Outline

- What sort of compounds may affect fertility?
- What areas of reproduction are we assessing in this test?
- How do we do preclinical testing?
- What is the outcome of our work?
- How does this relate to clinical testing?
Agents affecting male fertility

- Steroids - affect feedback control
- Alkylating agents - affect spermatogenesis
- Tranquillisers - affect sexual performance
Agents affecting female reproduction

- Ethanol: affects puberty/cycling by activity on ovary/pituitary feedback
- Estrogens/progesterone: affect gamete transport
- Prostaglandins: affect uterine environment
- Aminogluthethimide: affects steroidogenic enzymes
- Tranquillisers: affect maternal behaviour/lactation
What areas are we looking at?

- Estrous cycles and mating behaviour
- Implantation
- Early embryonic survival
- Male reproductive organ weights
- (CASA – computer assisted sperm assessment)
ICH - fertility study

**Males**
4 weeks before pairing

**Females**
2 weeks before pairing

**Mating**

**Day 7**

**Kill at Day 14 of pregnancy**

**Kill after necropsy of females**

**Male treatment period**

**Female treatment period**
Treatment period

- Males minimum 2 weeks - only effects on epididymal sperm detected at mating but look for testicular effects histopathologically after longer period
- Recommend 4 weeks but may extend to 10 weeks if general toxicity studies suggest effects upon male reproductive system
Fertility study endpoints

- **Estrous cycle:** the female rat normally has a 4-day cycle between ovulations with no luteal phase after ovulation.

- A luteal phase may be induced (pseudopregnancy) by sterile mating or stimulation of the vaginal cervix.

- The vaginal stimulation is also needed to convince the rat that pregnancy should occur and to allow implantation.
# Cell types and estrous staging – Rat

<table>
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<th>Stage</th>
<th>Code</th>
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<th>Large epithelial</th>
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Estrous cycle smears

D4: Estrus

D1: Metestrus

D3: Proestrus

D2: Diestrus
# Cycle classification

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<th>Female number</th>
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<th>Day of pairing</th>
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<th>Cycle classification</th>
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X = Mated  IC = Irregular Cycle  Ac = Acyclic  AP = Acyclic during pairing  EE = Extended estrus
Cycles/ovulation affected

- Longer cycles
- Irregular cycles
- Continuous diestrus
  (pseudopregnancy?)
- Continuous estrus
- Reduced numbers of corpora lutea
- (increased numbers of corpora lutea)
Mating

- Takes place overnight, starting before ovulation as the cycle moves from Proestrus to Estrus
Mating behaviour

Pre-ejaculatory intromissions

- Initiate the neuroendocrine reflex resulting in elevate progesterone levels – allows implantation and pregnancy (if mating fertile).
- Important for trans-cervical sperm transport into uterus.
- If female receives ejaculation without preceding intromissions, won’t be pregnant.
- If intromissions occur too soon after ejaculation, e.g. from rival male, will disrupt sperm transport (2nd mating male will sire more pups).
- Time interval between intromissions also critical.
Mating behaviour

- Successful mating depends on a complex set of physical and behavioural interactions between male and female – needs more than just good sperm and eggs.
- If only one plug, females have reduced pregnancy rate, and some females go acyclic without mating evidence when paired after E (intromissions without ejaculation)
- Repeated ejaculation improves pregnancy rate and inhibits mating by a rival males which can disrupt 1st male’s sperm transport.
Relevance to humans

Obvious differences in female reproductive physiology and behaviour BUT there are:
- Several common cross-species mechanisms regulating hypothalamic-pituitary-ovarian axis, especially those mediated by estrogen receptors.

AND
- Estrous cycles very sensitive indicator for female rodent reproductive toxicity involving changes in hormone levels – better than attempting to measure hormone levels?!
Mating evidence – the morning after

- Copulation plugs (on tray and sometimes in vagina), sperm in smear, cycle stage and smear appearance.
- Plug formed by secretions from seminal vesicles and coagulatory gland – stays in vagina until it falls out naturally or is dislodged by further mating.
- Absence of sperm doesn’t mean female hasn’t mated and single plug easily missed.
- Conversely, tray plug(s) can occur without mating, and sperm contamination of smears can occur accidentally.
Aftermath for the male

- Once mated, the male’s role in the fertility study is over, but usual to continue treatment until female necropsy confirms fertility.
- Males may then be necropsied for examination of reproductive organs or remated with untreated females to check if effects related to male or female.
Computer Assisted Sperm Assessment (CASA)

- An useful adjunct to studies on male fertility
- Allows assessment of
  - sperm motility
  - sperm numbers in the epididymis
  - homogenisation resistant spermatids in the testis
- (Sperm morphology by microscopy)
Sperm tracks as recorded on screen
Rat spermatozoon
Mating affected

- Reduced numbers of copulation plugs
- Reduced sperm numbers
- Missed mating opportunities
- Low numbers of pregnancies
Sperm transport and fertilisation affected

- No direct measure in screening studies
  – may be inferred from outcome
Counts of implantation sites compared to corpora lutea counts
Conclusions

- Fertility testing in animals can provide valuable insights into possible effects that might occur in man.
- BUT – man is inherently much less fertile than the rat.
- Care needed to determine if effects attributable to male or female.
- No real prospects of replacing animals in these studies.