ICHS6(R1): Reproductive Toxicity

Options to address reproductive toxicity with biotechnology-derived products: appropriate study design and data interpretation

Assessment of Fertility and Early Embryonic Development

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Outline of presentation

- Overview of session on reproductive toxicity
- J Sims
  - Overview of ICH S6 (R1) guidance on reproductive toxicity
  - Assessment of effects on fertility and early embryonic development (FEED) and timing aspects
  - Outstanding issues to resolve
- P Martin
  - Placental transfer and developmental toxicity

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ICH S5: Assessment of toxicity to reproduction

A. Premating to conception (adult male and female reproductive functions, development and maturation of gametes, mating behavior, fertilisation).

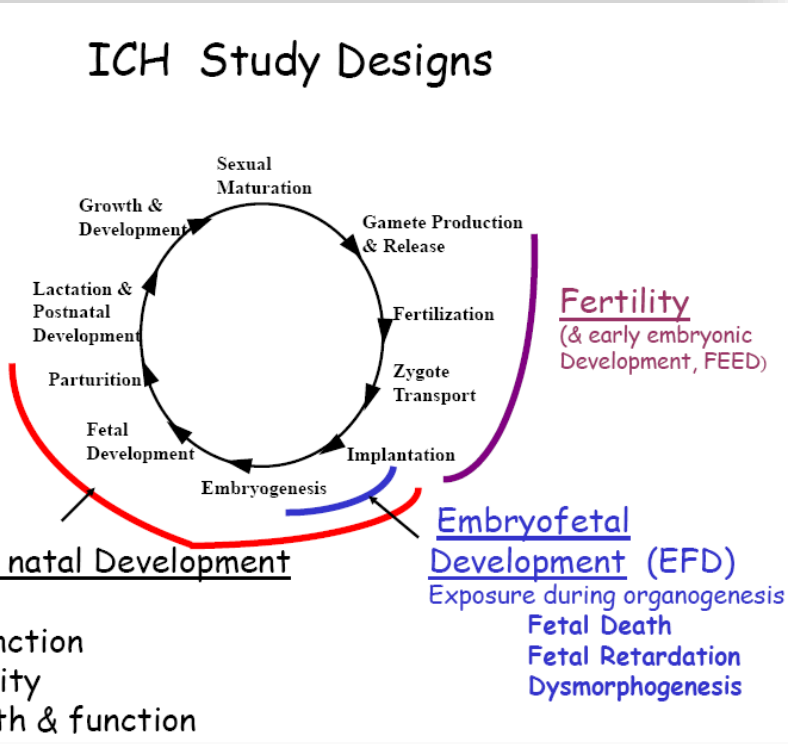
B. Conception to implantation (adult female reproductive functions, preimplantation development, implantation).

C. Implantation to closure of the hard palate (adult female reproductive functions, embryonic development, major organ formation).

D. Closure of the hard palate to the end of pregnancy (adult female reproductive functions, fetal development and growth, organ development and growth).

E. Birth to weaning (adult female reproductive functions, neonate adaptation to extrauterine life, preweaning development and growth).

F. Weaning to sexual maturity (postweaning development and growth, adaptation to independent life, attainment of full sexual function).



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ICH S6(R1): Reproductive/developmental toxicity

- General principles
  - Reproductive toxicity studies should be conducted in accordance with ICH S5(R2)
  - But specific study design and dosing schedule can be modified as appropriate
  - Preference for studies with the clinical candidate
  - Evaluation of reprotox should be conducted in pharmacologically relevant species
  - If there are >1 relevant species, one species is sufficient under defined conditions (no preference for rodent or non-rodent)
  - If pharmacologically active only in NHP still preference for clinical candidate, but possibility for alternative approaches.
  - No relevant species – consider alternative approaches



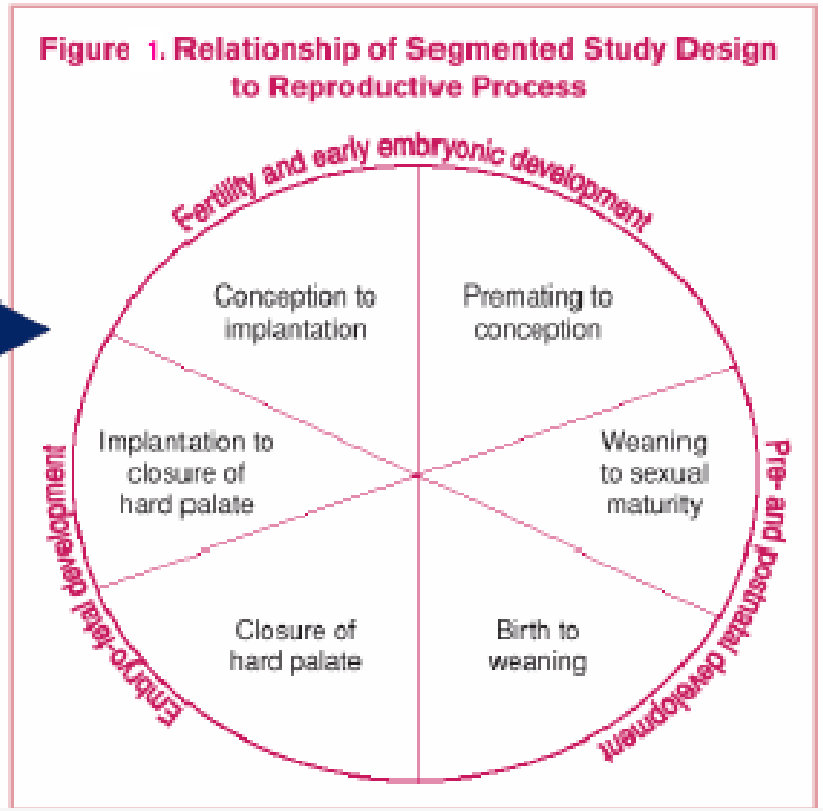

Limitations of Non Human Primate Model

Nonhuman Primate Model

Conception to Implantation Segment is not easily covered

F1/F2 generation study:
- Cynomolgus: **5- 8 yrs**
- Marmoset: **~ 4 yrs**

Prenatal loss



- Mating with females is an insensitive means of detecting effects on spermatogenesis
- Consideration for mating studies with non human primates
  - Litter size is one for macaques (twin rate approx 0.1%)
  - Fertility rate is 20-50% for macaques

Reaching consensus regarding assessment of fertility: discussions along the way...

- Male fertility
- Female fertility
- Timing

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ICH S6(R1): Reproductive/developmental toxicity

- Fertility
  - Can be assessed by mating studies in rodents (if relevant species)
  - It is recognised that mating studies are not practical for non human primate (NHP)
  - When the NHP is the only relevant species, the potential for effects on male and female fertility can be assessed by evaluation of the reproductive tract (organ weights and histological examination) in repeat dose toxicity studies of at least 3 months duration using sexually mature NHPs."
    - agreement on histopath
  - Further evaluations (menstrual cyclicity, sperm count / morphology / motility, reproductive hormones) when cause for concern exists
  - Data to be provided to support Phase III clinical trials

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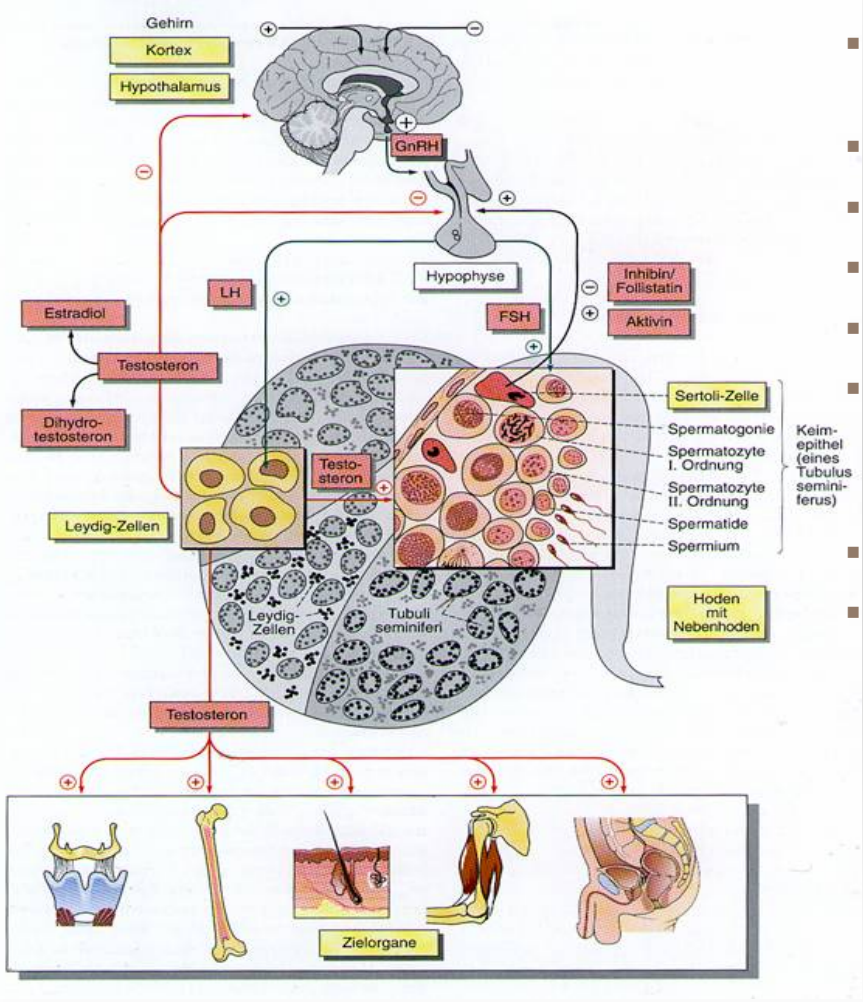
Male Reproductive System: Available techniques in cynomolgus match human



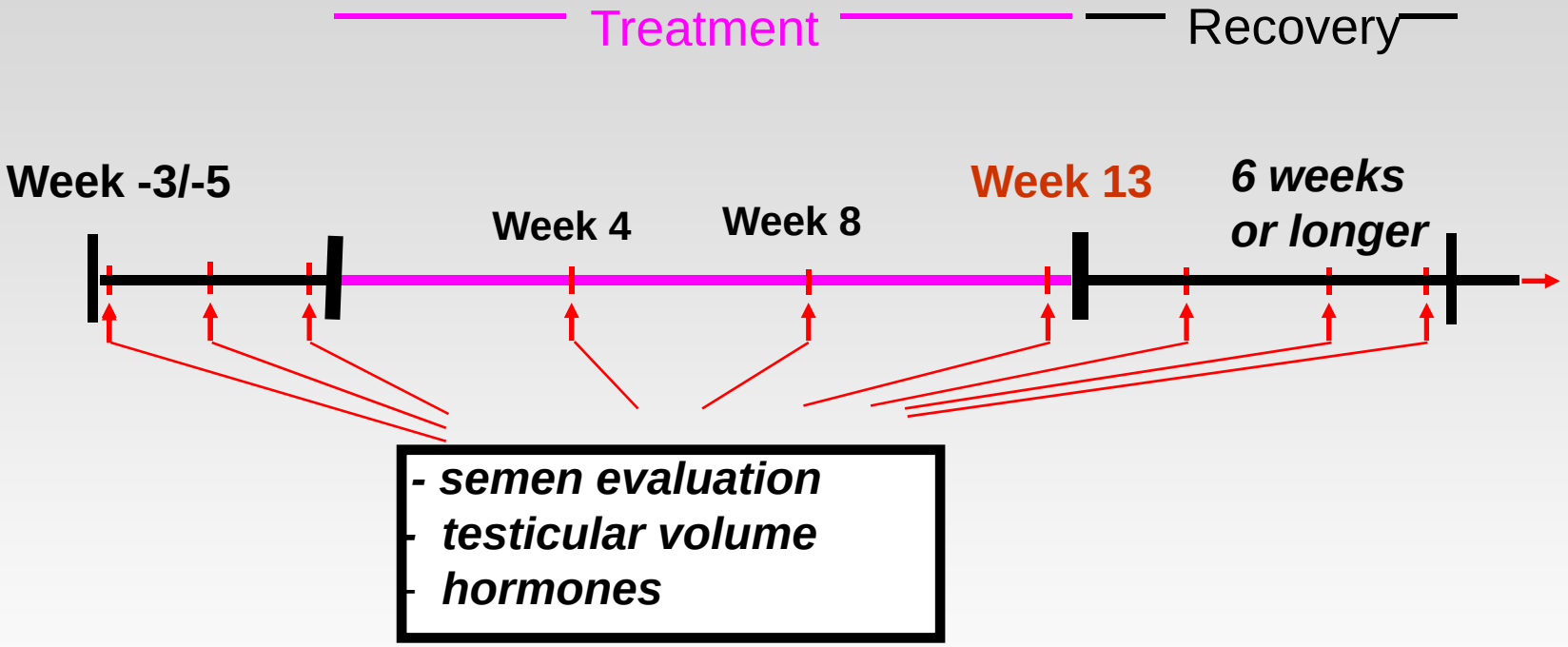
- **Spermatogenesis** (histology, spermatogenic stages, flow cytometry)
- **Sperm maturation** (epididymis)
- **Semen parameters** (WHO based)
- **Testis size** (caliper, ultrasound)
- **Testis biopsy**
- **Endocrinology**

  (LH, FSH, prolactin, estrogens, DHT, progesterone, androgens, inhibin B, SHBG)
- **Prostate status** (volume, uroflow)
- **Fertility** (mating)

Slide provided by Covance

(Covance) Standard Design: Male Fertility Study in the Cynomolgus Can combine with 3 or 6 month general toxicity study in sexually mature animals

Predose



Duration of spermatogenesis from stem cell to sperm is 40 days (Weinbauer et al (1998), Aslam et al (1999): Recommended duration of treatment is 13 weeks, i.e. at least twice the period required to produce sperm from stem cells.

Slide provided by Covance

MALE Fertility Assessment In Cynomolgus Monkeys

- Majority of male reproductive toxicants cause adverse effects on gonads and / or accessory sex organs
- ***Most sensitive endpoints are testicular & epididymal weights and histopathology using sexually mature animals***
  - *Male cynomolgus monkeys are considered to be* ***reproductively mature*** *if they are older than 5 years of age and weigh more than 4.5 kg, and if in addition testicular volume is greater than 10 (20) ml, testicular weight is > 10 g, and their testosterone level at least 1-10 ng/ml (Novartis definition of sexually mature agreed with CROs)*

- Add longitudinal sperm analysis (plus endocrine measurements) into combined 3 / 6 month general toxicity studies in sexually mature animals provides adequate screening for male reproductive toxicants
  - ***current view is only when cause for concern exists***

- Libido: can assess presence of masturbatory ejaculations

- **Does not assess conception or embryofetal viability**

BUT unlikely to have a male reproductive toxicant which does not provide signal from above parameters.

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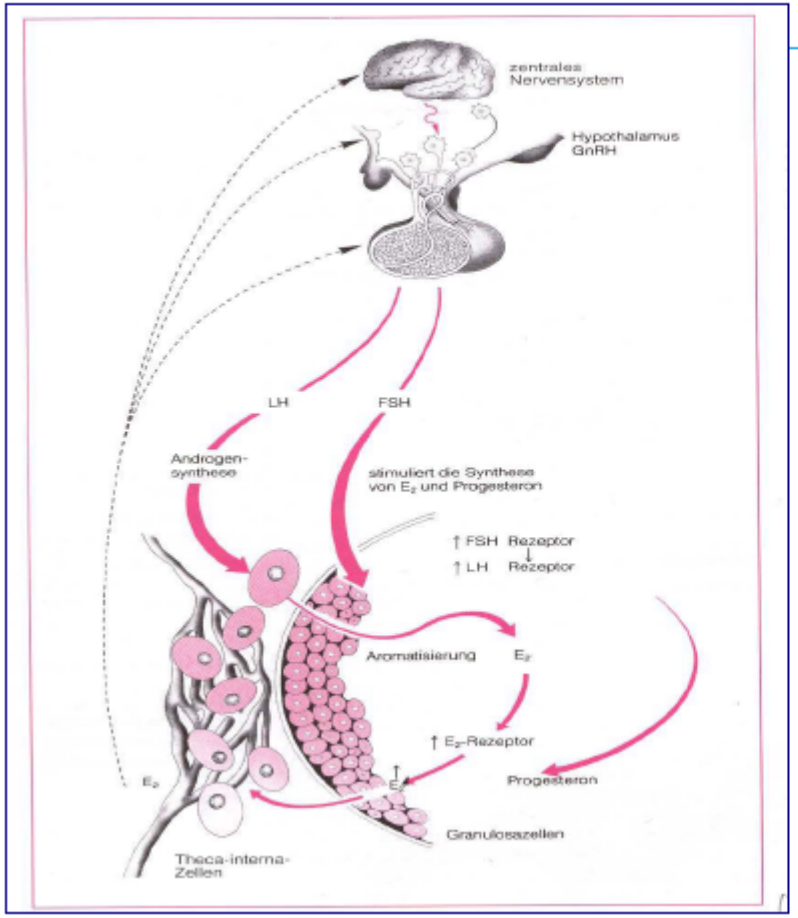
Female Reproductive System: Available techniques in cynomolgus match human

Female Reproductive System



- **Oogenesis** (histology)
- **Ovarian cyclicity** (28 – 30 days) (regular, irregular, amenorrhea)
- **Endometrial changes (biopsies, ultrasound)**
- **Ultrasound examination** (follicular growth, cysts)
- **Endocrinology** (LH, FSH, CG, prolactin, estrogens, progesterone, androgens, inhibin A & B)

Slide provided by Covance

FEMALE Fertility Assessment In Cynomolgus Monkeys

- Sexually mature animals
  - ***female sexual maturity*** *has been reached if the individual female cynomolgus monkey is older than 3years of age, at least 2.5 kg in body weight and has shown at least 3 consecutive regular menstrual cycles (Novartis working definition agreed with CROs)*
- Histopathology of female reproductive tract probably not as sensitive as histopathology of testes at detecting problems with gamete production
- 1 month study may not be adequate to detect ovarian changes
- Menstrual cycle tracking can be done in studies >3 months duration

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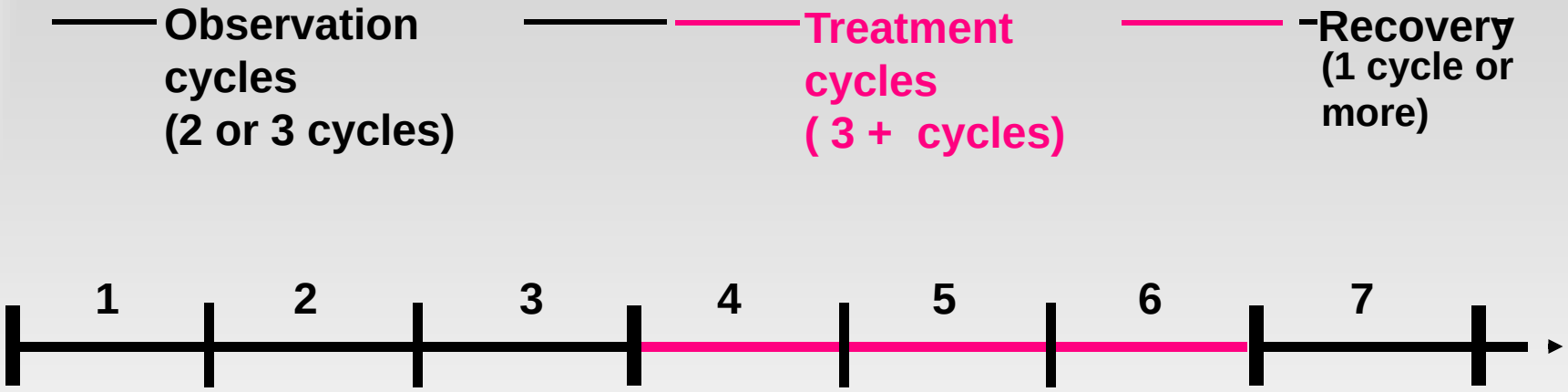
Female fertility endpoints within chronic toxicity study



Regular cycler selection ≈ 3 months, *(from established groups)*

- **Daily Vaginal smears**

Menstruation pattern shows normal cycling

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Slide adapted from Covance slide

Menstrual Cycle data v Endocrine monitoring

- Enright et al (2009) BDR 86:29-39
  Comparative effects of Interferon alpha-2b and pegylated interferon alpha-2b on menstrual cycles and ovarian hormones in cynomolgus monkeys

- n=7 per group, tracked for 1 cycle pre-dose, 23 doses every other day, then tracked for another 60 days. Blood samples every 3rd day.

- IFN: Lengthened menstrual cycles
  82+/-7.4 versus 31 +/- 1.3 days
- Delay in reaching peak E and lowered peak E
  - **Time to peak 34 versus 13.5 days**
- Delay in reaching peak P and lowered peak P
  - **Time to peak 32 v 22.5 days**

- Menstruation pattern – sensitive, simple - **ideal apical screen BUT...**

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Issues with menstrual cycle as a fertility endpoint in sexually mature monkey toxicity studies

- What is the practicality of measuring cycling in the context of repeat dose studies >3 months duration
  - Variability of users in calculating menstrual cycles
    - Need to establish a standard method to calculate menstrual cycle
  - Variability of monkeys in presenting menstrual cycles
    - Irregular cycling / spotting makes cycles difficult to calculate
    - Effect of housing conditions (single vs pair-housing)
  - Length of time necessary to establish baseline menstrual cycle
    - Pre-study procedures can affect menstrual cycles e.g. transportation, change in cage-mate
    - Recommended 3-6 months pre-dose (or 3 months post-randomisation)
  - Interpretation of drug effects on menstrual cycle
    - Y/N or prolonged / shortened cycle or specific cycle duration or statistics on frequency of monkeys outside normal range...
- Does cycling add information beyond histopathology?

Summary: It is Difficult to Conclude if Menstrual Cycle or Histology is Most Sensitive Endpoint

Agent	Menstrual Cycle	Histology of Female Reproductive Organs
PEGASYS® IFN-A(2a)	1-mo menstrual cycle study Menstrual cycle changes	3-mo tox study No findings
Rebif® IFN-B(1a)	Menstrual cycle evaluation as part of 6-mo tox study No changes in menstrual cycle	6-mo tox study No findings
PEG-Intron® IFN-A(2b)	1-mo menstrual cycle study Menstrual cycle changes	1-mo tox study No findings
Betaseron® IFN-B(1b)	3-mo menstrual cycle study No changes in menstrual cycle	1-mo tox study No findings
Avastin® VEGF Ab	Menstrual cycle evaluation as part of 6-mo tox study Menstrual cycle changes at high dose	6-mo tox study Effects on ovary and uterus at low, mid and high dose
Vectibix® EGF Ab	3-4 mo Fertility study Menstrual cycle prolonged (or amenorrhea) at low, mid and high dose (secondary to poor clinical condition/stress)	6-mo tox study No findings

Jeanine Bussiere, Amgen

Female Fertility Assessment In Cynomolgus

- Assessments in repeat dose toxicity studies not informative about conception, tubal transport, implantation.
- ***Situation no different from small molecules which are not pharmacologically relevant in rodent…..***
- "…..A homologous product or transgenic model could be the only practical means to assess potential effects on conception or implantation when those are of specific concern. However, it is not recommended to produce a homologous product or transgenic model solely to conduct mating studies in rodents"

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ICH S6 (R1): Issues for further discussion at EWG meeting in Japan, November 2010

- One or two species for EFD studies
  - Are there some examples where a rabbit has additional value over the rat?
  - Examples to be presented if available for discussion of impact
- Revised enhanced PPND design (combining EFD And PPND)

  Still not resolved: "Developmental toxicity studies in non human primates can only provide hazard identification. The number of animals per group should be sufficient to allow meaningful interpretation of the data as described in ICH S5 (see Note 4)."

  - Number of animals per group 12-20
  - Level of reassurance (detection of 5-fold or 3-fold increase in hazard)
  - Number of groups 1 or 2 dosed groups
- Note 3 (endpoints in pups and treatment after delivery) needs further discussion.

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Thank You