

# Emerging reproductive technologies that enhance genetic resource use

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# Overview

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- Introduction to artificial insemination (AI)
- Cooled semen
- Frozen-thawed semen
- Challenges for sheep and goat assisted reproduction
- Conclusions and future directions

# Fertility of AI in Agricultural Species

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- Cattle
  - Beef: > 40%; Dairy: 35%
- Goats
  - Fresh or frozen: > 50%
- Aquaculture
  - > 40%
- Swine
  - Fresh: 95%; Frozen: > 60% (decreased litter size)

# Current Status of Sheep Industry

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- Improvements difficult because of:
  - Necessity to 'move' rams to transport their genetics
  - Inability to efficiently utilize AI
  - Little or no AI industry

# Artificial Insemination

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- Estrus synchronization
  - CIDRs or sponges: 5-14 days
  - Gonadotropin e.g. eCG, PMSG, PG-600: 200-500 IU
  - PGF<sub>2α</sub> a.k.a. Lutalyse cocktail
  - MGA
  - Ram effect
- Differences
- Access to hormones
- Cost

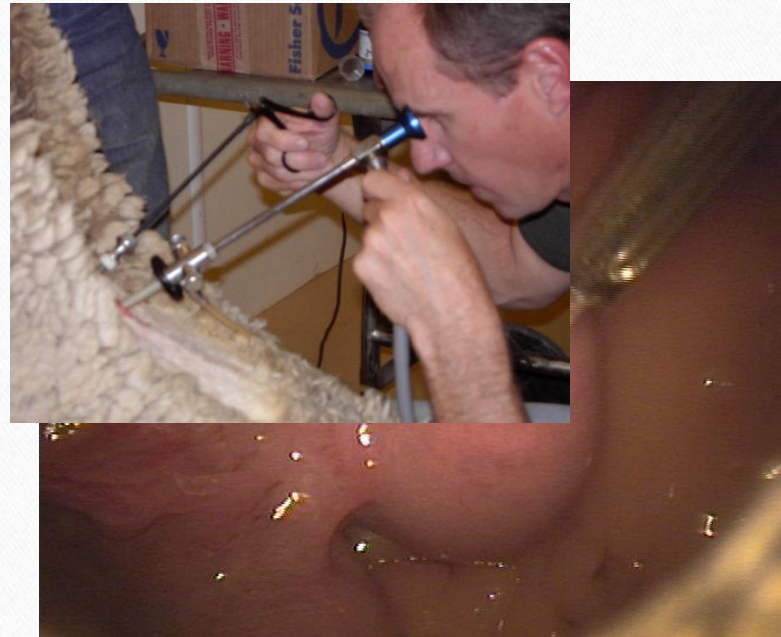
# Artificial Insemination

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- Semen preservation
  - Cooled or frozen-thawed
  - Milk or TRIS
  - Egg yolk
    - 2-20% by volume
  - Glycerol
  - Species specificity

# Artificial Insemination

- Laparoscopic insemination
  - \$25 + per head : semen, hormones, technician
  - Frozen semen
  - > 50% fertility



# Artificial Insemination

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- Cervical insemination
  - < \$10 per head
    - semen, hormones, technician (?)
  - Fresh/cooled semen:
    - $\geq 40\%$  fertility
  - Frozen-thawed semen:
    - 0-80% fertility
  - Variations on technique
    - Guelph, Gourley





# GOAL:

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Improve our ability to collect, preserve, transfer and inseminate quality semen;

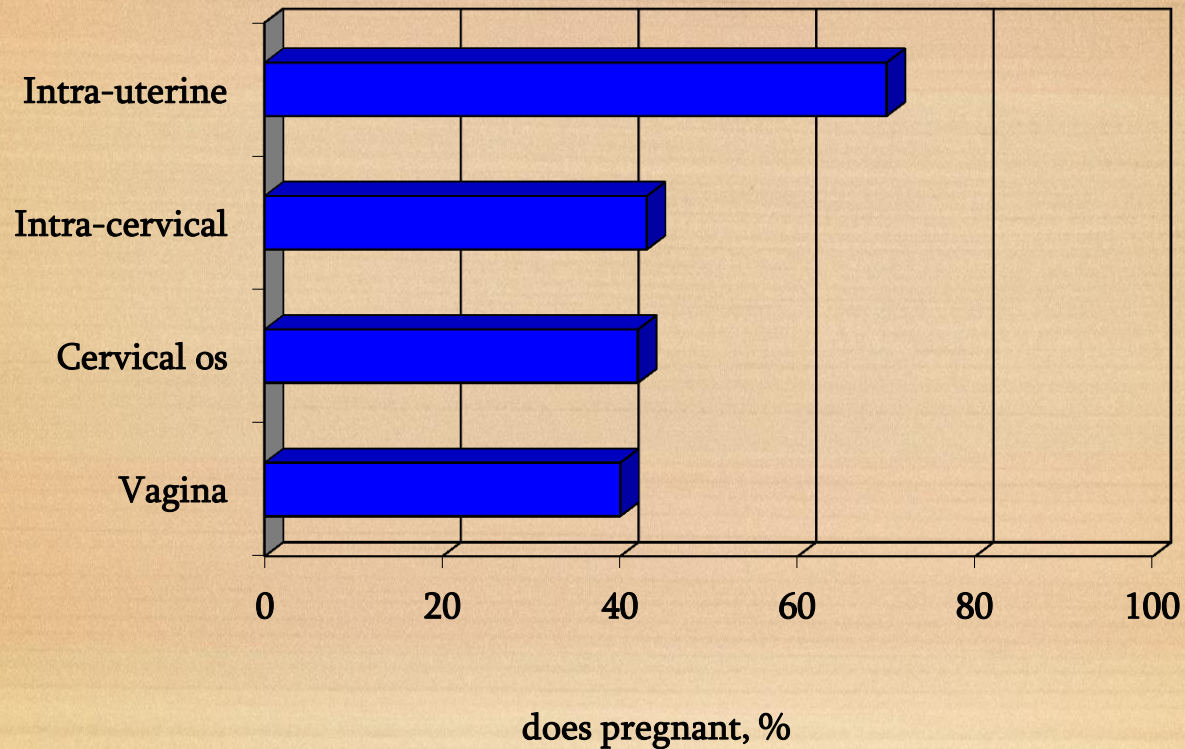
-i.e. improve our ability to utilize germplasm.

# Fresh and cooled semen research-an emphasis on ease of utilization

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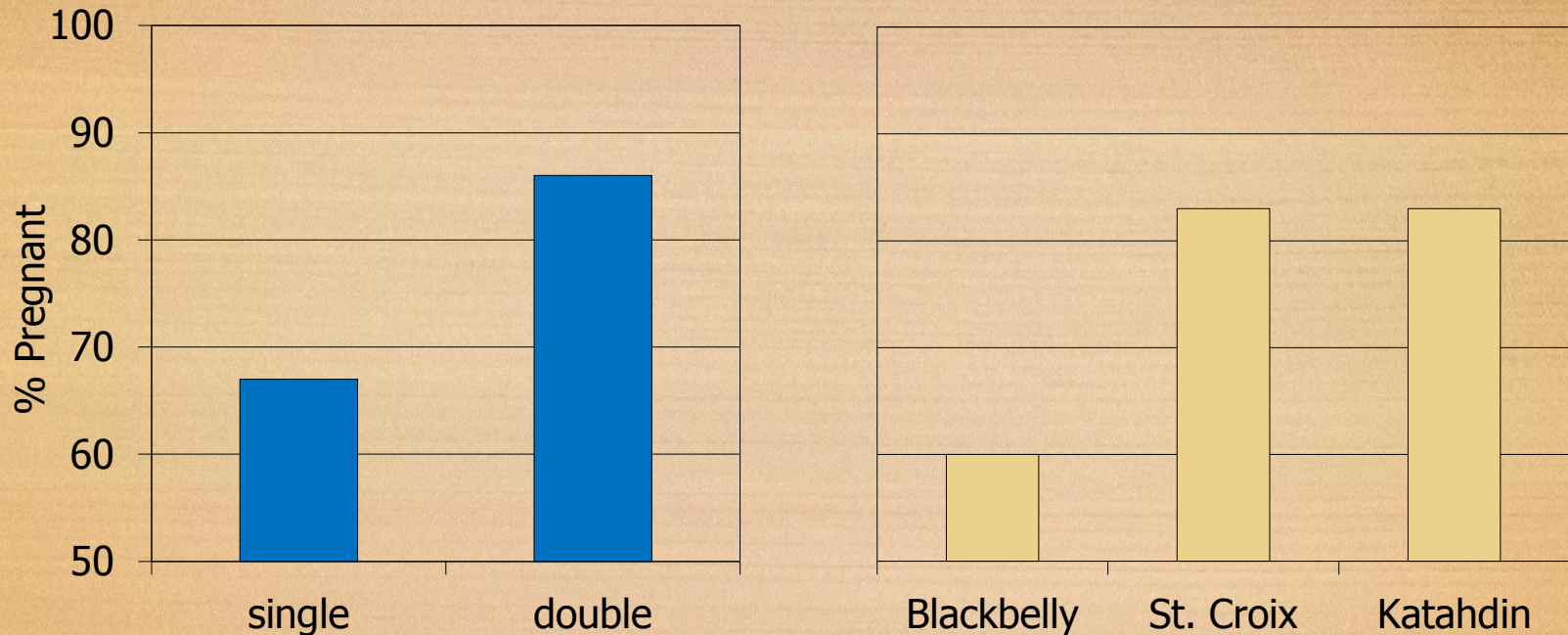


# Effect of site of deposition of chilled semen on pregnancy rates in *goats*



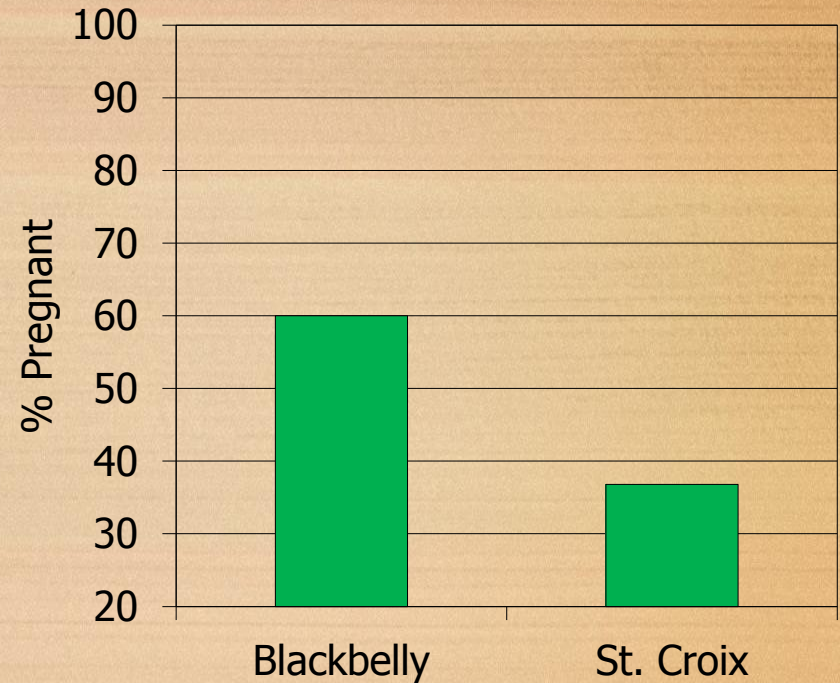
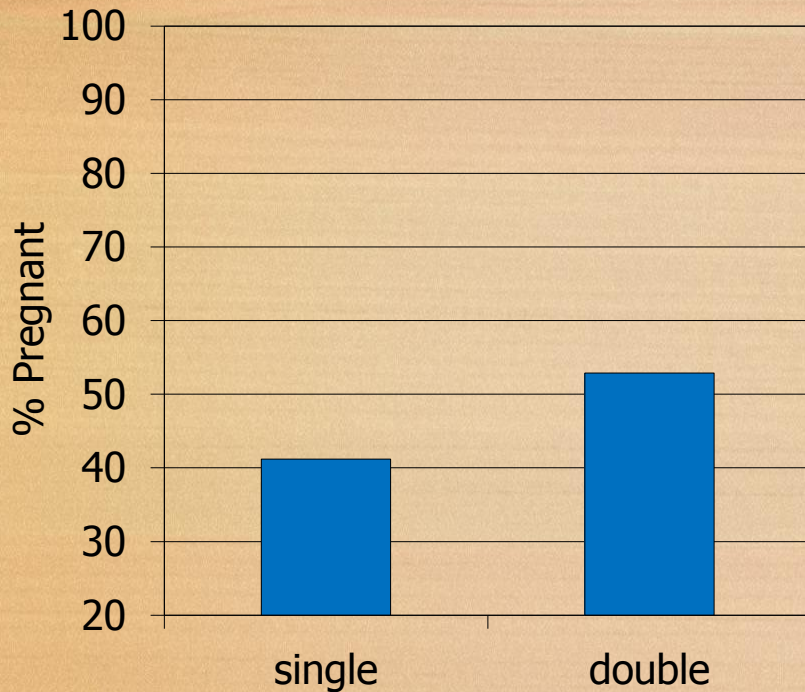
\*Frozen-thawed semen: 0 to 75%

# Cooled semen fertility -2 hr hold, mature ewes



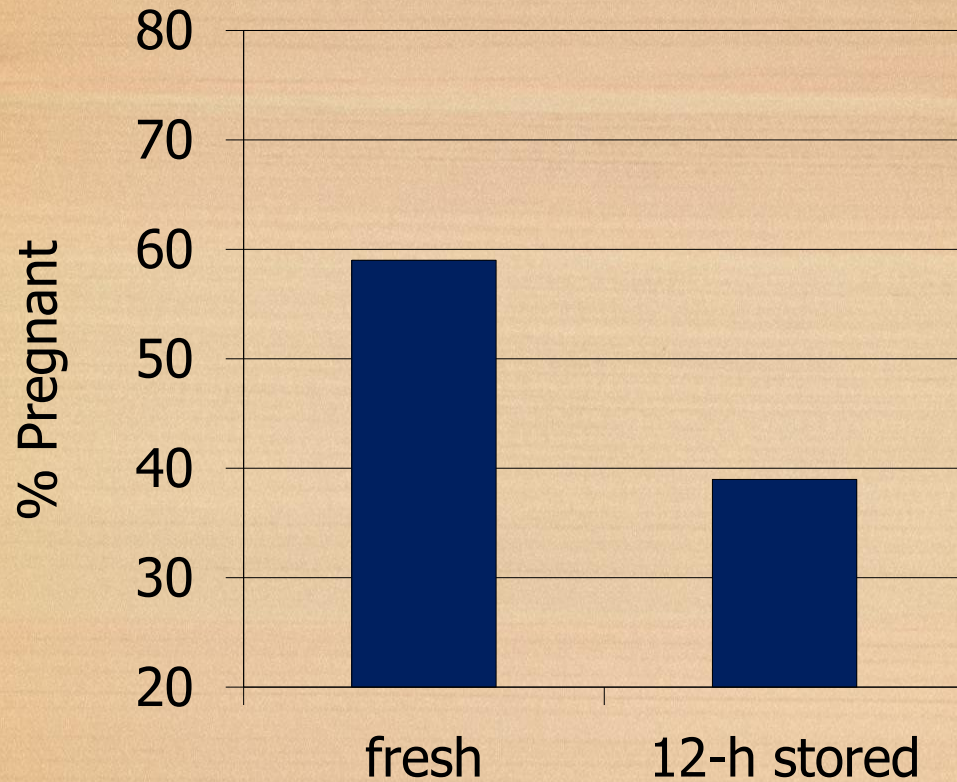
N = 39 ewes, MGA, fresh semen, AI 10-14 hours post-estrus

# Cooled semen fertility -12 hr hold, yearling ewes



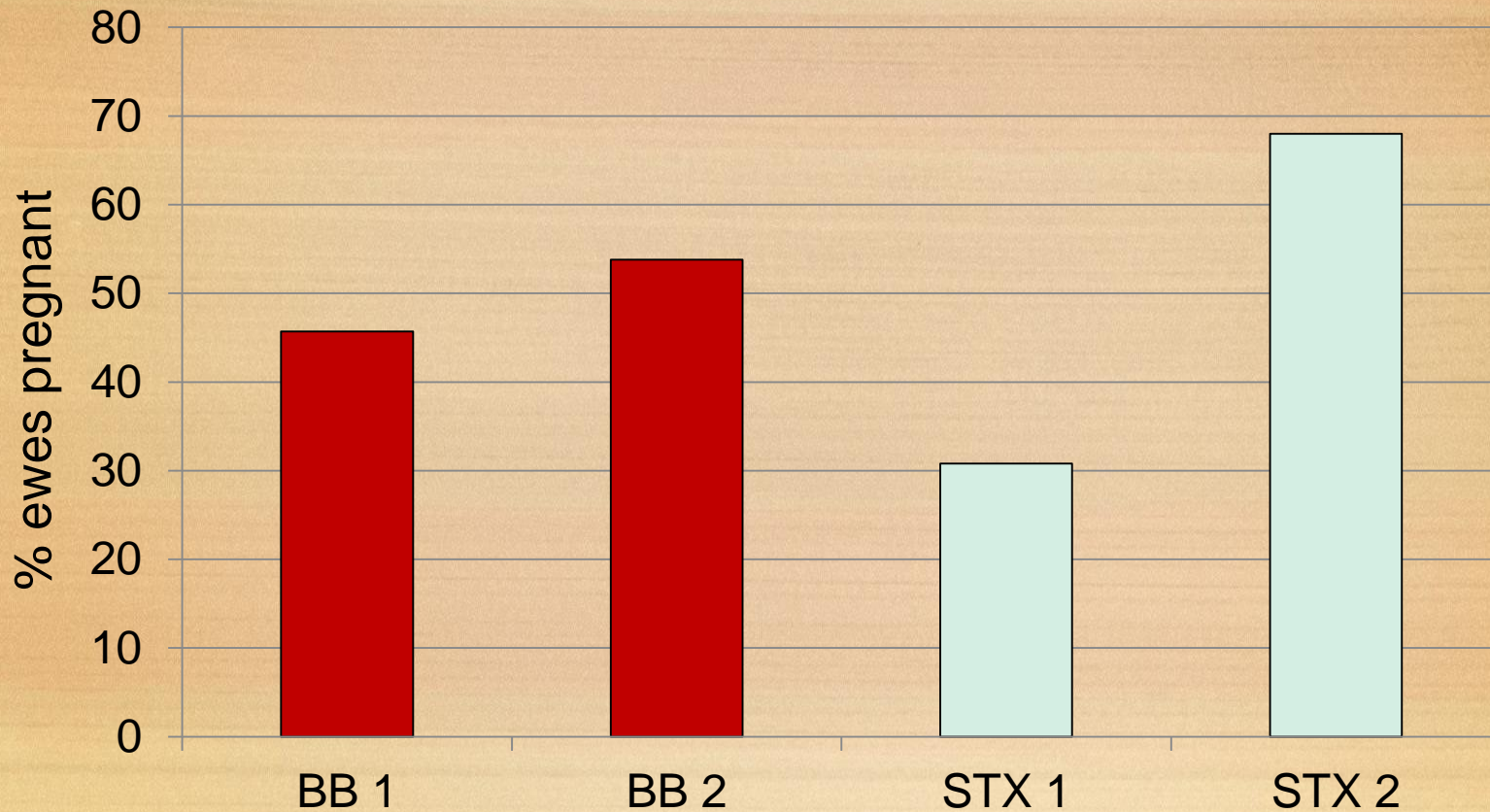
N = 40 ewes, CIDRs, spontaneous estrus, 12/24 hr hold, AI at 12/24 hr post estrus

# Cooled semen fertility -2 hr vs 12 hr semen hold



N = 110 ewes, MGA, spontaneous estrus, AI at 10-14 hr post estrus

# Sire effects (> 12 AI/ sire) -2 hr vs 12 hr semen hold

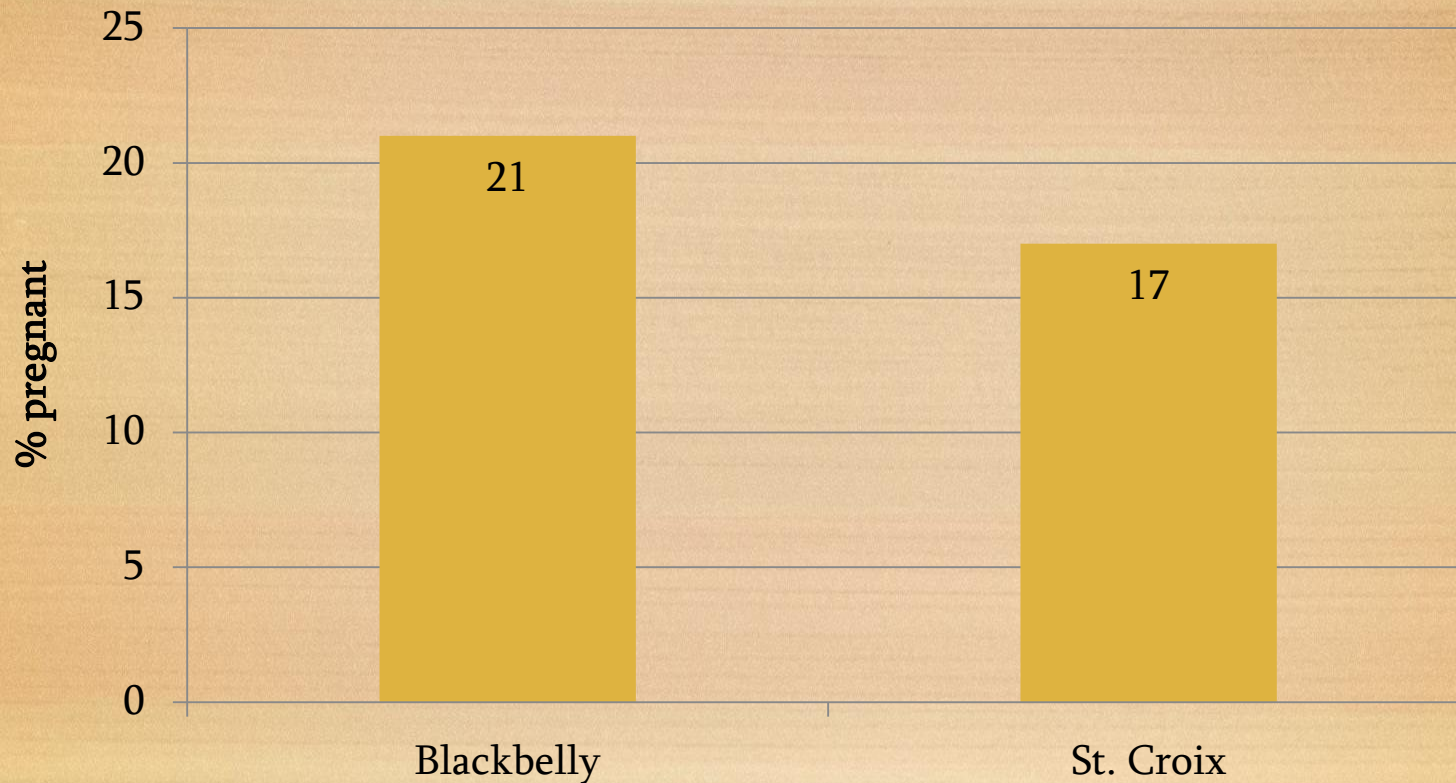


# Objective:

- To explore the factors that cause variation in fertility with this cooled semen model?



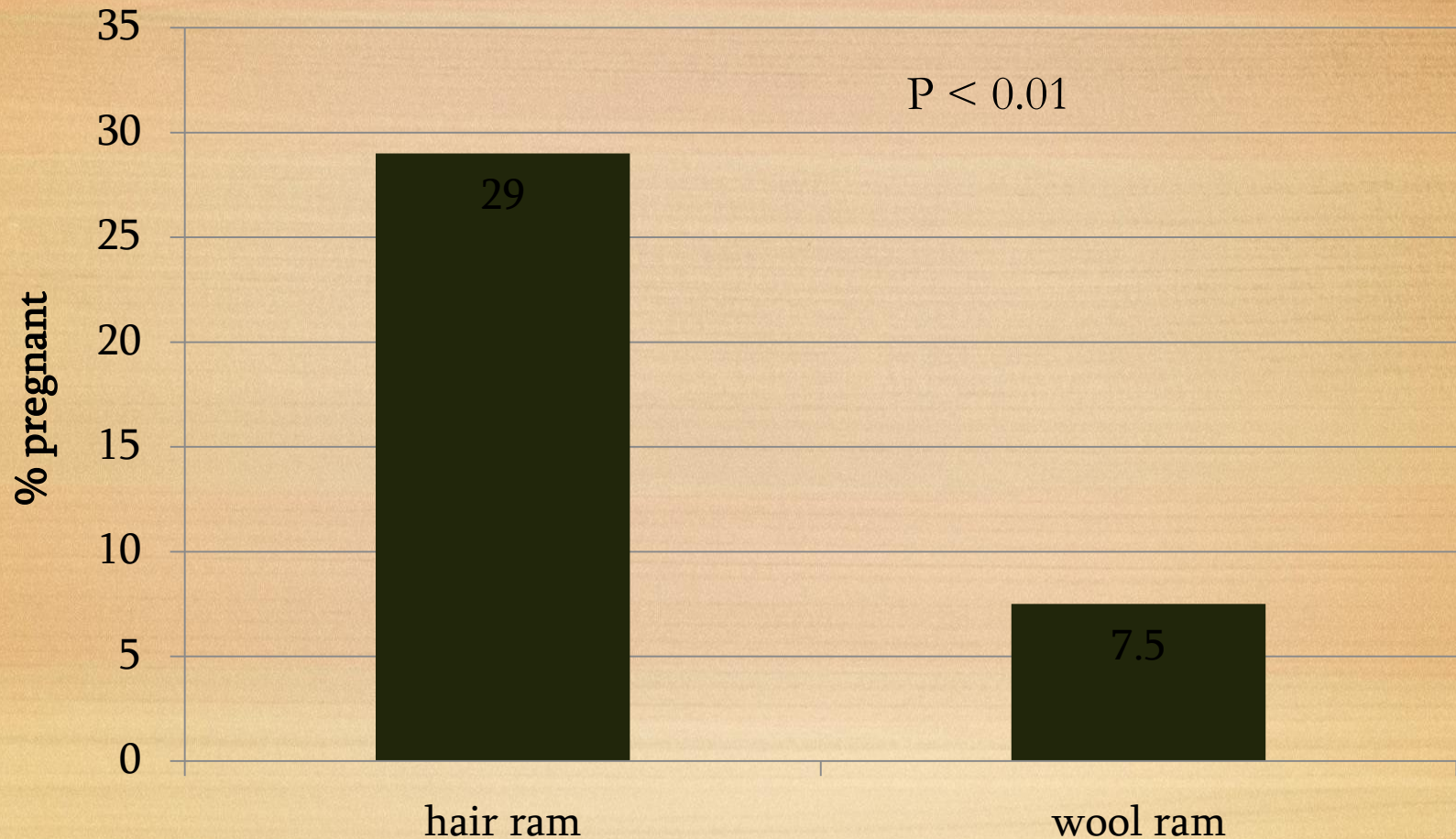
Breed of *dam* effects  
-Cooled semen stored at 5°C for 12 hr



N = 122 ewes, CIDRs, spontaneous estrus, AI at 12 hr post estrus, wool and hair ram semen

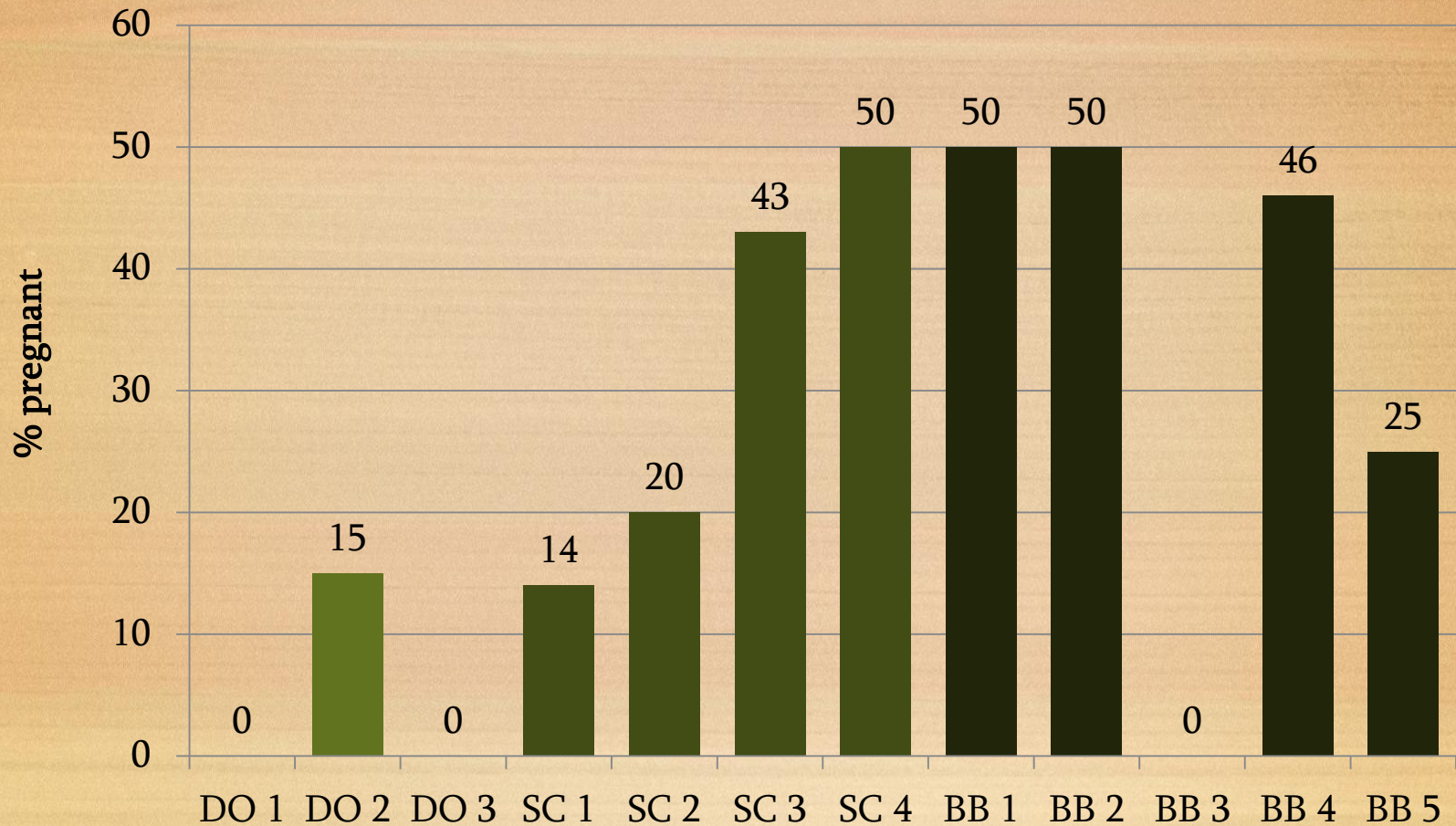
# Breed of sire effects

-Cooled semen stored at 5°C for 12 hr



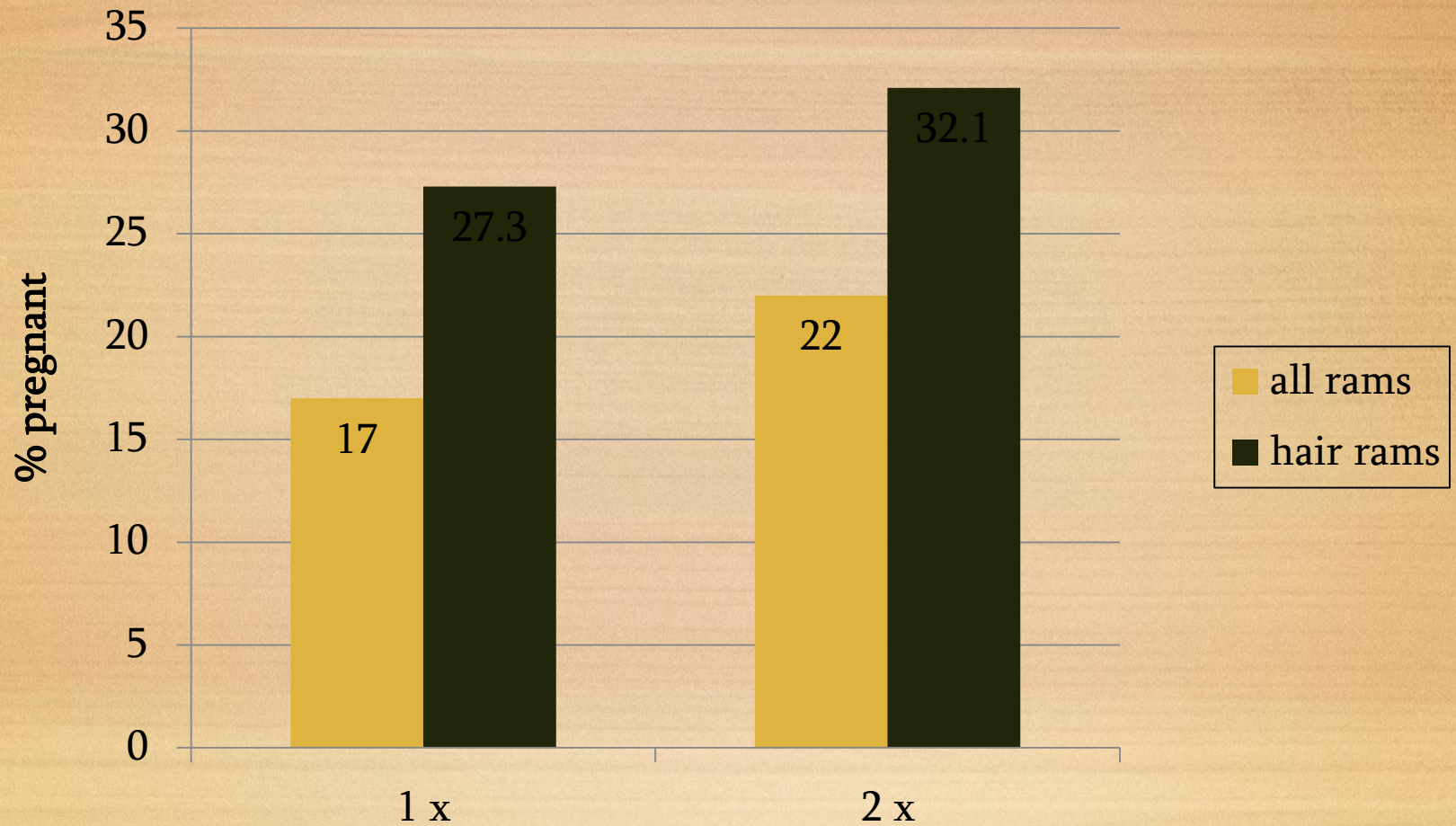
# Individual sire effects

-Cooled semen stored at 5°C for 12 hr

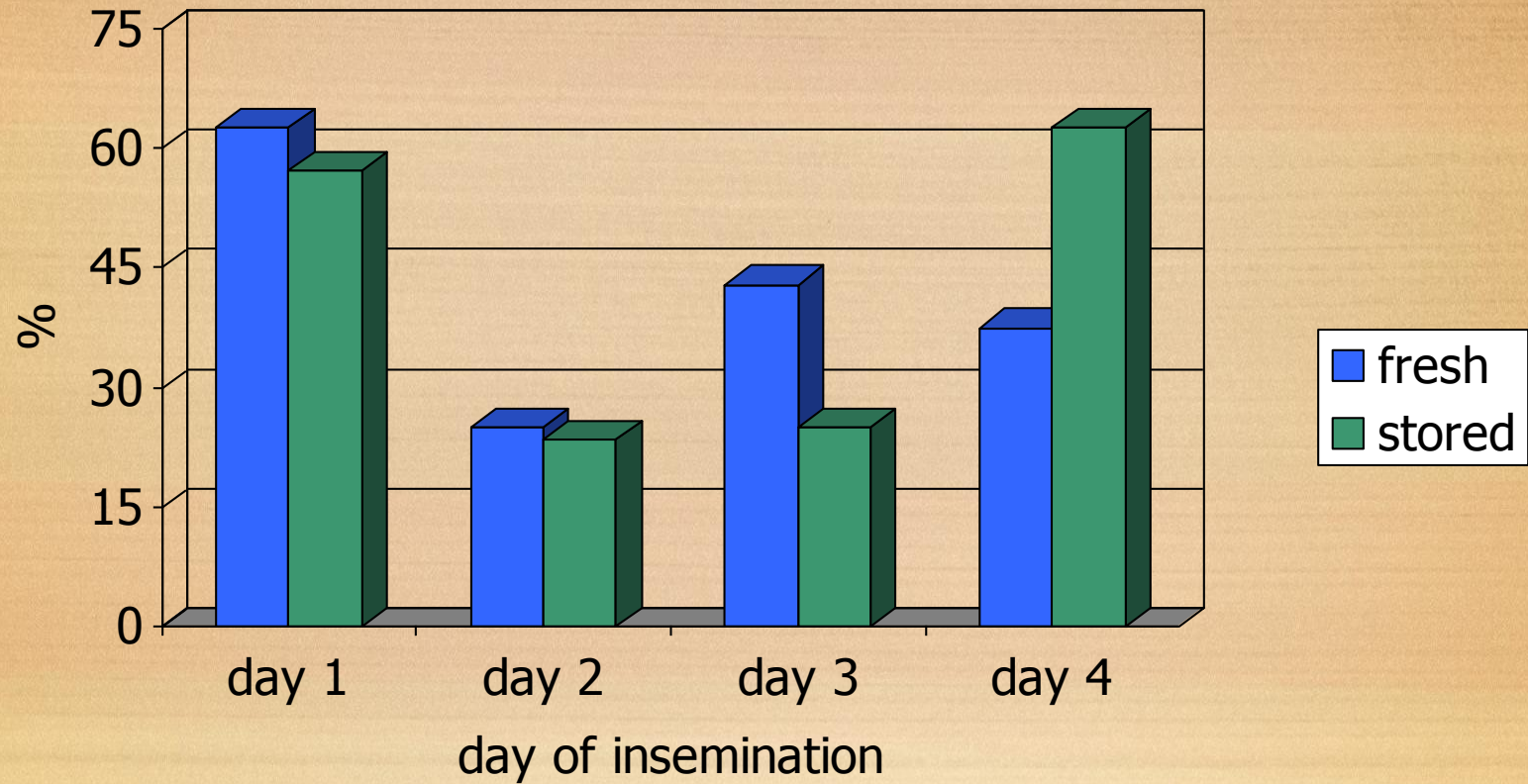


# Effect of number of inseminations

-Cooled semen stored at 5°C for 12 hr



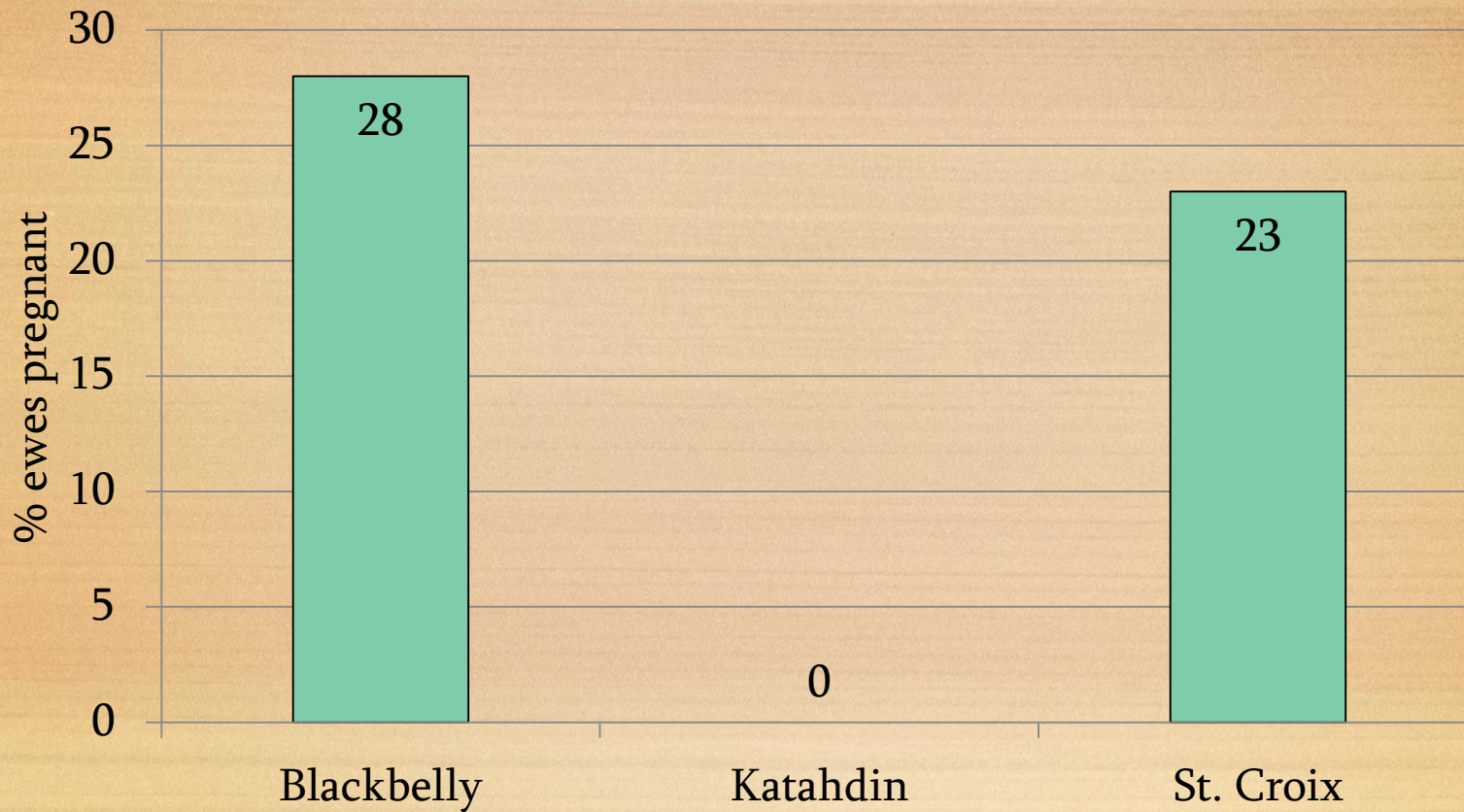
## Effects of holding semen -Cooled semen stored at 4°C for 72 h



N = 64 ewes, FGA sponges + eCG, AI at 54 hr post sponge removal

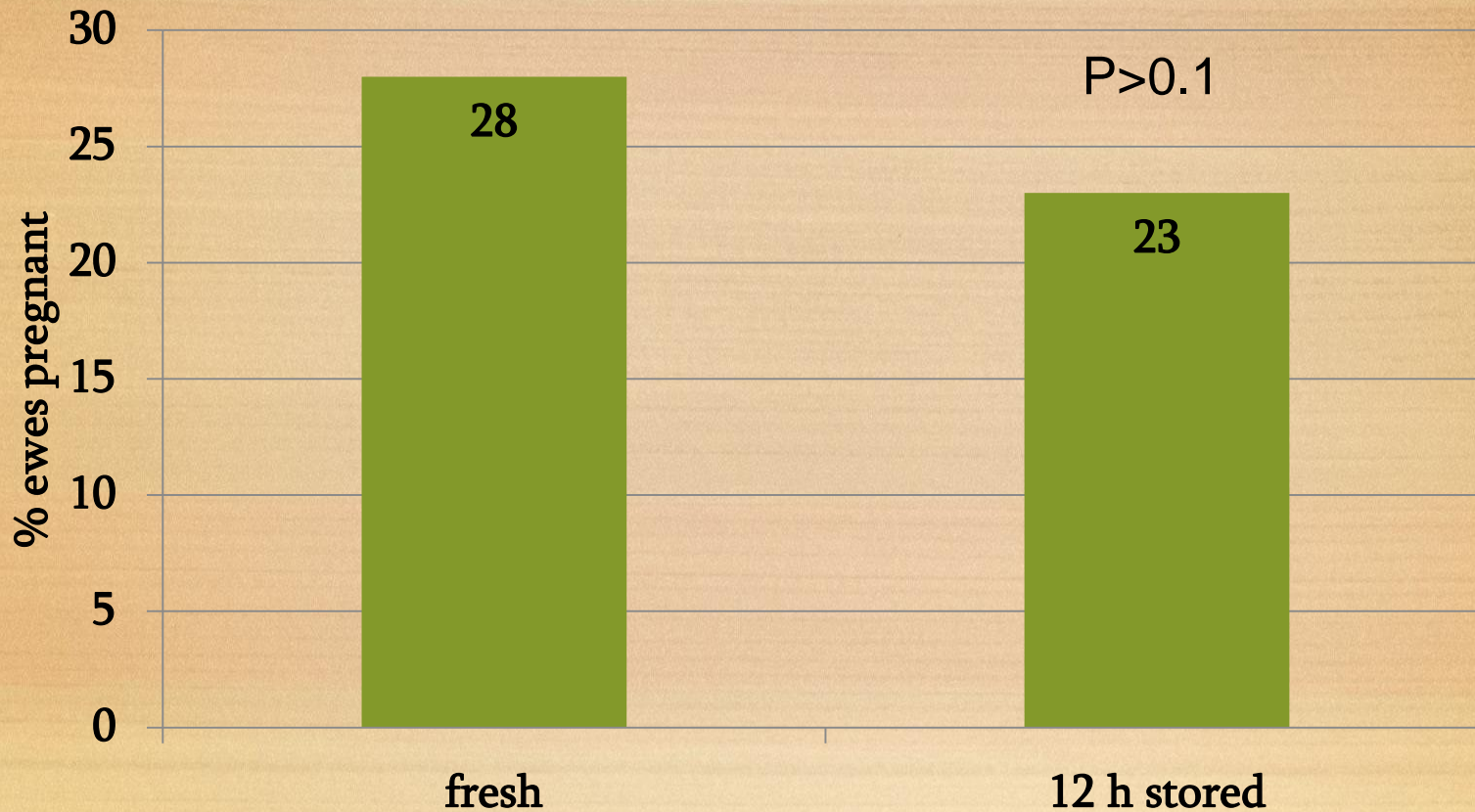
# Seasonality

## -2 hr vs 12 hr semen hold



# Seasonality

## -2 hr vs 12 hr semen hold



# Factors affecting fertility in sheep

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- Ewe age
- Semen age
  - Fresh vs 12 hr or 72 hr hold
- # of AIs
- Sire
- Dam
- Breed
- Season

Demonstrates an ability to move genetics without moving rams!



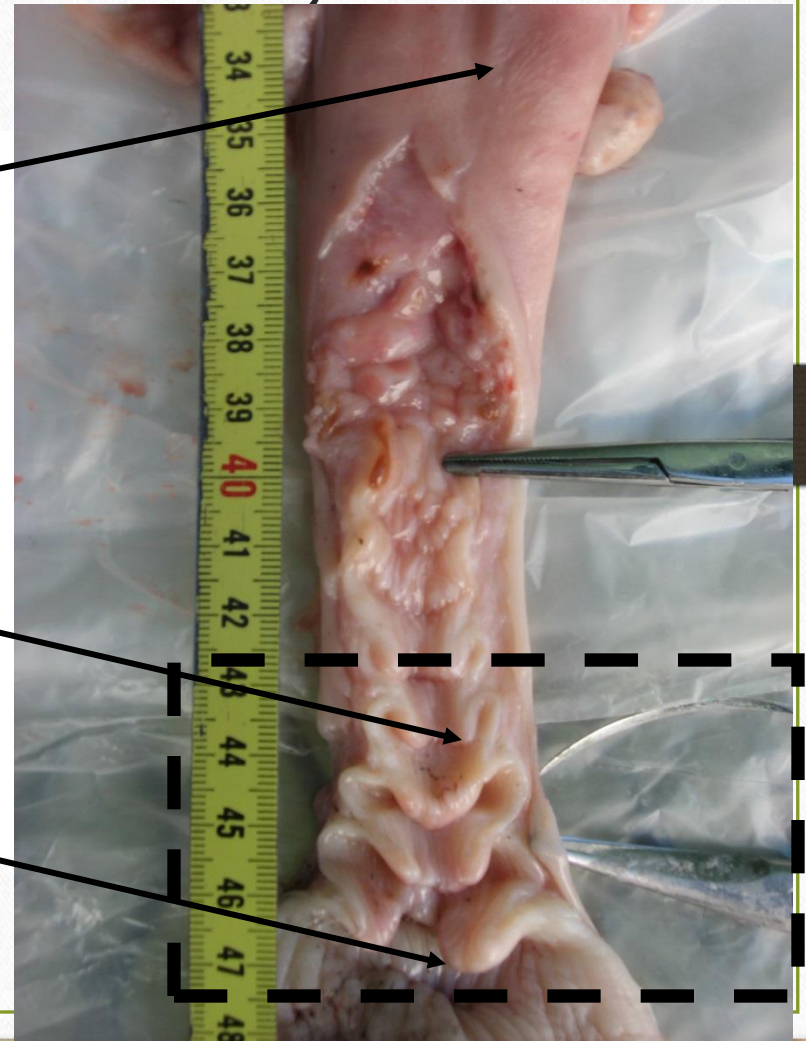
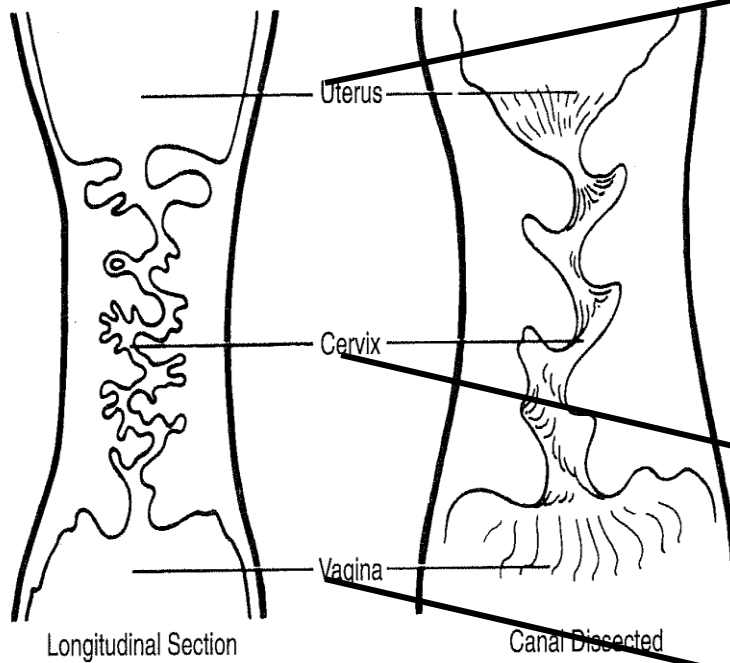
# Cryopreserved semen research

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# Laparoscopic AI

Experiment	Treatment	Fertility % (# ewes)	Prolificacy
1	Fresh	25 (27)	1.14 <sup>a</sup>
1	T0	20 (35)	1.27 <sup>a</sup>
1	T24	16 (35)	1.72 <sup>b</sup>
1	SEM	4.2	.08
2	Fresh	37 (33)	1.45
2	T24	45 (29)	1.2
2	CLC	41 (36)	1.48
2	SEM	5	.07

# Cervical anatomy



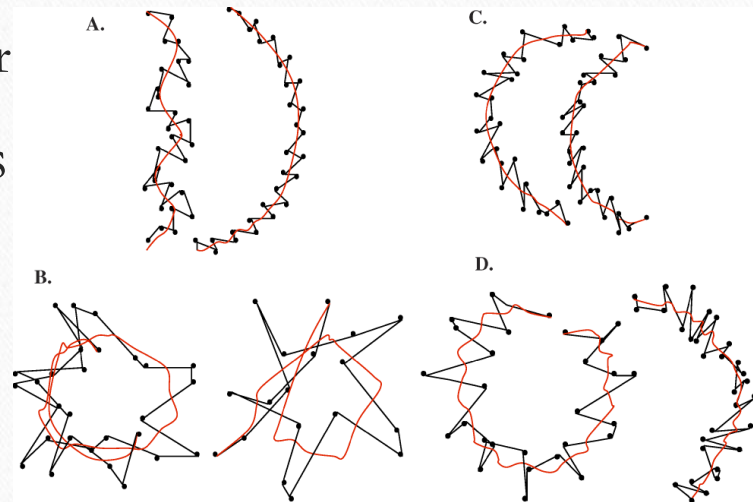
# Past research-since 2003-ewe factors

- Compare:
  - AI devices
  - Estrous synch. techniques
    - Sponges vs CIDRS
  - Cervical depth- BWM vs Suffolk



# Past research-since 2003-ram factors

- Ram semen holding time
  - Prior to freezing-0, 24, 48 hr
- Semen diluent comparisons
  - Milk vs TRIS AI
  - Holding time Milk vs TRIS
    - Age, season, diluent



# NAGP methods-Cervical AI

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- Semen preservation: Milk + Egg yolk + Glycerol
- Estrous synchronization
  - CIDRs or Sponges-12 days
  - PMSG 400 IU 24 hr prior to P4 removal
  - Ram effect!!!
- Insemination technique
  - Cervical at 53 and 57 hours post P4 removal
  - Sheep AI gun
  - $\geq 100$  million motile sperm

# Black Welsh Mountain sheep fertility

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Year	Pregnant	Open	% Lambing
2011	8	18	44
2012	10	10	50
2013	12	12	50
2014	?	?	?



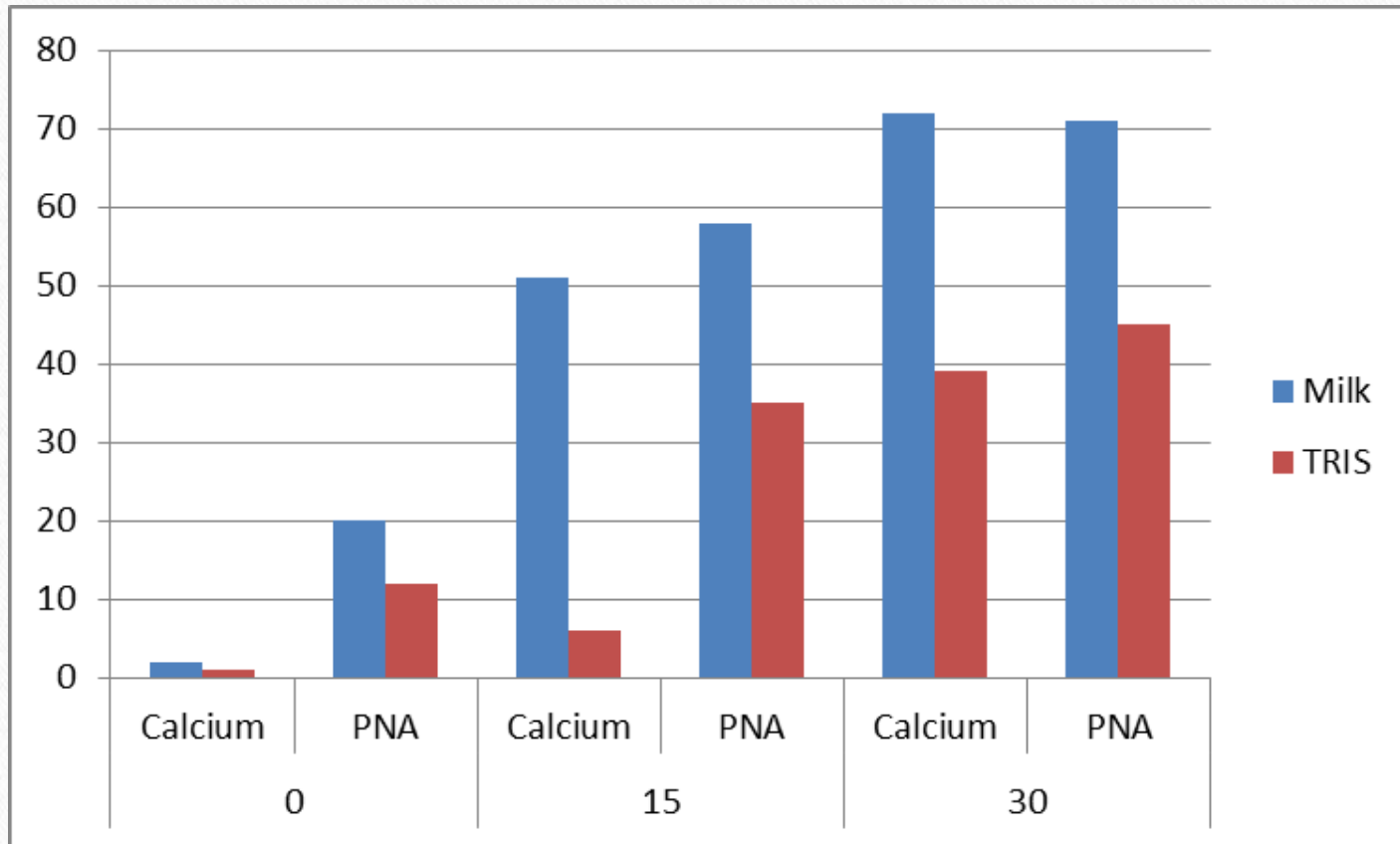
# Sheep AI-ram and diluent effects

Ram	Year	Breed	Treatment	Diluent	Preg/AI	%
Pool	2008	WWF	FT	TRIS	9/19	45 (Lap)
Pool	2008	WWF	FT	TRIS	3/20	15
Jerrold	2012	BWM	FT	SMEY	3/4	75
Natham	2012	BWM	FT	SMEY	1/4	25
Natham	2013	BWM	FT	SMEY	6/12	50
Kimm	2013	Suffolk	FT	SMEY	1/8	12.5
1396	2013	Hamp	FT	SMEY	4/8	50
Natham	2013	BWM	LC	SMEY	6/12	50

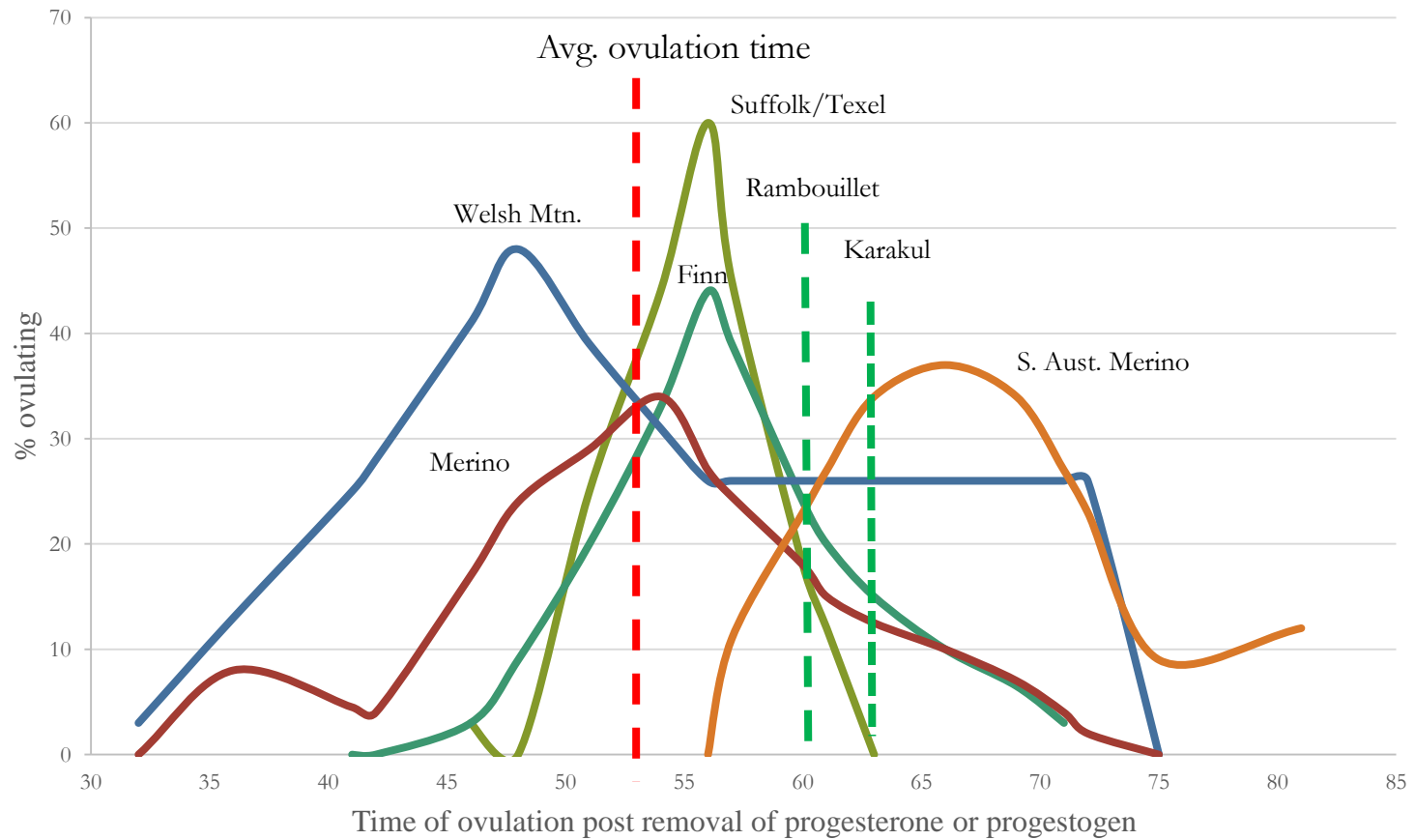
- All breeds: SMEY:42% TRIS: 15%
- Why?



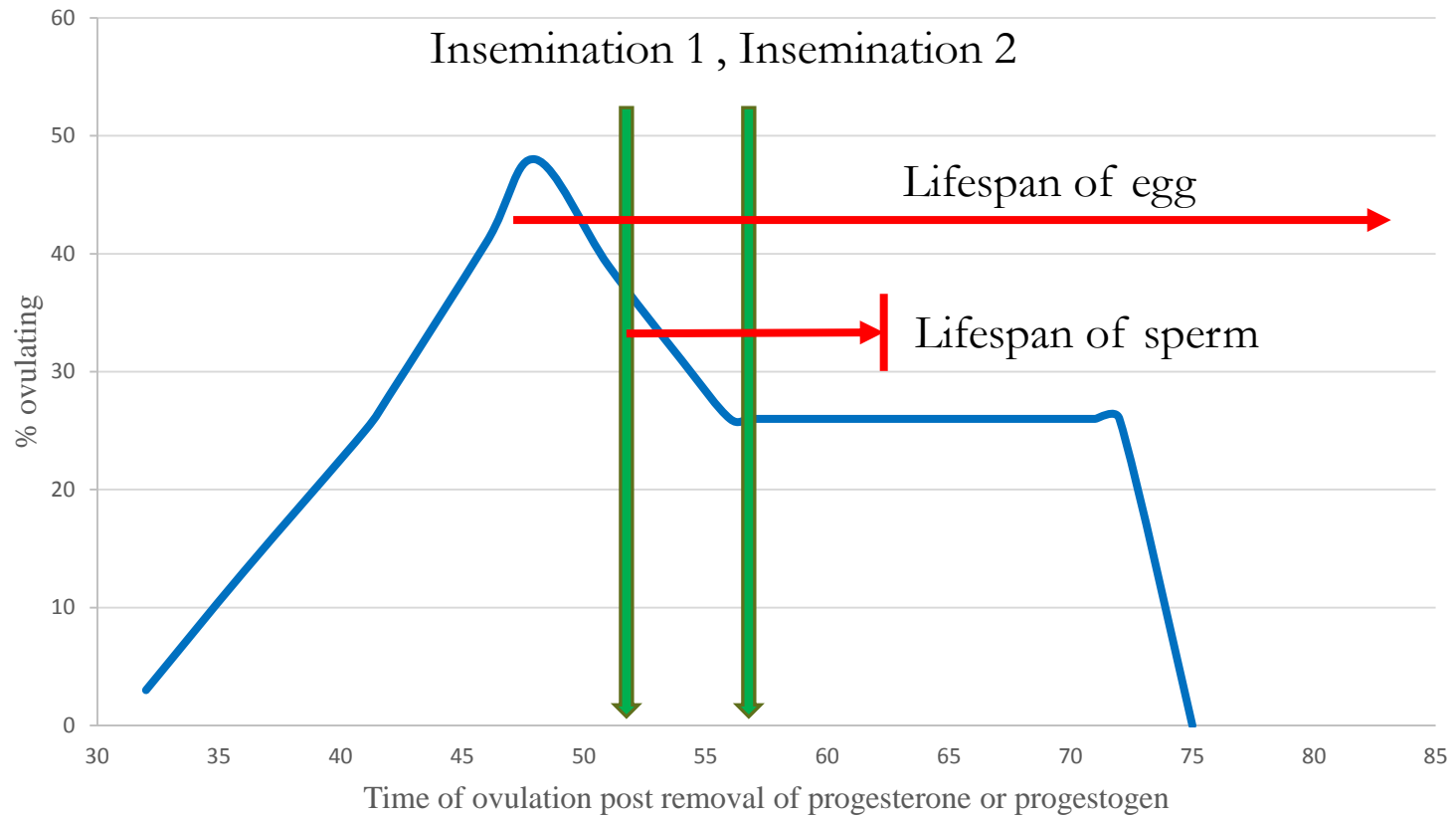
# MILK vs TRIS – Post-thaw



# Issues-Time of ovulation



# Time of ovulation and AI-Welsh Mountain



# Difficulties with AI

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- Variable fertility: ewe, ram, breed

- Non-optimized:

- estrous synchronization protocols

- semen cryopreservation protocols



Interaction

*-Is this different from other species?*

# Take home message:

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- Similarities to swine model
- Utility of:
  - Cooled semen-72 hour fertility
  - Frozen-thawed semen
    - How long can a ram or buck live?
- It works
  - Come and see!

# Contacts

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