



The Cavalier King Charles Spaniel Club UK
Summer Health Seminar – Peopleton 22nd July 2023

Part 2: Modern Techniques of Reproduction in Dogs

from collecting and handling of semen
to Artificial Insemination (AI)



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What to expect in this talk (I)

Practical advises for breeders who might want to perform collecting and/or AI themselves with as little scientific „mumbo-jumbo“ as possible

- ◆ **Planning an Artificial Insemination (AI)**
 - Benefits vs potential weakness of Semen Shipping or Storage
 - Recommendations before thinking about AI
 - Which kind of AI do I want to use:
 - different types of insemination
 - different forms of semen to be used: fresh / chilled / frozen
 - Timing of insemination
 - Preparing the bitch: drugs to help to increase breeding success
- ◆ **Semen Collecting**
 - Preparing the male – training, increasing semen quality
 - Practical advice / Fractions of the semen
 - How to keep the semen in best condition before conservating

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What to expect in this talk (II)

- ◆ **Chilled Semen : Handling and preparation for shipping**
 - Use of Extender – the „magic solution“ to keep the semen alive during shipping, maximum days of storage
 - Preparing the extended semen before shipping
 - Recommendations and limitations for shipping
 - Estimated costs
- ◆ **Chilled Semen : Handling and preparation after arrival**
 - Warming up and possible „at home“ evalation
 - Vaginal insemination

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◆ **Benefits vs potential weakness of Semen Shipping or Storage**

Potential benefits	Potential weakness
<ul style="list-style-type: none">• bigger gene pool• use of dogs that live far away• protection of the male against infections• frozen storage – conservation of genes that might have:<ul style="list-style-type: none">⇒ got lost⇒ turned out to be very useful because of longlivity of the dog / turned out to be clear of deseases that are found out after the death / or children turned out to be extra special in type and/or health	<ul style="list-style-type: none">• perfect timing necessary• good management both on dogs / bitch owners side• limitation of shipping• risk of decreasing libido• bitches get inseminated that are unable to get mated on natural way• frozen semen:<ul style="list-style-type: none">⇒ high costs⇒ invasive methods of insemination⇒ semen quality must be excellent

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Planning an Artificial Insemination (AI)

◆ Recommendations before thinking about AI

- check about the **rules of your national kennel club**
- check if both dog and bitch owner has a **veterinary** that is on standby
- dog and bitch should have had a **litter naturally way before** (within FCI mandatory)
- can I perform **progesteron testing** ? (which is mandatory as semen is not living as long as fresh semen)
- which **sort of insemination** do I want to use resp. which **form of semen** do I want to use

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Planning an Artificial Insemination (AI)

◆ Which kind of Insemination do I want to use ?

- different types of insemination -

① Vaginal Insemination

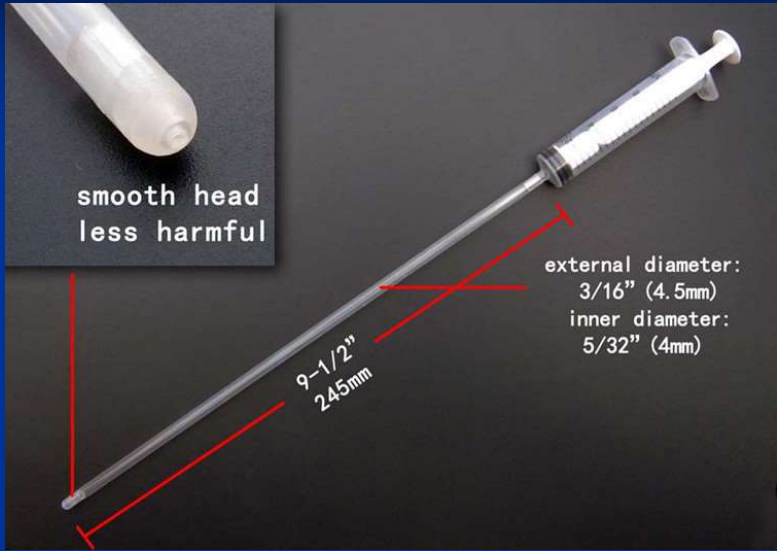
- semen is placed directly into the bitch's vaginal canal, just before the opening into the uterus
- usually used when using fresh semen which has just been collected or chilled semen shipped overnight – not to be used for frozen semen

Procedure:

- done using an insemination tube or better a balloon catheter
- gently inserted into the vaginal canal by going up first and then horizontal
- when not using a balloon catheter female's hind legs are elevated to help the semen move forward to the opening into the uterus with the aid of gravity

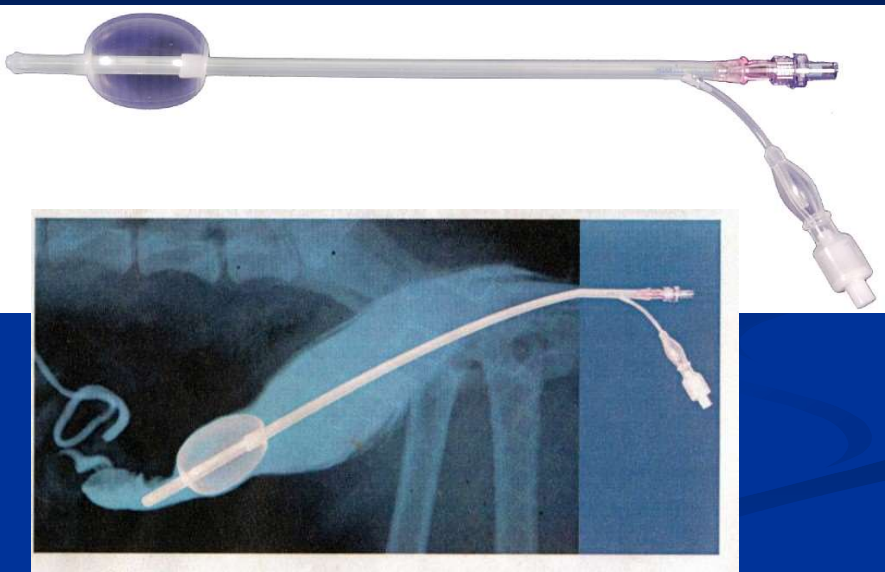
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Insemination Catheter (simple)



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Insemination Catheter (Balloon)



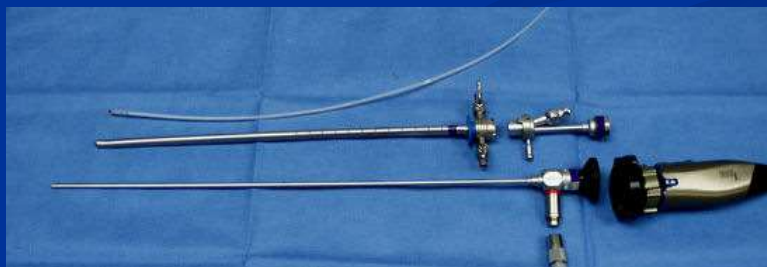
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② Transcervical Insemination (TCI)

- semen is placed directly into the uterus
- has to be performed by an experienced vet (additional costs ?)
- no anesthetic needed
- usually used when using chilled or frozen semen

Procedure:

- with the aid of a long, slender scope the cervical opening is visualized and a steril, flexible catheter is passed through the opening into the uterus



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③ Surgical Insemination

- semen is placed directly into the uterus
- visualizing the reproductive tract on the inside allows check for cysts, tumors, blockages etc. – to be used in bitches with history of losing pregnancies, conception problems or older bitches
- has to be performed by an experienced vet (additional costs ?)
- general anesthetic needed
- usually used when using chilled or frozen semen

Procedure:

- incision made on the midline below her umbilicus
- uterus is located and the semen is deposited directly into the uterus through a small catheter with a fine needle



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◆ Which kind of Insemination do I want to use ?

- different forms of semen to be used -

① Fresh Semen

- Male is **collected side by side** to the bitch in season that will be inseminated
- Semen is inseminated **without adding of Extender** shortly after collecting

Longevity of Semen: should be inseminated shortly after collecting

Use:

- to protect the male dog against **infections**
- **reluctant bitch**, „narrow“ bitch resp. male cannot get in properly

Timing / Type of insemination:

- 1-4 days after ovulation (resp. 3-6 days after LH Peak)
- Vaginal Insemination (transcervical insemination, surgical insemination)

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② Chilled Semen

- Male is collected in the absence of the bitch that will be inseminated, preferable with a teaser bitch or a cotton swap from a bitch in season
- Added Extender (solution that contains nutrients, buffers, antibiotics and cell wall protectants) helps to preserve the sperm quality and integrity during chilling and shipping
- Shipping overnight in a simple styropor box
- Collecting, preparation and shipping can be done by an experienced breeder with excellent results

Longevity of Semen: depends on used Extender

- **Extender without egg yolk 2-3 days** (up to 5 days)
- **Extender with egg yolk 3-5 days** (up to 10 days)

Use: easy way to use male dogs that live far away but where semen can be shipped with 48 hours

Timing / Type of insemination:

- 2-4 days after ovulation (resp. 4-6 days after LH Peak)
- Vaginal Insemination / Intrauterine Insemination

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③ Frozen Semen

- Male is collected in the absence of the bitch that will be inseminated, preferable with a teaser bitch or a cotton swap from a bitch in season
- Added Extender (solution that contains nutrients, buffers, antibiotics and cell wall protectants) helps to preserve the sperm quality and integrity during chilling, freezing and storage
- After chilling the semen is loaded into multiple straws and then put in liquid nitrogen at -196°C , after freezing a sample straw has to be warmed up and evaluated to see what percentage of good quality semen survived the freezing process to calculate how many straws will be needed to give one "portion"
- Semen must be of high quality, at least 80% normal as you will lose some of it during the freezing process
- Storage in liquid nitrogen in special containers at -196°C
- Shipping in multiple use containers or single used containers
- Collecting, preparation and storage has to be done by an experienced vet
=> higher costs

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Longevity of Semen:

- several decades, if stored correctly - theoretically forever

Use:

- To conserve semen of dogs that have the genetic potential to improve the breed for later use even after the dogs death
- In busy stud dogs that might not be available for stud at any time (due to show tours or extended stays or or or)
- Most reliable form of semen when shipping overseas where it may take a bit longer to clear customs and get to its destination

Timing / Type of insemination:

- 3-4 days after ovulation (resp. 5-6 days after LH Peak)
- Intrauterine Insemination (either transcervical or surgical)

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Planning an Artificial Insemination (AI)

♦ Timing of insemination

There are multiple hormones that help to regulate the estrus (heat) cycle and pregnancy in dogs. Important for timing of insemination are:

Estrogen: Stimulates the ovaries to produce eggs.

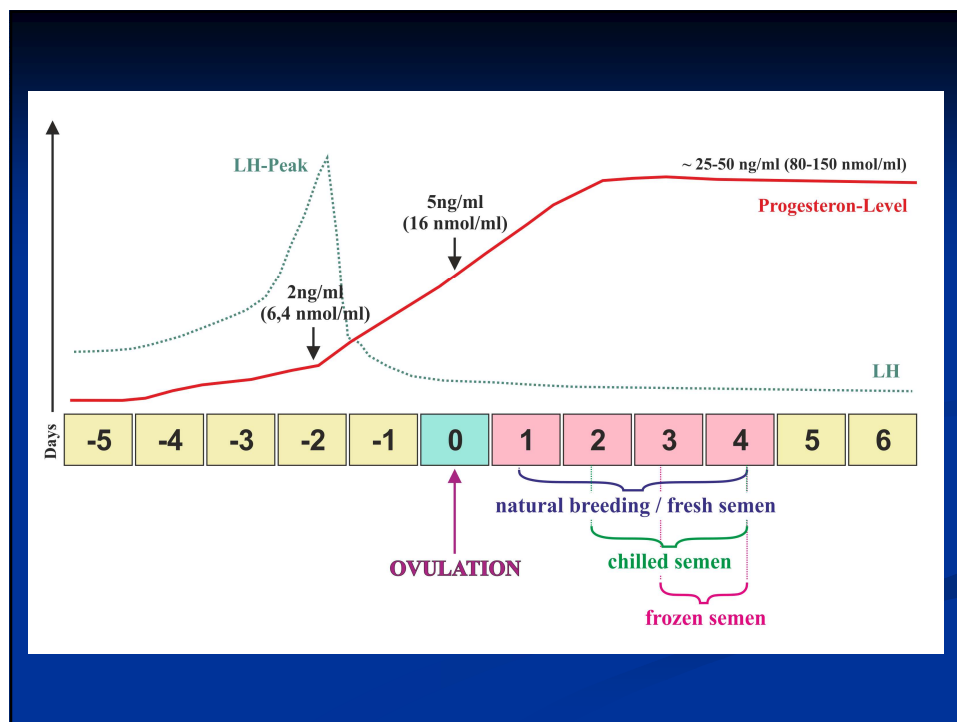
Luteinizing Hormone (LH): Stimulates the ovaries to release the eggs.

Progesterone: Maintains a pregnancy.

- **LH test:** needs to be done daily as LH-Peak occurs typically for less than 24 hours, on top its species specific and cannot be run in a human laboratory
- **Progesteron test:** is not species specific and can be run in a human lab as well, Progesteron test can be run every 2-3 days (or even longer depending on result) starting on day 5-6 of the cycle => Progesteron is the most easiest value to determine the optimal time of insemination

For performing an AI a proper Progesteron test is mandatory !!!

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Progesteron-Level:

- base progesteron level is less than 1 ng/ml (3,18 nmol/ml)
- interesting time starts when Progesteron raises above 2 ng/ml (6,4 nmol)

2 ng/ml (6,4 nmol/ml):

approx. 2 days before ovulation = LH-Peak = most likely receptive to male
estimated mating time in 3-6 days
when using chilled or frozen semen run another test in about 2 days !!

5 ng/ml (16 nmol/ml):

Ovulation
eggs need another 24-48 hours to mature and can be fertilized and live for approx. 2 days
mating / insemination in 1-4 days (exact timing depends on form of semen)

after ovulation:

progesteron level raises individually up to 25-50 ng/ml (80-150 nmol/ml)

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Day of insemination depending on form of used semen:

Natural Mating / Fresh Semen:

- Sperm survives 3-4 days inside the bitch (5-7 days just theoretically)
- **Insemination: 1-4 days after ovulation** (3-6 days after LH Peak)
- Personal note: in my own experience best 1-3 days after ovulation because opening to the uterus is closing and semen might not come through

Chilled Semen:

- Sperm survives 48 to 72 hours (2-3 days) inside the bitch
- **Insemination: 2-4 days after ovulation** (4-6 days after LH Peak)
- Personal note: when doing vaginal insemination best 2-3 days after ovulation because opening to the uterus is closing and semen might not come through

Frozen Semen:

- Sperm survives less than 24 hours inside the bitch
- **Insemination: 3-4 days after ovulation** (5-6 days after LH Peak) directly in the uterus

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Semen	Dosis	Expected spz survival	Insemination schedule	Expected fertility
Fresh	150-200x10 ⁶ spz/mL (extended)	4-6 days	- Every other day, when P ₄ rise above 4ng/mL, up to 3 times. - Day 1 to 4 post-ovulation - P ₄ levels between 8 and 15ng/mL	- 80-90% (either with transcervical or vaginal deposition)
Chilled	150 - 200x10 ⁶ spz/mL (extended)	24-72hrs	- Breeding once or twice 2-4 days post ovulation (P ₄ = 4 -10ng/mL). - Day 2 to 4 post-ovulation - P ₄ levels between 8 and 15ng/mL	- 80-90% (either with transcervical or vaginal deposition)
Frozen	50 - 300x10 ⁶ spz/mL (extended)	12-24hrs.	- Twice, at P ₄ levels above 8ng/mL and estrus vaginal cytology - Day 5 to 7 post-ovulation - P ₄ levels between 18 and 28 ng/mL	- 45% if vaginal deposition - 67 -84% if transcervical or intrauterine

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Planning an Artificial Insemination (AI)

◆ Preparing the bitch: drugs to help to increase breeding success

Drugs to shift the season of a bitch

Postpone the beginning of a season:

Medroxyprogesteronacetat 5mg (brand: e.g. Sedometril / Perlutex)
1 Tbl / Cavalier / day

- should only be used carefully and short-term and just when the following season is not used for breeding
- risk of reduced fertility or mucometra / pyometra rises when used after season has already started



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Trigger the beginning of a season:

Cabergolin (brand: e.g. Galastop) 2,5 - 5 µg / kg / once a day orally

- should only be used earliest 120 days (4 months) after the last season
- fertile season usually starts within 10-14 days
- continue treatment until bleeding starts, then immediately stop
- small intern study: 28 bitches treated, 28 came in season, 26 in whelp



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Drugs to increase breeding success

L-Tyrosine (Aminoacid):

1g / Cavalier / once a day on days 3 to 6 of the season

- simple amino acid, no prescription needed
- body uses this amino acid (NO hormone) to synthesize hormones itself that support fertility, exact mechanism not fully studied
- bitches seem to be „more happy“ and are more ready to welcome the male



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L-Lysin:

1g / Cavalier / once a day from day 1 of season until due date

- simple amino acid, no prescription needed
- known to reduce the risk of break-out of herpes virus

Vitamins:

Vitamins A, E, B complex, folic acid

- known to be helpful for optimal fertility and to avoid e.g. cleft palates
- mostly included in commercial products



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Semen Collection

◆ Preparing the male – training, increasing semen quality

Training:

- start as early as possible
 - using a teaser bitch in season, let the boy mount the girl (of course you always have to put your hand in between) => praise him to the skies for every attempt
 - when he is confident, collect him and give him a positive experience
 - male must get used to you sitting side to side to the bitch
- perform always the same rituals (e.g. a special blanket, has to go in to a separate cage 10 minutes prior the practise or or or)

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Increasing semen quality – environmental factors:

- use a teaser bitch in season or a frozen cotton swap of a bitch in season
- best if the male can mount a bitch using the practised rituals
- be aware of the rank order within the pack, do the collecting in the absence of other males in a quiet room
- best semen quality at the age of 1-5 years
=> freeze Mr Superduper when he is young !
- semen quality is reduced after a longer break since the last mating
=> best quality when collected / mated on natural way twice a month
=> 2 collections once a week prior to a collecting for freezing recommended

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Vitamins and Drugs to increase semen quality

Vitamin E / Selen:

1 mg Vitamin E in combination with 0,1 mg Selen / Cavalier / day

- use for at least 6 weeks prior to a collecting to improve semen quality
- especially needed when using a food with high rate of omega-3/6-acids
- best in combination with Zink, Vitamin C and Vitamin B complex



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GlycoFlex:

500 mg GlycoFlex / Cavalier / day

- use for at least 6-12 weeks prior to a collecting to improve semen count
- one of the main ingredients is Perna mussel who is doing its „magic“

L-Carnitine: (e.g. Canine Motility Plus)

500 mg L-Carnitine / Cavalier / day

- use for at least 6-12 weeks prior to a collecting to improve semen motility
- stimulates lipid oxidation in the mitochondria of the sperm and increases energy production which can help in dogs with low motility



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Homeopathic Drugs to increase the libido:

Damiana D1 & Acidum phosphoricum D6

- start latest 3 days (up to 3 weeks) prior of the mating / collecting
- 3 times a day 1 Dose (that means 1 tablet or 5 Globuli) with or without water placed at inside the lip
- on the day of the mating / collecting 1 Dose in the morning and second one 1 hour before the mating / collecting

Hormons: for Veterinary Use Only !!!

● **Dinoprost Tromethamine:**

0,5 mg s.c. / Cavalier / 15-20 minutes before collecting

- Hormon Prostaglandin F2 α
- may advance emission and ejaculation

● **GnRH:**

15 μ g i.m. / Cavalier / 1-3 hours before collecting

- Gonadotropin-Releasing-Hormon
- may increase libido and the chance that ejaculation will occur

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Semen Collection

Collecting Process in detail:

- most common method is by digital stimulation
- under ideal conditions, this procedure is performed in the presence of an estrous bitch or a frozen cotton swap or an estrous bitch
- best if the male can mount a bitch using the practised rituals
- be aware of the rank order within the pack, do the collecting in the absence of other males in a quiet room
- initially, the dog's penis is vigorously massaged through the prepuce at the level of the bulbus glandis until a partial erection develops
- then the prepuce is quickly retracted past the bulbus glandis and firm constant pressure is applied to the penis behind or at the bulbus glandis by squeezing the penis between index finger and thumb
- pelvic thrusting may occur following application of pressure behind the bulbus glandis during the development of a "full" erection

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Collecting tubes:

- cone or plastic sleeve
- funnel
- special collecting vial



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There are 3 fractions of the semen:

- 1st fraction: presperm fraction, which is a small volume of clear fluid, is emitted in 0.5 to 1 minute => sperm-poor, do not use for shipping
- 2nd fraction: cloudy fluid, dog will usually thrust vigorously, also rapidly completed (1-2 minutes) => sperm-rich, use for shipping
- 3rd fraction: clear prostatic fluid, prior to ejaculating this fraction, the dog will usually dismount and attempt to step over the arm of the collector, it may take up from 5 to 30 minutes to be completed
=> do not use for shipping

In most dogs, semen can be collected twice at 30 minutes interval, although the second sample is usually diluted



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Characteristics	1 st fraction	2 nd Fraction	3 rd Fraction
Volume	0.1-2 mL (average 0.33 mL)	0.1-3 mL (average 1.17 mL) Sometimes larger volume	1-2 to >20 mL Quite variable depending on the animal.
Colour	clear or opaque	greyish-white, white, milky-white	clear, transparent
Consistency	watery	watery-milky, milky	watery
Character	prostate secretion with admixture of epithelial cells, urine, bacteria and sperm cells	sperm cells suspended in seminal plasma	prostate gland secretion
pH (average)	6.37	6.10	7.20
Duration	5-90 sec. (average 13.5 sec)	5-300 sec. (average 52.4 sec.)	60 sec-20 min. (average 6 min. 55 sec.)

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Practical advises:

- best semen quality at the age of 1-5 years
=> freeze Mr Superduper when he is young !
- after a prolonged sexual rest dogs may ejaculate many dead, immotile spermatozoa of abnormal morphology
=> ideal intervals between collections are 2 to 5 days, whilst intervals longer than 10 days may result in an increased number of morphological abnormalities and decreased motility
=> it is advisable to perform one or two previous collections, if semen is to be chilled or frozen for shipment
- AI with freshly collected semen can be performed without fractioning the ejaculate, for chilling and freezing only use the second fraction
- do not use latex gloves or syringes that contain latex as this may decrease sperm quality
- keep the semen always warm (at about 37-38°C), best in a water bath or in your hands

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Chilled Semen : Handling and preparation for shipping

- only use the 2nd fraction of the semen and keep it warm !!
- to keep the semen “alive” and to preserve the sperm quality and integrity during chilling and shipping you have to add extender (solution that contains nutrients, buffers, antibiotics and cell wall protectants)
- warm up the extender prior of collecting, ideally in a water bath at 37/38°C
- avoid any temperature variation during preservation
- SLOWLY dilute the semen with the pre-warmed extender – the ratio depends on the used Extender, in Minitube CaniPlus Chill 1 part semen to 2-3 parts Extender
- fill the diluted semen in tubes or syringes, seal and label them
- store the semen at +5°C in the fridge for at least 2 hours before packing and shipping

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Preparing the extended semen before shipping:

- a specific styropor transport container (e.g. Minitube Semen Shipper)
- put in two thermal packs that were stored in the freezer overnight, at low outside temperatures its recommended to use only one pack from the freezer, the second one should be at fridge temperature
- ideally put the tube with the semen into another bigger tube and seal to avoid variations in temperatur during transport by providing an air buffer
- close the transport container, add needed paperwork and put it into the cardboard box, add the shipping documents and attach the address label
- constant temperature of semen below +10°C is maintained for approx. 24 hours (ordinary Styrofoam boxes) to 40 hours (Minitube Semen Shipper), however the duration of constant temperature depends on the outside temp



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Recommendations and limitations for shipping:

- use an overnight shipping, preferable morning express
- shippings longer than 40 hours are at risk for not performing correct temperature
- shipping companies e.g. FedEx or DHL Express
- check shipping times before accepting an order for chilled semen shipping
- be aware of limitation of shipping over the weekend !
- normally a shipping is only possible monday to thursday, arriving tuesday to friday – usually no arrivings on saturday, sunday or monday possible
- be aware of possible customs services that might delay the shipping
- shipping within the US or within the EU is no problem, outside of the EU or the US chilled semen is often not possible due to longer shipping times, in that cases better go for the more expensive procedure of frozen semen
- DHL Express rates are usually 30-50 GBP for domestic services, 100-150 GBP for international services within the EU

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Estimated costs:

no responsibility is taken for the correctness of this information

Collecting, evaluation and preparation (incl. Box) at a repro vet	~ 200-250 GBP
when collecting yourself: costs of the box and extender	~ 20-30 GBP
Shipping costs	DHL Express: ~ 30-50 GBP domestic service ~ 100-150 GBP international service within the EU
Total:	
collecting at a repro vet	~ 230-400 GBP
collecting yourself	~ 50-180 GBP

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Chilled Semen : Handling and preparation after arrival

- only open the transport container at time of insemination !
- if you open and do not want to use all semen included at the same time, store the not used semen immediately in the fridge at +5-7°C
- at time of insemination warm the diluted semen to room temperature (best to body temperature by warming up in your hands) and perform if wanted a motility evaluation
- depending on the semen quality at the time of insemination perform either a vaginal, transcervical (TCI) or surgical insemination
 - Vaginal insemination:** preferable with a balloon catheter, max. total volumes:
 - 2-5 ml for bitches <10kg (<22lbs)
 - 5-10 ml for bitches between 11 and 19kg (22lbs to 44lbs)
 - up to 20 ml for bitches more than 20kg (>44lbs)
 - TCI or surgical insemination:**
 - 1-3 ml of extended semen depending on size of the bitch

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Possible “at home” evaluation:

- **Volume:** not a specific marker as it all depends on the used ratio of extender and how much prostatic fluid was included
- **Colour:** when analysing the colour, one should be aware of the method of collection, as colour varies with volume of collected third fraction of ejaculate
 - any kind of semen contamination, such as hair or mud, exclude the semen from further procedures including AI
 - the presence of sediment consisting of sperm cells at the bottom of the tube is a normal feature if the semen is left at the same position for several min.
- **Microscopic evaluation:** use pre-warmed slide, objective of x20 to x40
 - **Motility:** assessment is based on the evaluation of the average percentage of progressively motile spermatozoa, normal dog semen contains at least 70% of progressively motile spermatozoa
 - **Concentration/total sperm count:** at home just subjective evaluation possible
 - **Sperm morphology:** semen is smeared on a glass slide, air dried and stained (e.g. DiffQuik, Spermac stain), percentage of morphologically normal spermatozoa in canine semen should be greater than 70%
 - **‘Live-dead’ spermatozoa:** normal dog semen consists of maximal percentage of 30% of dead sperm cells

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Vaginal Insemination of Chilled Semen:

- semen is placed directly into the bitch's vaginal canal, just before the opening into the uterus
- usually used when using fresh semen which has just been collected or chilled semen sipped overnight – not to be used for frozen semen

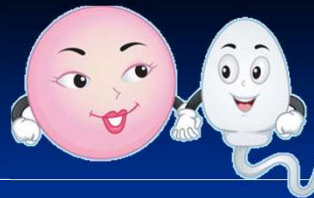
Procedure:

- done using an insemination tube or better a balloon catheter
- gently inserted into the vaginal canal by going up first and then horizontal
- when not using a balloon catheter female's hind legs are elevated to help the semen move forward to the opening into the uterus with the aid of gravity



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THANK YOU
for your attention



If you have further questions, feel free to contact me:

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Sources:

- Dr. Konrad Blendinger, www.blendivet.de
- Rita Payan-Carreira, Sónia Miranda and Wojciech Niżański:
Artificial Insemination in Dogs
- Minitube Germany: www.minitube.de

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