The Ultimate Value of Breeding Soundness Evaluations

George A. Perry and
Saulo Menegatti Zoca

Why do we test bulls?

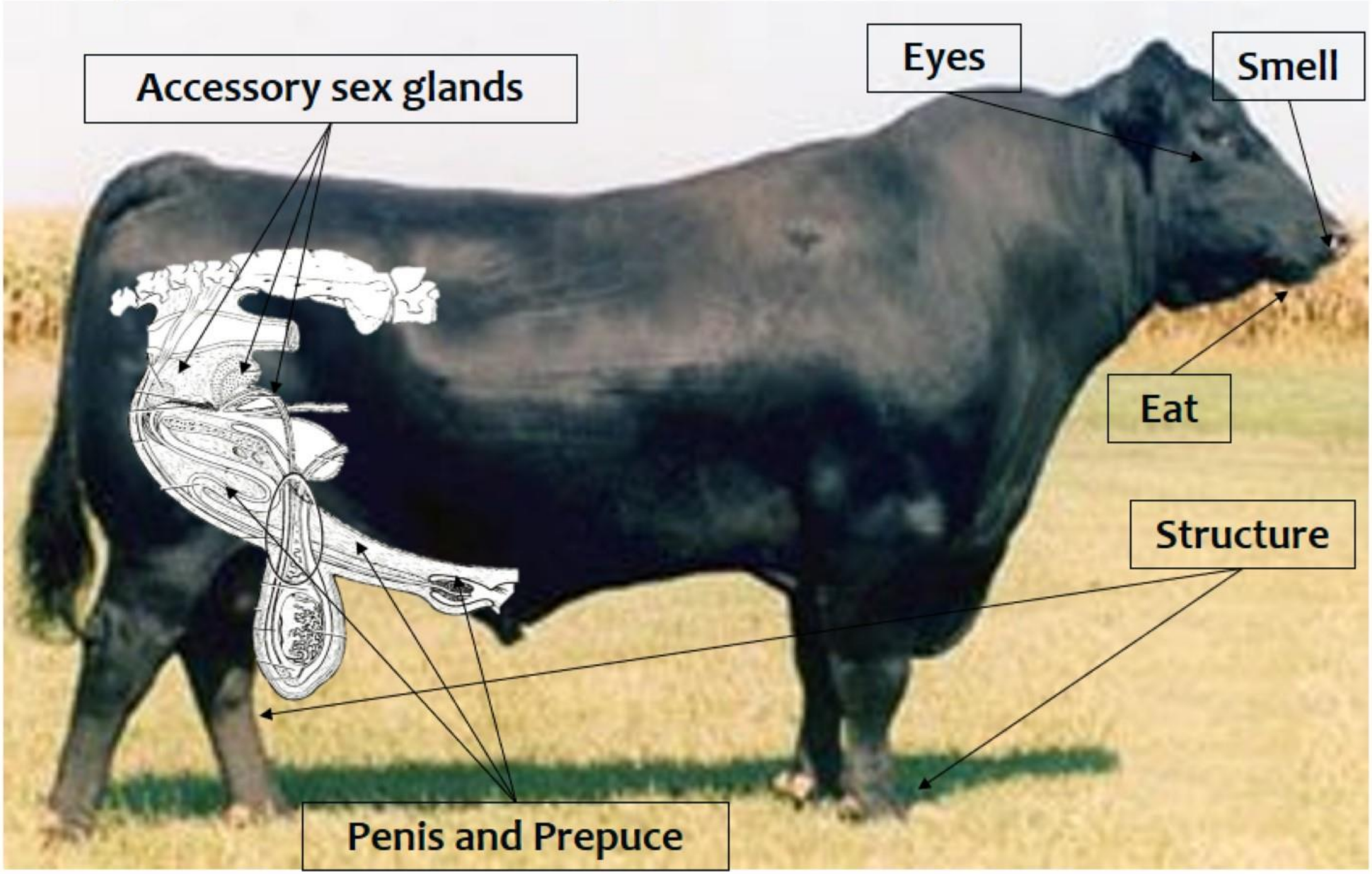
- Reproduction is 5x more important economically than growth performance and at least 10x more important than product quality (Trenkle and Willham, 1977)
- A single bull can affect the genetics of a herd in a much greater proportion than a single cow
  - Half of a calf's genetic makeup comes from the bull
  - A bull can sire 30-60 cows in a year
  - A bull stays in the herd for ~4 years
- Influence of herd bulls in overall herd fertility
  - Loss of Fertility = Loss of Calf Crop
  - 20% of unselected bulls are sub-fertile or infertile

How do we test bulls' fertility?

- The standard method to analyze bull fertility is through a breeding soundness exam (BSE)
- BSE includes:
  - Physical soundness
  - Estimation of sperm production (scrotal circumference)
  - Assessment of sperm quality

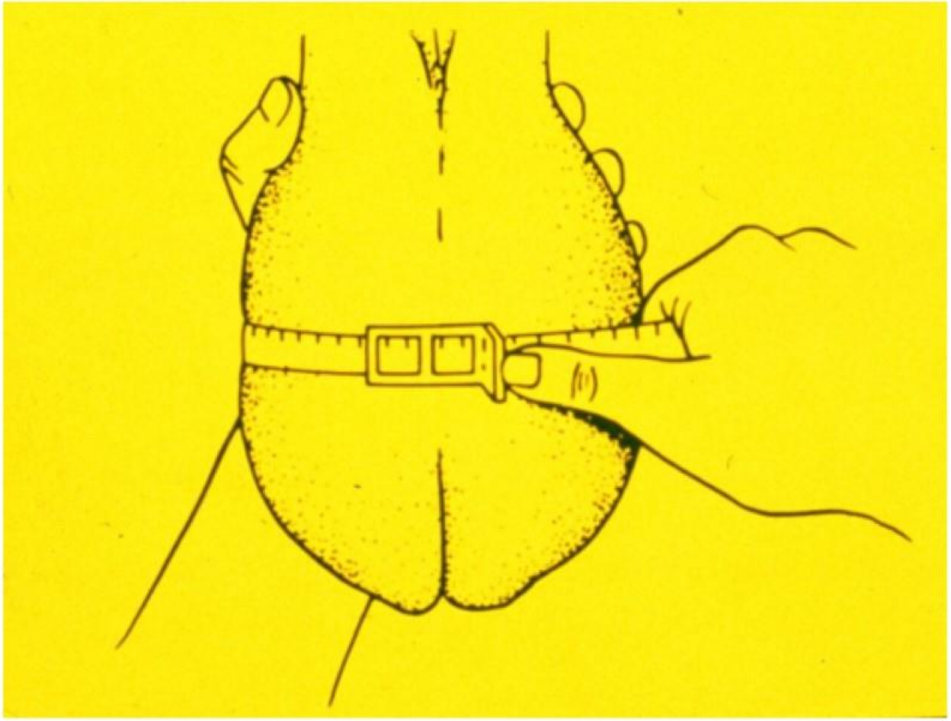
Physical Exam

- Physical soundness + body condition score



Scrotal Circumference

- Estimation of sperm production
- Bulls with larger scrotums have
  - Sons with larger scrotums
  - Daughters that reach puberty at a younger age



Scrotal Circumference

- Scrotal circumference
  - Highly heritable
  - Breed effects: Simm > Angus > Charolais > Hereford > Shorthorn > Limousin > Longhorn
  - Studies: 7 – 13 % disqualified due to low SC

Minimum requirement

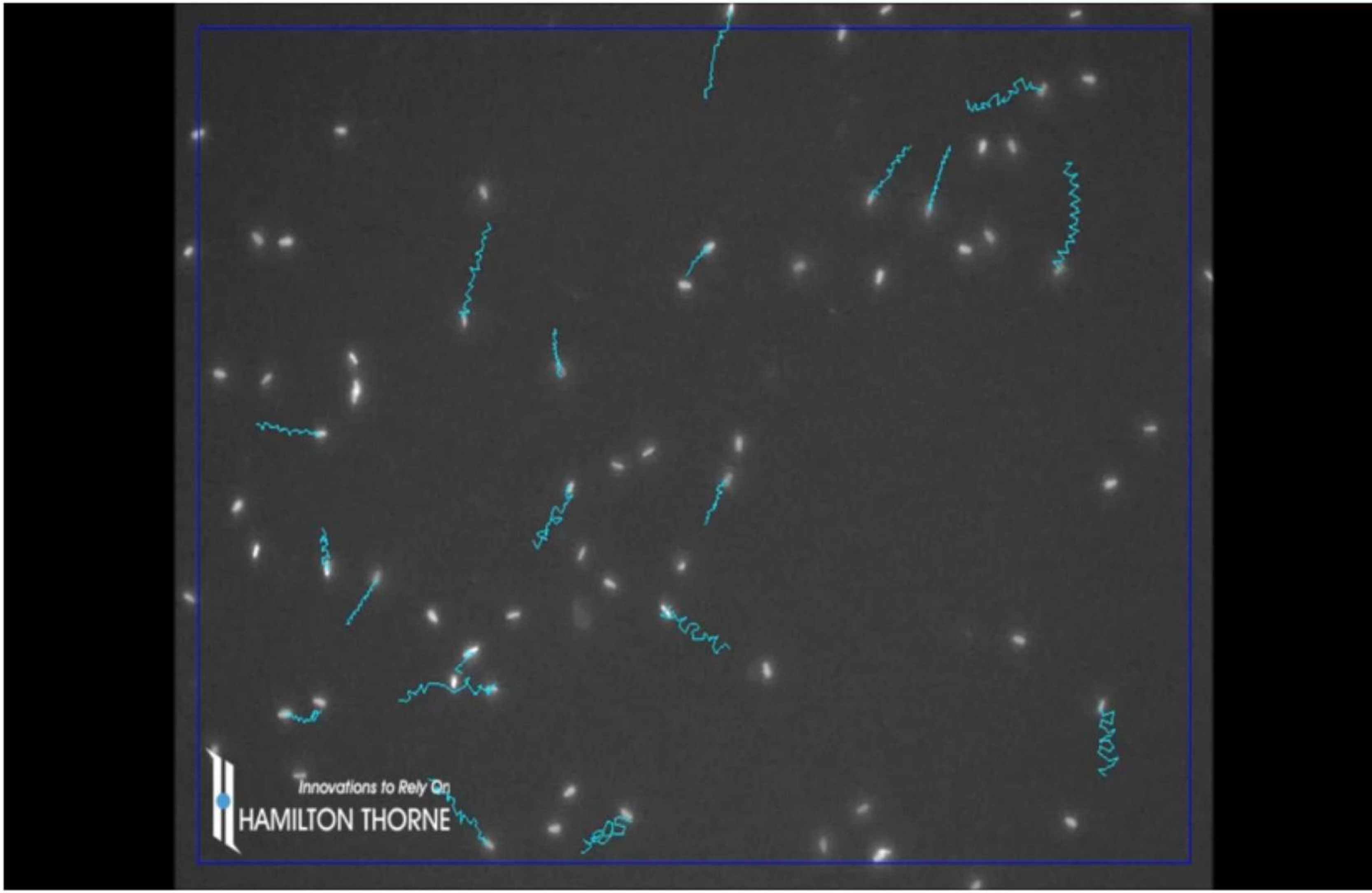
Age	Scrotum Circumference
< 15 mo	30 cm
15-18 mo	31 cm
18-21 mo	32 cm
21-24 mo	33 cm
> 24 mo	34 cm

Sperm quality

1. Ejaculate volume
2. Sperm concentration
3. Percentage of progressive motility ≥ 30%
4. Morphology ≥ 70%

- Can semen quality change?
  - Substandard nutrition
  - Temperature
  - Injury
  - Disease

Progressive motility



Sperm morphology

	Year 1		Year 2	
	Random Group	≥ 80% Normal Sperm	Random Group	≥ 80% Normal Sperm
Cows Exposed	655	675	1282	808
No. of Bulls	26(~1:25)	27(~1:25)	51(~1:25)	33(~1:25)
No. Pregnant	571	656	1179	769
% Pregnant	87%	93%	85%	90%
% Increase		6%		5%

(Wiltbank and Parish, 1986)

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Breeding soundness exam

- Kennedy et al., 2002 Theriogenology
- Bulls evaluated 3,648 (10 -19 mo)
- 10 breeds
- 5 performance test stations
- 10.2% classified as unsatisfactory without semen collection
  - 7.1% inadequate scrotal circumference
  - 3.1% physical abnormalities
- 13.6% classified as unsatisfactory because of motility, morphology or both

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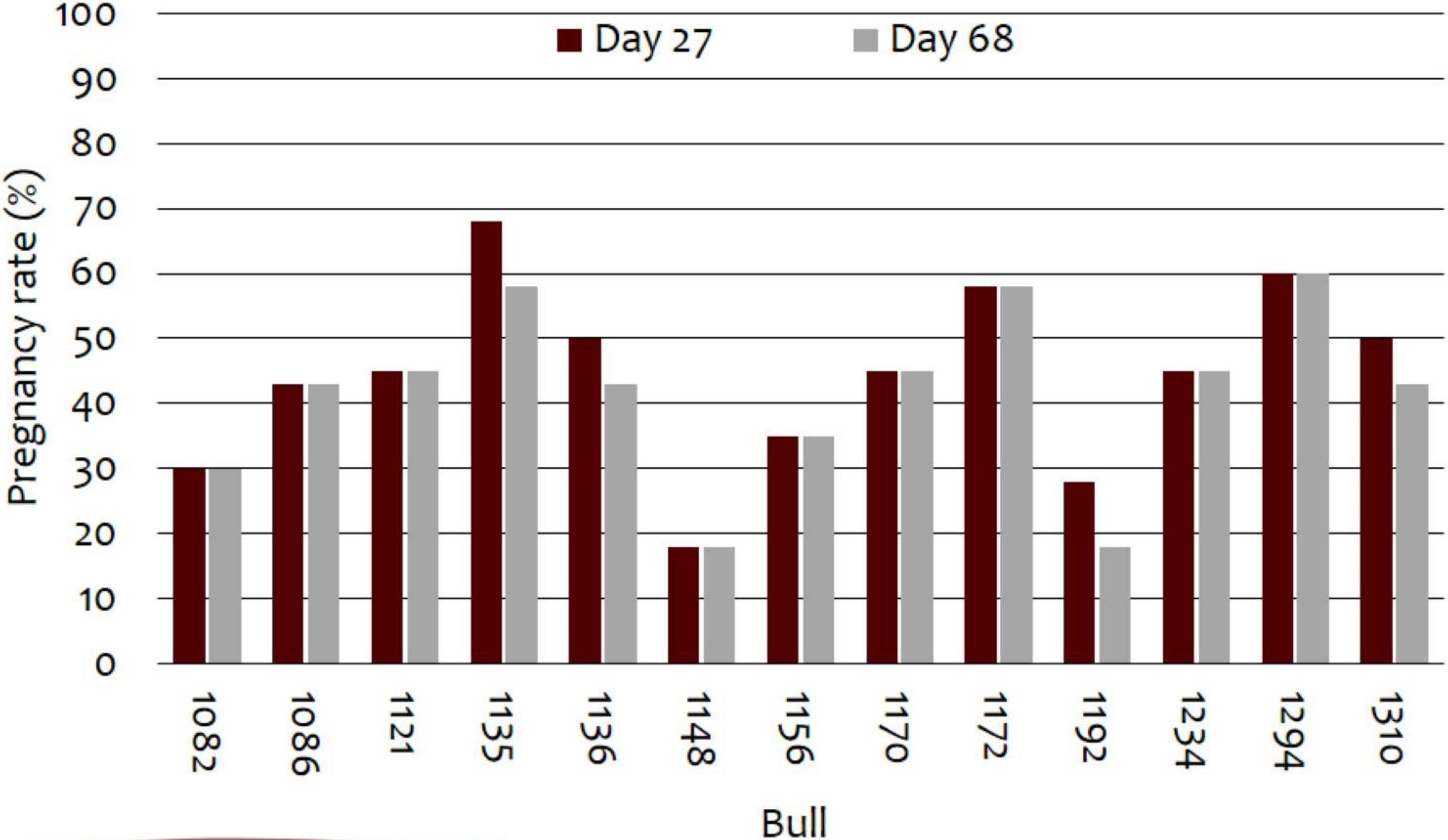
EFFECT OF BULL ON PREGNANCY RATES - TAI



Sire x Treatment

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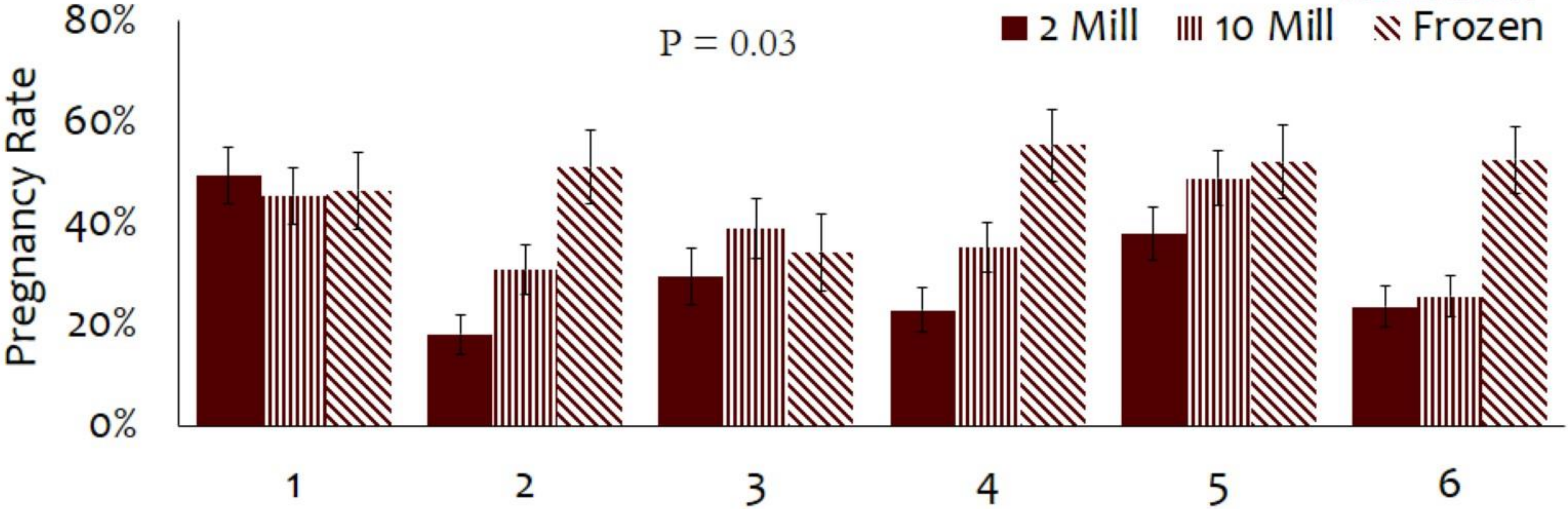
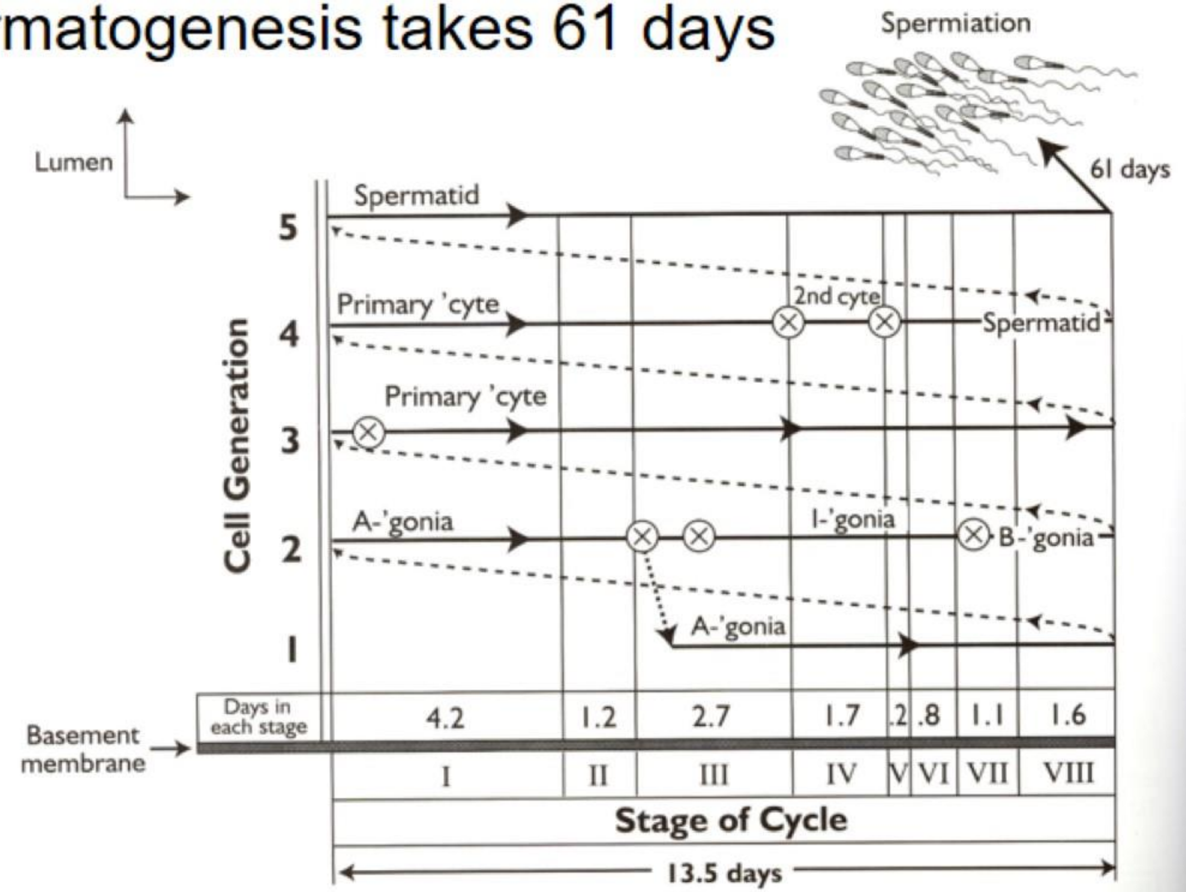


CASA data	1			2			3			4			5			6		
Total Motile (%)	62	61	57	43	65	48	43	40	36	38	43	42	63	64	49	56	59	44
Prog. Motile (%)	23	28	43	27	40	44	16	24	26	16	21	31	30	37	35	12	20	33
Velocity (µm/s)	111	133	123	93	114	116	90	124	87	88	102	92	105	126	100	87	125	107
Straightness (%)	75	79	82	81	79	92	77	84	87	75	76	87	80	83	84	69	72	80

	1		2		3		4		5		6	
Morph. Normal (%)	66	66	65	68	59	59	66	67	67	62	64	62

- **REMINDER: BSE is a snapshot of a bull's fertility**
  - Represents the semen and physical condition on the day of test
  - Spermatogenesis takes 61 days



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What is not included in a BSE?

- Mating ability
  - Ability for a bull to complete service
  - 4.8% of 165 mature bulls with a satisfactory BSE were physically unable to service cows (Barth et al., 2004)
- Serving capacity
  - Recommendations range from 1:10 to 1:60
  - Yearling < older bulls
  - Synchronization places greater pressure on bulls and lowers serving capacity
  - Multiple sire pastures decrease serving capacity since multiple sires will mate an individual cow

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Libido (Sex Drive or Willingness)

- Poor Libido (Sex Drive or Willingness) can compromise reproductive performance
  - Libido is highly heritable (as high as 0.59)
- Libido can practically be measured by closely watching a bull after introducing him to a herd of cows

Libido

Libido

A. Barth

Bull fertility

- **BSE is a great tool to detect sterile and infertile animals**
- Below average and low fertility bulls are often classified as satisfactory
- High fertility is not guaranteed
- Several tests have been developed and failed to further explain differences in bull fertility when compared to traditional morphology and progressive motility analysis

PREGNANCY RATE OF ANGUS BULLS IN NELORE COWS

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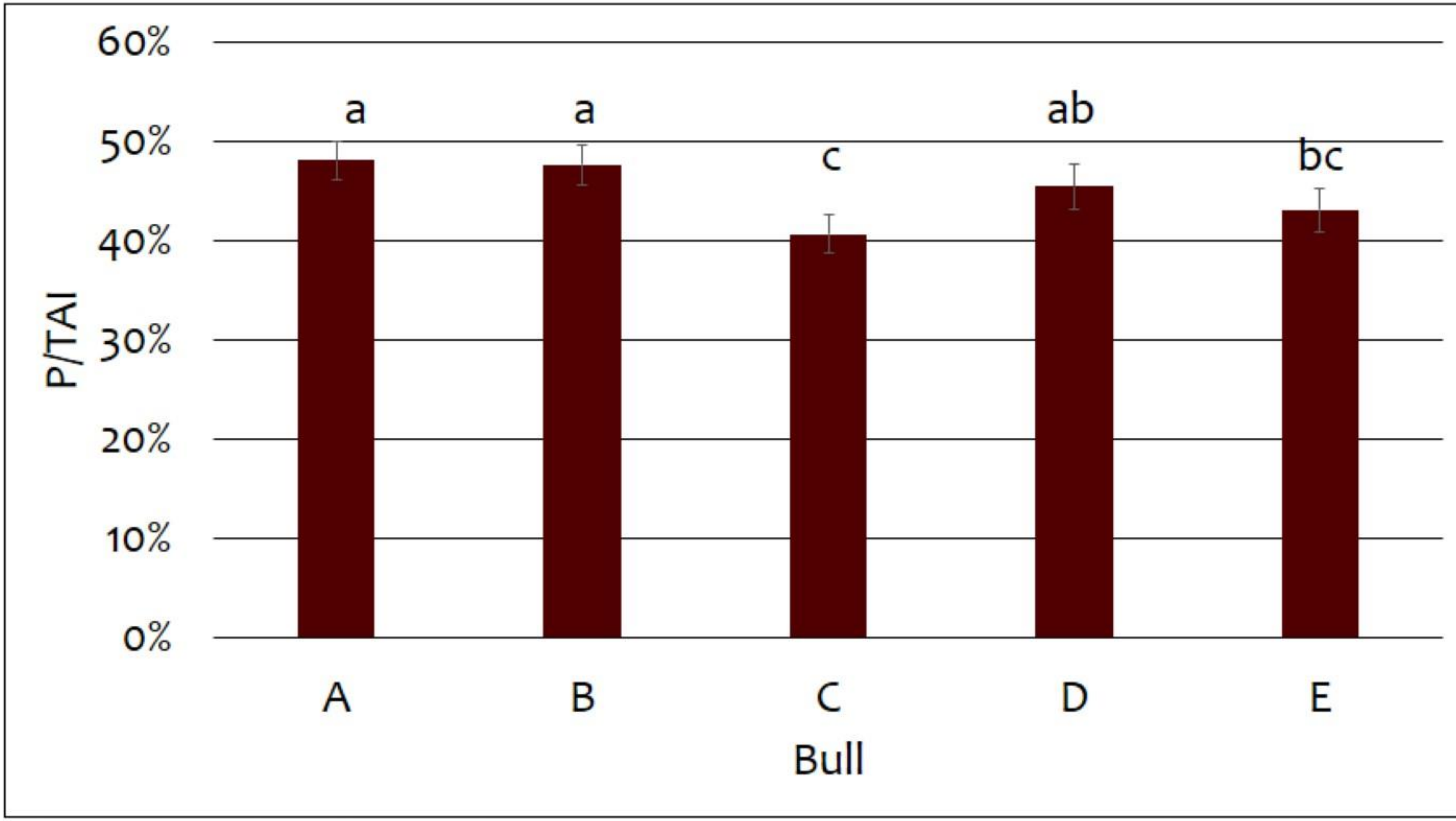

Zoca et al., Theriogenology 2019: n=4866 (range from 747 to 1206 inseminations per bull) *P*<0.05

EFFECT OF BULL ON PREGNANCY RATES - TAI

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	Bull				
	A	B	C	D	E
Morphology					
Normal[2], %	66.7	72.5	70.5	75.7	70.8
SM, %	42.1 ± 2.3[c]	54.8 2.5[ab]	50.8 ± 2.2[b]	60.6 ± 1.9[a]	38.3 ± 1.5[c]
CASA					
TM, %	31.8 ± 2.2[bc]	33.0 ± 2.4[b]	26.5 ± 2.0[cd]	51.6 ± 2.0[a]	24.2 ± 1.4[d]
PM, %	21.8 ± 2.0[b]	23.7 ± 2.2[b]	19.8 ± 1.8[b]	36.5 ± 2.0[a]	19.0 ± 1.3[b]
VAP, µm/s	87.3 ± 2.4[d]	93.3 ± 2.5[cd]	107.1 ± 2.2[b]	99.5 ± 2.0[c]	114.0 ± 1.6[a]
VCL, µm/s	154.3 ± 5.3[d]	165.3 ± 5.7[cd]	192.4 ± 5.0[b]	177.5 ± 4.5[c]	206.4 ± 3.7[a]
VSL, µm/s	73.7 ± 2.1[c]	76.9 ± 2.2[c]	89.9 ± 2.0[a]	84.1 ± 1.7[b]	92.8 ± 1.4[a]
STR, %	83.8 ± 0.9[abc]	82.4 ± 1.0[bc]	85.3 ± 0.8[a]	84.6 ± 0.7[ab]	82.1 ± 0.6[c]
LIN, %	50.9 ± 1.2	49.1 ± 1.3	51.0 ± 1.1	50.4 ± 1.0	48.4 ± 0.8
Flow Cytometer					
Viable, %	48.4 ± 2.0[c]	55.1 ± 2.1[b]	41.6 ± 1.8[d]	66.2 ± 1.6[a]	30.5 ± 1.2[e]
Acrosome, %	59.7 ±2.1[b]	76.7 ± 1.8[a]	64.3 ± 1.9[b]	77.5 ± 1.4[a]	53.2 ± 1.5[c]
VA, %	49.4 ± 2.0[c]	58.1 ± 2.2[b]	44.8 ± 1.9[c]	68.1 ± 1.6[a]	31.8 ± 1.3[d]
VNCa, %	45.9 ± 2.1[c]	53.2 ± 2.3[b]	40.1 ± 2.0[d]	62.9 ± 1.7[a]	29.1 ± 1.3[e]
ANCa, %	91.6 ± 0.9[a]	92.1 ± 0.9[a]	91.7 ± 0.8[a]	91.8 ± 0.7[a]	87.7 ± 0.8[b]
VANCa, %	44.1 ± 2.0[c]	50.7 ± 2.2[b]	38.1 ± 1.8[d]	60.6 ± 1.7[a]	27.1 ± 1.2[e]
DFI, %	37.7 ± 2.0[c]	26.1 ± 1.8[b]	36.6 ± 1.9[c]	21.5 ± 1.4[a]	47.8 ± 1.5[d]

Zoca et al., Theriogenology 2019

Comparison of fertility varying the interval from CIDR removal to insemination

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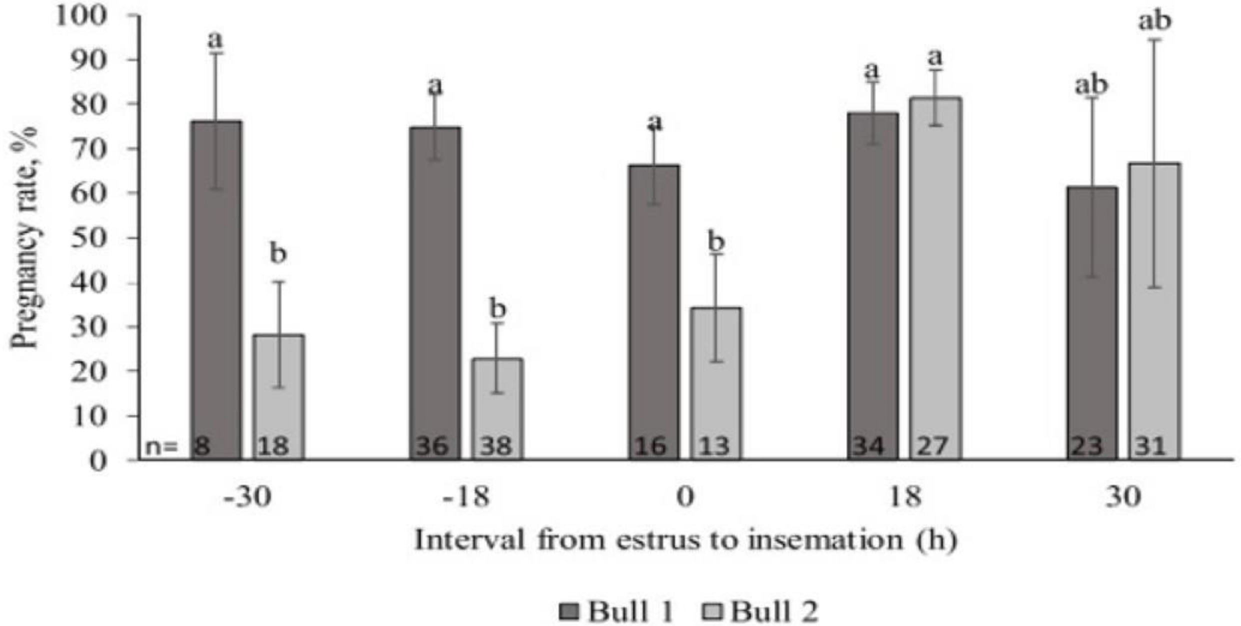

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Table 1

Analysis of sperm plasma membrane viability, DNA stability, and percent total and progressively motile sperm at 0 h and 3 h post-thawing for frozen semen and in liquid semen on Days 2 and 3 post-collection.

Bull	Semen Type	% of total (flow cytometry)				% motile (CASA)[a]			
		Membrane viability		DNA Stability		Total/Progressive (0 h)		Total/Progressive (3 h)	
		d 2	d 3	d 2	d 3	d 2	d 3	d 2	d 3
1	Frozen	53	55	67	65	51/39	51/39	46/29	45/29
1	Liquid	64	76	88	90	64/55	59/49	61/52	58/50
2	Frozen	46	49	59	61	33/22	44/28	42/24	41/24
2	Liquid	83	84	93	95	74/65	60/49	69/59	63/53

[a] Computer-assisted sperm analysis.

Richardson et al., Anim Reprod Sci. 2017

What does the future holds?

- In order to traverse the female barriers and complete fertilization the spermatozoon requires several traits
  1. Progressive motility
  2. Normal morphology
  3. Intact membranes (acrosome and plasma membrane)
  4. Stable DNA
  5. Ability to undergo capacitation

- However there are other traits that are unknown
  - Surface proteins?
  - miRNAs?
  - Others

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Characterization of proteins in the bovine epididymal and seminal fluid and proteins attached to epididymal and ejaculated sperm.

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[2]Department of Animal Science, ISU, Ames, IA
[3]USDA, ARS MARC, Clay Center, NE

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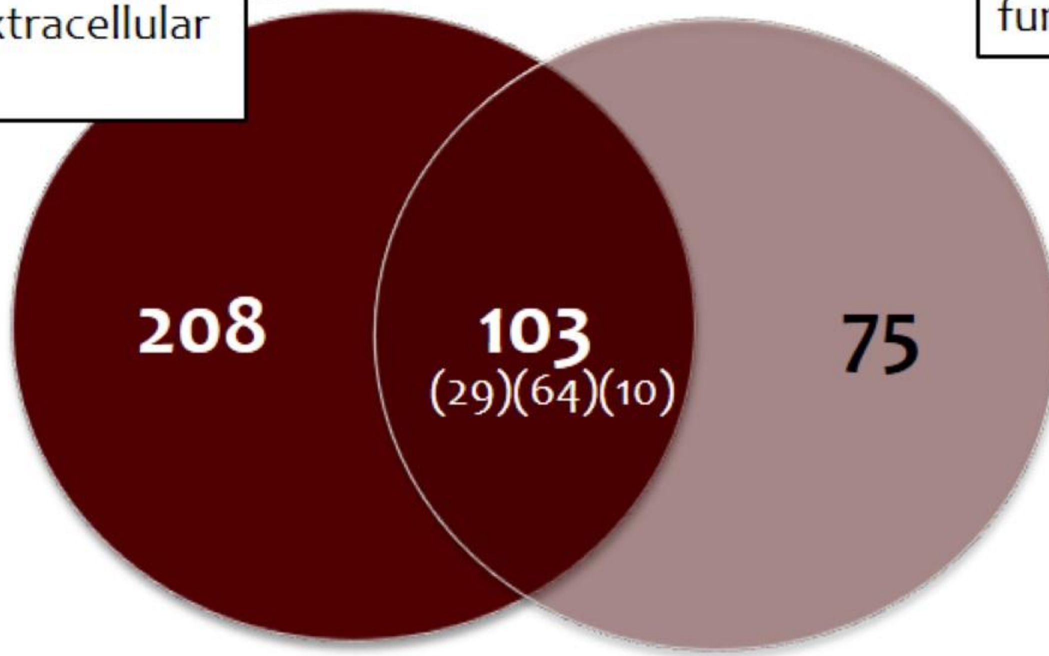
Epididymal Vs. Ejaculated Fluid

Fluid up in epididymis
GO term: Extracellular exosome

Take Home: 3/17 role in motitility

Fluid: Epididymis only
GO term: Myelin sheath (Second behind extracellular exosome)

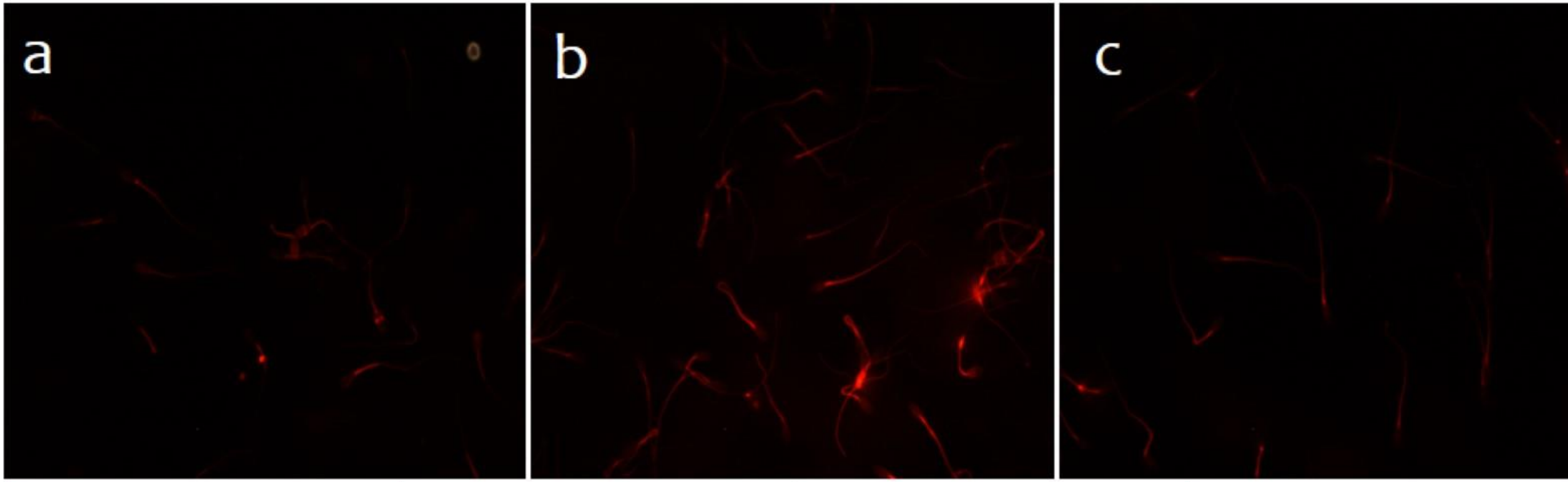
Take Home: Mitochondrial function



Fluid: Ejaculate only
GO term: Extracellular region

Take Home: Very little interaction

Mitochondrial membrane potential



	Ejaculated pH 7.3	Epididymal pH 7.3	Epididymal pH 5.8
Fluorescent intensity	1.8 ± 0.32[a]	3.4 ± 0.32[b]	4.3 ± 0.34[c]

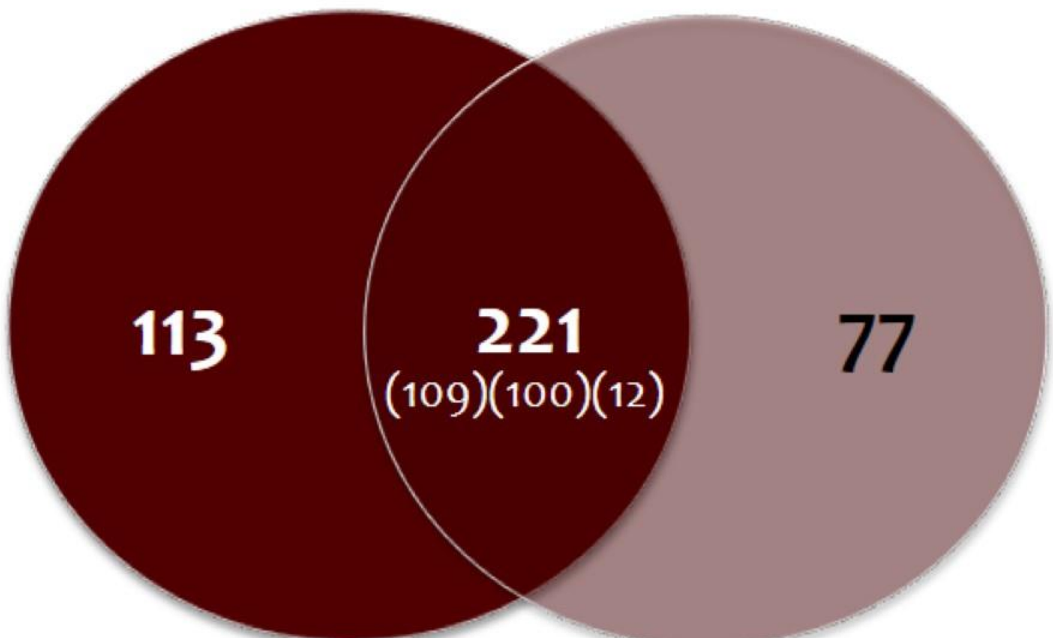
Values with different superscripts are different [a vs b] $P < 0.01$

Perry et al., 2016

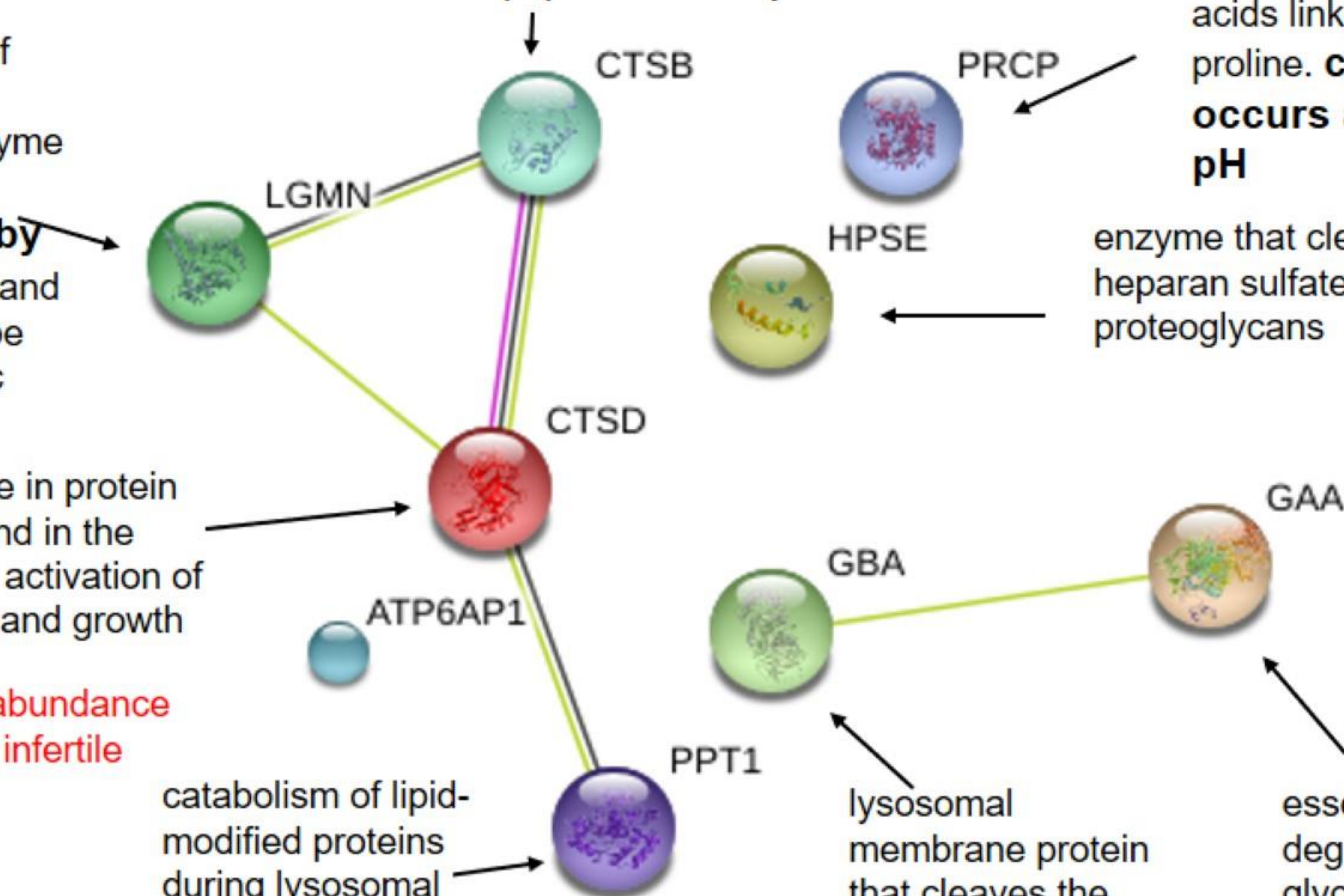
Epididymal Vs. Ejaculated Sperm

Sperm up in epididymis
GO term: Threonine-type peptidase activity

Take Home: catalytic subunits of proteasomes (the main function of proteasomes is to degrade unneeded or damaged proteins)



Sperm ejaculate only
GO term: Vacuole



Bovine Cervical Mucus

- a hydrogel with 92 to 95 % water content

Soluble substances are

- lactoferrin
- Immunoglobulins
- glucosidases
- matrix metalloproteases
- carbohydrates (i.e. fructose and glucose)
- amino acids
- Lipids (i.e. cholesterol)

Non-Soluble substances are

- Mucins (highly glycosylated proteins)

Cortes et al., 2014

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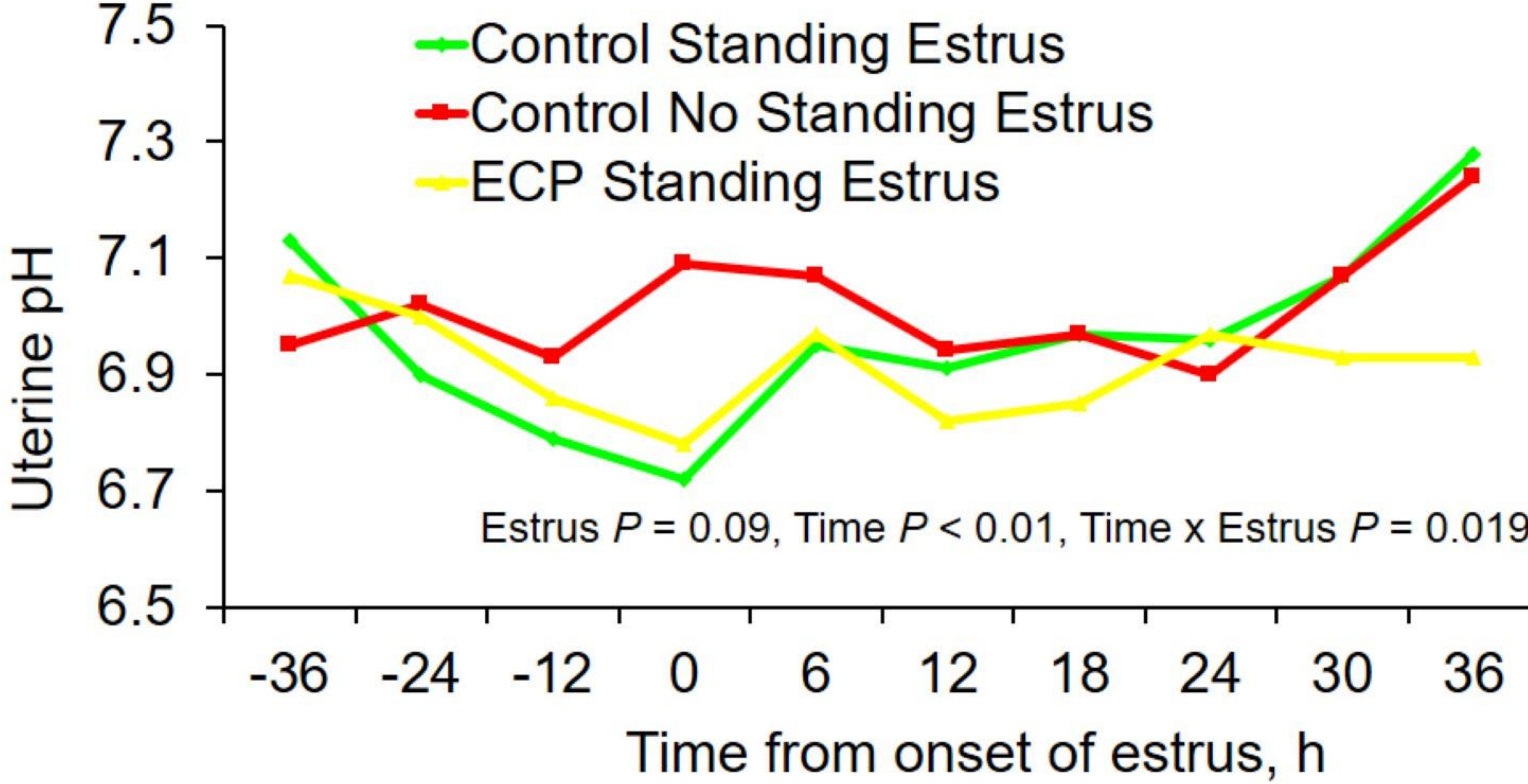
Uterine pH around the onset of standing estrus



Perry and Perry, 2008 J. Anim. Sci. 86: 2928-2935.

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What does the future holds?

Use of Sperm Proteins as a Putative Fertility Marker

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[1]Department of Animal Science, South Dakota State University, Brookings, SD

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Use of Sperm Proteins as a Putative Fertility Marker

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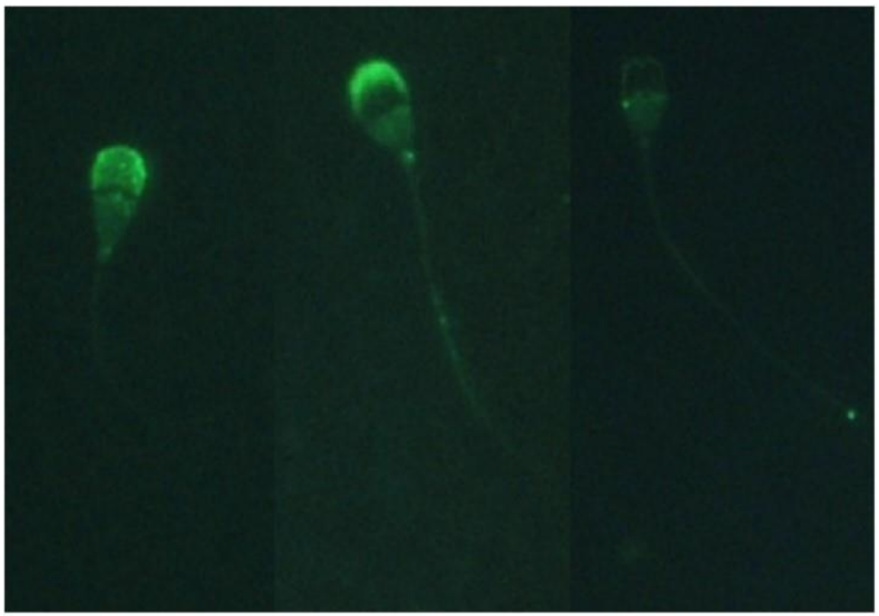
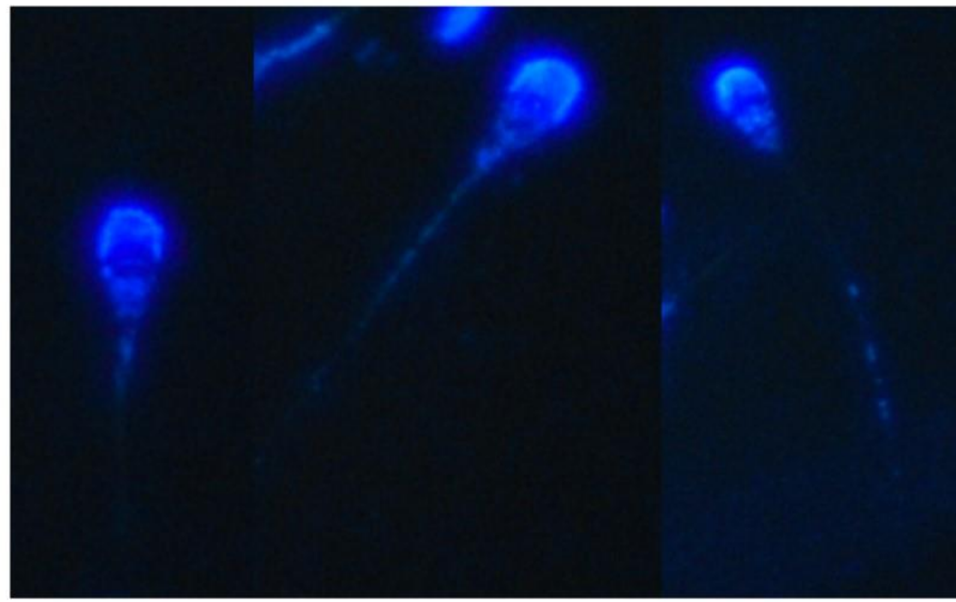
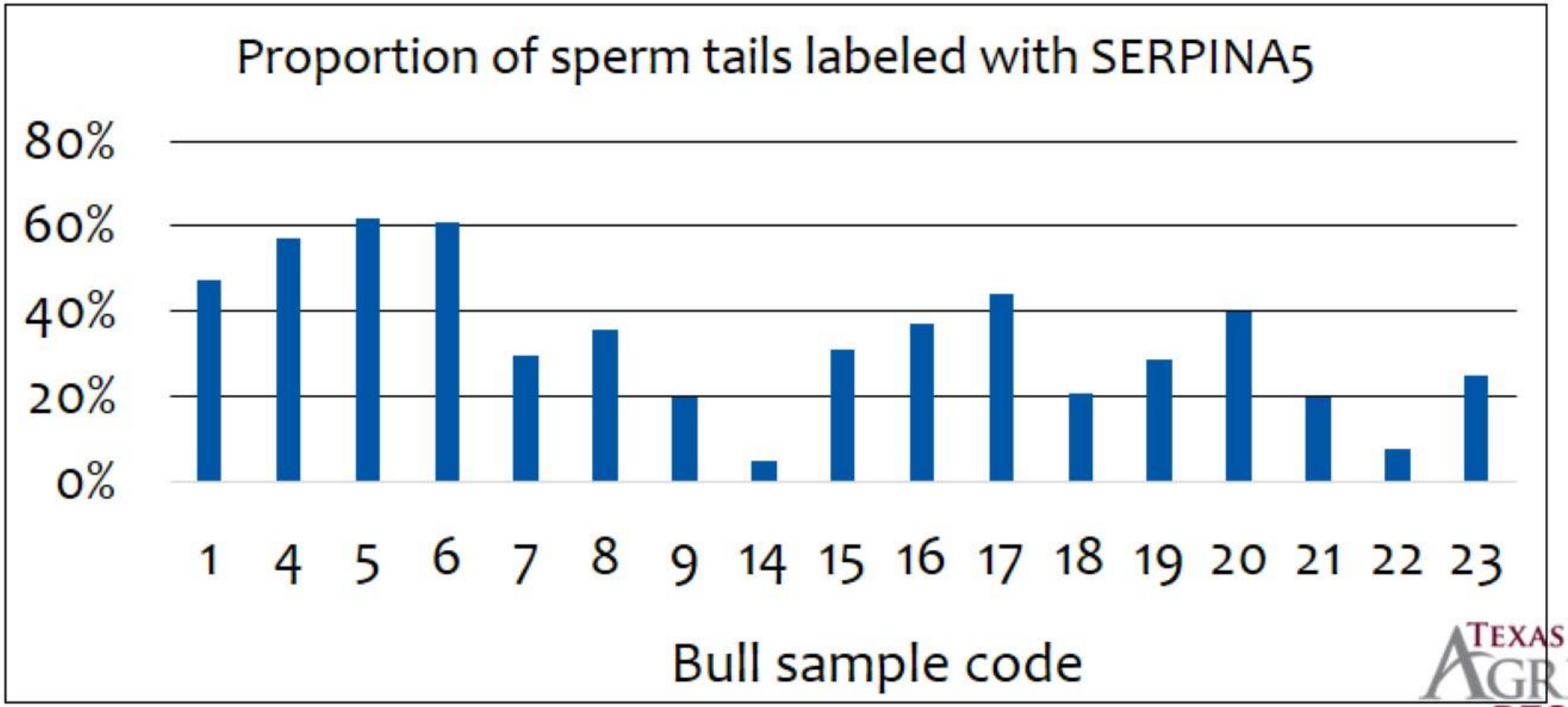
- Both CD9 and SERPINA5 were localized to the sperm head





Use of Sperm Proteins as a Putative Fertility Marker

- SERPINA5 was also detected on the proximal region of the sperm tail among all bulls
  - 33.5 ± 4.1% of sperm tails among all bulls
  - Range from 4% to 61%



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What does the future holds?

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Influence of microRNAs from Semen on Bovine Fertility

S.D. Perkins[1], B.N. Keel[2], E.J. Northrop[1], T.G. McDaneld[2], B.R. Harstine[3], J.M. Dejarnette[3], M.D. Utt[3], & G.A. Perry[1]

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[3]Select Sires Inc, Plain City, OH

Introduction

- MicroRNAs (miRNA) are small noncoding RNAs ranging from 18 to 26 nucleotides that can change genomic expression by altering translation of mRNA (Hutvagner, 2005).

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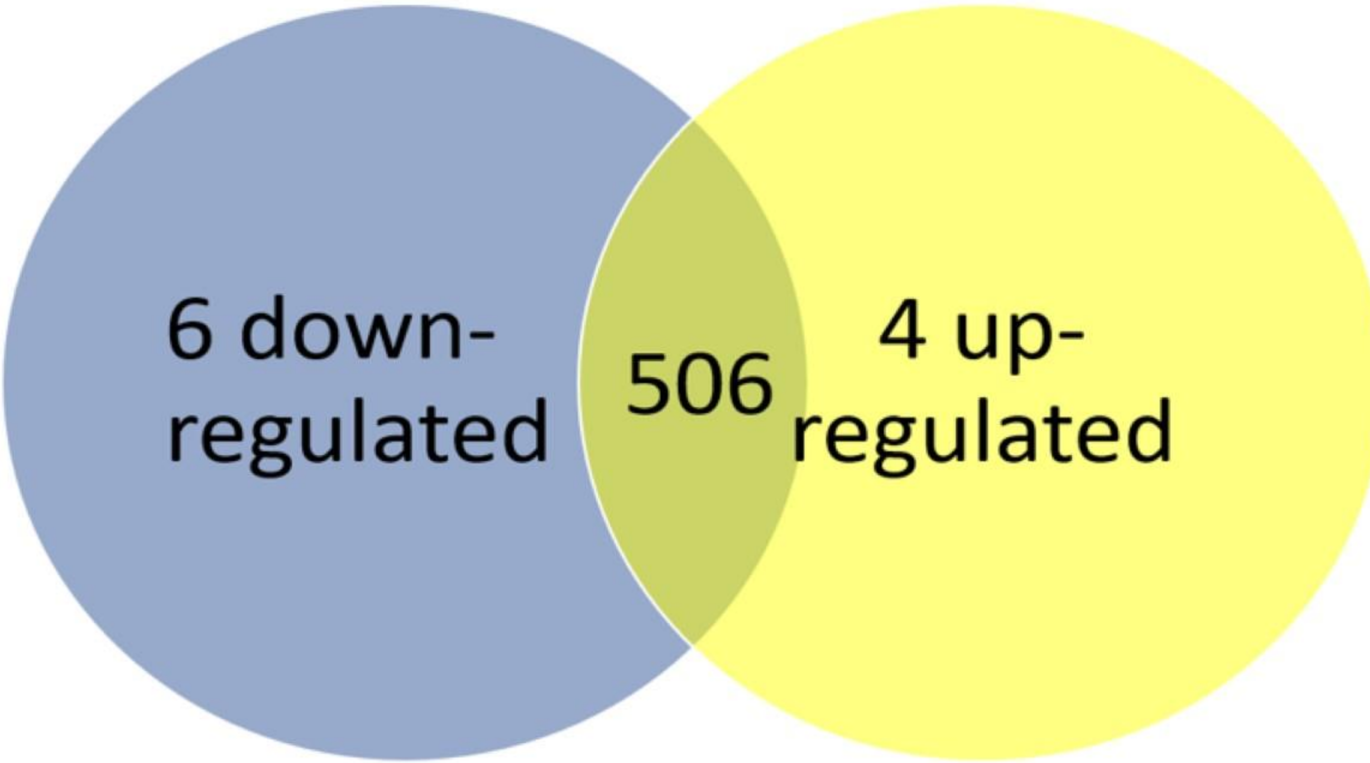
Influence of microRNAs from Semen on Bovine Fertility



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Influence of microRNAs from Semen on Bovine Fertility

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miRNA	Up or Down Regulated in Low Bulls	Fold Change
miR-9-5p	Down	-0.88
miR-98	Down	-1.63
miR-126-5p	Down	-0.93
miR-142-5p	Down	-0.95
miR-182	Up	0.29
miR-296-3p	Up	1.03
miR-329a	Down	-2.36
miR-449a	Down	-0.63
miR-1839	Up	0.41
miR-2284y	Up	0.32

In vitro Embryo development by sire conception rate

Bull SCR class	Sire	Oocytes (n)	Cleavage at Day 3 (%)[1,2]	Blastocyst at Day 8 (%)
High	3	384	88.7 ± 2.2^{a}	43.7 ± 2.5^{a}
	5	388	90.4 ± 2.3^{a}	43.6 ± 2.5^{a}
Low	8	368	87.6 ± 2.3^{a}	43.6 ± 2.5^{a}
	9	374	85.5 ± 2.4^{a}	31.7 ± 2.5^{b}
	10	385	90.8 ± 2.2^{a}	33.6 ± 2.5^{b}
Overall	High	762	89.3 ± 1.1^{a}	42.6 ± 1.7^{a}
	Low	1127	88.2 ± 0.9^{a}	35.6 ± 1.5^{b}

[1]Values are presented as least-squares means ± SEM.

[2]Values with different superscripts differ ($P < 0.05$).

Ortega et al., 2018 Biology of Reproduction

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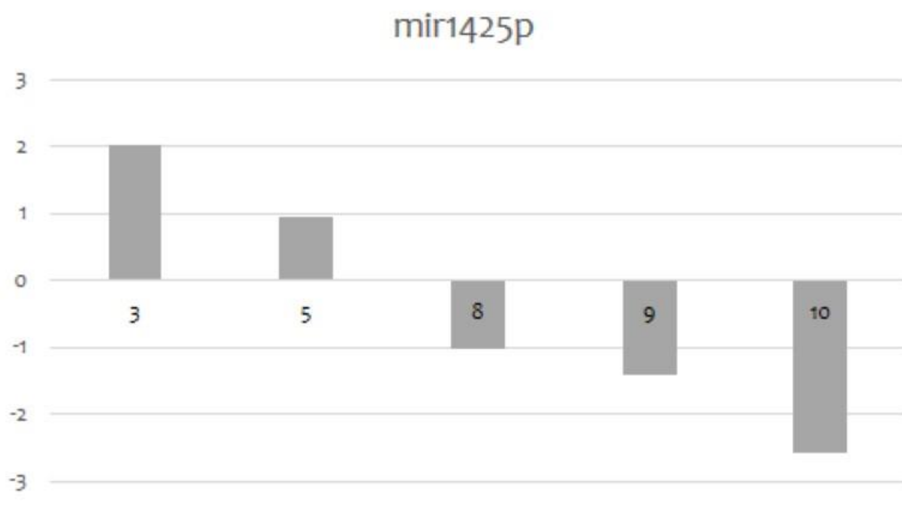
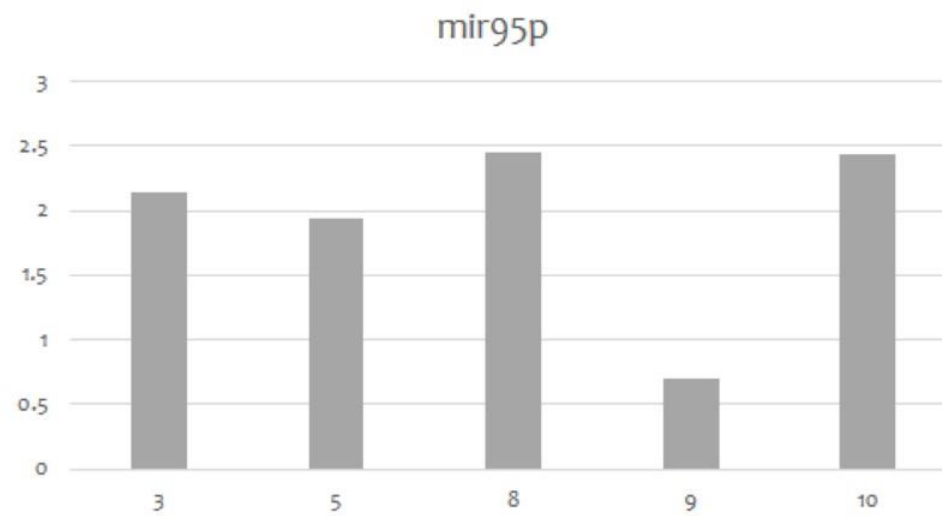
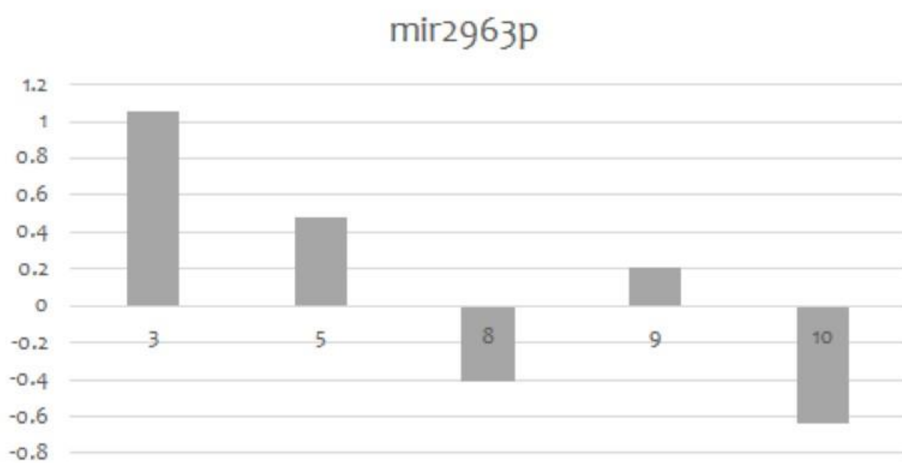
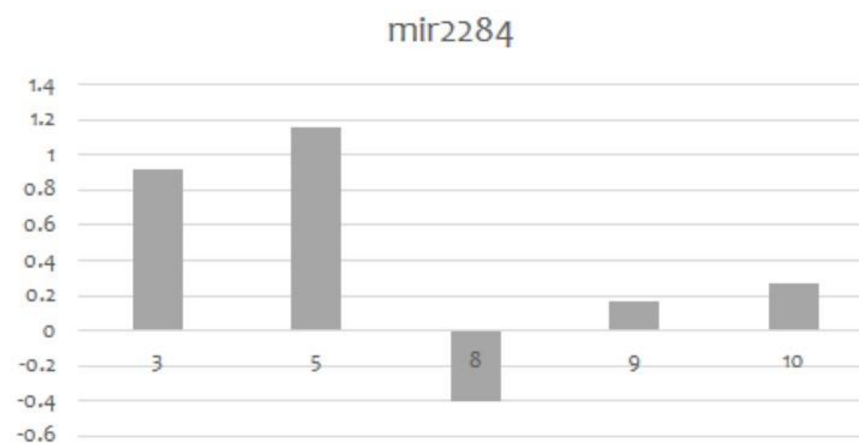
What about micro RNAs









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In vitro Embryo development by sire conception rate

		Expanded blastocysts			Hatched blastocysts[2,3]		
Bull SCR class	Sire	n	Trophectoderm	ICM[1]	n	Trophectoderm	ICM
High	3	52	150 ± 5^{a}	60 ± 6^{a}	34	232 ± 14^{a}	79 ± 6^{a}
	5	23	164 ± 10^{a}	42 ± 8^{c}	19	252 ± 17^{a}	59 ± 7^{c}
Low	8	14	$122 \pm 9^{b,c}$	$59 \pm 6^{a,b}$	32	184 ± 16^{b}	$74 \pm 7^{a,b,c}$
	9	20	$144 \pm 8^{a,b}$	$45 \pm 8^{b,c}$	20	225 ± 16^{a}	$64 \pm 7^{b,c}$
	10	25	$131 \pm 7^{b,c}$	$50 \pm 8^{a,b,c}$	30	243 ± 15^{a}	63 ± 6^{c}
Overall	High	75	144 ± 7^{a}	62 ± 5^{a}	53	227 ± 13^{a}	78 ± 3^{a}
	Low	59	121 ± 7^{b}	57 ± 5^{a}	82	216 ± 11^{a}	73 ± 3^{a}

[1]ICM: inner cell mass.
[2]Values are presented as least-squares means ± SEM.
[3]Values with different superscripts differ ($P < 0.05$).

Ortega et al., 2018 Biology of Reproduction

TEXAS A&M AGRILIFE RESEARCH

Influence of microRNAs from Semen on Bovine Fertility

SUMMARY:

- There were 516 miRNA identified in bulls with high and low fertility designation
- 10 miRNA were differentially expressed
- Supports the idea that a small proportion of sperm miRNAs may have a direct impact on fertility/embryo development

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Take home message

- **BSE will always be the first step for bull fertility**
  - Identification of sterile and infertile bulls
  - Can be done in the field with minimum equipment
- Protein and miRNA tests will be a further selection
- Bulls with problems in sperm transport or embryo development may be identified

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Thank You!!!

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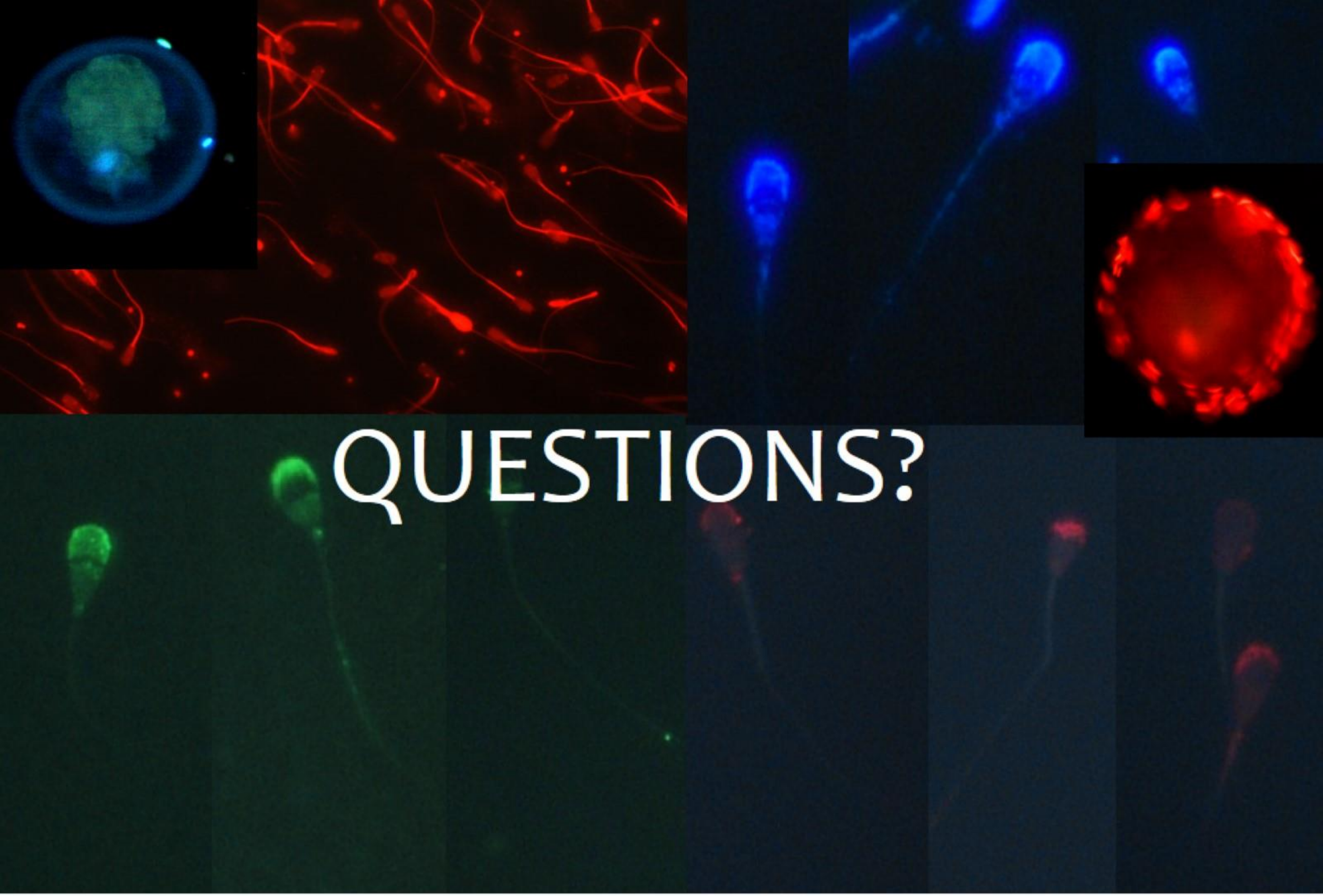

QUESTIONS?

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