The Dog as Alternative Species in Developmental Toxicology Studies

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Introduction

- Rodents (rats and mice) and rabbits are traditional animal models for use in developmental toxicity studies
- These animals models have short gestation period, large litter size, and are easy to breed
- However, in some cases traditional animal models may not be relevant for various reasons
- Alternative animal models are needed

Introduction -continue

- There is a wide variety of species from which the toxicologist may choose (background data, biological characteristics, cost, sensitivity, etc.).
- The dog is the primary nonrodent species used in toxicology
- Understanding of laboratory animals' biology, and physiology is important
- As with any animal model, dogs must be shown to be relevant species (i.e., drug metabolism and pharmacology must be determined) prior to initiating development toxicity study

Dog as an Alternative Species for Dev Tox

- There are references in the literature for the use of dogs as an animal model for developmental Toxicology (Robertson et al. 1979).

- It is common practice to use beagle
- Beagle has some advantages:
    - Medium size
    - Adaptability to group housing
    - Easy handling
    - Extensive knowledge on the reproductive physiology
- Beagle has several advantages:
    - Smaller litter size compared to rodents
    - Expensive
    - Need exercise
    - Special housing requirements
    - Variable body weight and size
    - Natural tendency to vomit
    - Require large amount of test material
    - Ethical issues

Robertson RT, Allen HL,and Bokelman DL. Aspirin: teratogenic evaluation in the dog. Teratology. 1979 Oct;20(2):313-20.

Dogs as an Alternative Animal Species in Dev Tox

- Reasons to why beagle dogs may be used as an alternative species in Developmental & reproductive testing:
  - When the compound is not tolerated in rats and rabbits
  - When the metabolism in rats and rabbits is completely different from that in humans
- Dogs are not used as a second species for developmental toxicity testing; they are only used in circumstances where no alternative methods are available
- Although safety testing in preclinical studies represents one of the major uses of dogs, in reality rarely compounds are tested in dogs for developmental toxicity

Physiology of the Ovarian Cycle in the Bitch

- **Puberty:**
- In most bitches begins at **6 to 9** months of age.

- **Dogs are monoestrous: The** Interval from cycle to cycle varies among breeds

  Average: **7 Months**
  Range: **4 to 13 Months**

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Sexual Cycle in Dogs

Cycle Stage	Length	Hormonal changes	Behavior of the Bitch	Clinical Signs
Proestrus	~9 days	Estrogen	Attractive to the male, but won't stand for mating	Vulvar edema and swelling, bloody discharge
Estrus	~9 days	Progesterone	Accepts male and will stand	Less edema, discharge becomes clearer
Diestrus	~60 days	Progesterone	Ceases to accept male	Little discharge, Edema decreased
Anestrus	70-80 days for CL to regress and 130days to repair endometrium.	Progesterone	No outward signs	Scanty, tenacious secretions

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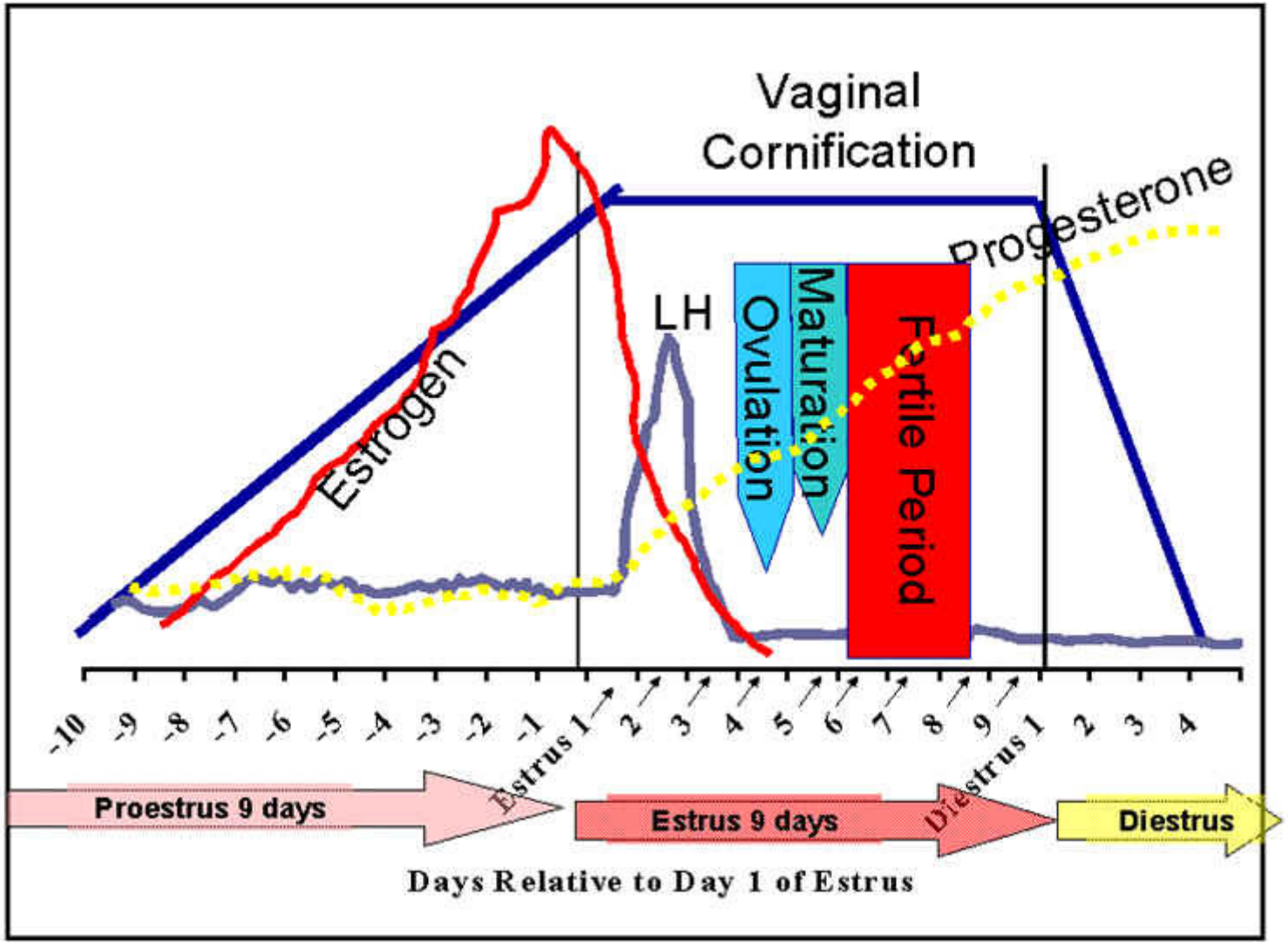
A Canine Cycle



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Ovulation

Ova are released from the follicles approximately two to three days following initiation of LH surge

Fertilization:

Ovulated ova are not ready for fertilization (must complete Meiosis I) and therefore are not fertilized until Day 4 to 7 following the LH surge

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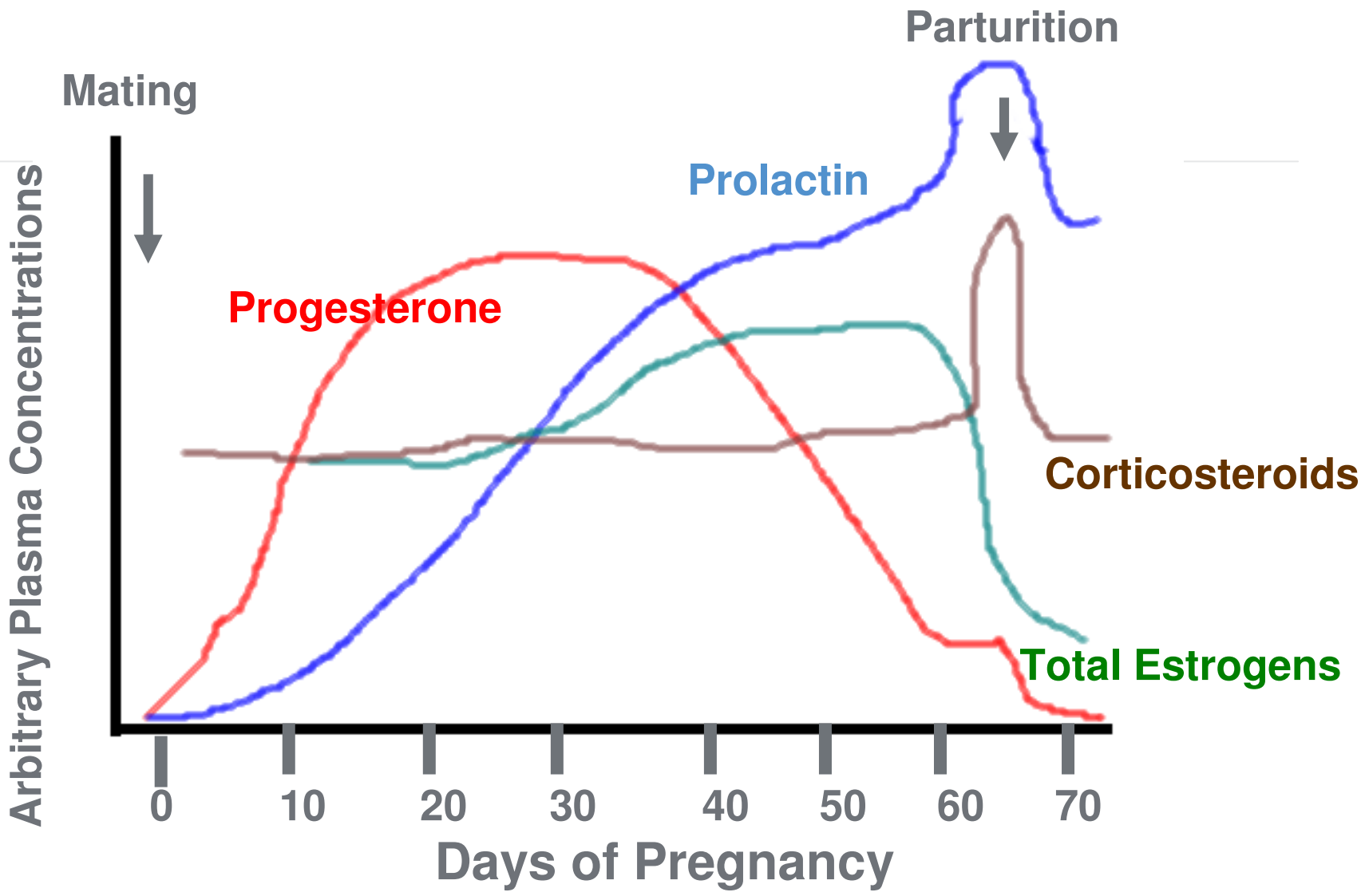
Hormone Concentrations During Pregnancy and Parturition



http://www.avs.uidaho.edu/avs-222/powerpoint/Dog%20Reproduction.ppt#257,1,Slide 14

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Species Comparison of some Reproductive Physiology

Species	Puberty	Cycle Length	Estrus Length
Mouse	28 – 36 days	4 – 5 days	Approx. 13 hours
Rat	33 – 44 days	4 – 5 days	Approx. 14 hours
Rabbit	90 – 120 days	15 – 17 days (variable)	Ovulation after mating
Minipig	140 – 170 days	21 – 22 days	3 days
Monkey	2.5 – 3 years	30 – 37 days	–
Dog	6 – 12 months	18 – 20 days	4 – 13 days

Modified from Jorgensen (1998)

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Species Comparisons of some Reproductive Physiology

Species	Blastocyst Formation	Implantation	Organogenesis Period	Length of Gestation
Mouse	3 – 4	4 – 5	6 – 15	19
Rat	3 – 4	5 – 6	6 – 15	22
Rabbit	3 – 4	7 – 8	6 – 18	33
Minipig	5 – 6	11 – 13	11 – 35	114
Cynomolgus monkeys	5 – 7	9 – 11	20 – 50	164
Dog	-	17 – 19	18 – 35	62 – 64
Human	5 – 8	8 – 13	21 – 56	267

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Comparison of key Female Reproductive Parameters in Dogs and Minipigs

	Dog	Minipig
Sexual maturation (months)	6- 12	5
Reproductive cycle	Mono-estrus	Poly-estrus
Gestation period (Days)	62 – 64	112- 114
Period of organogenesis	18-35	11-35
C-section Day	GD 57	GD 110-112
Mean Pregnancy rate (%)	77%	80%
Mean litter size (n)	5-6	5-6
Fetal weight (g)	202- 230	350-400
Background incidence of malformations/ variations (%)	Low birth defect rates	4*`

*n= 2504 fetuses examined with 106 abnormal fetuses
Note: minipig data obtained from Jorgensen, 1998

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Breeding Procedure

- The bitch is exposed to a male dog (stud) in the studs' cage on or before the eighth day after a dam is identified as being in proestrus, (the day the dam is noted in pro-estrus is considered day 1)

- If a locked mating is not observed, the bitch is exposed daily Monday through Sunday, until a lock is observed, or until physical examination proves her to be out of estrus.
- If a locked mating does not occur after one day of placement with a stud and physical examination indicates that the dam is still in estrus, a new stud may be used.
- If the dam and her assigned stud appear to be incompatible (i.e., fight or exhibit a lack of interest), another stud may be selected.

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Breeding Procedure- cont.

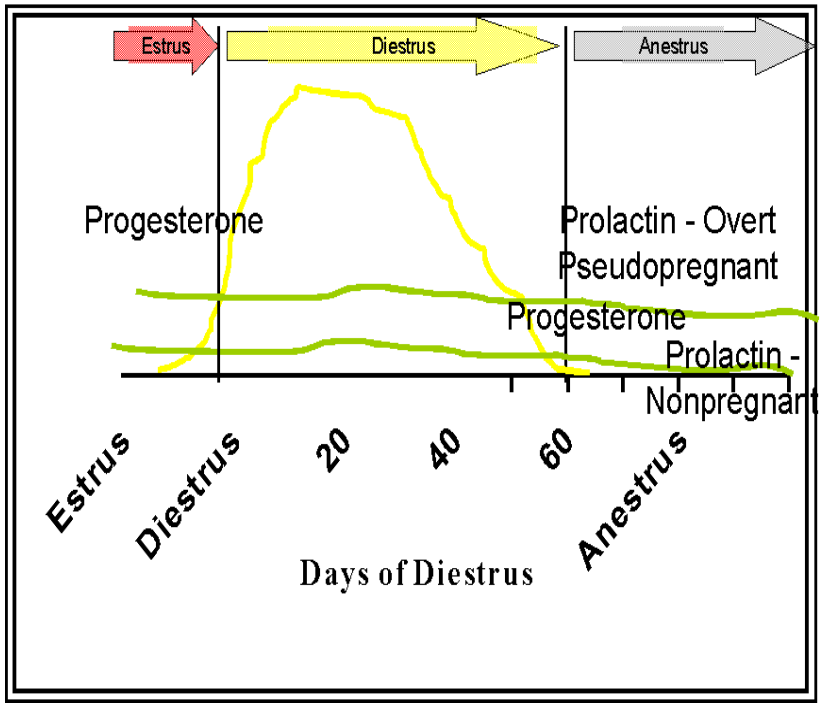
- After the first mating (locked mating as observed by breeding tech), the dam is removed from the stud's cage and returned to her cage.
- Approximately 48 hours after the first mating, the dam is again placed with the same stud.
- If second locked mating is observed, approximately 48 hours later, the dam is again placed with the same stud for the third mating.
- The dam is continually placed with same stud until 3rd locked mating is observed or until physical signs of estrus has ceased.
- After third positive mating, the dam is considered pregnant and the date of the first mating is recorded as Gestation Day 0.

Pregnancy Determination

- There are no practical blood or urine tests available to confirm pregnancy in the dog

- The earliest reliable and definitive method is ultrasound detection of fetal heartbeats, typically visible by Day 25.

Canine Pseudopregnancy



- Pseudo pregnancy, or pseudocyesis, is a normal physiologic process that occurs in female dogs.

- It is seen 45-60 days after a normal estrous (heat) period

- It occurs when progesterone levels, which rise after ovulation, begin to fall

- Decreasing progesterone leads to an increase of the hormone prolactin

Female will have nest building behavior and enlarge mammary glands**.**

•Prolactin is responsible for most of the behaviors seen during a pseudopregnancy episode

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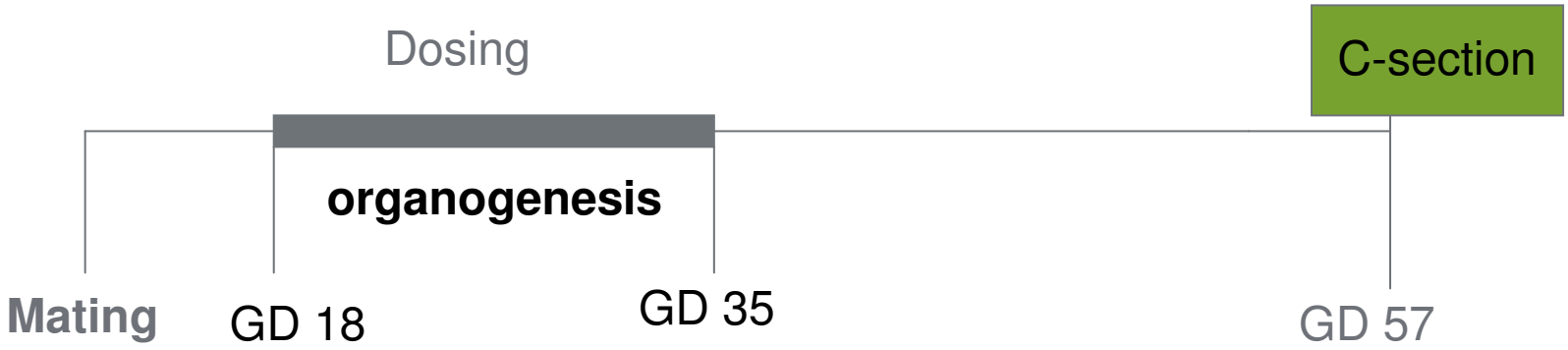
Embryo-fetal Development Study in Canine
Study Design



- Age: 2 - 5 years
- BW: 8.0 -12.0 kg

Fetal Evaluation:
External, Visceral, and
Skeletal

= Dosing period

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PREGNANCY DATA

No. of Studies	3
Number of fetuses evaluated	184
No. of Females on Study	33
No. Pregnant	28
No. Not Pregnant	5
No. Died Pregnant	0
No. Abortions	0
No. Early Deliveries	0
No. Females with All Resorptions	0
No. Females with Viable Fetuses	28

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UTERINE DATA

Parameter	Mean	Range of Values		
Pregnancy Index (%)	77.53	66.7	-	90.9
Corpora Lutea (Mean No. per Animal)	6.97	6.5	-	7.6
Implantation Sites (Mean No. per Animal)	6.53	6.0	-	6.9
Pre-implantation Loss (Mean % per Animal)	6.170	2.08	-	9.29
Viable Fetuses (Mean No. per Animal)	6.23	5.5	-	6.7
Fetal Sex Ratio (Mean % Males per Animal)	51.13	40.8	-	61.7
Post-implantation Loss (Mean % Implants per Animal)	4.08	1.8	-	8.3
Nonviable Fetuses (Mean No. per Animal)	0.00	0.0	-	0.0
Litter Size (Mean No. per Animal)	6.23	5.5	-	6.7
Resorptions: Early and Late (Mean No. per Animal)	0.30	0.2	-	0.5
Resorptions: Early (Mean No. per Animal)	0.27	0.1	-	0.5
Resorptions: Late (Mean No. per Animal)	0.03	0.0	-	0.1
Mean Fetal Body Weight - Males (grams)	221.04	205.3	-	240.0
Mean Fetal Body Weight - Females (grams)	205.66	201.1	-	211.8
Mean Fetal Body Weight - Total (grams)	215.59	202.2	-	230.2

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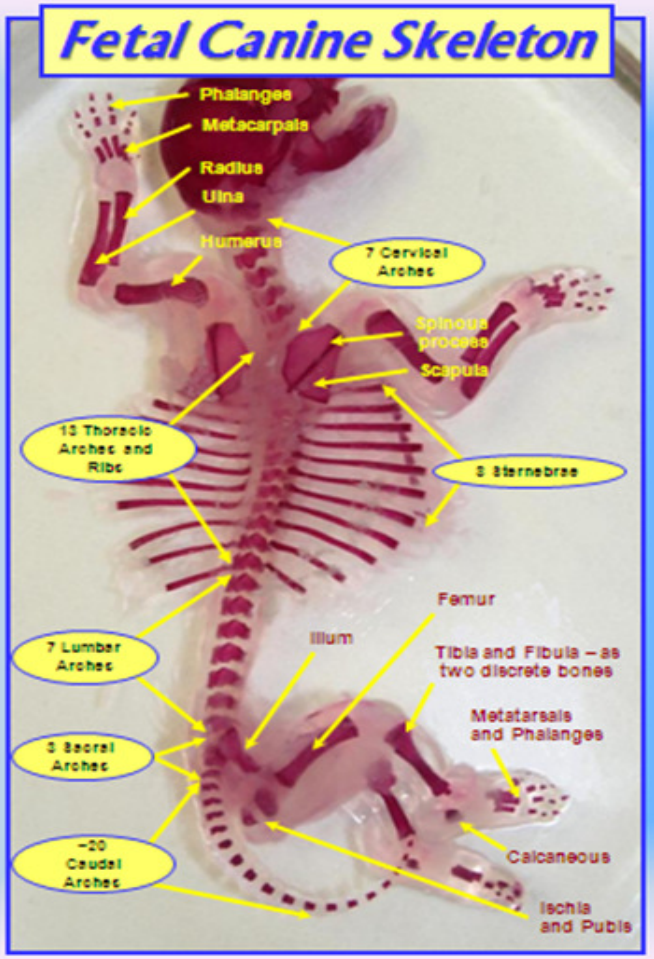
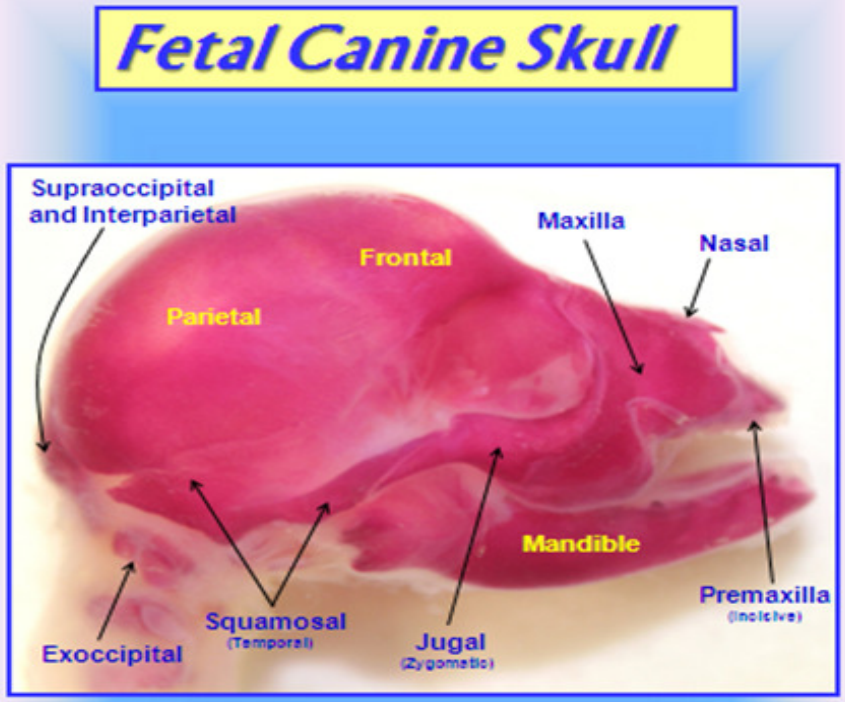
Skeletal Evaluation





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Resorptions or Abortions?

- Resorptions of partial or entire litters can occur with little or no evidence between days 28 and 36 in apparently healthy and otherwise normal pregnant bitches.
- Resorptions and abortions after day 36 with some related vaginal discharges can occur without detection when bitches lick away or ingest discharged materials.
- Resorption likely occurs in 10-15 % of pregnancies.
- Many instances of resorption are proceeded by early embryonic development retarded in relation to the expected time course.
- Abortions after day 40 are less common and morel likely to represent disease or infection, and merit evaluation of brucella, herpesvirus, or other suspected agents.

Table 5. Summary of species-to-species extrapolation.[a]

Species	n[a]	NT/T, %[a]	Best response, %[b]	Sensitivity, %[c]	
				−	+
Rat	322	60	71	30	29
Mouse	289	53	59	37	33
Rabbit	270	74	50	50	40
Hamster	93	38	71	31	17
Primate	59	72	49	53	18
Dog	44	52	43	39	33
Guinea pig	24	48	50	33	23
Pig	21	62	62	0	0
Cat	8	25	0	100	83
Ferret	5	40	50	50	33

[a] n = Number of reports evaluated for particular species; NT/T = ratio of nonteratogenic reports of selected nonteratogenic agents in humans.

[b] Most similar to humans in nonteratogenic response = highest nonteratogenic dosage.

[c] Lowest nonteratogenic dosage (−), lowest teratogenic dosage (+).

- Rabbits and monkeys offered greater predictability

Schardein et al. 1985

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Conclusion

- Dog has relatively short gestation period compared to the other non-rodent species
- Pregnancy rate ranging from 77% to 90% (pigs, 90%; monkey= 45%)
- Mean litter size of 5-6 fetuses
- Low spontaneous malformation rate
- Dog is relatively a good model for developmental toxicology

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