



ARTIFICIAL INSEMINATION IN THE BITCH

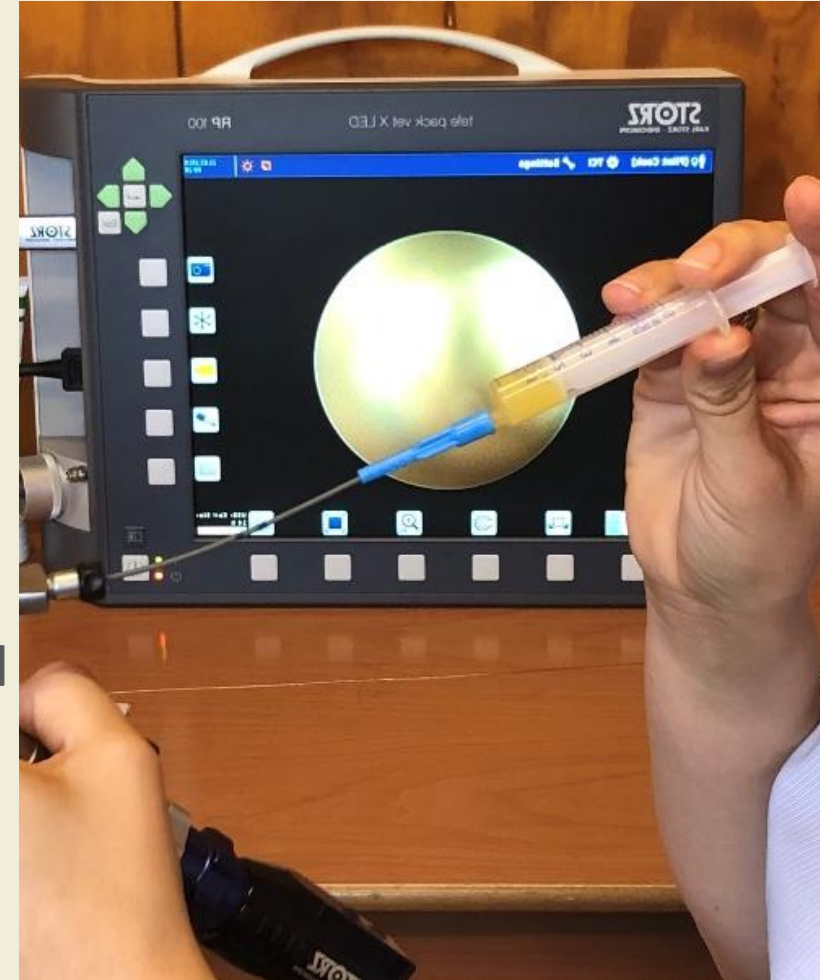
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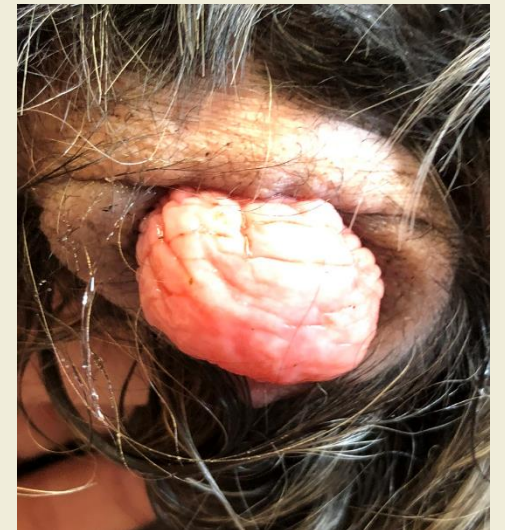
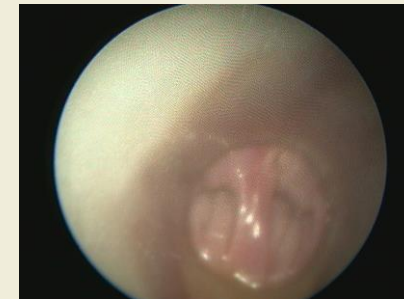
OVERVIEW: ARTIFICIAL INSEMINATION

- Indications for AI
- Unique anatomy of the bitch
- Vaginal AI
- Intrauterine AI
- Factors that affect canine reproductive success after AI



INDICATIONS FOR AI

- Behavioral issues – poor libido or bitch aggression
- Male and female dogs located at a distance
- Frozen semen: introduction of valuable and new genetics
- Compromised semen i.e. older stud dog
- Health problems with the male – HL weakness, neurological deficits – unable to mount
- Health problems with the bitch: overcome vaginal abnormalities
(care-often heritable defects and therefore should not be breeding)
- Breed: bulldog, giant breeds



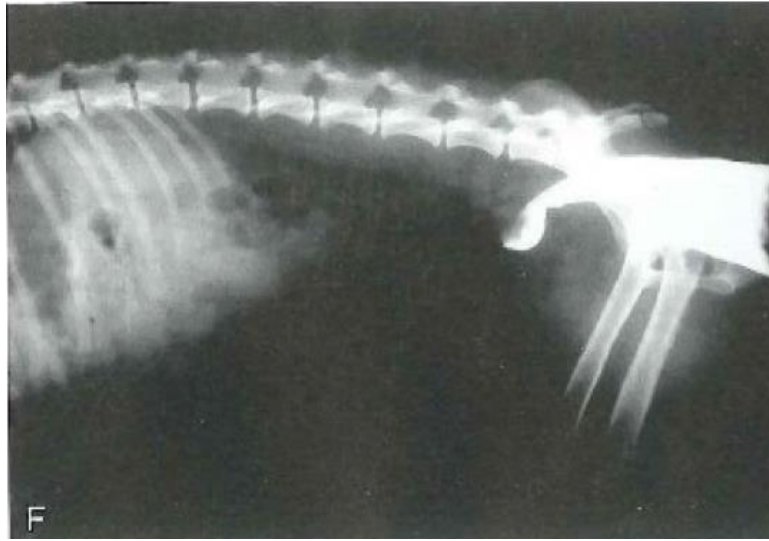
UNIQUE FEATURES OF THE ANATOMY OF THE REPRODUCTIVE TRACT OF THE BITCH:

OBSTACLES TO OVERCOME FOR SUCCESSFUL AI

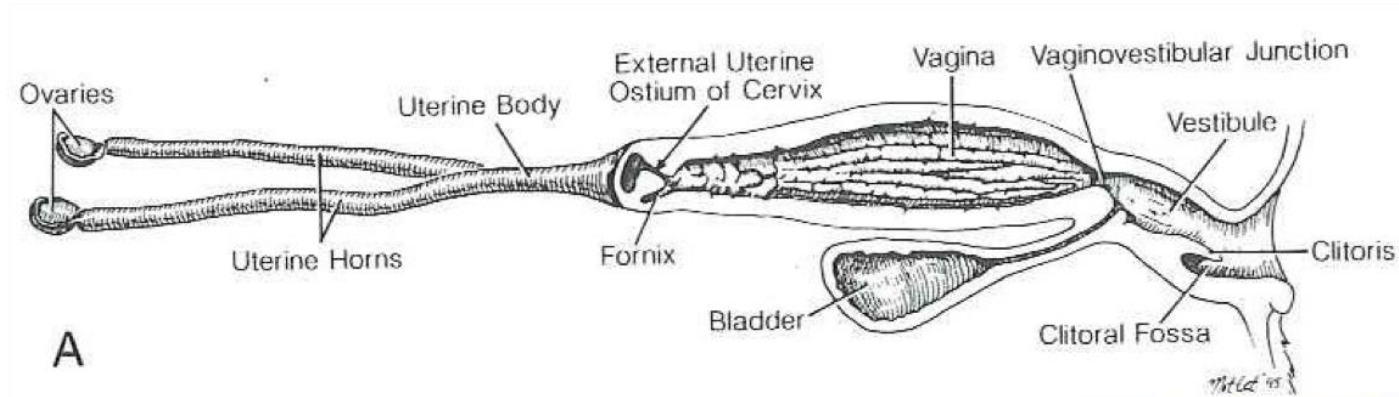
1. Length of the vagina: long
2. Narrow space under the DMF
3. Limited space in paracervical area
4. Position of the ventrally facing/ mobile os cervix
5. Angle (and diameter) of the cervical canal



REPRODUCTIVE TRACT: LONG!



Contrast
vaginograms In
dioestrus where
the cervix is closed



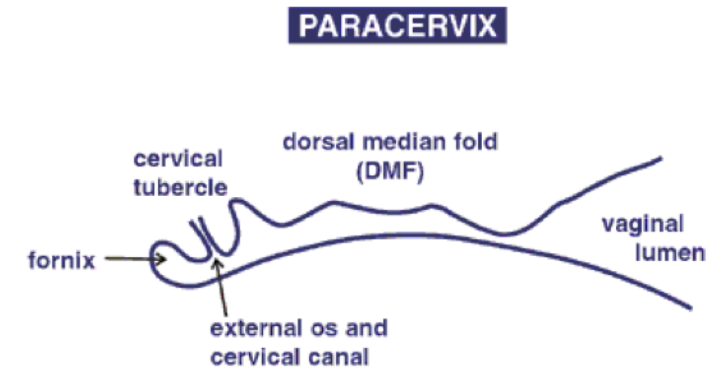
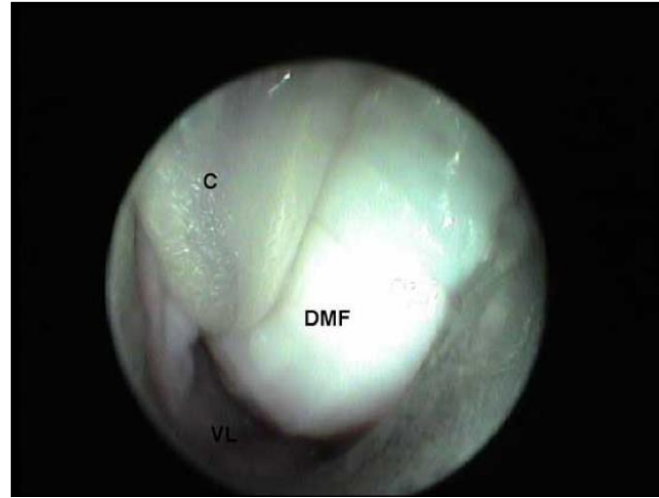
1. **Very long vagina coupled with a being an acidic and hostile environment for sperm:** important consideration for natural mating and vaginal insemination



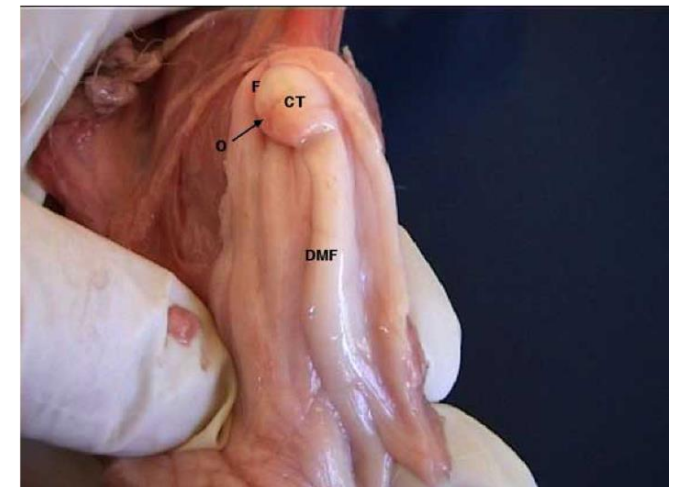
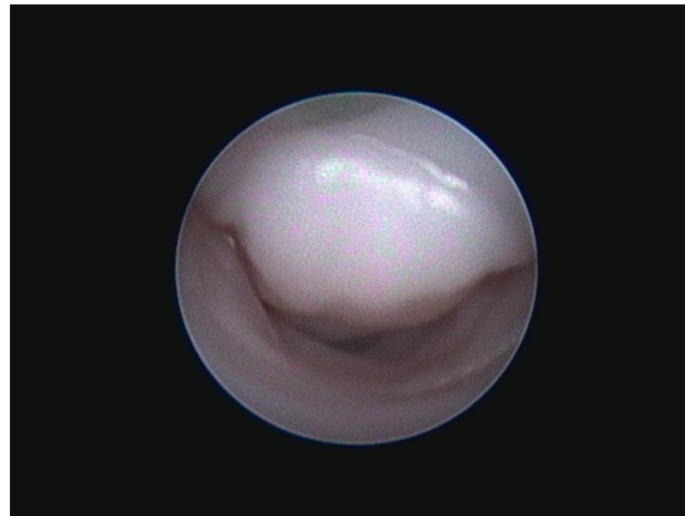
PARACERVICAL AREA: NARROW

2. Dorsal Median Fold (DMF) and Paracervical area narrow

DMF creates an 'obstruction' in front of the cervical tubercle and os: important consideration when carrying out an endoscopic transcervical AI



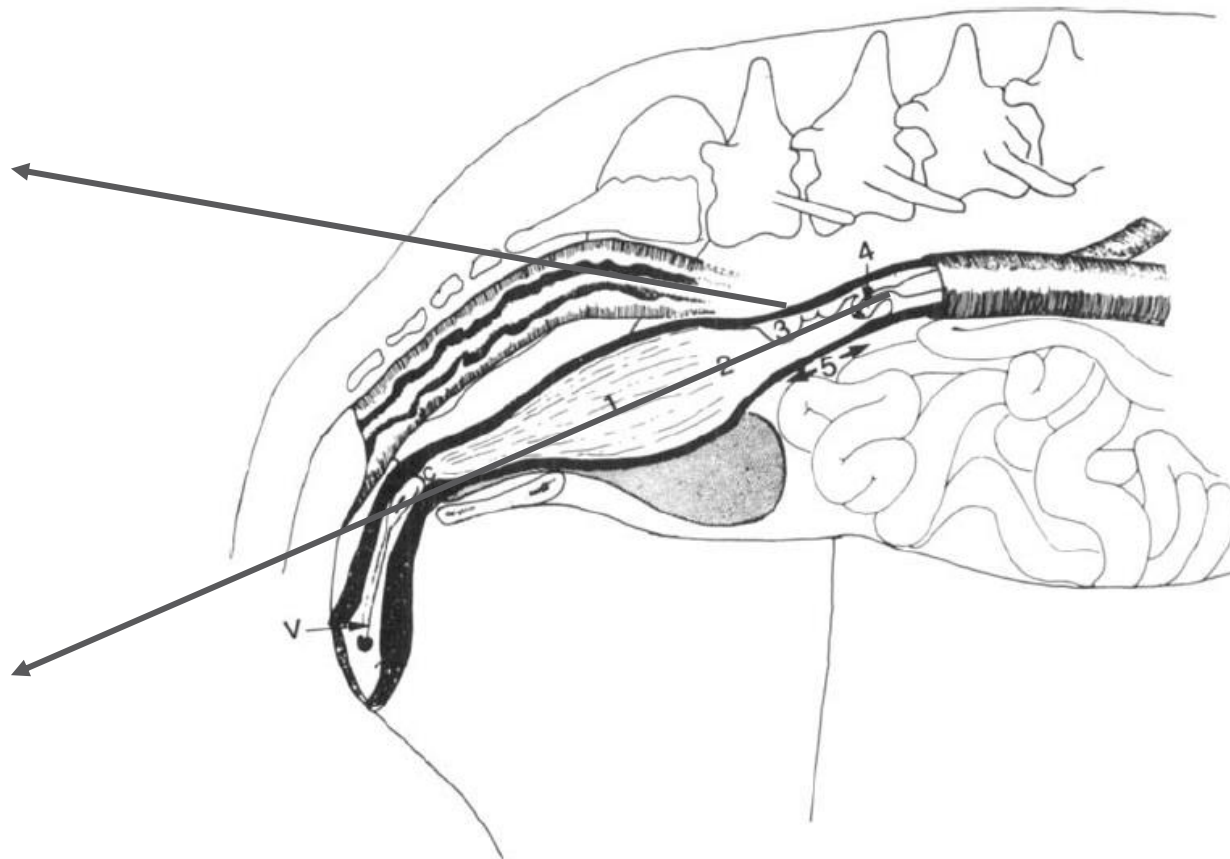
Note the crescent shaped narrowed lumen under the DMF



3. Cervix hangs from roof of cranial vagina, mobile and the opening faces ventrally downwards



Appearance of the DMF -> paracervix through an endoscope



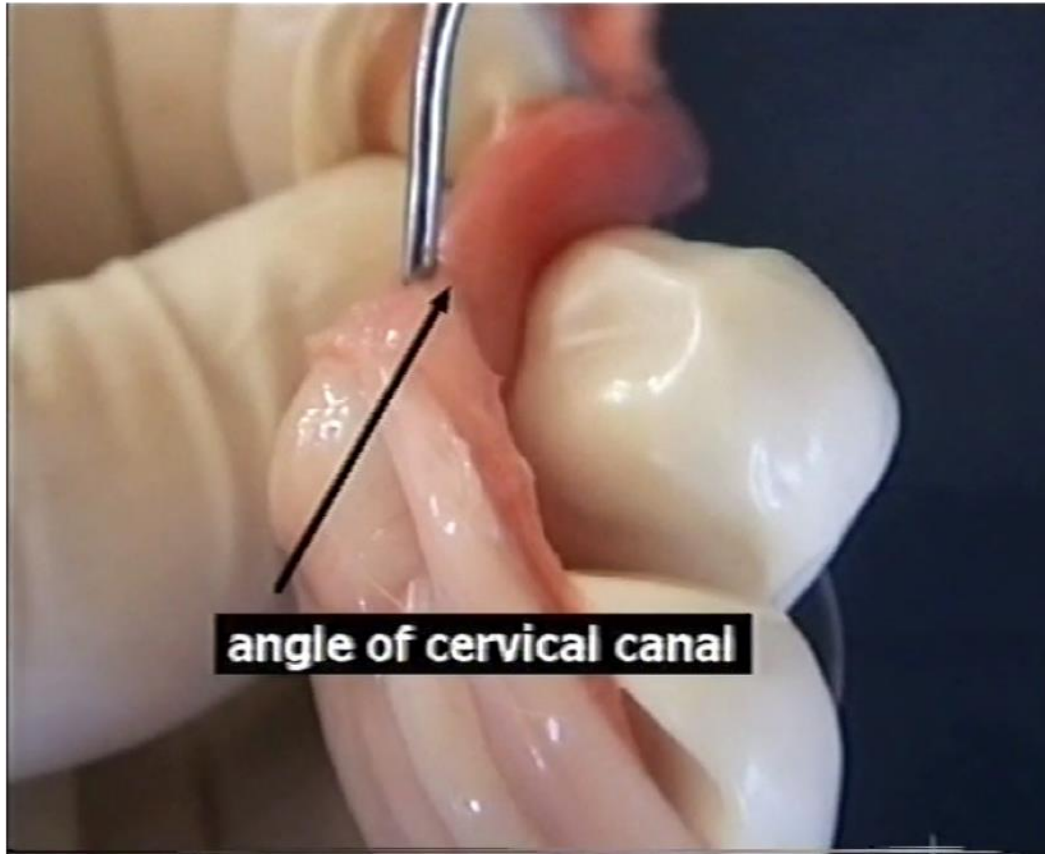
Appearance of the mobile cervix with the opening to the cervix facing downwards

Caudal reproductive tract of the bitch. 1 and 2, vagina; 3, dorsal median fold (dmf) of paracervix: number is placed on caudal tubercle of dmf; 4, external os of cervix; 5, paracervical area/paracervix *J Small Anim Pract* 24:1-15; 1983





4. CERVICAL CANAL ANGLE AND LENGTH



1. ANGLE: Cervical canal is **directed cranio-dorsally** from the cranial vagina to to the body of the uterus= opening faces vaginal floor



2. Image of inside the cervical canal: it is **long**-must ensure you pass the catheter all the way into the uterine body

Vaginal AI

Aim: Overcome anatomical barriers
by mimicking natural mating

Key aspects to vaginal AI:

1. **Long + flexible AI pipette** = overcome long vagina and paracervical barrier
2. **Foley catheter/balloon:** Bulbus glandis = prevent backflow + stretching vaginal wall stimulate oxytocin release -> vaginal contractions -> movement of semen towards the cervix
3. **Flush** with large volumes of canine extender (10-30ml) SLOWLY (over 10-15min): Prostatic fluid = wash sperm along the long vagina and protect sperm in hostile vaginal environment
4. **Feathering:** Tie= stimulate vaginal and urethral contractions

Requirement for hindleg elevation??



'Feathering technique' stimulates vaginal and uterine contractions-moves sperm forward towards uterine horns.

Has been shown to improve PR by up to 10% (England et al 2012)

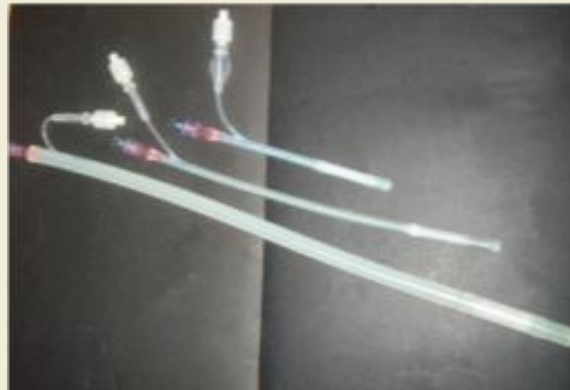


MAVIC VAGINAL AI CATHETERS (Minitube®)



Advantages:

- Long-almost reach cervix even in large breeds
- Flexible and soft but with stilet so can manipulate under DMF
- Balloon/foley- prevent backflow and stimulate vaginal contractions



VAGINAL AI VIDEO



Vaginal AI is most successful when:

- Use a foley catheter
- Use a large volume of “flush”(prostatic fluid or canine extender) after depositing semen (i.e 30ml in large breed!)
- Take time injecting flush (i.e 10-15 minutes): feathering
- Use high quality and quantity of fresh or chilled semen

Vaginal AI is not successful when:

- Poor Quality Semen – motility and morphology
- Low Quantity Semen
- Frozen-thawed Semen
- Closed Cervix – Late in Estrus



Intrauterine AI

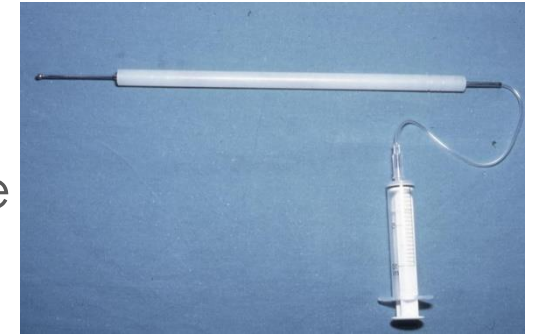
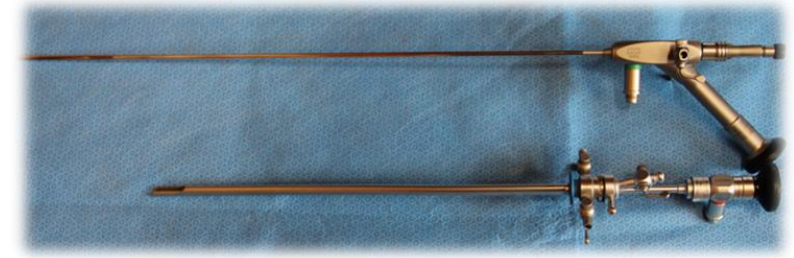




Intrauterine AI Techniques

INTRAUTERINE AI TECHNIQUES ARE CLASSIFIED AS INVASIVE OR NON- INVASIVE

- Transcervical AI:
 - Endoscopic (TCI)
 - Scandinavian / Norwegian device
- Surgical AI
- Laparoscopic AI



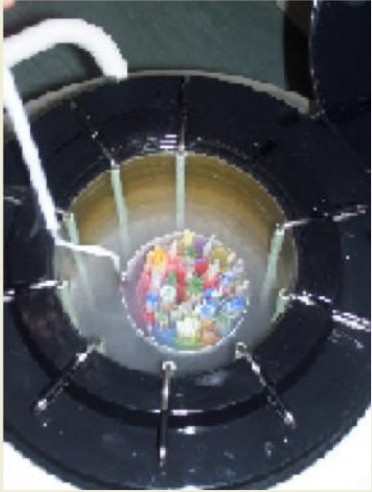
Reproduction in Domestic Animals, Volume: 49, Issue: s4, Pages: 56-63, First published: 03 October 2014, DOI: (10.1111/rda.12395)



Dr Bill Swanson, Cincinnati Zoo performing laparoscopic AI on a Thai Fishing Cat



INDICATIONS FOR IUI



- Frozen-thawed semen
- Poor quality chilled or fresh semen
- Low numbers of fresh or chilled semen
- Advantage in certain breeds: Bulldogs and Giant breeds





Advantages: Non Invasive

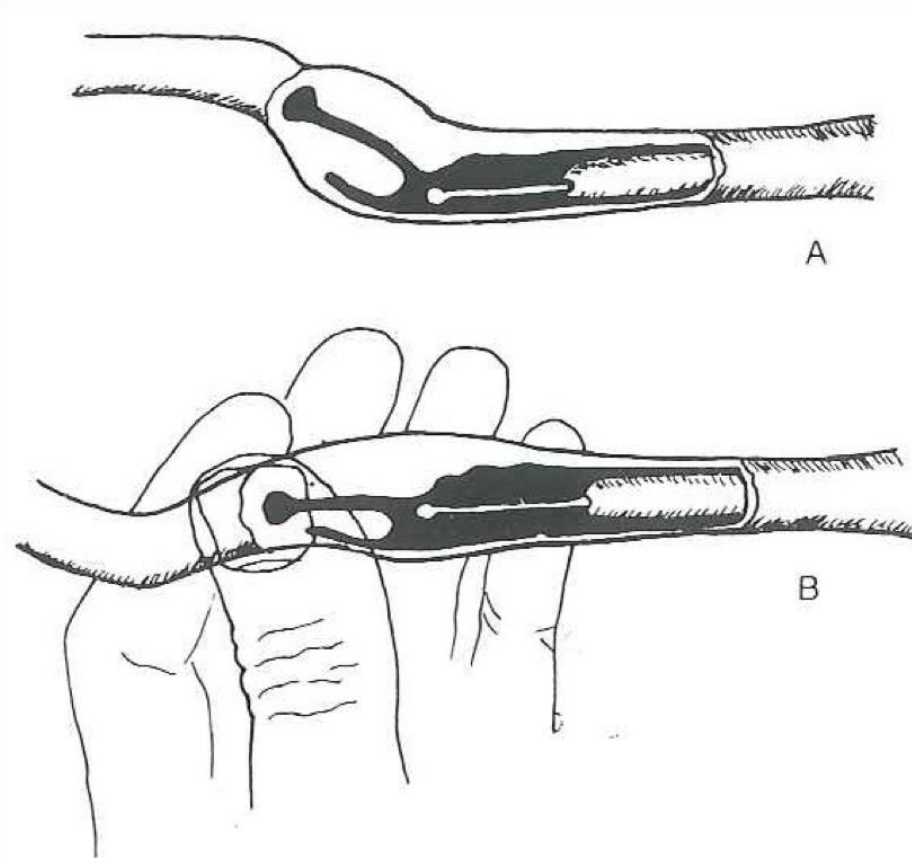
Disadvantages:

- significant skill and training required
- cervix is not visualized but palpated
- Limitation in bitches can perform procedure on
- Risk of trauma: penetration of vaginal wall



Image of different sized catheters all with outer plastic nylon sheath and dinner steel catheter

Slight Alternative: Norwegian catheter



Transabdominal, blind manipulation of the reproductive tract to locate the cervix by abdominal palpation and catheterize the cervix : some similarities to insemination in the cow.



TRANSCERVICAL INSEMINATION (TCI)

Advantages:

NON - INVASIVE

- no anesthesia and associated risks, no surgical laparotomy and surgical recovery
- Rare to need sedation
- Perform multiple AI's per heat
- Visualization (vaginocopy)-see vaginal pathology
- Fast-out patient consultation (1 min) once technique mastered
- Pregnancy rates similar or better than other intrauterine AI techniques*



Bitch stands (in standing heat) on the table and requires no sedation. The anatomy and catheterization of the cervix is visualized on the TV monitor.

*Hollinshead and Hanlon (2017) Factors affecting the reproductive performance of bitches





ADVANTAGE : MOST CLINICS HAVE ALL THE EQUIPMENT TO PERFORM A SURGICAL AI AND LOW SKILL LEVEL REQUIRED

- Invasive
- Non-repeatable
- General anaesthesia
- Potential post –operative side effects and complications
- Higher running costs
- Ethical concerns?
- Slower procedure with G/A and recovery time
- In- patient procedure

SURGICAL INSEMINATION

- Not currently performed in many countries around the world
- Evidence supports TCI has better or equal pregnancy rates as surgical insemination
- We do not recommend surgical insemination



Factors affecting the reproductive performance of bitches: A prospective cohort study involving 1203 inseminations with fresh and frozen semen

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ABSTRACT

The aim of this prospective cohort study was to utilize multivariable statistical methods to identify factors that significantly affected whelping rate, litter size and gestation length in a large population of bitches of many different breeds, presented for routine breeding management. In addition, we aimed to determine the incidence of dystocia and the proportion of bitches undergoing a caesarian section procedure. A total of 1146 individual bitches representing 84 different breeds contributed 1203 inseminations over the 9 year (2007–2015) study period. Bitches were inseminated with either frozen-thawed ($n = 645$), fresh ($n = 543$) or chilled ($n = 15$) semen from 1371 different males. The mean (SD) whelping rate was 74% (4%) and the mean litter size was 5.8 ± 3.1 pups per litter for all bitches in the study. The whelping rate was significantly lower in bitches inseminated with frozen-thawed semen compared with bitches inseminated with fresh semen (71% vs 80% respectively; $P < 0.001$). Semen that was classified as having poor motility ($<30\%$ progressive) resulted in a significantly lower whelping rate (37%) than semen classified as good (30–65% progressive; whelping rate = 67%) or excellent ($>65\%$ progressive; whelping rate = 79%). There was a linear decline in whelping rate with advancing age. Greyhounds and Labradors demonstrated a significantly higher whelping rate (88% and 94% respectively) compared with all other breeds (71.3%; $P < 0.001$). Bitches inseminated with frozen-thawed semen had significantly smaller litter sizes than bitches inseminated with fresh semen (5.4 ± 3.1 vs 6.2 ± 3.0 pups per litter respectively; $P = 0.02$). Smaller breeds had significantly smaller litters (4.4 ± 2.1 pups) than medium (5.2 ± 2.9 pups), large (5.9 ± 2.9 pups) or giant (6.7 ± 3.8 pups) breeds. For each advancing year of age, litter size decreased by 0.13 pups per litter. The mean (SD) gestation length from LH0 was 65 ± 1.9 d. Greyhounds had a significantly longer pregnancy duration (68.0 ± 1.5 d) than other breeds. For each additional year of bitch age, gestation length increased by 0.11 days ($P < 0.01$), and for each additional pup per litter, gestation length was reduced by 0.08 days ($P < 0.05$). Of the 890 bitches for which whelping outcomes were recorded, 409 (46%) whelped normally without assistance, 249 (28%) had an elective C-section, 205 (23%) underwent an emergency C-section and 27 (3%) were medically managed or required veterinary assistance for dystocia. Brachycephalic breeds were 11.3 (95CI = 9.3–17.9; $P < 0.001$) times more likely to have a C-section compared to all other breeds. Bitches with litter sizes of one or two pups had a C-section rate of 83%, whereas bitches with litter sizes of three or more pups had a C-section rate of 43% ($P < 0.001$). This study provides important clinical information to optimise whelping rates, litter size and the prediction of whelping in certain breeds for clinicians working in canine reproduction.

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Comparison of endoscopic-assisted transcervical and laparotomy insemination with frozen-thawed dog semen: A retrospective clinical study

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ABSTRACT

The objective of this retrospective clinical study was to compare pregnancy rates obtained after the use of endoscopic-assisted transcervical catheterization (EIU) or laparotomy (SIU) for insemination of frozen-thawed dog semen. Healthy bitches from various breeds were inseminated with semen from multiple donors processed by different freezing centers. Data from 118 inseminations (78 EIU and 40 SIU) performed between 2009 and 2011 were analyzed. Insemination timing was based on vaginal cytology, serum progesterone concentrations, and vaginography. A ureterorenoscope and a CH-5 Transcervical insemination catheter were used for EIU; 28 of the bitches in this group were inseminated twice with the second insemination less than 12 hours after the first. The numbers of live morphologically normal sperm (LMNS) were determined to characterize insemination doses. Overall, pregnancy rate was greater ($P < 0.05$) in the EIU group (65%) than in the SIU group (45%). Pregnancy rates were greater ($P \leq 0.06$) when more than 100×10^6 LMNS were inseminated regardless of insemination method; the greatest pregnancy rate was observed in the EIU group when this insemination dose was used (38/49; 78%). There was no significant difference in pregnancy rate whether one (69%) or two inseminations (64%) were performed in the EIU group. Complications in the SIU group included anesthetic-induced bradycardia during surgery, significant postoperative pain, seroma formation over the abdominal incision, and delayed wound healing. No complications were noted during or after insemination in the EIU group. In conclusion, these results support the use of EIU as a noninvasive alternative to laparotomy for insemination of frozen-thawed dog semen. In addition, use of more than 100×10^6 LMNS is also recommended for insemination.

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SMALL ANIMALS

Coeliotomy-assisted intrauterine insemination in dogs: a study of 238 inseminations

DM Burgess^a, KE Mitchell and PGA Thomas

Objective (1) To report whelping rates and litter sizes following coeliotomy-assisted intrauterine inseminations (CAII) performed commercially and (2) to identify factors that may influence these outcomes.

Design Retrospective single cohort observational study.

Procedure All oestrous cycles in bitches that presented to the study hospital for CAII between 1 January 2005 and 31 December 2010 were included. One insemination was performed per oestrus. Whelping and litter size following CAII were recorded. Potential determinants of these outcomes were assessed.

Results Of 238 inseminations performed, 174 (73.1%) resulted in whelping. The known litter size ranged from 1 to 16 pups (mean \pm SD 6.12 ± 3.12 pups). From univariable analyses, progressive moti-

efficiency, convenience and quality assurance, as well as control of breeding-associated infectious disease.¹⁻⁴ Disadvantages include potential welfare and ethical concerns, the potential for heritable disease selection, over-use of a particular male's genes and confusion over parentage.¹

Sperm for insemination are most commonly collected through ejaculation; however, use of epididymal⁵ or testicular⁶ sperm has been reported. Semen may be fresh, fresh-chilled or frozen-thawed and may be inseminated into the vagina, cervix, uterus or uterine tubes.⁴⁻¹⁰

The fertility observed with intravaginal insemination of frozen-thawed semen is generally poor and it is reported that higher levels of fecundity are achieved with intrauterine insemination.¹⁴⁻¹⁶ Several intrauterine insemination techniques have been developed, includ-



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REGARDLESS OF
THE METHOD
USED IT ALL
ENDS UP IN THE
UTERUS!

Contrast media in both
uterine horns after intra-
uterine deposition using a
TCI endoscope (courtesy of
M.Wilson, 1993)



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- There are many techniques in which semen can be deposited into the uterus.
- There is NO difference in pregnancy rates or litter size after either surgical or transcervical insemination in the bitch (*Hollinshead and Hanlon 2017*)
- This is because the semen is deposited into the SAME site i.e the uterus
- There are MANY other factors that affect reproductive performance

FACTORS THAT AFFECT CANINE REPRODUCTIVE PERFORMANCE:

What really matters?!

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WHAT FACTORS AFFECT REPRODUCTIVE PERFORMANCE?

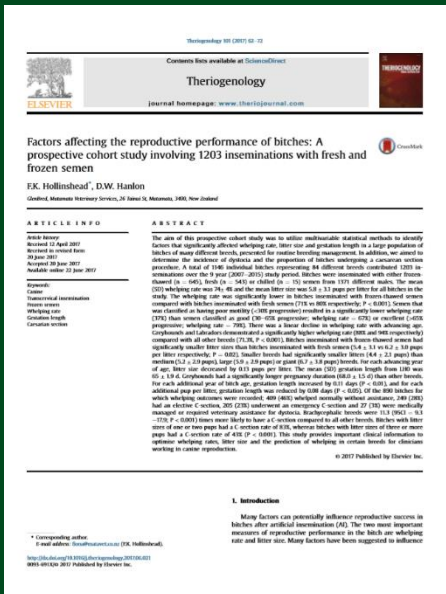
FACTORS CONTRIBUTING TO PREGNANCY RATE AND LITTER SIZE AFTER AI :

- Semen type (fresh v frozen v chilled)
- Semen motility/quality
- Inherent male and female fertility
- Age and mating status of dog at time of AI or semen freezing
- Age, BW and parity status of bitch at time of AI
- Freezing method and operator skill
- Timing of AI in relation to LH 0/ovulation
- Number of sperm inseminated (1 vs 2 AI's per heat)
- Type of AI technique
- Site of semen deposition
- Breed, Season



CANINE REPRODUCTIVE PERFORMANCE STUDY:

- Prospective cohort study
- 2007-2016
- 1146 bitches (mean age: 3.9 +/-1.7 y)
- 1094 dogs (fresh =558, frozen-thawed=645)
- 84 breeds (bulldog, GSD, labrador, greyhound)
- 1251 inseminations/estrous cycles
- TCI (n=1103); surgical AI (n=36)
- 2 experienced operators
- **Multivariate analysis**



Hollinshead and Hanlon (2017) Factors affecting the reproductive performance of bitches: A prospective cohort study involving 1203 inseminations with fresh and frozen semen. *Theriogenology*, 101:62-67



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RESULTS: FACTORS AFFECTING WHELP RATE

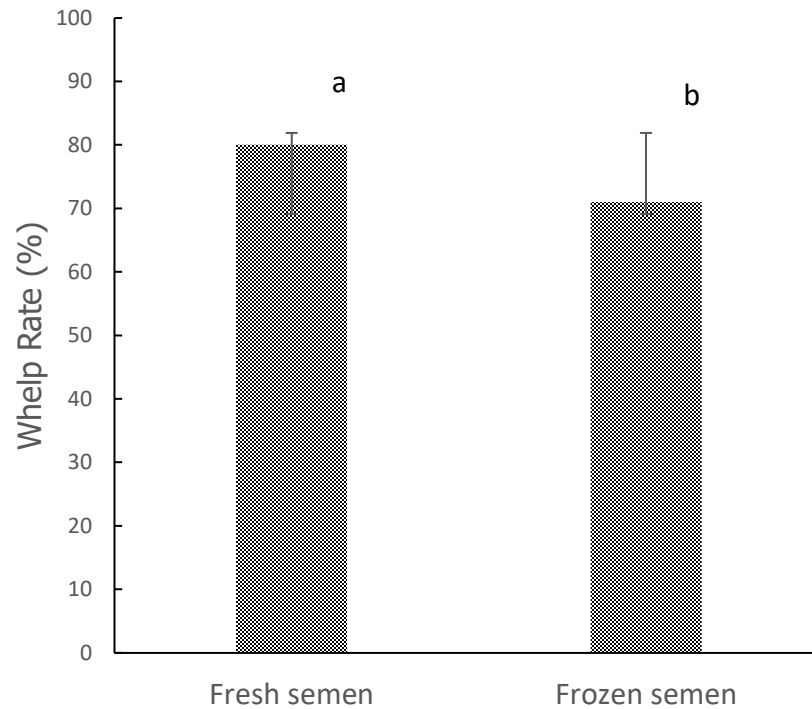
Whelp Rate=% bitches producing at least 1 live pup (mean 74%)

1. Semen Type (fresh vs frozen)
2. Semen motility
3. Semen source (frozen)
4. **Age of the bitch at the time of AI**
5. Breed of the bitch

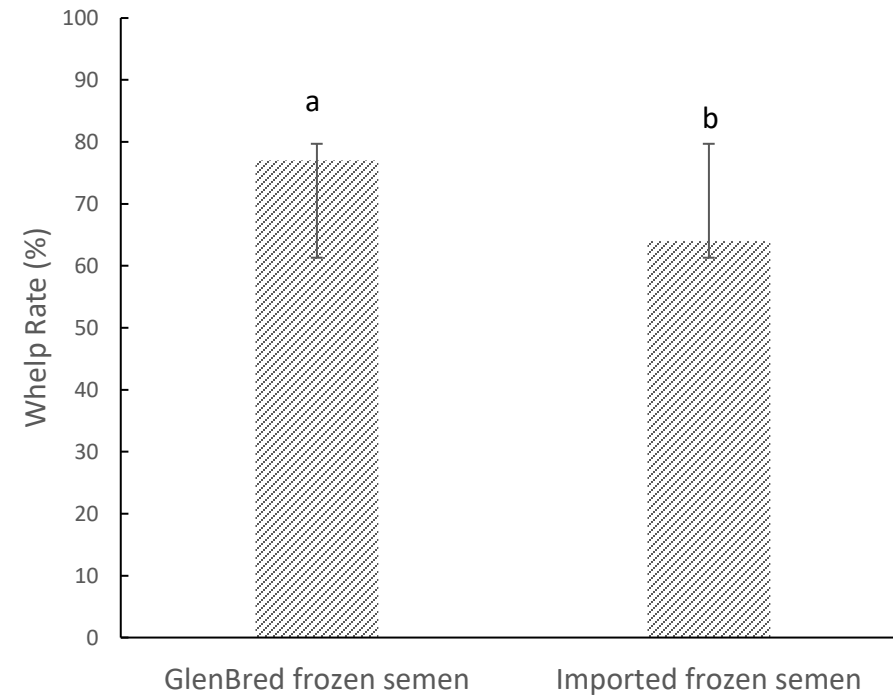




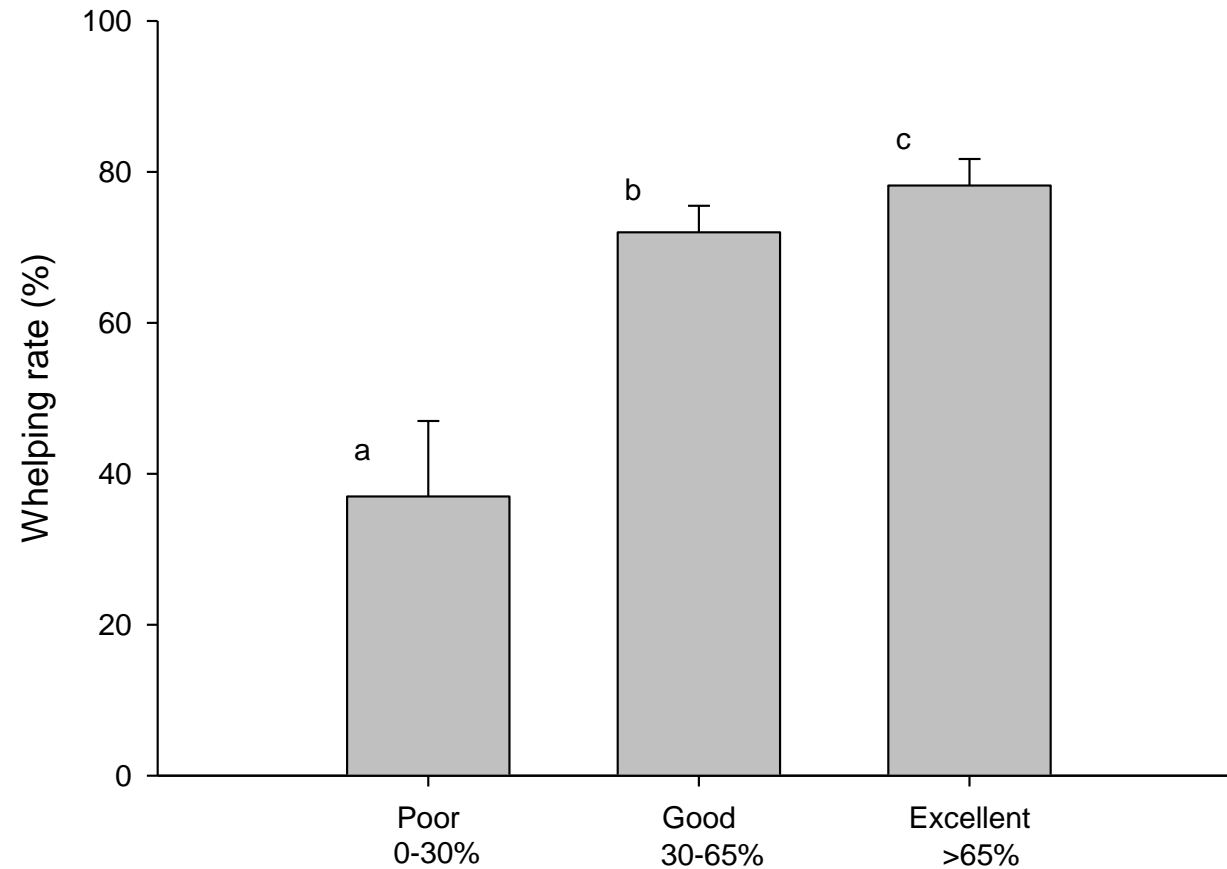
WHELP RATE: SEMEN TYPE AND SOURCE



The whelping rate was significantly lower in bitches inseminated with frozen semen compared with bitches inseminated with fresh semen (71% (95CI=65-75) vs 80% (95CI=77-84) respectively; $P < 0.001$)



The whelping rate was significantly lower for frozen-thawed semen imported into GlenBred compared with semen processed at GlenBred (whelping rate of 64% vs 77% respectively; $P < 0.001$).



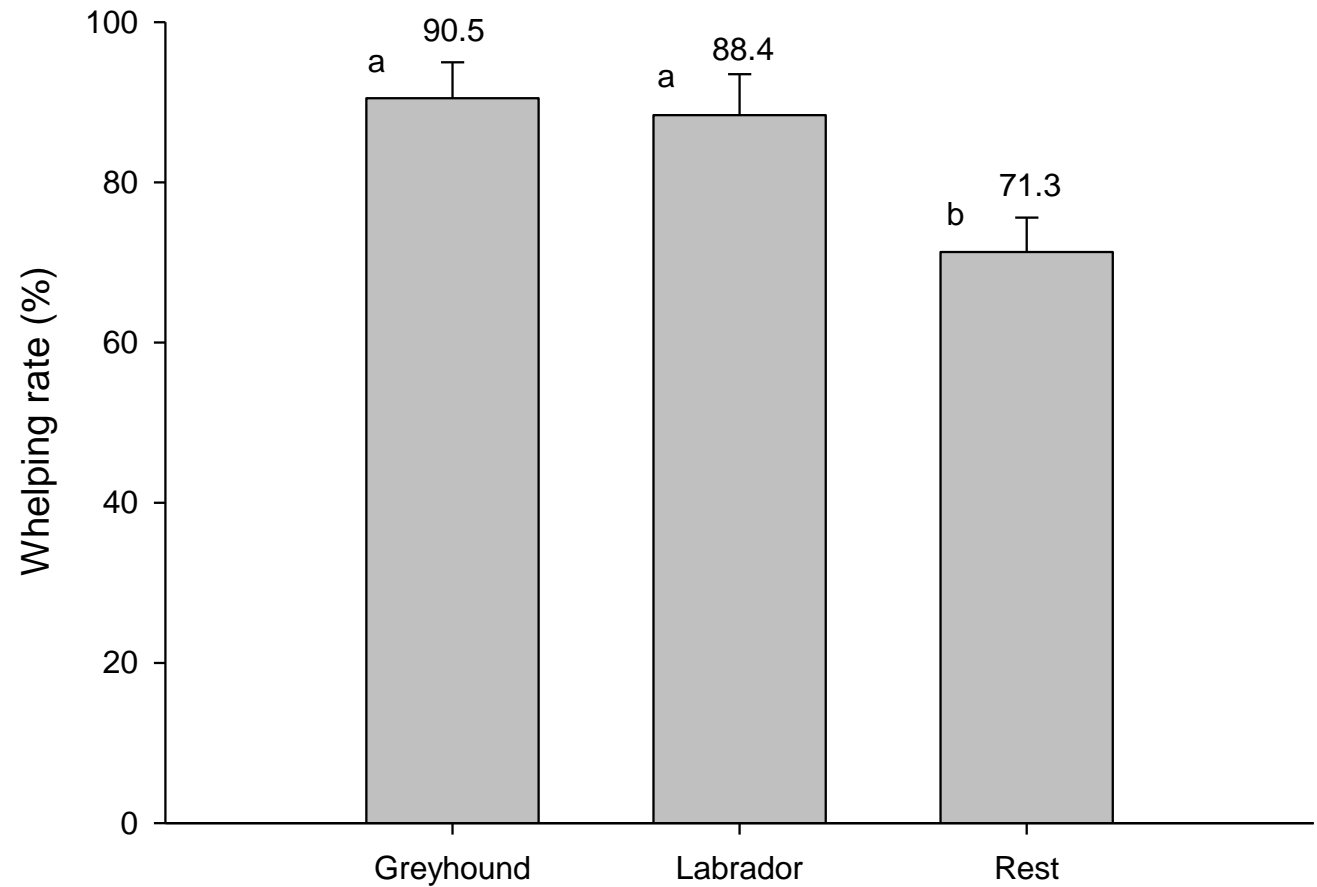
Semen that was classified as having poor motility (<30%) resulted in a lower whelping rate (37% (95CI=23-52)) than semen classified as good (30-65%; whelping rate = 67% (95CI=62-72)) or excellent (>65%; whelping rate = 79% (95CI=76-82)).

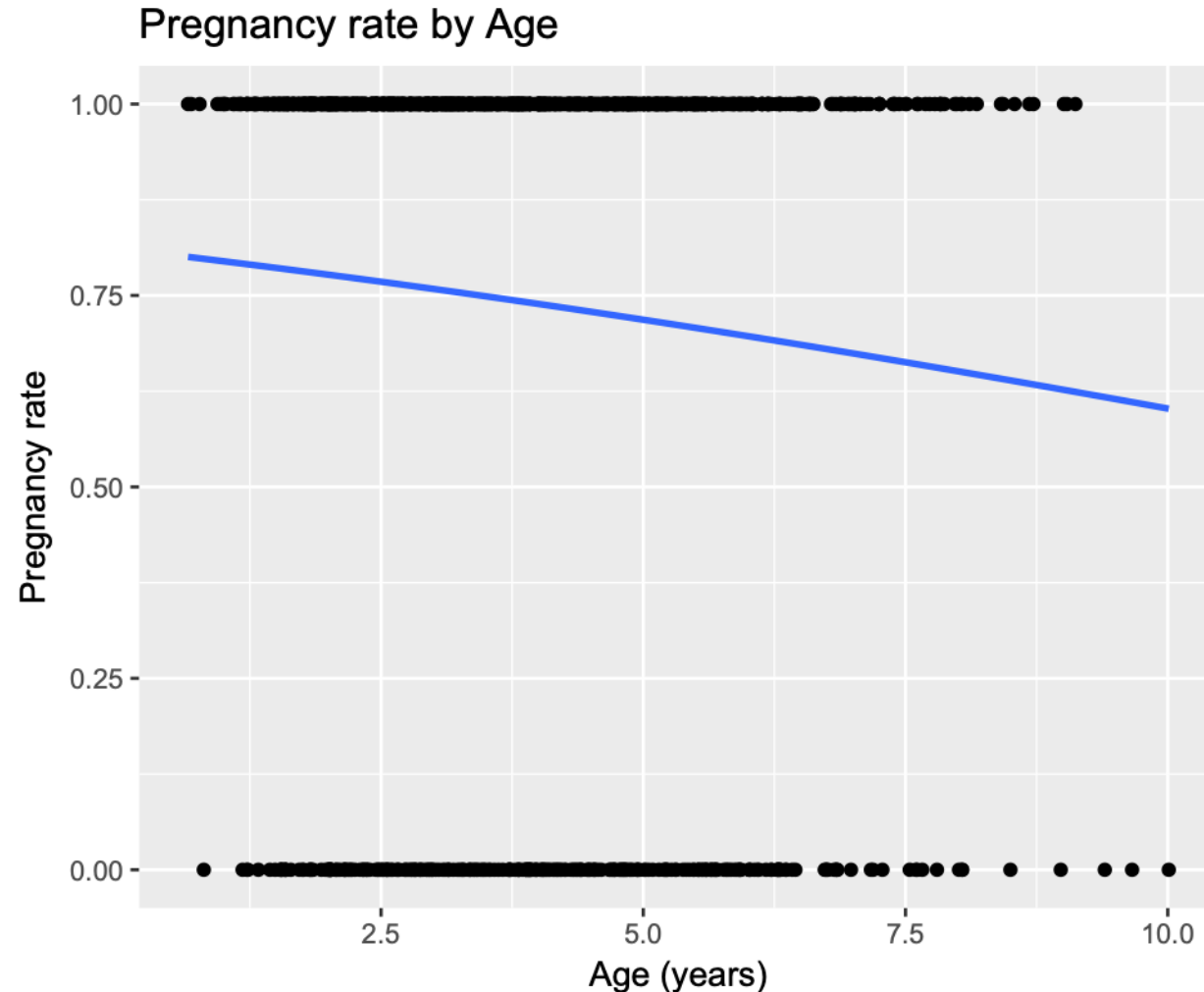
P<0.001

WHELP
RATE:

SPERM
MOTILITY

WHELP RATE: BREED





A linear decline was seen in WR with advancing age: for each additional year of age the odds of whelping declined by 0.93

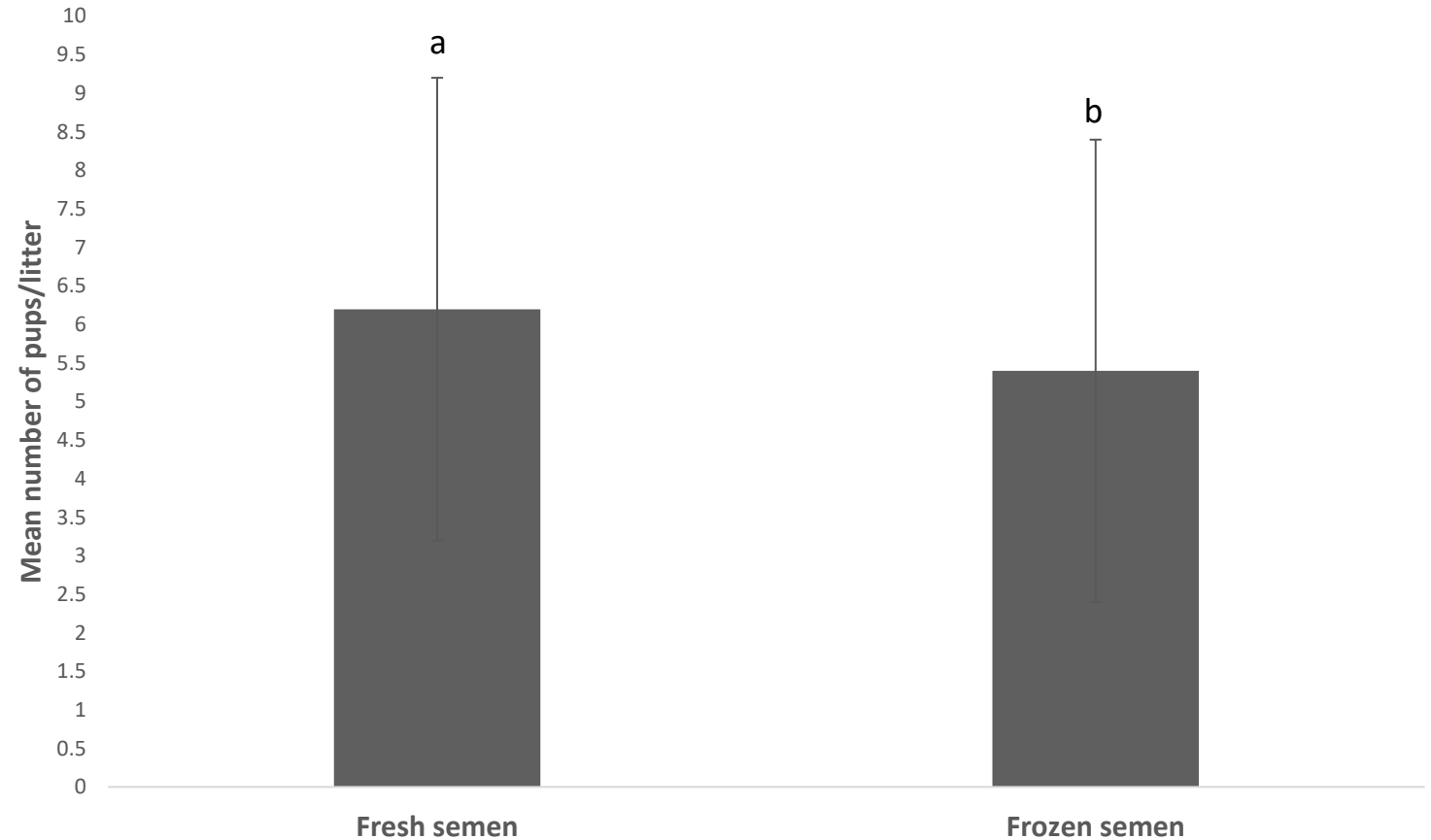
FACTORS THAT AFFECT LITTER SIZE

Definition= Number of live pups born (mean = 5.8 +/- 3.1)

1. Semen type
2. Bitch breed/body weight
3. Age of bitch at time of AI
4. Timing of AI and progesterone curve



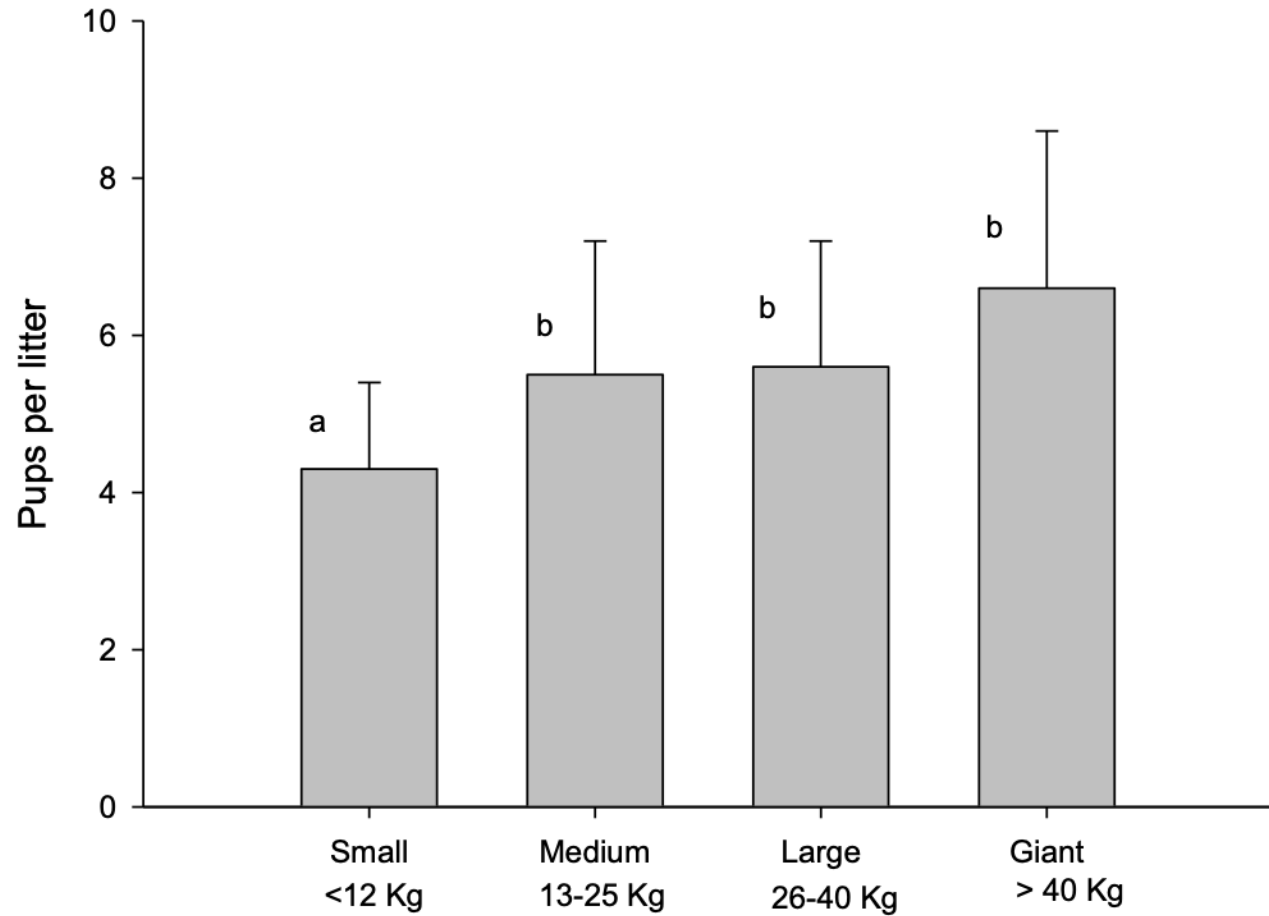
LITTER SIZE: SEMEN TYPE



^{ab} Bitches inseminated with frozen semen had significantly smaller litter sizes than bitches inseminated with fresh semen (5.4 ± 3.1 vs 6.2 ± 3.0 pups per litter for bitches inseminated with frozen and fresh semen respectively; $P = 0.02$)



LITTER SIZE: BREED





LITTER SIZE: AGE OF THE BITCH AT TIME OF AI

For each bitch year of age at the time of AI litter size declined by 0.13 pups.

(same graph as for age and whelping rate)

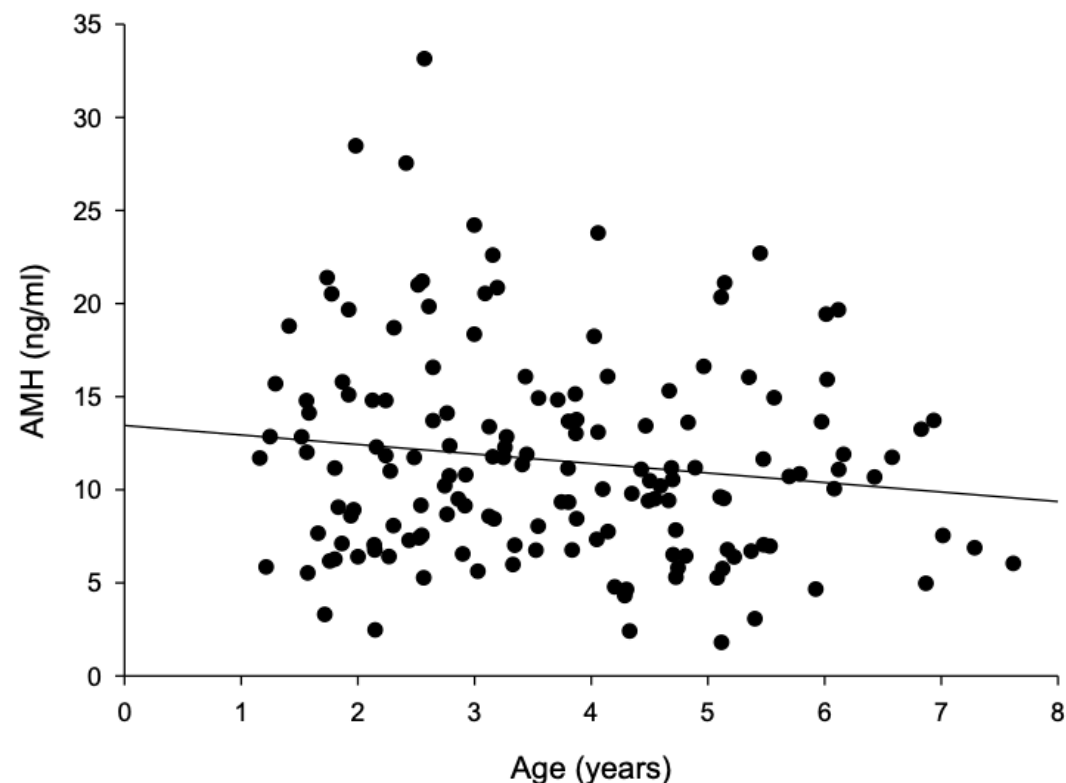
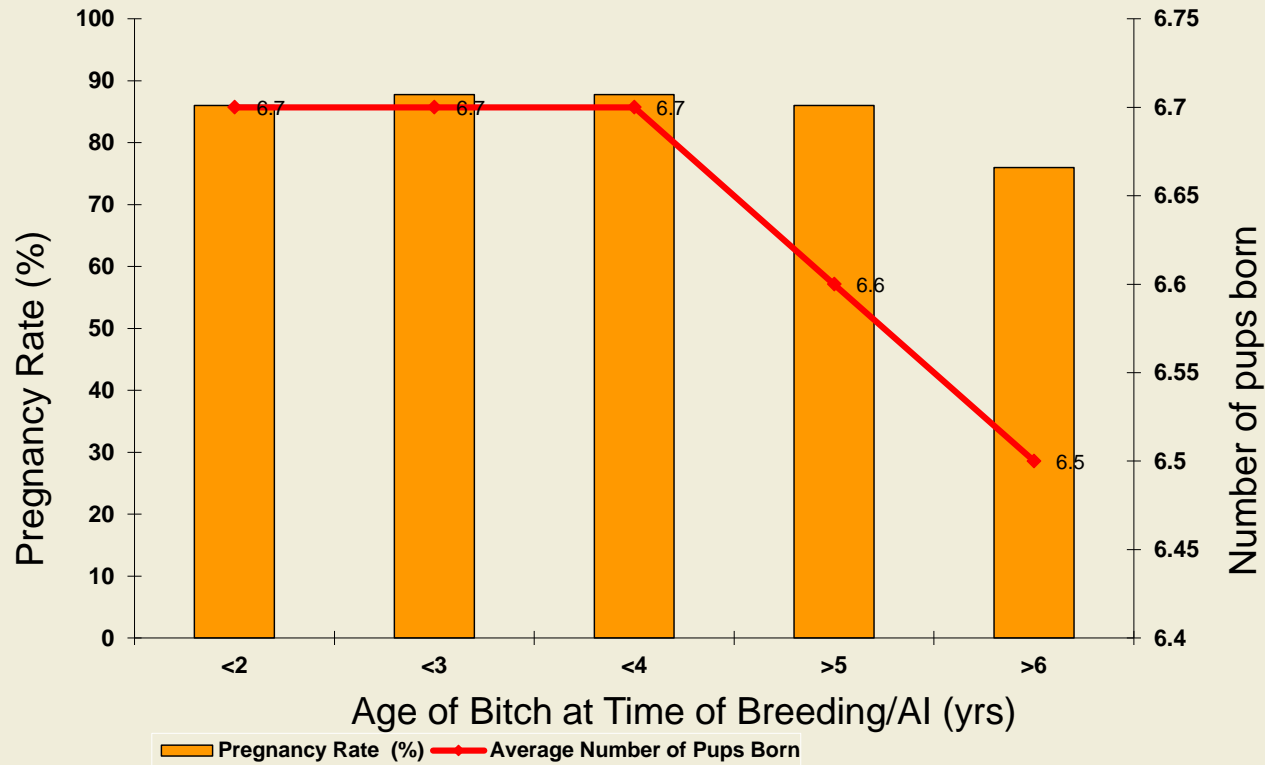


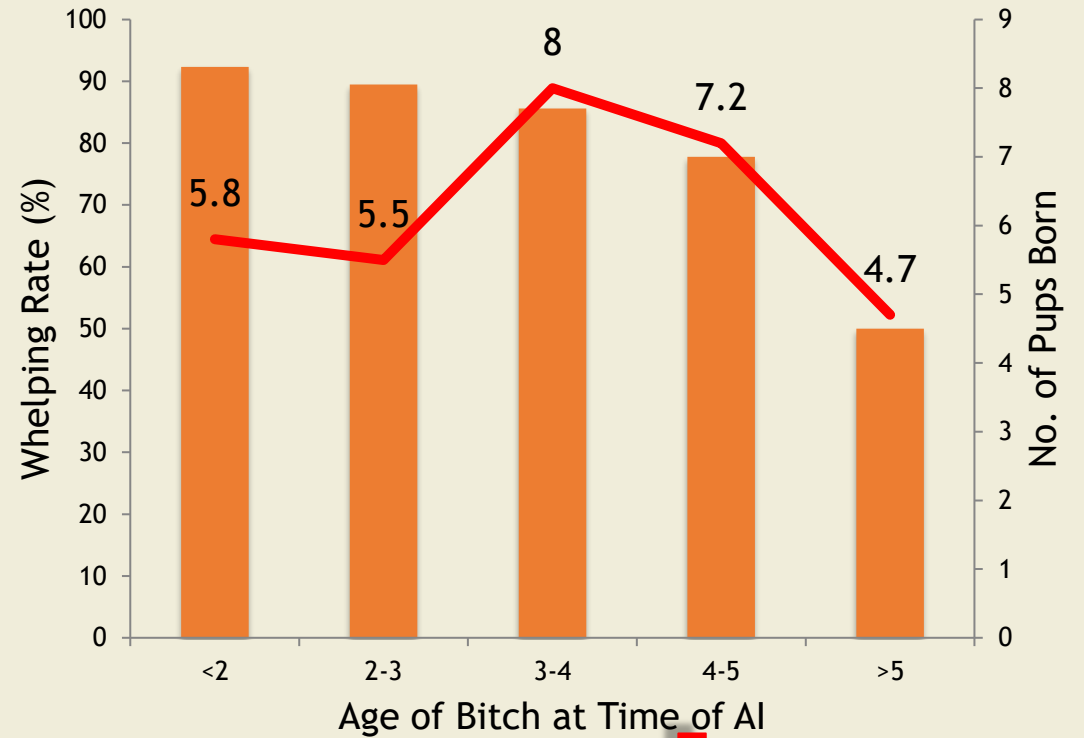
Fig: Regression graph of AMH concentrations (ng/ml) in 155 bitches of different ages. For each additional year of age, AMH concentrations fell by 0.5 ng/ml.

Ref: Hollinshead F.K., Walker, C. and Hanlon D. W. (2016) Determination of the normal reference interval for Anti-Müllerian Hormone (AMH) in bitches and use of AMH as a potential predictor of litter size. *Reprod. Dom. Anim.* 51; 3, 1-6

EFFECT OF AGE OF BITCH AT TIME OF BREEDING ON WHELPING RATE AND LITTER SIZE IN TWO WORKING DOG COLONIES



Labrador Retrievers



PR % Litter size
German Shepherds



CONCLUSION: WHAT REALLY MATTERS?



- **SPERM MOTILITY IS IMPORTANT!**
- **BITCH AGE AT TIME OF AI**
- **BREEDING MANAGEMENT (TIMING OF AI)**

THANK YOU!



- Staff at NZ Guide Dogs, NZ
Police Dog Breeding Centre,
MPI Breeding Centre



- CSU Small Animal Repro Team
- ARBL team at CSU

