Canine Semen: Collection, Frozen, Fresh Chilled, Insemination.

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North American Versatile Hunting Dog Association - January 23rd, 2011

Topics

- Bit about me…
- Semen collection
- Semen evaluation
- Fresh Chilled
- Frozen
- Bit about breeding/timing
- Questions

Semen Evaluation

OSGOODE VETERINARY SERVICES
Canine Breeding Soundness Evaluation

Date:				
Owners Name:				
Dogs Nickname:				
Dogs Registered Name:				
Tattoo:				
Microchip:				
Registration number:				
Collection by:				
Teaser used:	Y	N		

General Physical Examination			**Comments**
Prepuce:	Normal ____		
Penis:	Normal ____		
Scrotum:	Normal ____		
Prostate:	Normal ____		
Testes:	Normal ____		
Epididymides:	Normal ____		

Brucella canis:	Date:	
	Results:	

Semen Analysis	**Ref. Values**	**1st collection**	**2nd collection**
1st Fraction	0.25-3		
2nd Fraction	0.4-8		
Gross appearance (2nd)	milky white		
pH			
Sperm Concentracion/ml ($x10^6$/ml)			
Total Sperm/Ejaculate	$>200x10^6$		
% Motility (total progressive)	60% to 90%		
Motile Sperm/Ejaculate			
Diluent (if used)			
Speed: Slow, Moderate, Fast			

Morphology	**Ref. Values**	**1st collection**	**2nd collection**
Method/stain			
% Normal Sperm	75%		
% Abnormal Heads			
% Abnomal Midpieces			
% Abnormal Tails			
% Loose Heads			
% Proximal Droplets			
% Distal Droplets			
Total Number Sperm/Ejaculate			

OVS - Canine Breeding Soundness Evaluation (page 2)

Semen Cytology	**1st Fraction**	**2nd Fraction**	**3rd Fraction**
Bacteria			
Epithelial cells			
White Blood cells			
Red Blood cells			
Other			

2nd Fraction Microbiology

Culture and Sensitivity	
Mycoplasma	
Ureaplasma	
Other	

Prostatic Fraction

Appearance	(clear)
pH	(6.0-7.4)
Cytology	
Culture and Sensitivity	
Mycoplasma	
Ureaplasma	
Other	

To the best of my knowledge, the results of this examination would indicate that the potential breeding capacity of this animal would be:

Satisfactory _____ **Questionable** ____ **Unsatisfactory** ___

Comments:

Fresh Chilled

Extender	
Shipping address	

Frozen

Extender			
#straws		Post Thaw Motility	
Sperm/straw		Storage Location	
Breeding unit		Storage start date	

Semen Collection

Breeding soundness evaluation

Artificial Vagina vs. Cones vs. other

Lubricant and Gloves

Quiet

Training

Female in estrus vs. swabs vs. pheromones

Semen Collection

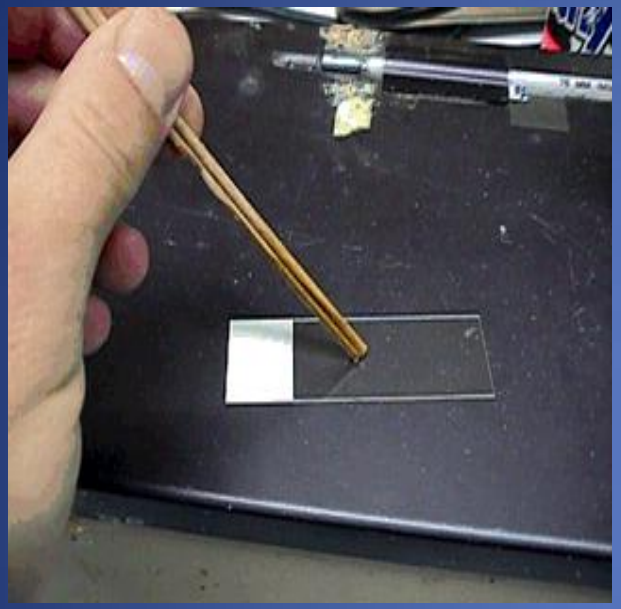
Moving the Prepuce

Placing the AV

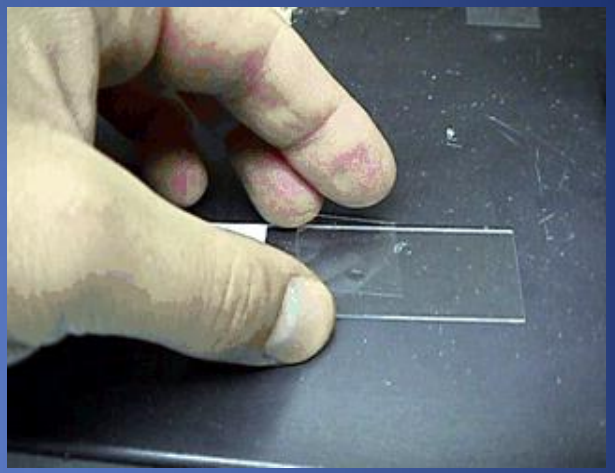
Engorged Bulbous glandis

Semen Evaluation

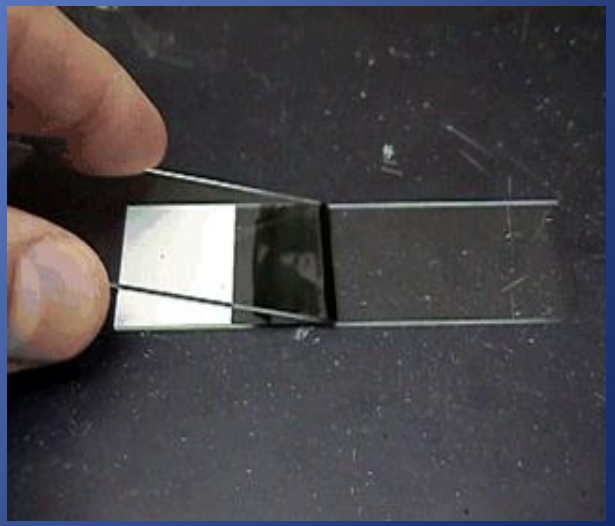
Semen Evaluation



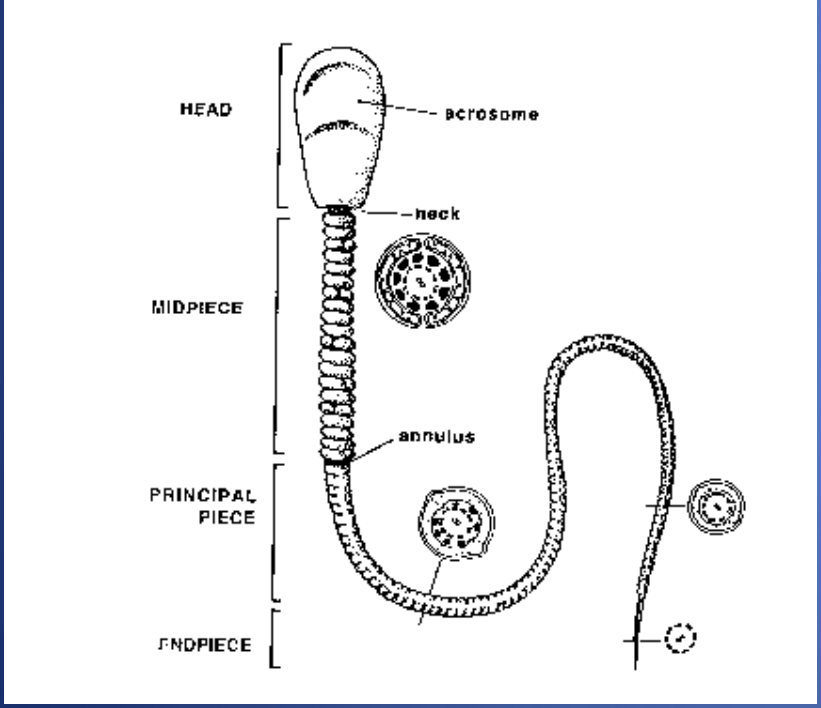
Motility

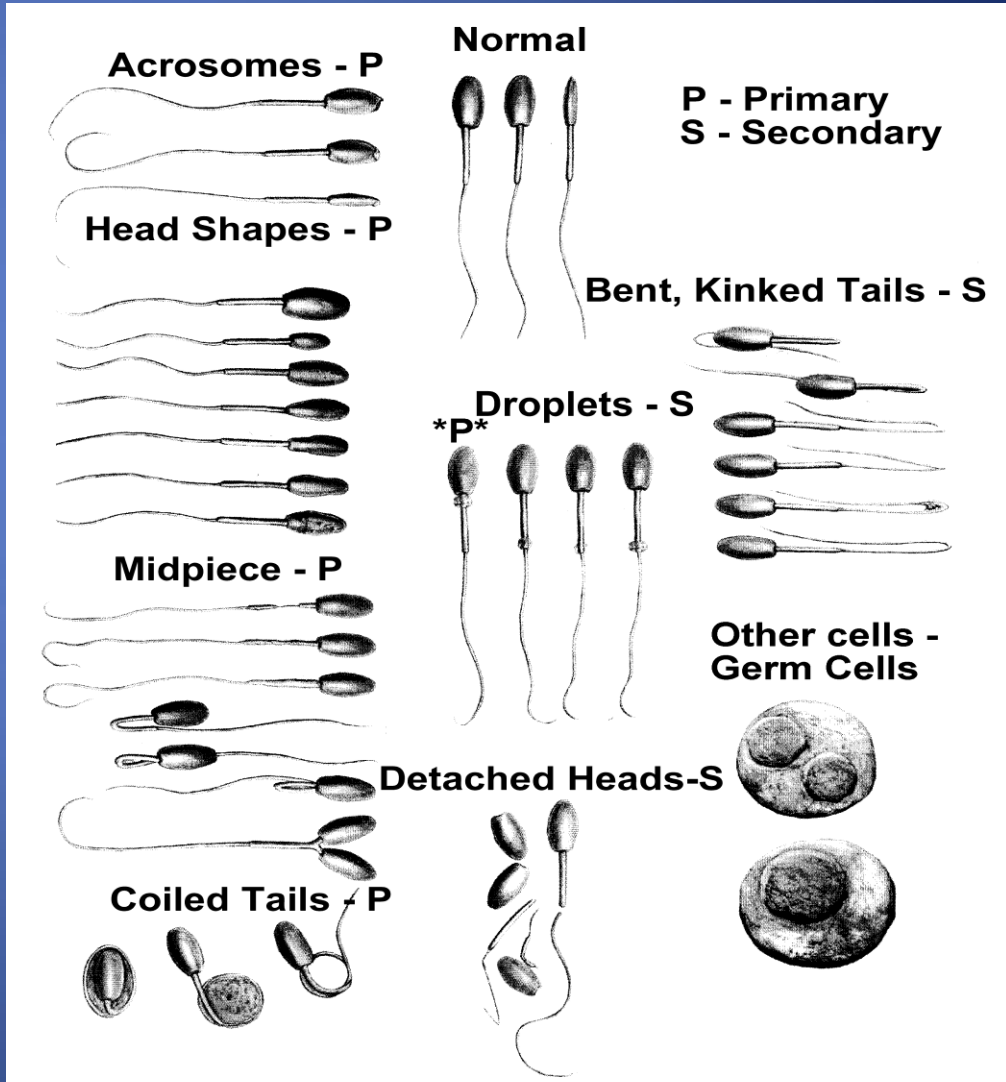


Morphology

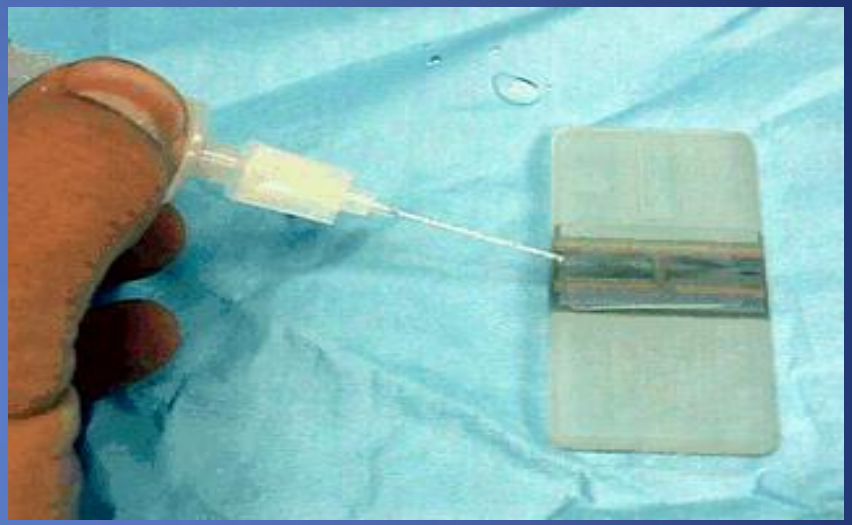


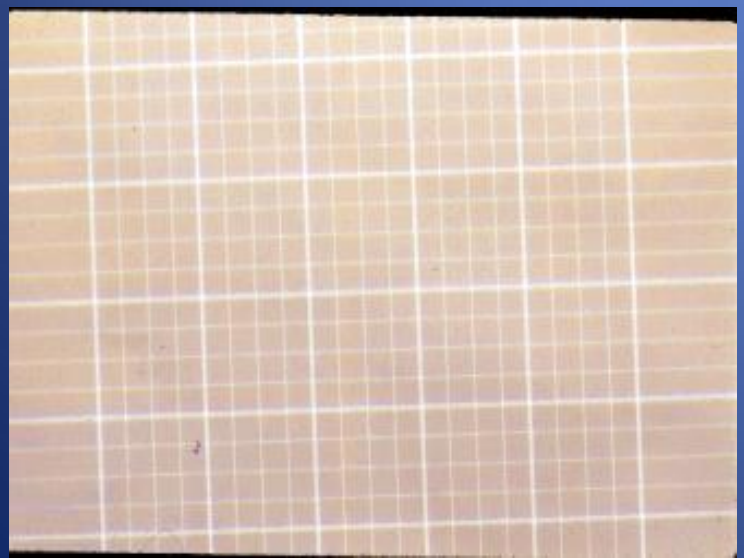
Semen Evaluation

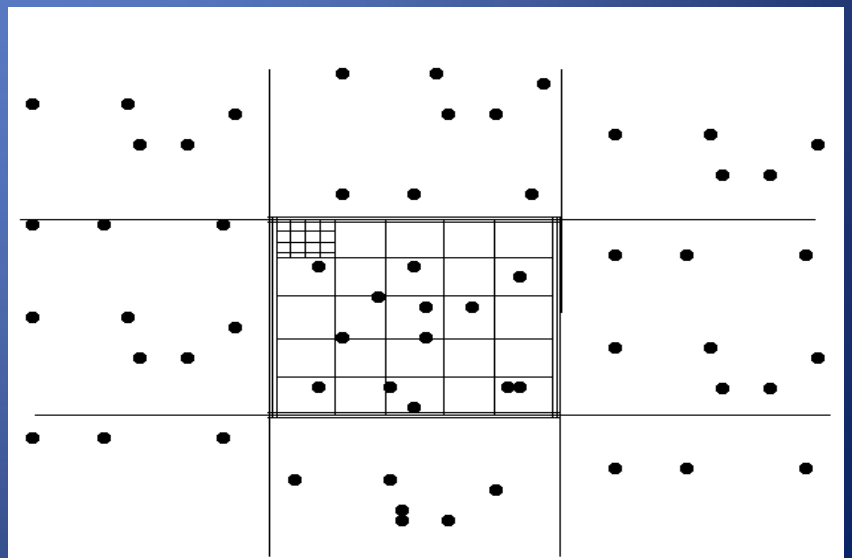




Semen Evaluation







Semen Evaluation

Semen Evaluation

- By the end of the analysis and calculations:
  - Assessed sperm motility
  - Assessed sperm morphology
  - Calculated the number of motile sperm in the ejaculate and per ml of ejaculate = the concentration.
  - In cases of infertility have also looked for specific problems with the semen, assessed concentration, looked for extra cells and started further tests.

What do you do with it?

- Artificial Insemination
  - Fresh – 4 to 6 days survival in utero
  - Fresh chilled – 24 to72hrs survival in utero
  - Frozen – 12 to 24hrs survival in utero

TIMING IS EVERYTHING!!!!!!!!

- Store frozen in liquid nitrogen
  - Lasts indefinitely

Fresh Chilled Semen

- cooking 101

- Why consider Fresh Chilled Semen?
  - Hard to ship female or male
  - Male on show circuit
  - Hard to take time off work when female is in estrus
  - Increased genetic options by shipping semen

Fresh Chilled Semen

Extenders

Using the biochemistry knowledge we have about semen, an extender is added to allow for sperm survival during the chilling process; buffers the solution and adds nutrients for the sperm during the time it is chilled prior to warming and insemination.

Fresh Chilled Semen

Minitube Chill 10

Mix with Egg yolk
Mix volume to volume
Chill and test
Package
Ship

Frozen Semen

Why?

1. for shipping distances (7 days grace)

2. to save genetic potential for the breed

3. insure the breeding potential of a stud dog against loss, death or infertility

Frozen Semen

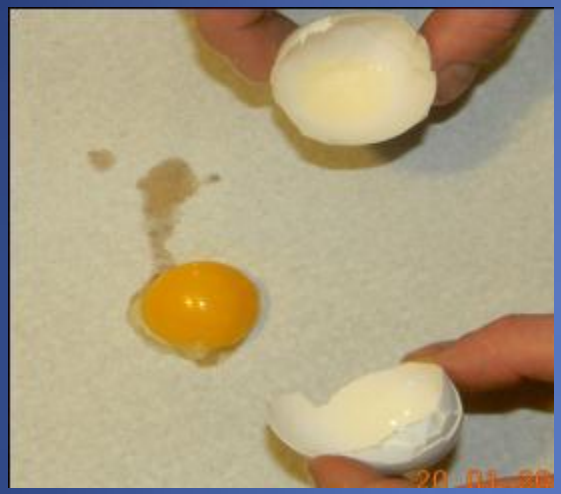
- Extender

Frozen Semen

- An ideal extender should have nutrients as an energy source, substances that buffer against harmful changes of pH, it provides a physiological osmotic pressure and concentration of electrolytes, that prevents bacterial growth and protects the cells from cold shock during the freezing and thawing processes

Frozen Semen

- Add 20% egg yolk to both Part A and Part B



Frozen Semen

- Part B and egg yolk to chill to 4^0C.
- If Sperm rich fraction is contaminated with Prostatic fraction – wash sperm with part A and egg yolk mix.
- Add Part A to semen in equal volumes – chill for 2hrs

Frozen Semen

- Over 3min. add equal amounts of Part B with yolk to the original volume of semen
- Fill straws already pre-cooled and labeled

Frozen Semen

Frozen Semen

Frozen Semen

Liquid Nitrogen

Frozen Semen

Frozen Semen

- Warm part A and B of extender
- Add % egg yolk
- Cool part B of extender to 4^0C
- Mix volume of sperm rich fraction with volume part A and egg yolk
- Cool this to 4^0C for 2hrs
- Add volume of part B with egg yolk
- Fill straws that have been marked and pre-cooled to 4^0C as well – seal and cool for 20-60min.
- Put above liquid nitrogen (4-5cm) for 20min then plunge into liquid nitrogen
- Test one straw for post thaw motility and store rest

Frozen Semen

Frozen Semen

Insemination/Timing

- Most important component of breeding, especially if using fresh chilled or frozen semen
- Knowing the female
- Vaginal cytology
- Progesterone (qualitative and quantitative testing)
- Luteanizing Hormone surge

Insemination/Timing

- Signs of Estrus starts
- Vaginal changes start (cytology) as estrogen increases
- Estrogen starts to drop and LH surge occurs
- Approx. 2-3days later Ovulation occurs
- Approx 2-4 days later Oocyte maturation occurs
- Approx 2-5 days later Diestrus occurs.

Insemination/Timing

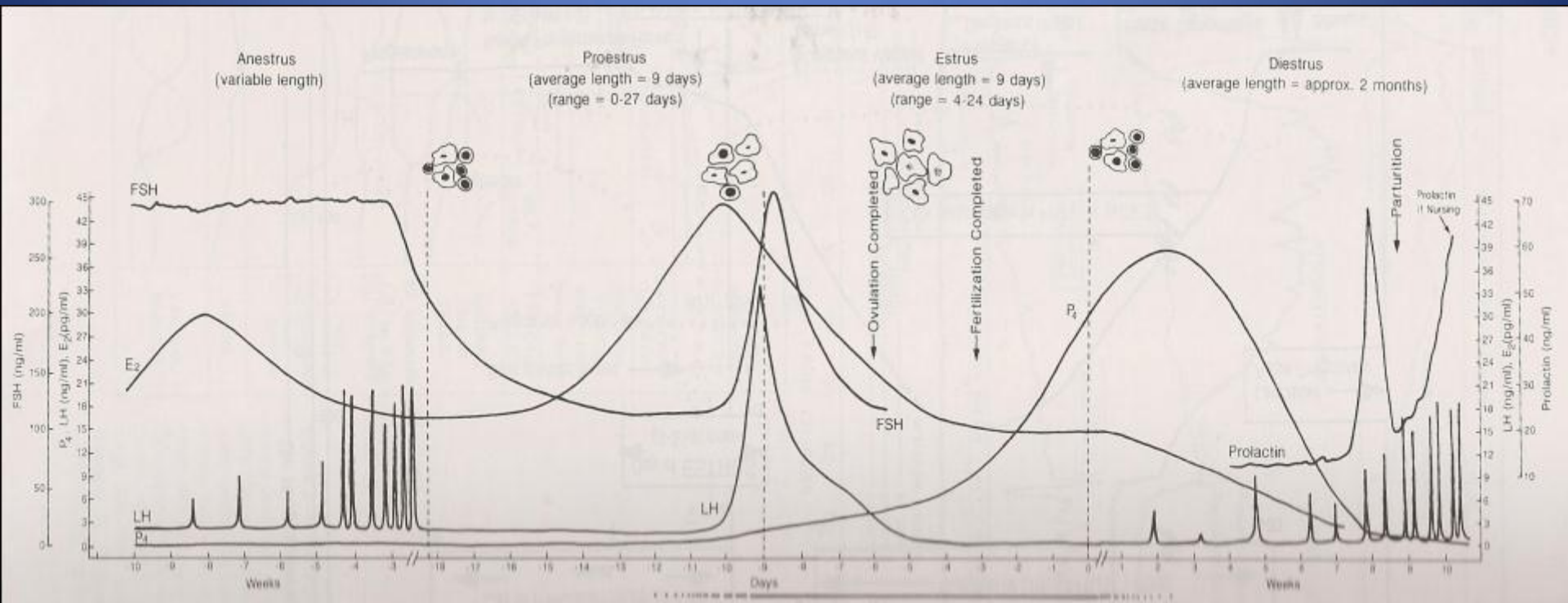
To confuse:

Ovulation does not always coincide with onset of sexual behaviour. Behavioural estrus in some bitches occurs 2-3 days before the LH surge, some 4-5 days after the LH surge and in some extreme cases males have been allowed by the female to breed in pro-estrus 4-5 days prior to the LH surge. Some have even refused a male until 6 days post the LH surge.

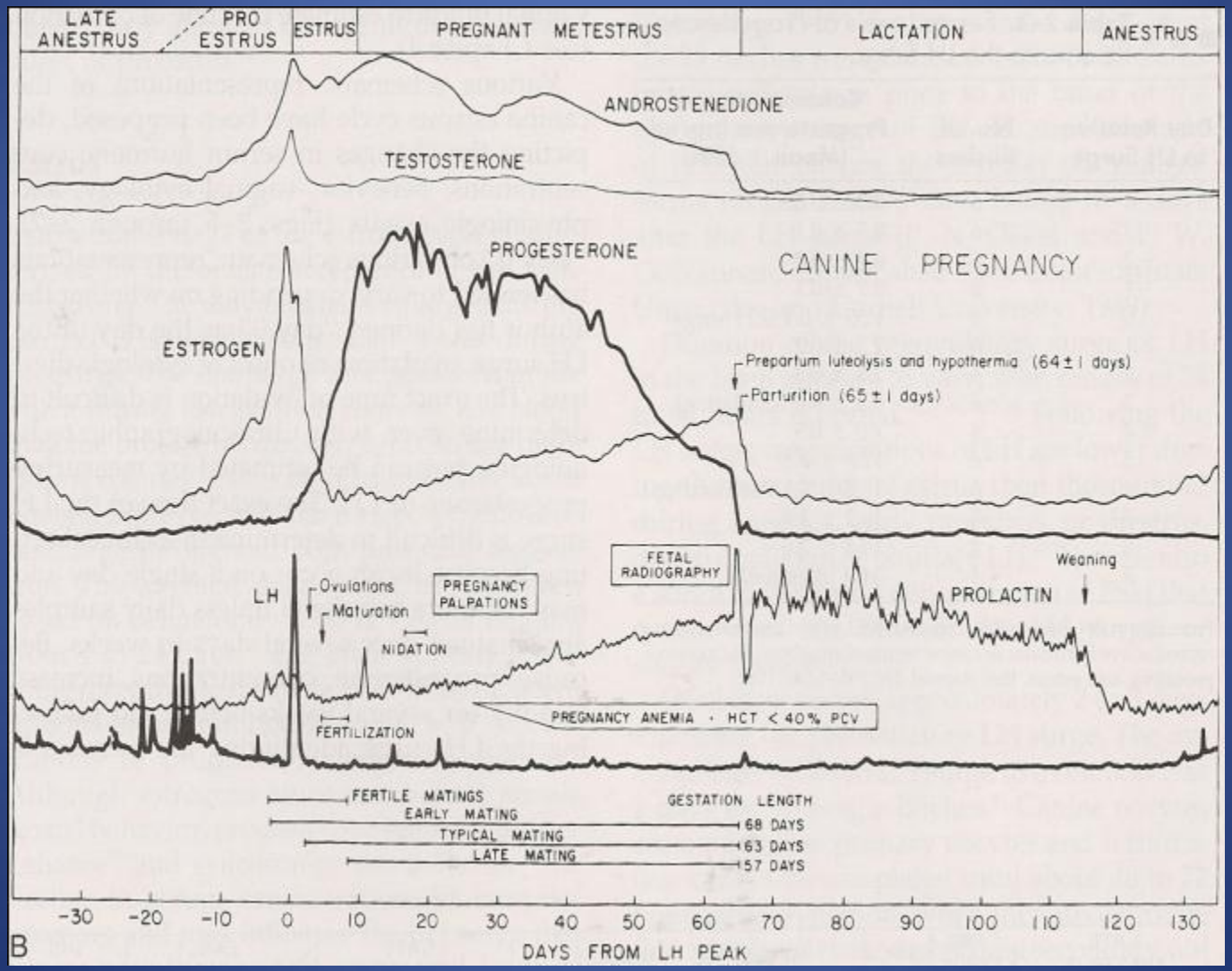
Insemination/Timing

Therefore:

Behavioural estrus vs. hormonal cycle and true ovulation etc.



Insemination/Timing



Insemination/Timing

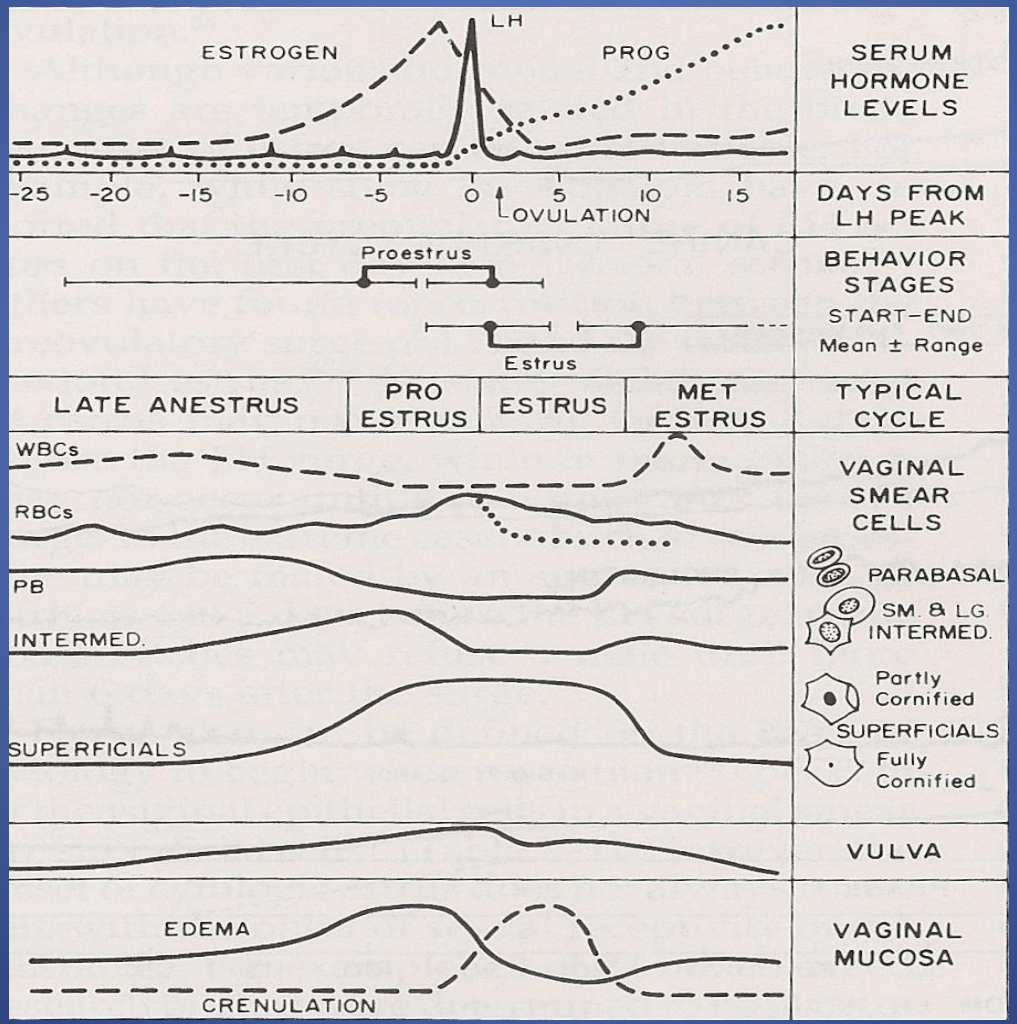
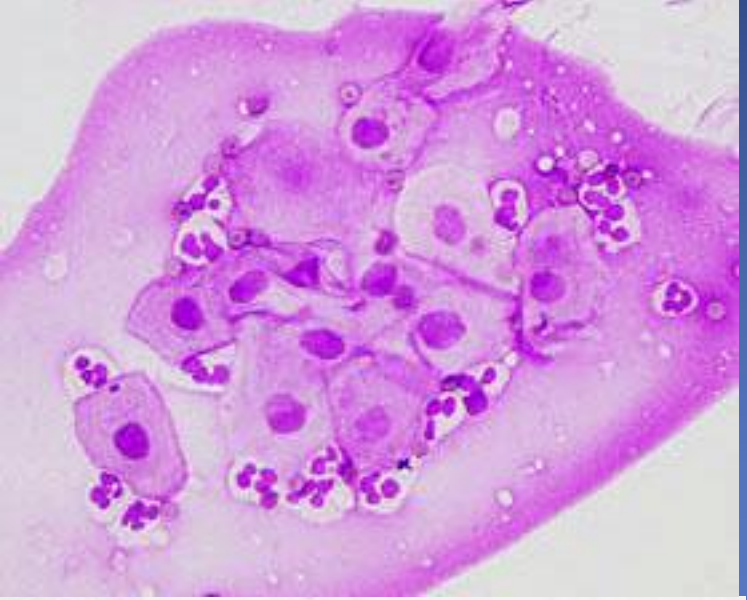


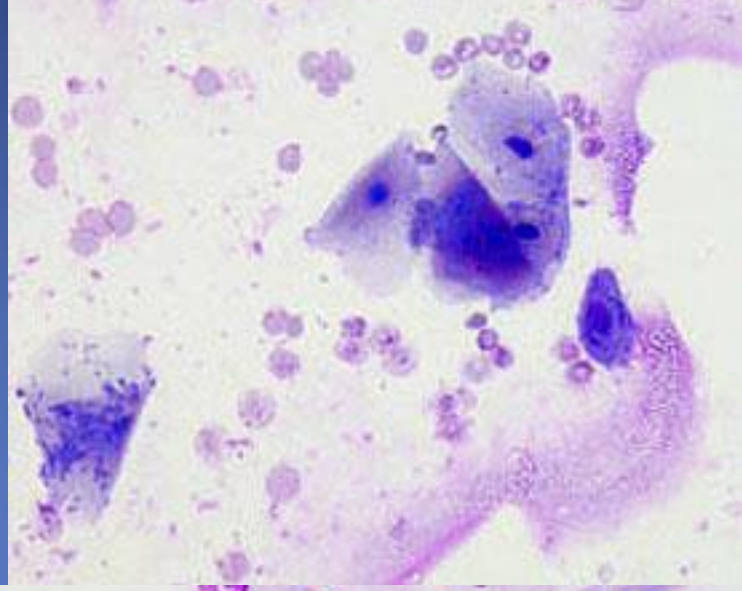
Table 1. Characteristics of the Canine Estrous Cycle

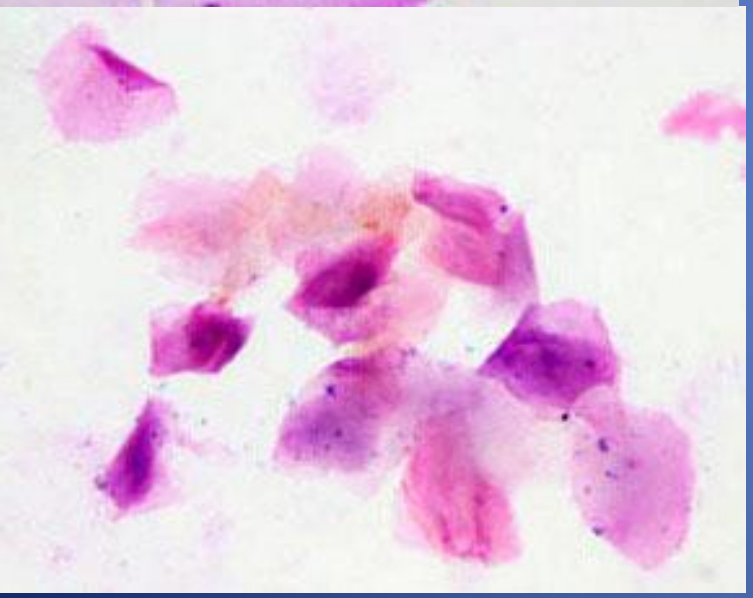
Cycle Stage	Length	Hormonal changes	Predominant Cell Types	Erythrocytes	Neutrophils	Behavior of the Bitch	Clinical Signs
Proestrus	~9 days	Estrogen ▲	Early: mixed population of cells Late: large intermediate and superficial cells	Early: present Late: ±	Early: present Late: decreased	Attractive to the male, but won't stand for mating	Vulvar edema and swelling, bloody discharge
Estrus	~9 days	LH (surge) ▲ Estrogen ▼ Progesterone ▲	90% superficial cells Bacteria usually present	±	Absent	Accepts male and will stand	Less edema, discharge becomes clearer
Diestrus	~60 days	Progesterone ▲	Abrupt change in relative # of epithelial cells. Superficial cells decrease by 20%	±	Few to none	Ceases to accept male	Little discharge, edema decreased
Anestrus	Depends on whether pregnant or not. 70–80 days for CL to regress and 130 days to repair endometrium.	Progesterone ▼	Parabasal and Intermediate cells	Few if any	Few if present	No outward signs	Scanty, tenacious secretions



Proestrus – early

Proestrus - late





Estrus

Diestrus

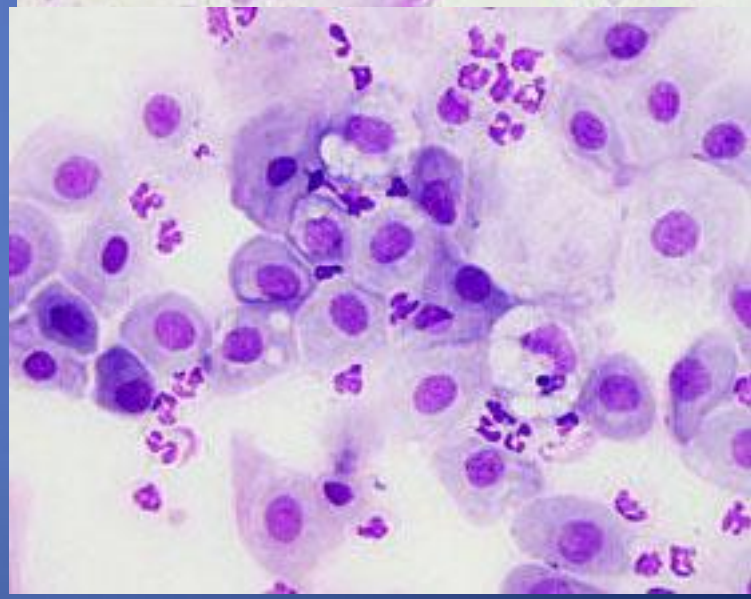
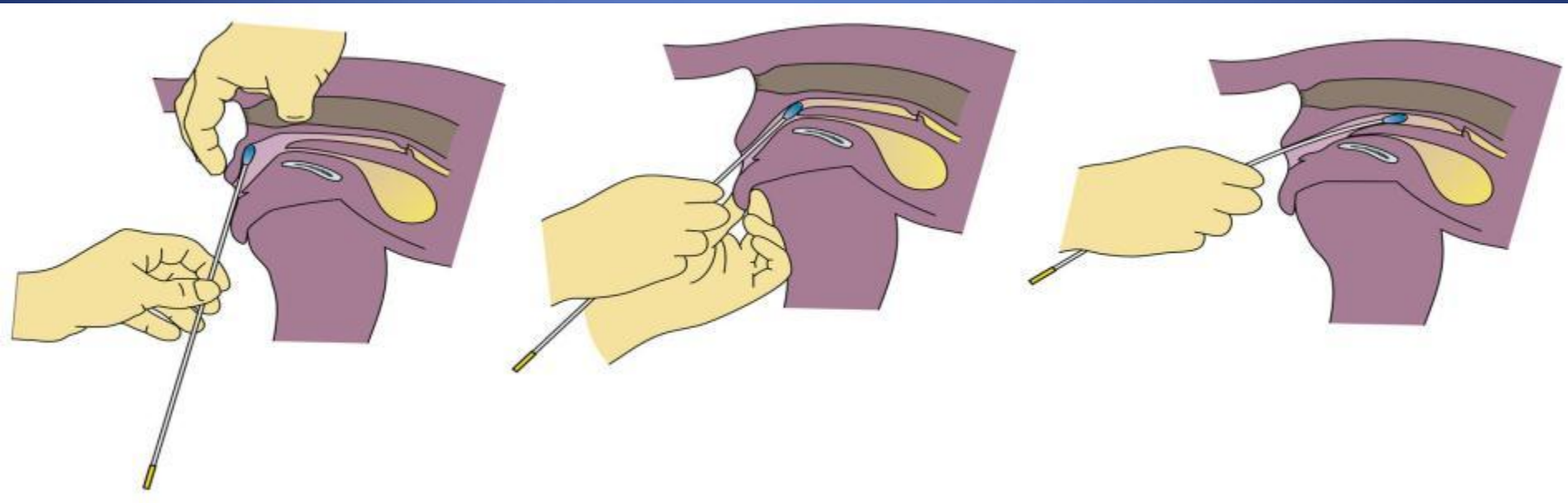


Chart and pictures from University of Georgia, College of Veterinary Medicine web site: http://www.vet.uga.edu/vpp/clerk/beimborn/

Insemination/Timing

Natural coverage or fresh AI

- Whenever female will accept the male; breed two days apart for at least 2-3 breedings. Semen lasts average 4-6 days.



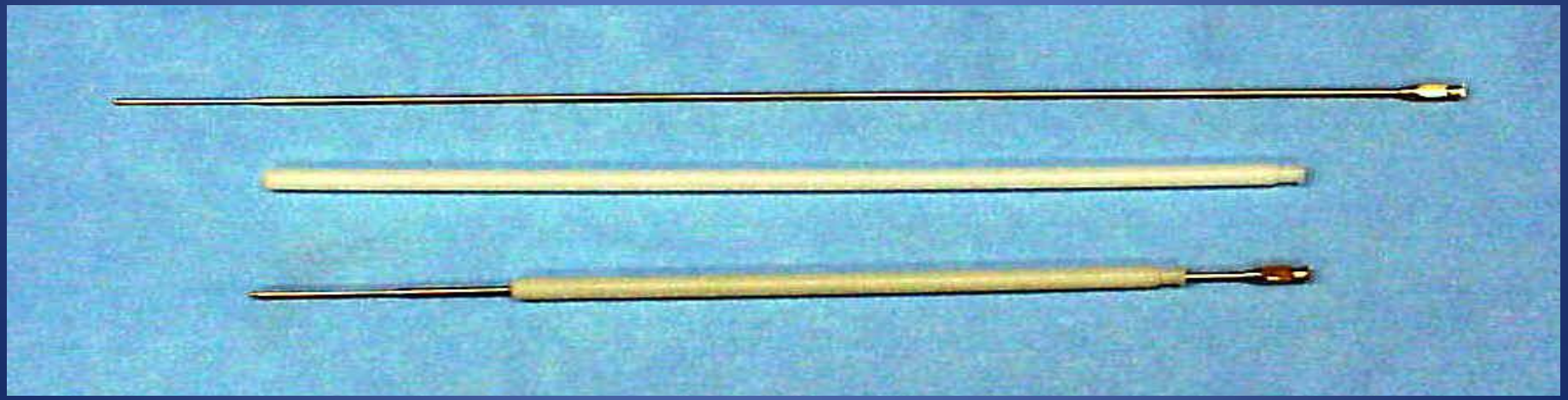
Insemination/Timing

AI rods

Mavic Catheter

Insemination/Timing

- Chilled Extended Semen
  - Knowing when ovulation has taken place helps and breeding once or twice 2-4 days post ovulation. Semen survival 24-72hrs.

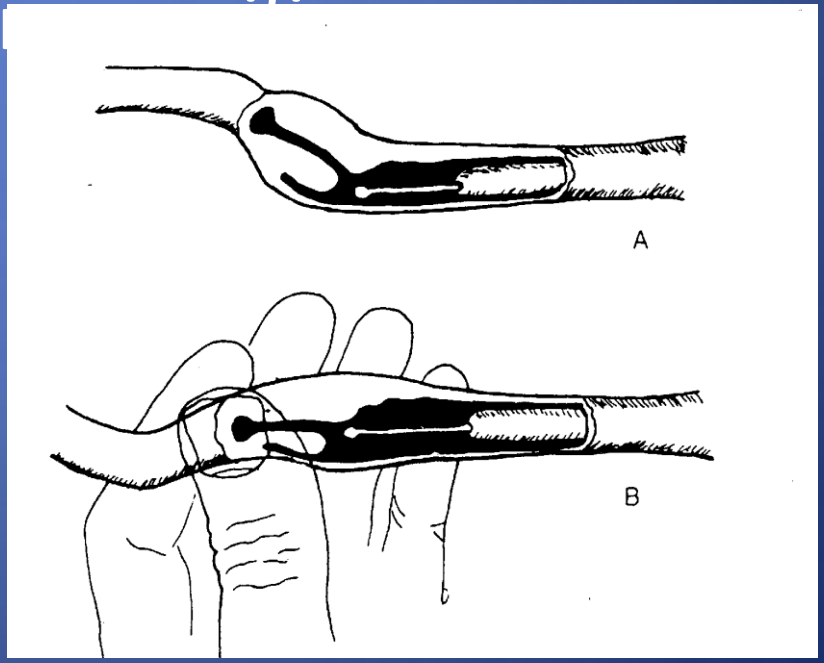


Norwegian Catheter – picture from Louisiana State University School of Veterinary Medicine web site: http://www.vetmed.lsu.edu/eiltslotus/theriogenology-5361/k9_breeding_management.htm

Insemination/Timing

Frozen Semen

- Timing critical as semen survival 12-24hrs only.
- Thaw instructions ve



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Insemination/Timing

Surgical Insemination vs.

Transcervical Insemination