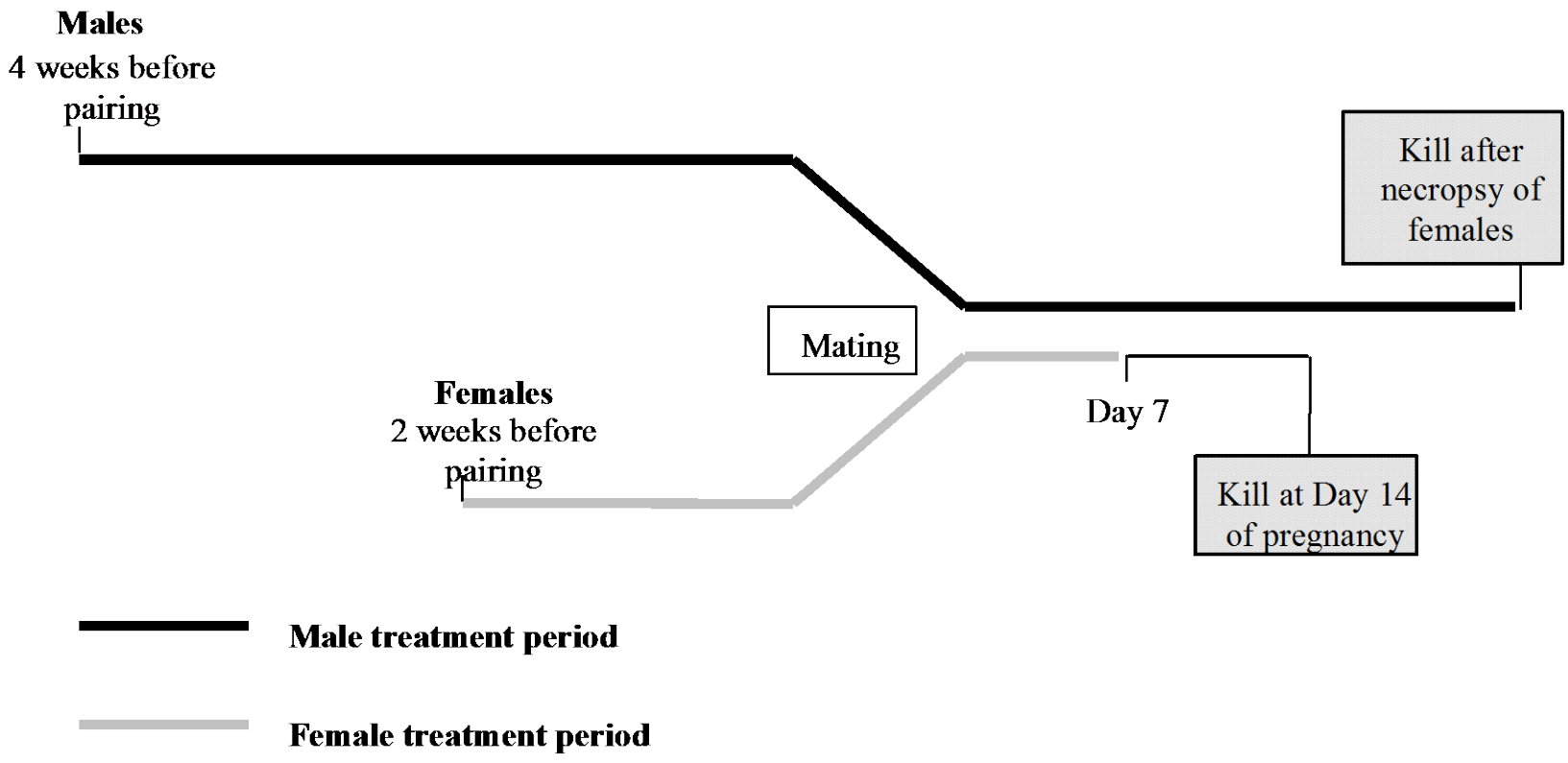
Basic tests for “pharma”

- Fertility - “SEGMENT I” covers phases A and B, both sexes combined or assessed separately
- Embryo-fetal - “SEGMENT II” covers phase C
- Pre- and post-natal study - “SEGMENT III” covers phases C through to F
- (Juvenile toxicity – direct post partum effects)

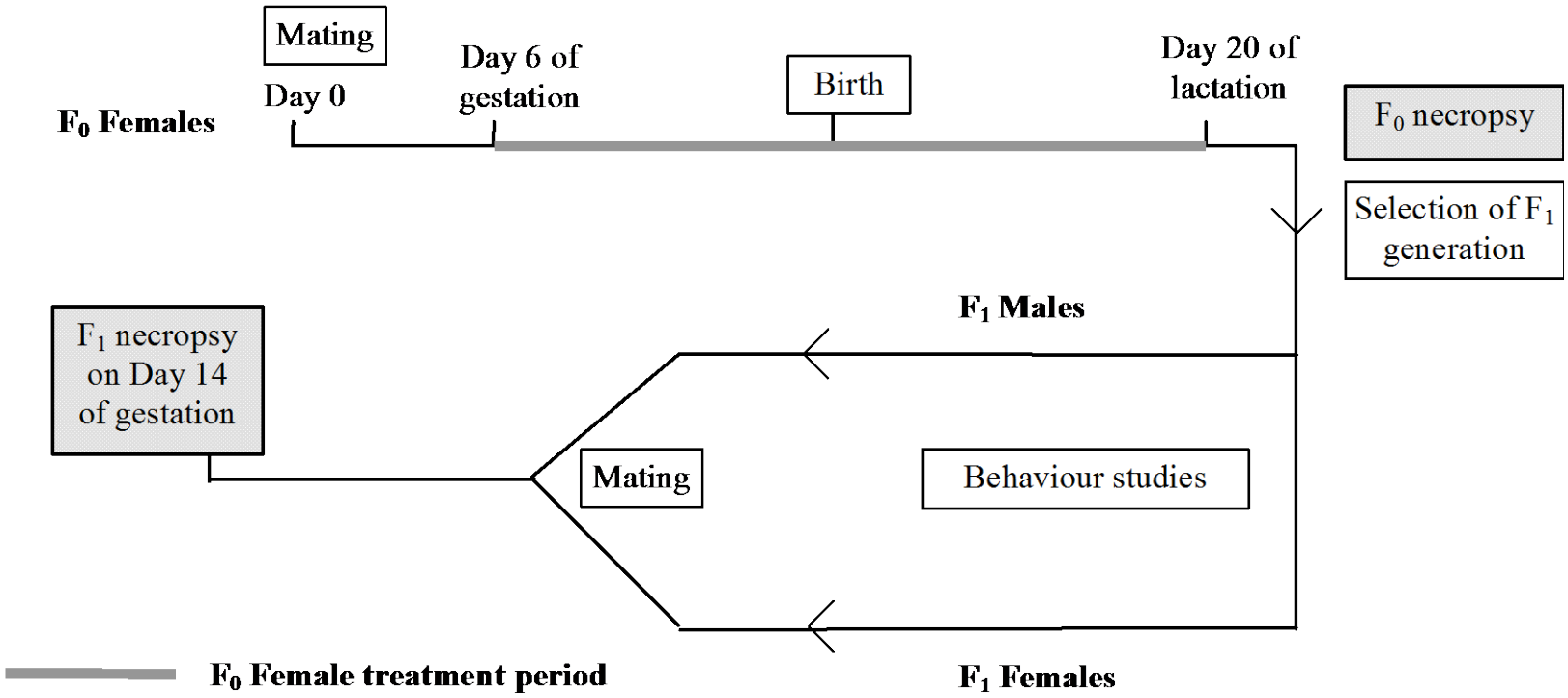
ICH embryo fetal study



ICH fertility study



ICH pre- and post-natal study



Juvenile toxicity studies

A blank screen

Treatment period and end points depends on drug type and planned human usage

Endpoints may include

Toxicokinetics at different ages

Bodyweight and bone growth

All general toxicity parameters

Behaviour and learning

Reproductive performance

Recovery assessment

How many animals to use?

- “Enough!” to reliably demonstrate effects occurring at a frequency considered to be biologically significant
- History and usage generally requires 20+ animals per group for reproductive studies
- For Embryo-fetal and littering studies “n” is the number of mothers, not the number of babies

Power to detect change

- Depends on basic data type
  - Nominal –Yes/No response, a not = b
  - Ordinal – data can be ranked, a<b<c
  - Interval – interval between ranks constant, 1,2,3
- And on the variation in data within the group
- Reproductive studies contain all types of data and from precise measures such as ovulation rates to inexact measures in behavioural assessment

Effects of variability on statistical power

Mean	Sd	CV	Difference	% difference	Group size for 80% power
12.0	1.2	10	0.6	5	48
12.0	1.2	10	1.2	10	24
12.0	1.2	10	2.4	20	12
12.0	1.8	15	0.6	5	72
12.0	1.8	15	1.2	10	36
12.0	1.8	15	2.4	20	18
12.0	2.4	20	0.6	5	96
12.0	2.4	20	1.2	10	48
12.0	2.4	20	2.4	20	24

Statistical power to detect fetal changes

Parameter	Change	Power (%)
13/14 ribs	50% > 60%	16
	50% > 75%	64
cleft palate	1%> 2%	11
	1%> 5%	84
Based on 20 litters per group, binomial data, Chi squared test at 5%		

Conclusions

- Reproductive toxicology aims to predict effects of medicines and chemicals on the ability of man to reproduce by assessing effects in animals
- Study designs divide the total process of reproduction in to a series of manageable units which can provide answers to specific challenges to the reproductive processes
- No real prospects of replacing animals in these studies.