

## **Assisted Reproduction: Insight Into Current and Future Techniques for the Next Generation of Puppies**

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### Pre-breeding Exam Male

- Health Record Review
- Breeding History
- Physical Exam
- Semen evaluation
- Brucellosis Test

### History Questions, Male

- What is the reason for the exam
  - Routine, Infertility, Pre-breeding
- Signalment
  - Age: Young, Mature, Old
  - Breed
- Family and kennel history
  - Reproductive history of males and females
  - Genetic problems in this line
- Brucellosis test(s) performed
- Diet and Supplements received
- History of training, stress, or high exercise levels
- Previous Breeding History
  - Last breeding when and how timed
  - How many litters and how long ago
- Mating behavior observations
  - Interested in estrous female
  - Mounts and ties normally
- Any other health or behavior issues
  - Arthritis, systemic illness, injuries, etc.
- Any previous semen evaluations
- Is there any frozen semen available on this male

### Pre-breeding Exam Female

- Health record review
- Breeding and cycle history review
- Physical exam
- Brucellosis test
- Vaginal cultures are not needed because the information they give tells us nothing in a bitch that is healthy. We expect to grow bacteria because the vagina is not sterile.

## History Questions, Female

- What is the reason for the exam
  - Routine, Infertility, Pre-breeding
- Age: Young, Mature, Old
- Breed
- Family and Kennel History
  - Reproductive history of males and females
  - Genetic problems in this line
- Diet
  - Current and previous
  - Supplements and vitamins (Joint meds, etc.)
  - Treats or rewards (liver, commercial, homemade)
  - Medications (HW, Flea and Tick, thyroid, NSAIDs, etc)
- Medical History
  - Previous illness
  - Systemic signs of current illness
    - Vomiting, diarrhea, coughing, sneezing, skin, drinking too much (PD), urinating too much (PU), activity level, body condition score, other
- Cycle history
  - First cycle
    - Can be erratic and may be too young to breed
  - Have cycles been normal
  - How often does she cycle and how long
  - Has she ever had a vaginal smear or series of smears
  - Has she ever has a progesterone or luteinizing hormone (LH) test
- Breeding history
  - Has she been bred before
  - Will she allow a male to mount and tie
  - How was her cycle timed when she was bred
  - Was she bred here or shipped to the male
  - Was she bred to a proven male
    - When was his last litter born
  - How was she bred
    - Natural – was a tie observed
    - AI or chilled – was the semen quality evaluated
    - Surgical or TCI – post thaw semen evaluation
- Pregnancy history
  - Has a pregnancy test been performed
  - How and when was the test done
    - Ultrasound, palpation, relaxin, x-ray

- Has she had a litter
  - How many litters
  - When was the last litter
  - History of dystocia or c-section
  - Size and health of litter
  - Maternal behavior
- History of abortion or fetal resorption (early pregnancy loss)
- Brucellosis testing
- Reproduction Treatments
  - Pyometra
  - Vaginitis
  - Thyroid
  - Mismatching treatments
  - Treatments to prevent or delay estrous cycles
  - Has she been treated with anything for infertility
    - Estrus induction, fertility drugs, homeopathic meds, supplements etc

#### Physical Exam

- Thorough Exam
  - Routine Physical
    - Systemic Medical Conditions may affect fertility
    - Conditions such as lameness or obesity may affect the ability to breed or give birth
  - Reproductive Organs
    - Examined for overall condition as well as presence of discharge (male or female)

#### Semen Evaluation

- Mating Behavior
- Sample Color, Cloudiness, Consistency
- Motility
- Morphology
- Number of Sperm
- Total Number of Normal Progressively Motile Sperm (NPMS) per collection

#### Ways to Use Semen

- Natural Service - Hand or Run Bred
  - Pros
    - Low cost
    - Often is the easiest for many breeders
    - Fresh semen can live in the reproductive tract for 4-9 days.
    - Mating can be done several times.
    - No damage to semen from handling

- Cons
  - Male must be alive, healthy and fertile
  - Number of females serviced is limited.
  - Male and female must be brought together
  - Semen can not be evaluated at time of insemination
  - Male can service only one female per ejaculation.
  - Temperaments of animals must be compatible
- Fresh - Manually collected and used immediately
  - Pros
    - Semen is evaluated
    - Accurate breeding dates recorded
    - Semen can be placed in uterus if male is sub-fertile
    - Semen may be split between females
  - Cons
    - Male and female must be in close proximity
    - Male must be alive healthy and fertile
    - Limited services per week
    - Rarely, males will not allow collection
    - Must be used immediately
- Chilled - Manually collected, extenders are added and it is cooled for use within 48 hours
  - Pros
    - Male and female do not have to be in close proximity
    - May be shipped overnight
    - May be inseminated in uterus or vagina
    - Semen is evaluated before use both when collected and before insemination.
  - Cons
    - Not all sperm survive chilling
    - A few dogs' semen does not chill well
    - Shipment timing can be difficult because of mail or flight scheduling weather, etc.
    - Male must be alive, healthy and fertile
    - Number of services limited
    - Semen may arrive in poor condition due to delay in shipment, temperature variation, or individual low tolerance to chilling process.
- Frozen - Manually collected, frozen and stored until needed
  - Pros
    - Can be stored for long periods of time
    - Multiple breeding units per collection

- Semen is evaluated
- Can be shipped
- Semen may be used after male is dead or unable to breed
- Many more bitches can be serviced
- Cons
  - Best results when placed in the uterus
  - Semen is partially damaged in freezing process
  - Bitch timing must be exact
  - Special advanced equipment required
  - Not all males have semen that is good enough to freeze

The Secret to Success: **TIMING !**

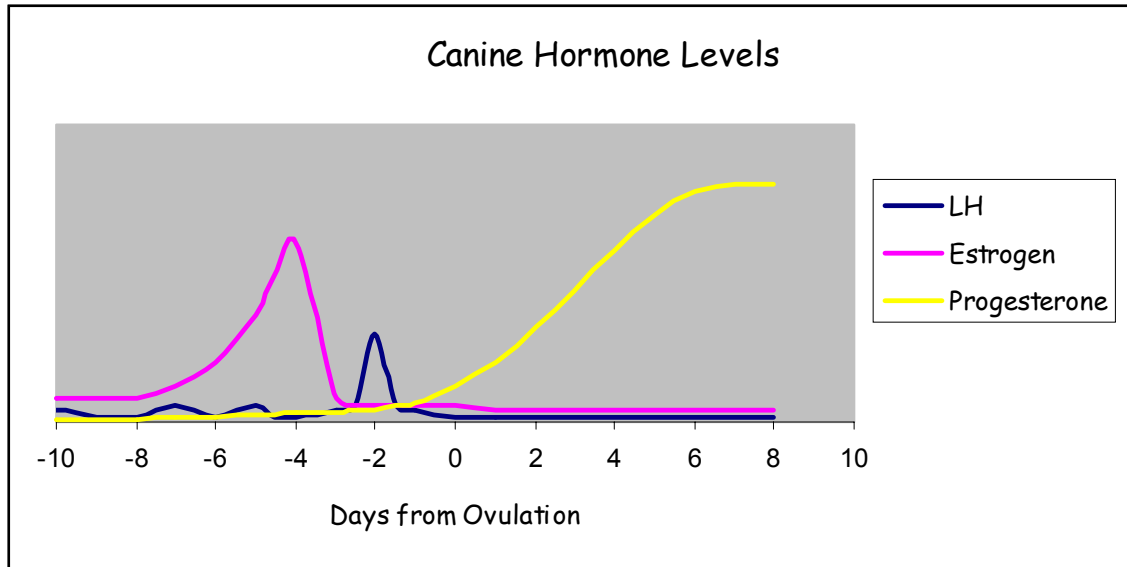
Put excellent quality semen into a fertile female at the right time for the best results.

Timing a Bitch's Season

- The Old Way – These techniques will get some bitches pregnant, they have worked for years. However when you want more than “some” of your bitches pregnant with the highest possible litter size then the new techniques are the best way to go. It is all a matter of how important **this** breeding is to the breeder.
  - Breed on Day 13
    - When do you count day 1?
    - Many bitches will be at the wrong point in the cycle.
    - This works a few times because fresh semen lives in the reproductive tract for a long period of time.
  - Breed when the male says she is ready
    - Some males will breed anything that moves
    - Some females “smell” or “act” ready at the wrong point in their cycle
  - Breed every other day as long as the bitch will stand
    - The most effective of the non-timed methods
    - Works because canine semen lives a long time in the female tract
    - Some bitches will not stand at all, some will stand at the wrong time, if semen less than ideal quality this may not work.

### Hormones

- Luteinizing Hormone (LH) – peak stimulates ovulation 2 days later
- Estrogen – causes maturation and cornification of vaginal lining cells, responsible for breeding behavior
- Progesterone – produced in pregnant AND non-pregnant bitches; maintains pregnancy, induces maternal behavior
- Relaxin – produced by the placenta, relaxes pelvic canal and cervix in late pregnancy. Can be measured to determine pregnancy.

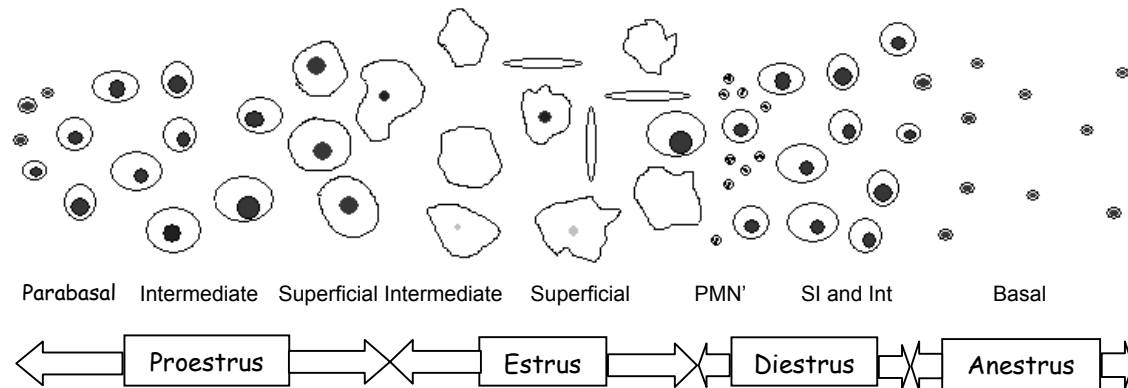


#### Current techniques for timing

- Luteinizing Hormone (LH) levels
  - Must be run every 12 hours to be sure to catch the surge
  - Very accurate predictor of ovulation
  - Best used in conjunction with progesterone levels
  - Can miss the LH surge if run too late in season. If the progesterone has already risen above the range we would expect for the LH surge then running LH level tests would be a waste of time and money.
  - Can become expensive
- Progesterone levels
  - Very accurate when run in a series
  - Single numbers are not as helpful as trends
  - Must be interpreted by someone experienced in canine reproduction and familiar with the lab running the tests.
- Vaginal Smears
  - Best used to gain a basic idea of where a bitch is in her cycle.
  - Should not be used alone as a timing tool if the breeding is important.
  - Are very useful when compared to progesterone levels to evaluate the stage and quality of the cycle.

## Vaginal Cytology

### Canine Vaginal Cytology Progression



#### The meaning of progesterone levels

Progesterone values are in ng/dl and the values here are only valid when run using a technique called RIA, other methods, such as in clinic machines, may give very different numbers.

- $< 0.5$  = anestrus or early proestrus
- $0.5-1.9$  = proestrus or early estrus
- $2 - 2.9$  = day of the LH peak
- $3 - 3.9$  = day before ovulation
- $4 - 10$  = ovulation day
- $> 10$  = has already ovulated
  - Remember not every female “reads the book”, rarely we need to adjust our thinking for a female that is having an unusual cycle
  - Bitches should be bred 4 and 6 days after the LH peak with fresh semen for the best results. This is because it takes 2 days for the egg to mature after ovulation before it will be ready to be fertilized.
  - Progesterone should be measured to be sure she has ovulated on the day of breeding.

#### Timing a Bitch's Season

- First exam - Day 6-7 of her cycle
  - Physical Exam, Vaginal Cytology
  - Progesterone testing if indicated
- Recheck based on previous results
  - If vaginal smear indicates early proestrus it may be 4-7 days before recheck
  - If progesterone test is run a recheck exam will be based on the results both that and the vaginal smear.

## Assisted Techniques

- Artificial Insemination (AI)
  - Semen can be fresh, chilled, or frozen
  - Best success with fresh semen
  - Semen is deposited in the vagina
- Surgical Insemination
  - Bitch is anesthetized and an incision is made in her flank or lower abdomen
  - Uterus exteriorized, semen is injected into each horn, uterus is replaced
  - Incision is sutured and bitch is allowed to wake up.
- Trans-cervical Insemination (TCI)
  - Fresh, chilled or frozen semen may be used
  - An endoscope is passed into the vagina to the cervical opening
  - A catheter is threaded through the cervix and semen is deposited in the uterine body
- Putting semen directly in the uterus may help some stud dogs with poor quality semen produce offspring
  - TCI or Surgical; fresh semen only
  - Consider that poor fertility can be hereditary

## The Age of DNA

- Genetic testing is now available
  - Paternity testing
    - Enables confirmation of sire
    - Allows for multiple sires for one litter
  - Genetic Disease Testing
    - Allows control of genetic diseases by testing potential parents for carrier status

## Cutting Edge

- Post Mortem Semen Harvest
  - Semen can be harvested from stud dogs that have recently died unexpectedly then frozen for future use.
- Sex Sorted Semen
  - Commercially available for Humans, Cattle, Horses. Not yet available for dogs. The availability will largely be based on demand for the service. It is very costly to set up the sorter and obtain the license to run it.
  - Sorted Female Sperm is 91% female
  - Sorted Male Sperm is 76% male

## On The Horizon

None of these techniques are available commercially right now but they may be coming very soon in the future.

- Oocyte Harvest
- Ovarian Cyropreservation – Eggcicles
- In Vitro Fertilization- test tube puppies
- Embryo Transfer – surrogate bitches
- Cloning- Copy Dogs

## Biographical Profile

**Dr. Traas** is currently the Medical Genetics Resident at the Matthew J. Ryan Veterinary Hospital at the University of Pennsylvania where she sees cases in genetics, pediatrics, and reproduction.

Most of her undergraduate work was completed at the University of Wisconsin – River Falls but she received her BS in 1999 from the University of Minnesota. She also received her DVM from the University of Minnesota in 2001.

The three years preceding her residency were spent at Symbioun Inc., a private practice in Kansas, where over 90% of her cases were reproduction related. Over the last three years she has assisted in the breeding of over 3,500 dogs with an average success rate between 93% and 95%.

She currently lives in Philadelphia with her husband Jeremy, her Border Collies, and Ragdoll cats.