

Semen Collection and Analysis



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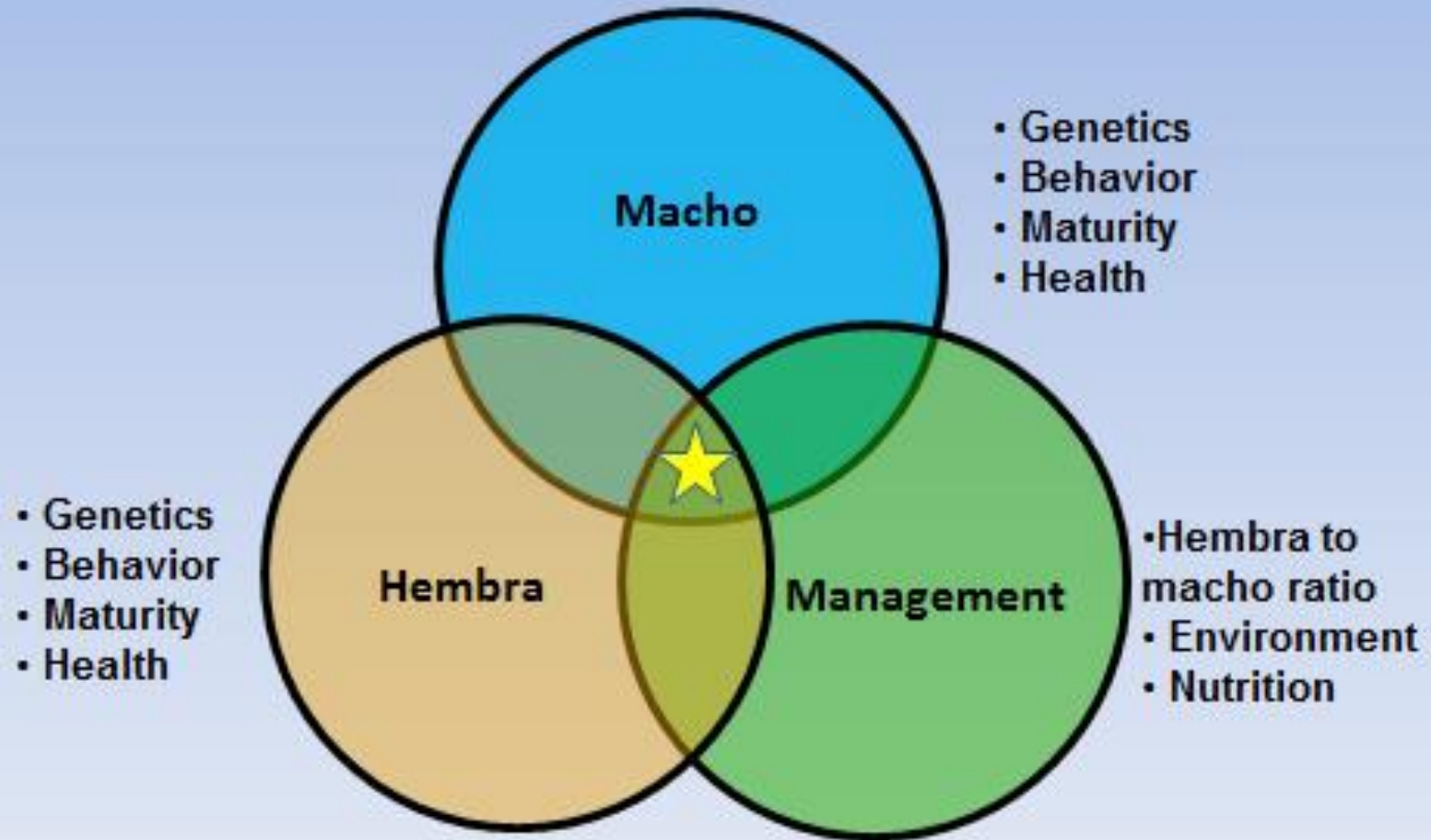
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Learning Objectives

- To discuss when semen evaluations should be performed
- To discuss the procedure for collecting alpaca and llama semen
- To discuss evaluation and interpretation of camelid semen results

Breeding Success





When should we evaluate semen in males?

- **At the start of the breeding season**
 - producing useful sperm?
 - Avoid herd repro failure
 - Males with low or no sperm may not show evidence of illness



➤ **During the breeding season**

- Does he need a rest to replenish sperm?
- information is available immediately after the male breeds
- No need to wait to see if females become pregnant and thereby lose production.



Case 1

- 5 year old male purchased 2 years prior as unproven with no BSE
- Bred spring- fall- spring to 30 females with no pregnancies!
- Owner suspected a problem after 1.5 years or perhaps did not want to admit to one sooner
- Semen evaluations 2 weeks apart =
 < 50 sperm found




Case 2

- 8 year old experienced male
- Last successful breeding November
- Semen evaluation January >> no sperm
- Same result monthly for the next 12 months
- No history of illness or other abnormal exam findings
- Libido and BCS normal
- Starting spring 2017= no sperm, normal libido, testes atrophied and soft



Case 3

- Experienced male purchased at auction
- Insured with no repro exam by company for \$200K for loss of use
- New owner complained that male was not functioning properly and wanted the insurance money, and to stop paying the seller
- BSE including semen analysis = normal
 - Pregnancy achieved in the test breedings
 - Insurance company relieved!

- 
- **All males** are good candidates for semen evaluation regardless of age during the breeding season
 - Especially if they are repeatedly breeding the same females
 - **Most females should become pregnant in the first 60 days of the season** if the male has good semen **and the management is good**
 - Not much breeding activity should be seen in the last 30 days unless poor semen, overuse, or problem females are still unbred.



How frequently can males be used for breeding?

- It depends on the male and semen evaluation will answer that!
- Overuse >> low conception rates!

Results from Nunoa, Peru: Jan- April 2013

- 1 male turned out with 20 females
- Initial semen sample results were good- sperm found
- Semen samples evaluated two days in a row (n = 6) after 1 month = no sperm in any sample!?
- When females are all bred and become non receptive, the male does not breed and therefore the sperm will be replenished!
- Pregnancy rate after 3 months with the females was 80%!

Results from another Nunooa farm:

- 2 unproven, young males used to breed 40 females over 90 days
- 25% pregnancy rate!
- Semen analysis or careful attention to repeat breeding would have identified this problem and allowed for correction **during** the breeding season.
- This manager had consistently seen 80 to 90% pregnancy rates in past years.

Breeding in Peru





Another Peruvian farm in 2016

- ▶ 100 females ultrasounded after 2 hour hike up a steep mountain at 14,000 ft
- ▶ 30 pregnancies found = 30%
- ▶ 15 crias in herd = 50% mortality??
- ▶ Why?
 - ▶ 1 male
 - ▶ Solutions?
 - ▶ Less females per male
 - ▶ More males
 - ▶ better food and water sources

Collecting Semen

- **Post breeding collection** with a vaginal speculum
 - Insert speculum to front of vagina right after male dismounts
 - Withdraw speculum and transfer sample to plastic tube.
- Blood contamination is seen in almost every sample
 - RBCs are not toxic to camelid sperm

Post breeding collection with a vaginal speculum



Post Breeding Semen Collection








Breeding dummy





Evaluation of the initial post breeding sample:

Sample volume

Semen viscosity

Gross blood

**Sperm motility
(total and progressive)**

Sperm concentration

Perform within 5 minutes of collection

- motility decreases over time

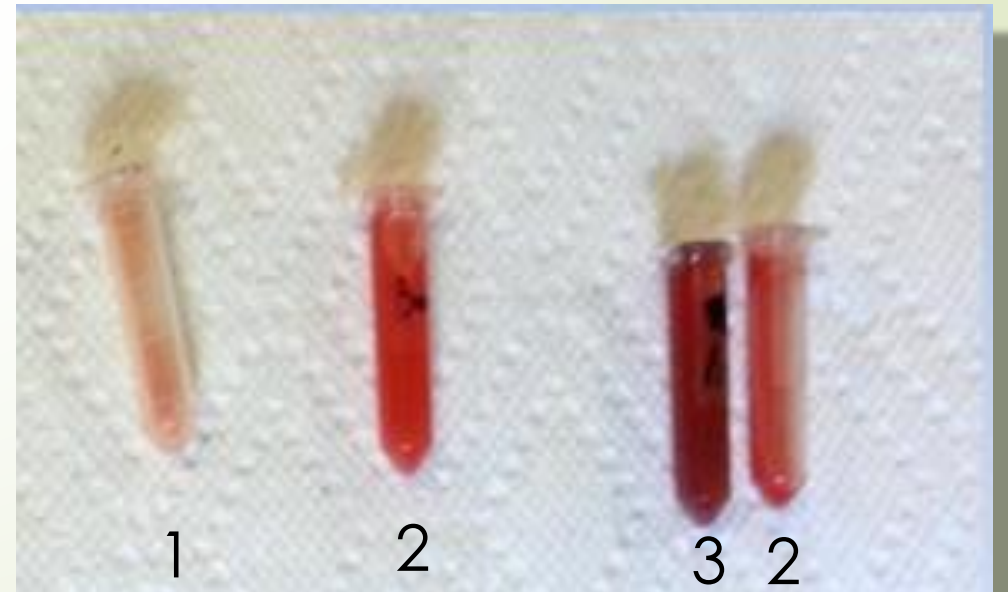
Make a morphology slide for later evaluation


Gross exam of semen: Semen Volume

- Small with a post breeding vaginal collection
- not representative of the total semen volume deposited in the uterus
- **Last portion of the ejaculate**
- Normal collection volume
0.1 - 1.0 ml
- average 0.25 ml (up to 3.0 ml on occasion).

Gross blood in the semen

- Seen in most samples- seen on slides in all samples
- Related to inflammation in the uterus prior to breeding?
- increasing gross blood leads to decreasing pregnancy rates
- Not consistent for the male or female





Initial Microscopic Evaluation of Semen: Sperm motility

- Place a drop of fresh ejaculate onto a microscope slide, apply a cover slip, and examine it under 400X magnification to estimate what percentage are moving.
- There is **very little to no progressive motility** in normal alpaca semen due to the high viscosity
- Estimate on farm from looking at 10 fields at 400X- determine how many out of each 10 sperm are motile.
- Average of 8 out of 10 active = 80% **total motility**
- Normal range 40 to 100%



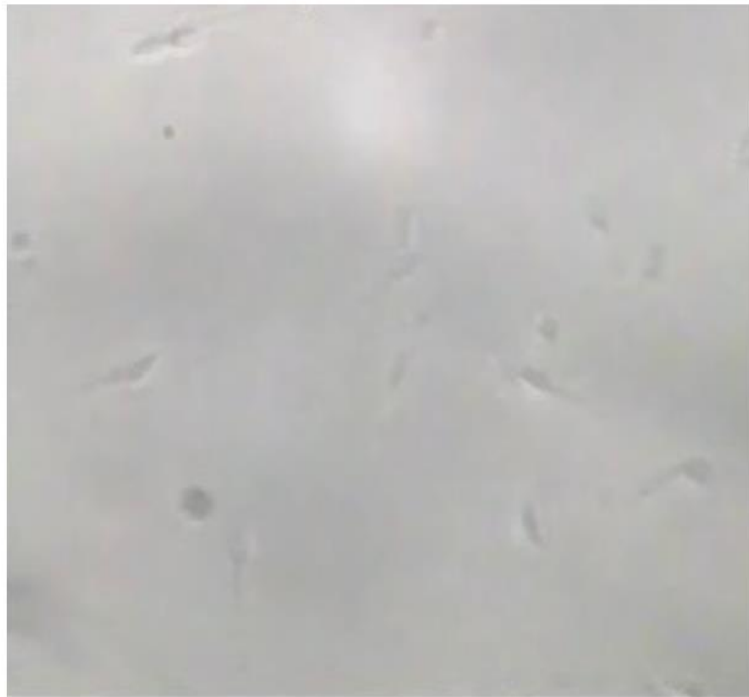


Sperm Motility videos



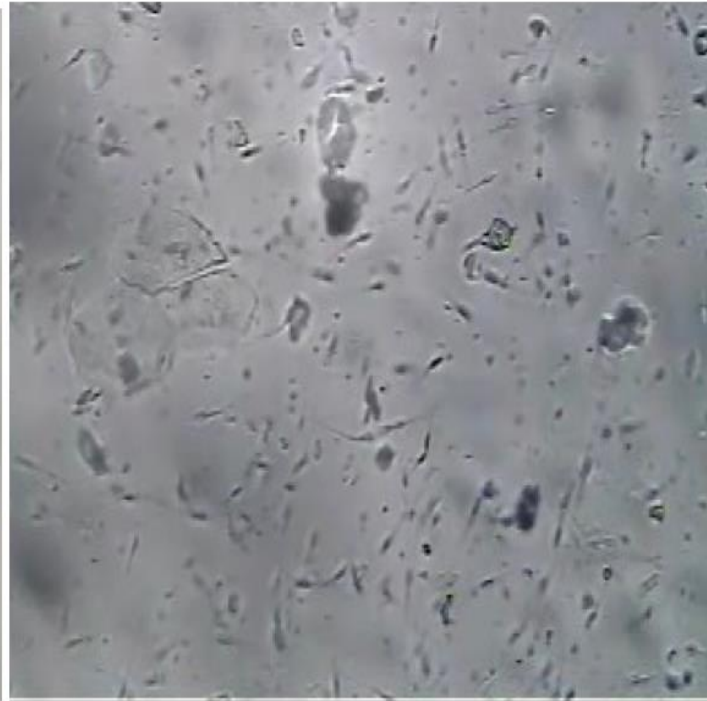
Sperm Concentration Estimate

Low 1



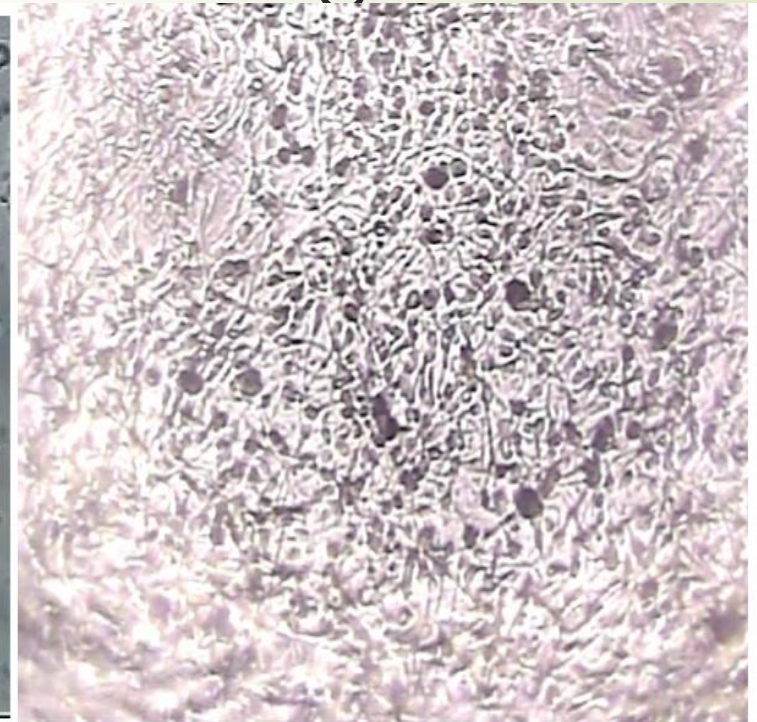
**Sperm in < 33 %
of fields**

Medium 2



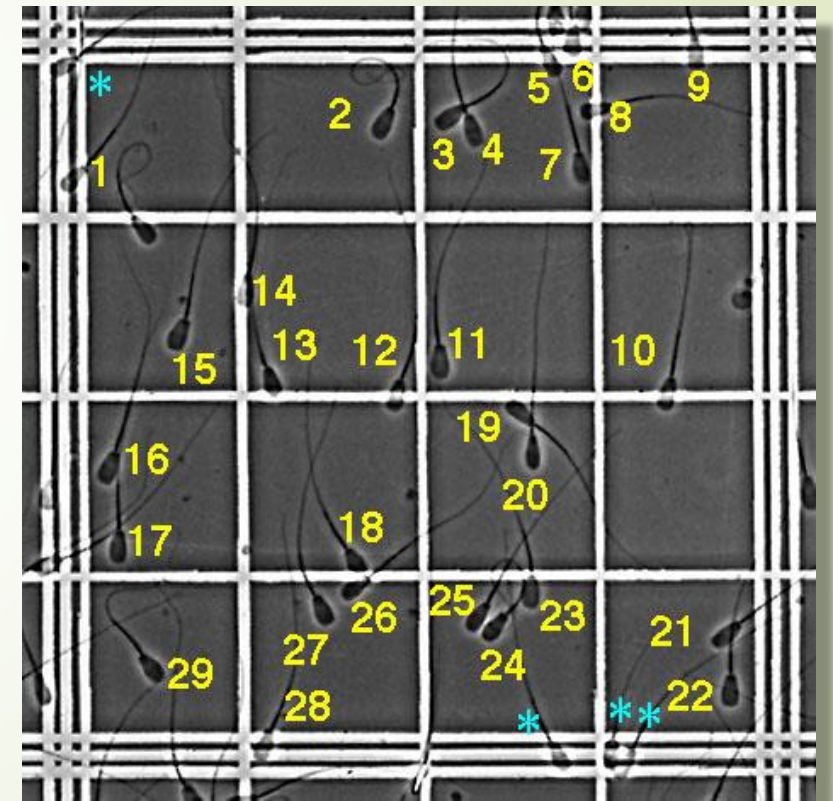
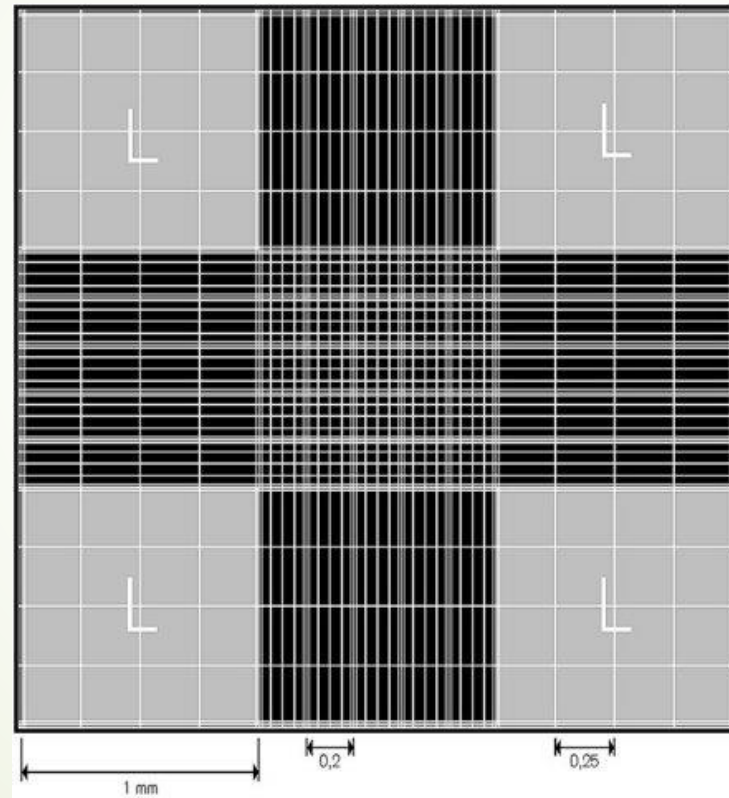
**Sperm in 33 – 67 %
of fields**

High



**Sperm in 68 - 100%
of fields**

Concentration with Hemacytometer

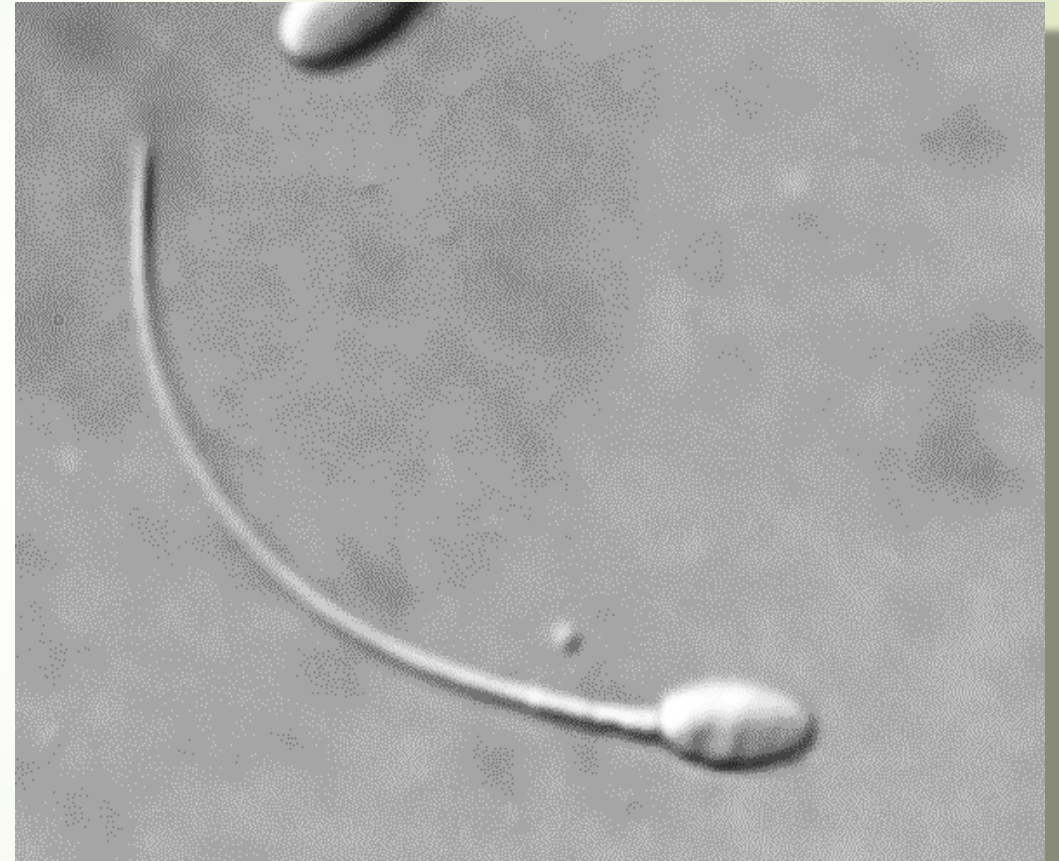


Sperm Morphology



- Determined from the stained slides made immediately after collection of the sample.
- Slides can be examined immediately or saved for later
- Abnormal morphology decreases the ability of the sperm cells to move and penetrate the egg.

Sperm Morphology

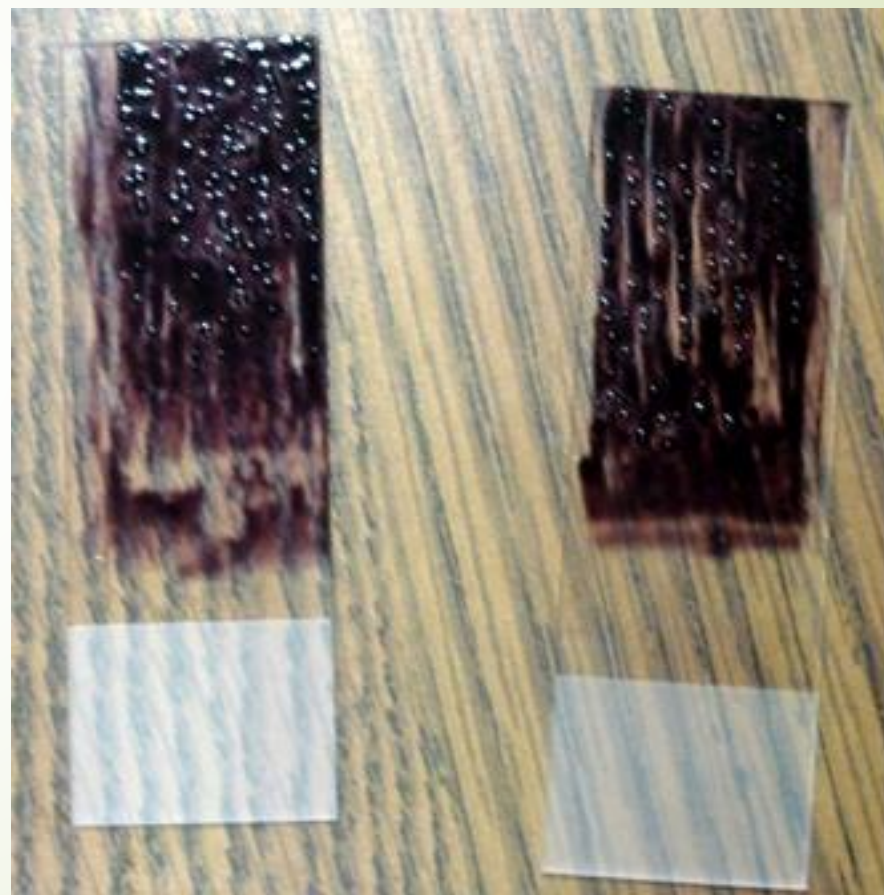


Woodcock



Making Morphology Slides

- Apply an equal sized drop of eosin-nigrosin (live-dead) stain to a drop of semen
- Apply a second slide over the mixture and draw them apart to mix the two drops
- Allow to dry
- View slides at 1000X (oil) magnification with light microscopy

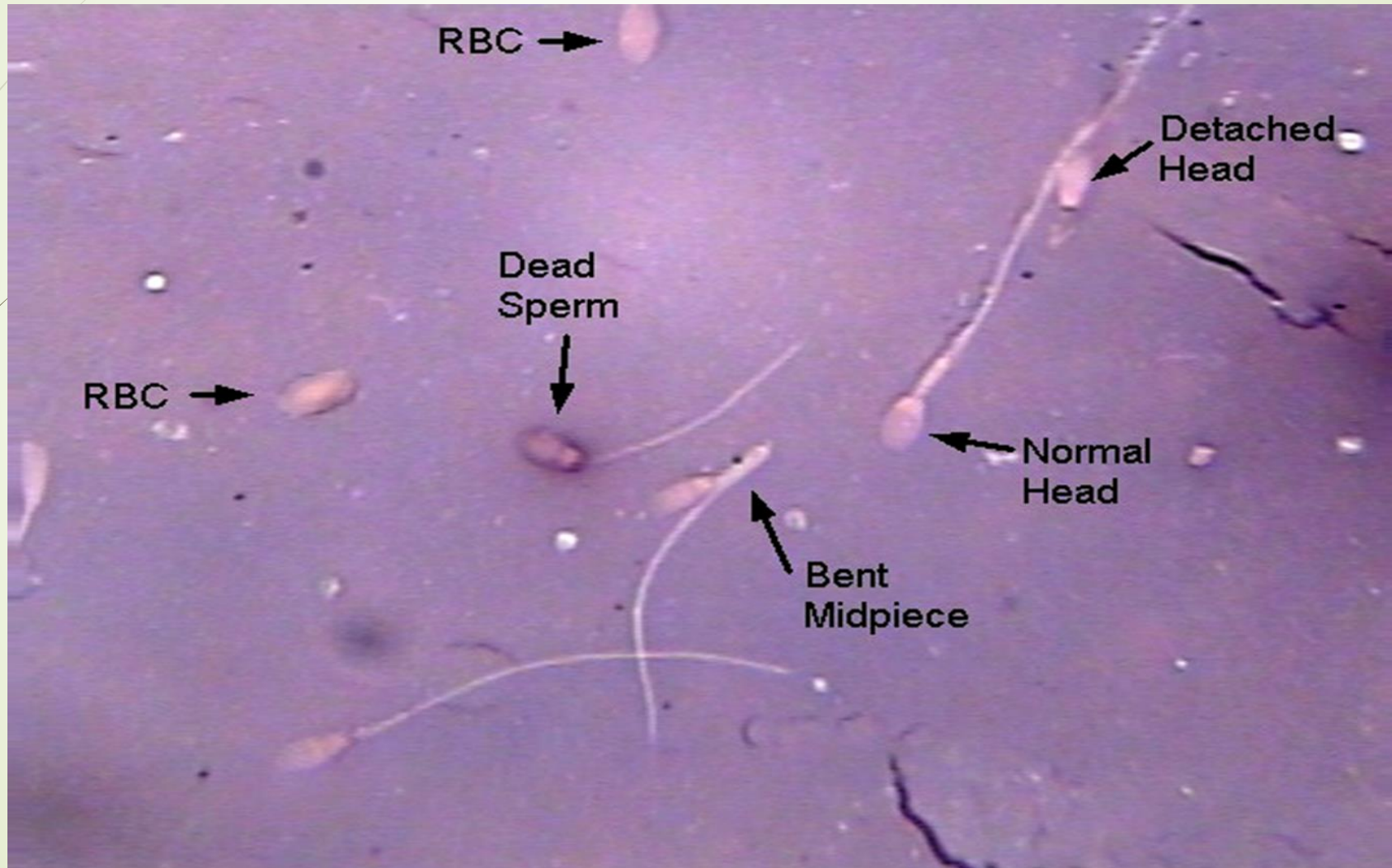




Percentage of live sperm

- ▶ The stain colorizes only the cells that are dead
 - ▶ dark = dead
 - ▶ light = live
- ▶ Dead sperm have a dark purple halo around the head and the head is dark in color
- ▶ Normal range is approximately 50 to 100%

Live vs. Dead Sperm





Normal Sperm

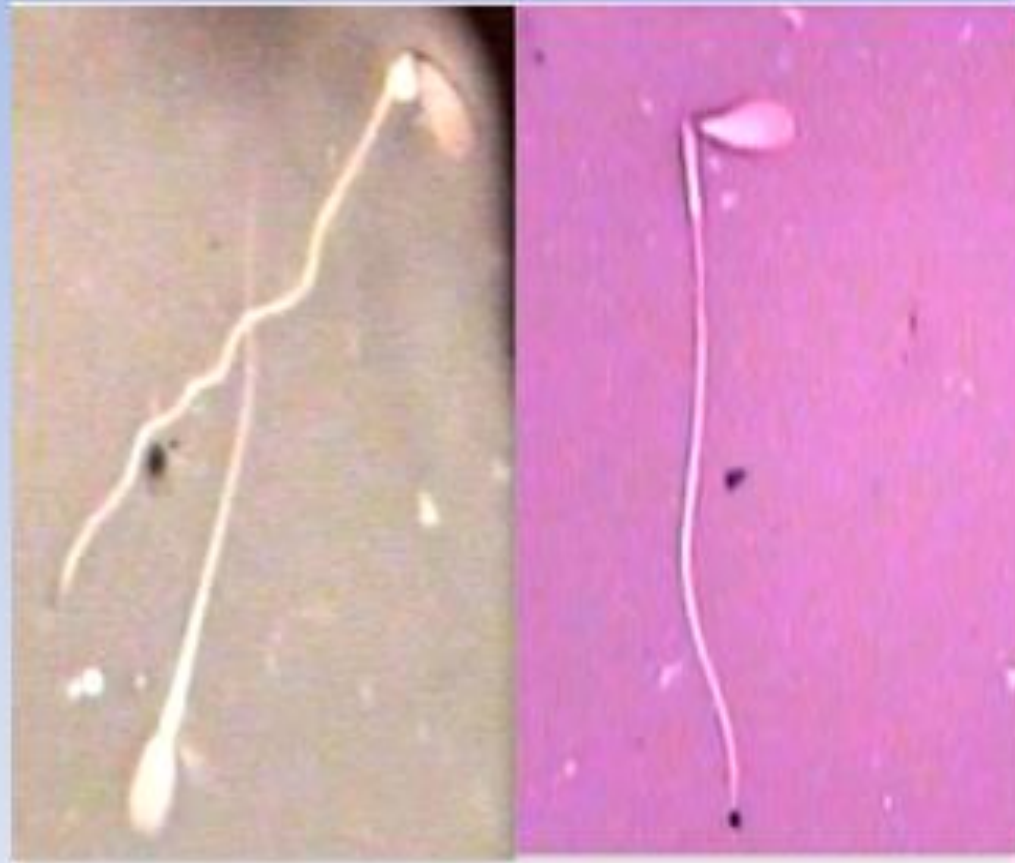


Abnormal Sperm

Head Defects: Headless tail



Decapitated heads
(1%)



Double heads (< 1%)

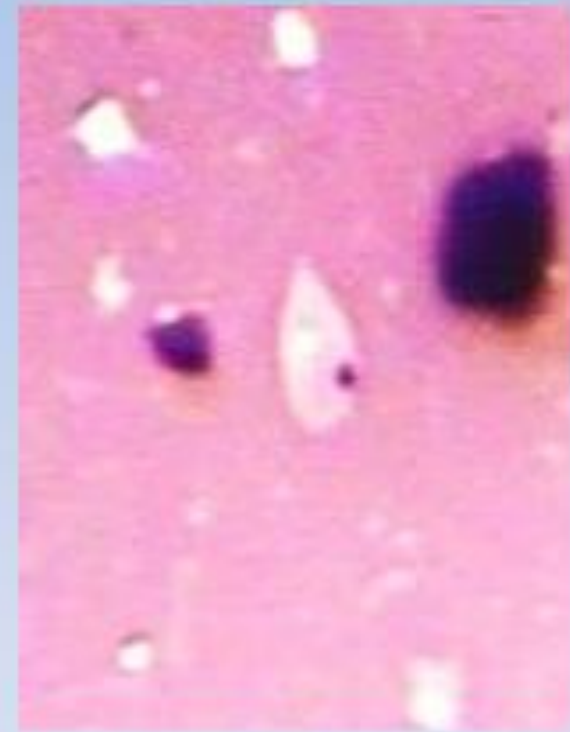


Microcephaly (<1%)



Misshapen heads

Pyriform Head



Midpiece Defects

Irregular midpiece



Thickened midpiece



Bent midpiece

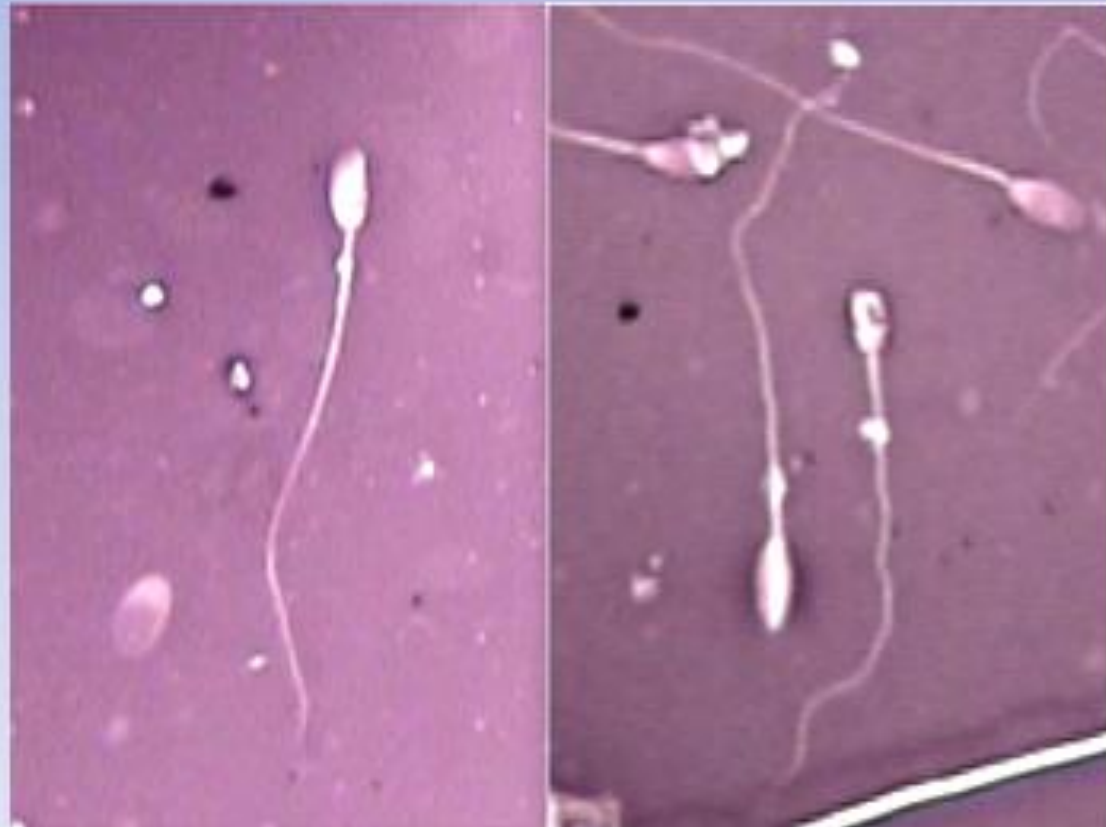


Thickened midpiece



Cytoplasmic droplets: proximal- 5-11%; distal- 1-9%

➡ = immature sperm and are seen in all ejaculates

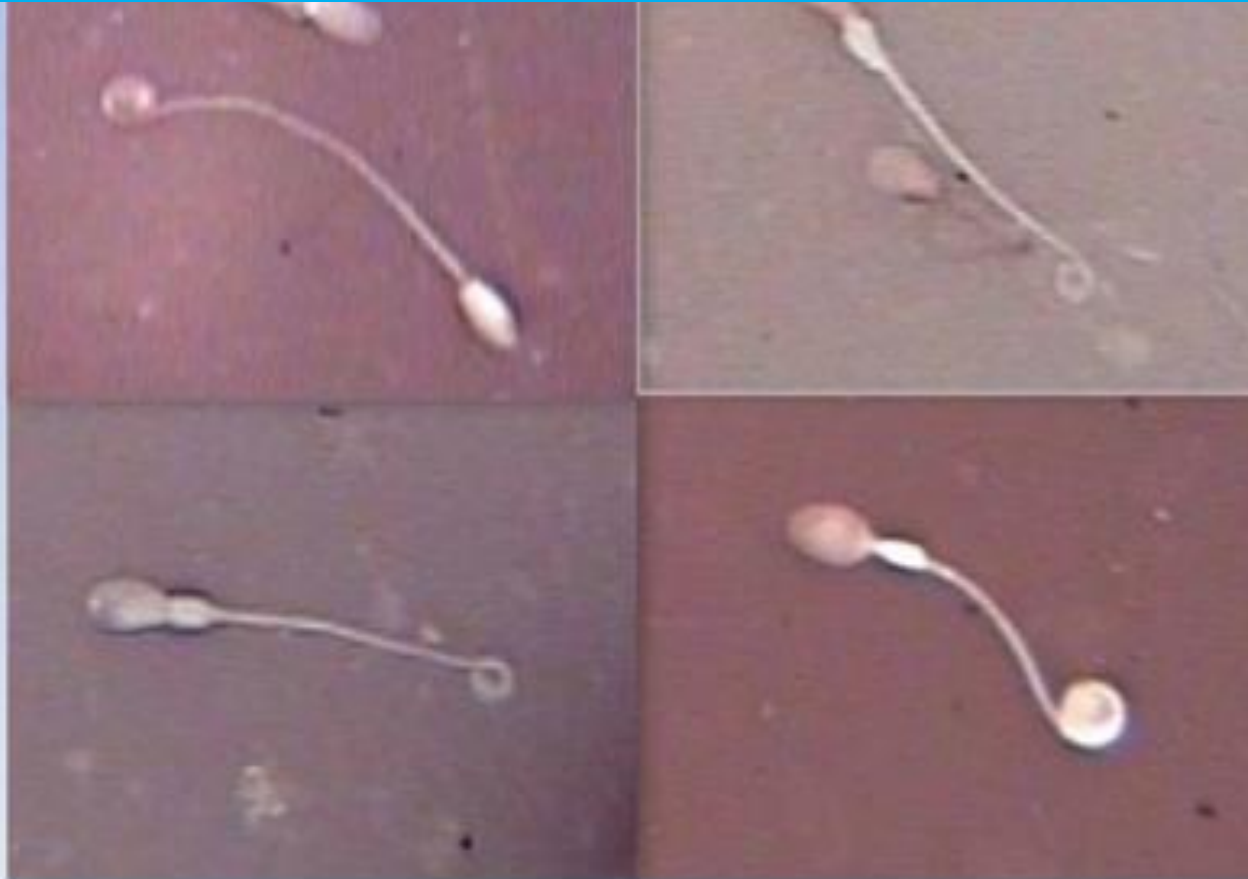


Proximal cytoplasmic droplets



Tail Defects:

Terminally coiled tail = not cold shock!



Severely coiled tail



Tailless heads (<math><1\%</math>)




Semen parameters

66 analyses (47 AV; 19 live cover)

Semen Parameter	Value (range or average)
Sperm concentration	10-800 x 10 ⁶ sperm/ml
Sperm total motility	0-85%
Live spermatozoa	65-99%
Semen viscosity	10-80 mm semen thread break length
Sperm Morphology	
Normal	30 - 90%
Cytoplasmic droplets - proximal	8%
- distal	2%
Midpiece defects	4%
Head abnormalities	4%
Tail abnormalities	11%

What we know about alpaca semen:

- Much variation among sequential ejaculates from the same male
- Sperm concentration **may** decrease with each sequential breeding
- Motility and % normal **may** also decrease
 - This results in decreased chance of pregnancy
- Much variation among males
- Seasonal variation in semen characteristics- not found to date
- ***Alpaca semen is consistently inconsistent!***



Alpacas are not a low
fertility species !!!

- ***If managed properly.....***
- Expect 80 to 90% pregnancy rate
- One well managed, northeastern US breeding farm had a 94% birthing rate over 3 years!!

Questions?

