



# Fertility and early embryonic development

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# 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

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- Steroids - affect feedback control
- Alkylating agents - affect spermatogenesis
- Tranquillisers - affect sexual performance

# Agents affecting female reproduction

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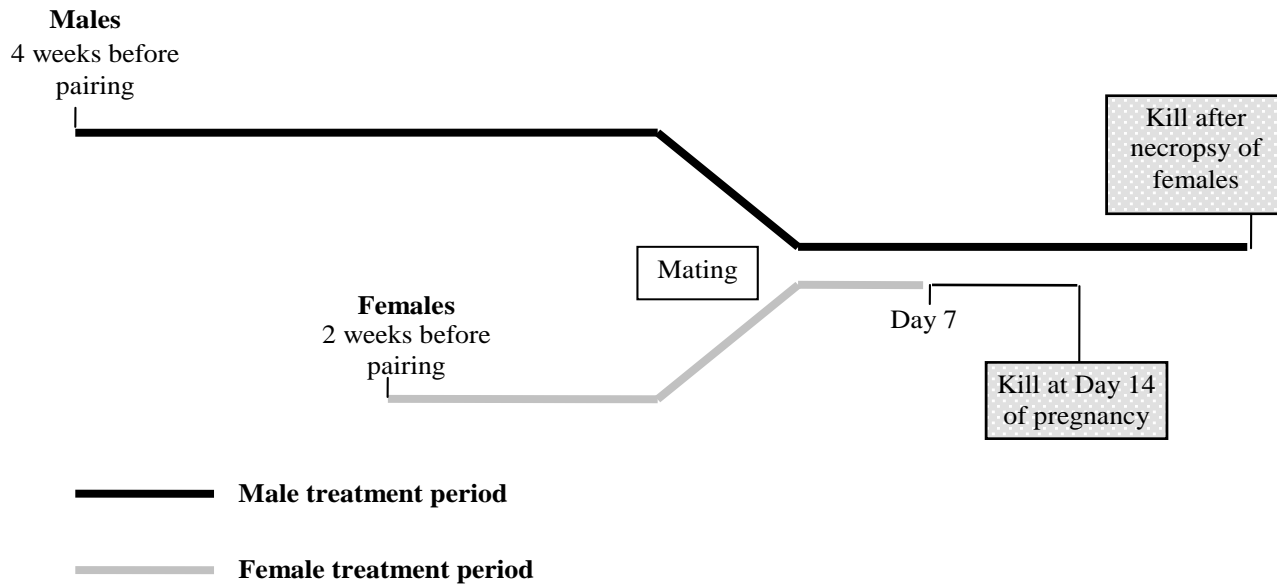
- Ethanol: affects puberty/cycling by activity on ovary/pituitary feedback
- Estrogens/progesterone: affect gamete transport
- Prostaglandins: affect uterine environment
- Aminoglutethimide: affects steroidogenic enzymes
- Tranquillisers: affect maternal behaviour/lactation

# What areas are we looking at?

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

# ICH - fertility study



# Treatment period

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- 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

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- **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

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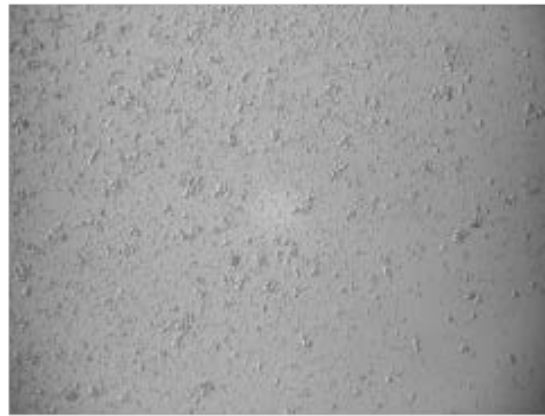
Stage	Code	Cell types				Duration
		Leucocytes	Nucleated epithelial	Large epithelial	Fully cornified epithelial	Hrs
	e/m	Y			Y	
<b>Met-estrus</b>	<b>M</b>	YYY		Y		<b>20</b>
	m/d	yy			y	
<b>Di-estrus</b>	<b>D</b>	Y(Y)		Y		<b>36</b>
	d/p	Y	Y			
<b>Pro-estrus</b>	<b>P</b>		Y(Y)			<b>10</b>
	p/e		Y	Y	Y	
<b>Estrus</b>	<b>E</b>				YYY	<b>30</b>

# Estrous cycle smears

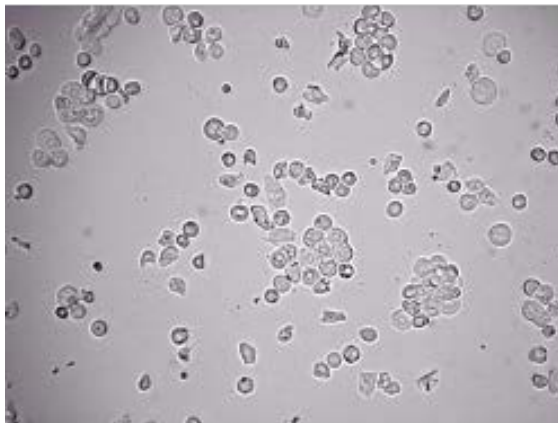
D4: Estrus



D1: Metestrus



D3: Proestrus



D2: Diestrus



# Cycle classification

Female number	Day of smearing										Day of pairing				Cycle lengths	Cycle classification
	1	2	3	4	5	6	7	8	9	10	1	2	3	4		
1	E	M	D	P	E	M	D	P	E	M	D	P	X		4,4,4	4
2	E	E	M	D	P	E	E	M	D	P	E	X			5,5	5
3	E	M	D	P	E	M	D	P	E	E	M	D	P	X	4,5,4	4/5
4	E	M	D	D	D	P	E	M	D	P	X				6,4	IC
5	E	M	D	D	D	D	D	D	D	D	D	P	X		12	Ac
6	E	E	E	E	E	M	D	E	E	E	X				N/A	EE
7	E	M	D	P	E	M	D	P	E	E	M	D	D	D	4,5,12	4/5 (AP)

X = Mated IC = Irregular Cycle Ac = Acyclic AP = Acyclic during pairing EE = Extended estrus

# Cycles/ovulation affected

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- 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

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## 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 (2<sup>nd</sup> mating male will sire more pups).
- Time interval between intromissions also critical.

# Mating behaviour

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- 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 1<sup>st</sup> male's sperm transport.

# Relevance to humans

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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

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- 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

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- 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

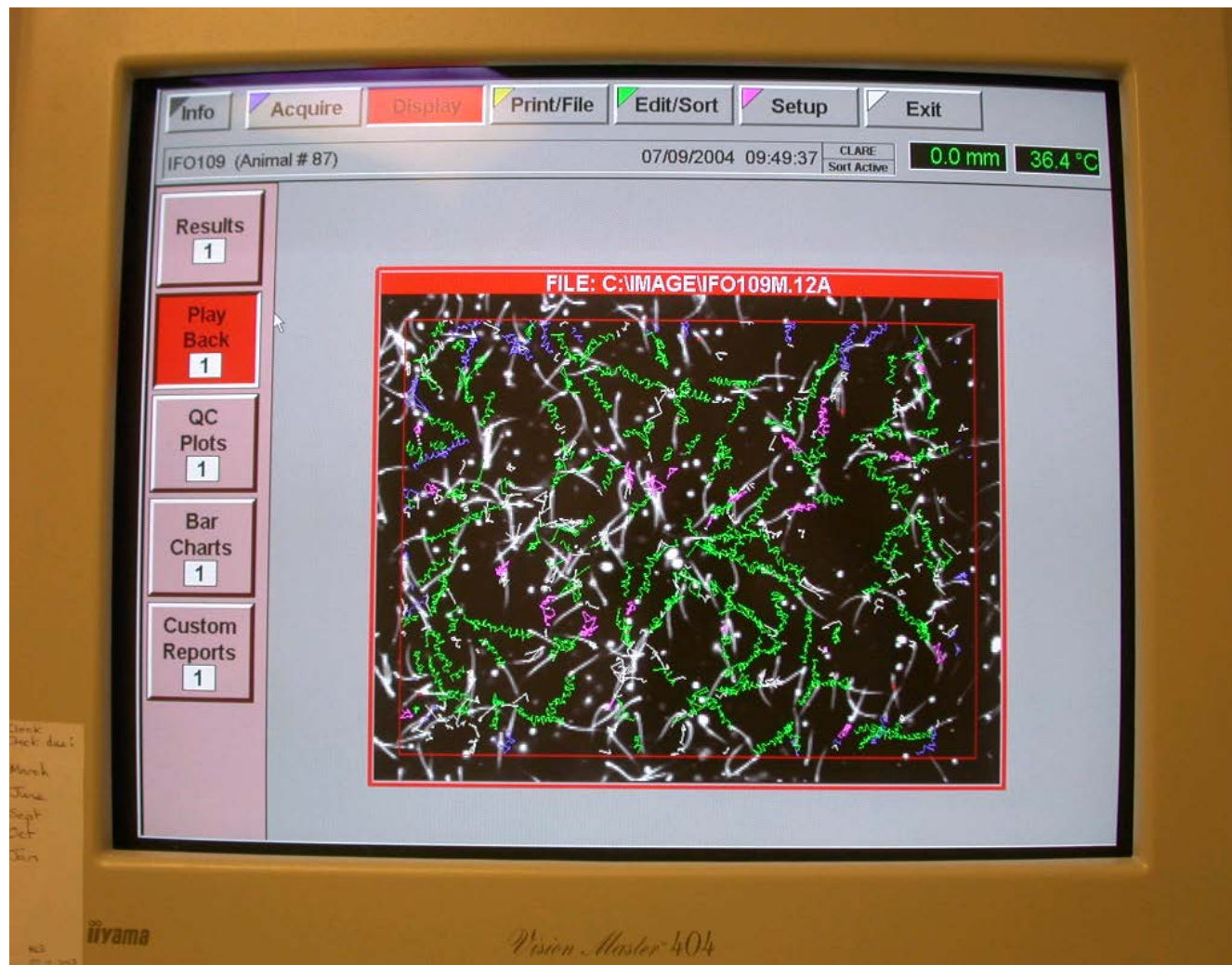
# Computer Assisted Sperm Assessment (CASA)

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- 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

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# Rat spermatozoon



# Mating affected

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- Reduced numbers of copulation plugs
- Reduced sperm numbers
- Missed mating opportunities
- Low numbers of pregnancies

# Sperm transport and fertilisation affected

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- No direct measure in screening studies
  - may be inferred from outcome

# Implantation and early embryonic survival

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- Counts of implantation sites compared to corpora lutea counts



# Conclusions

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- 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.