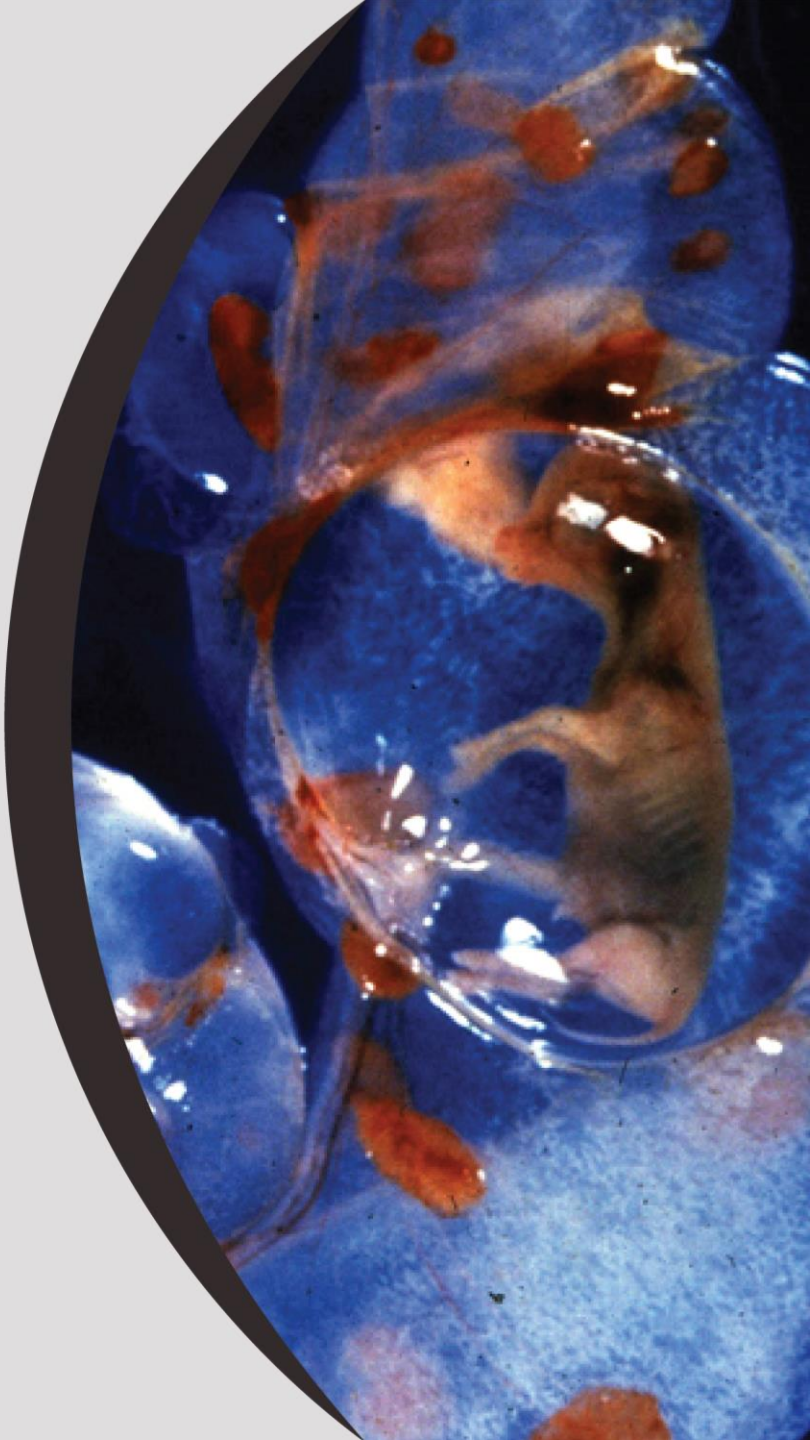
PREGNANCY & DEVELOPMENTAL PROGRAMMING

Utilizing Sexed Semen in AI and ET Programs



G. Cliff Lamb

Nicky Oosthuizen

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Sexed Semen

- **Advantages:**
  - Select calf gender with high accuracy (> 90%)
    - Replacement Heifers
    - Feedlot Steers
  - Can be used for embryo production
  - Defective sperm removed in sorting process
- **Disadvantages:**
  - Expensive ($25 vs $45 per straw)
  - Lower fertility (10 – 20% lower)
  - No official TAI protocols developed yet

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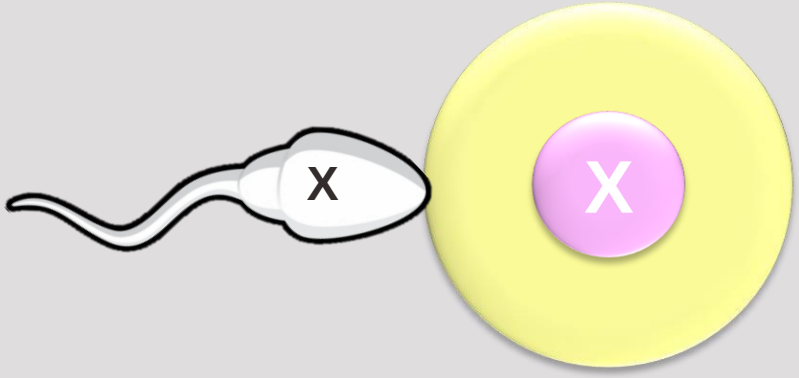
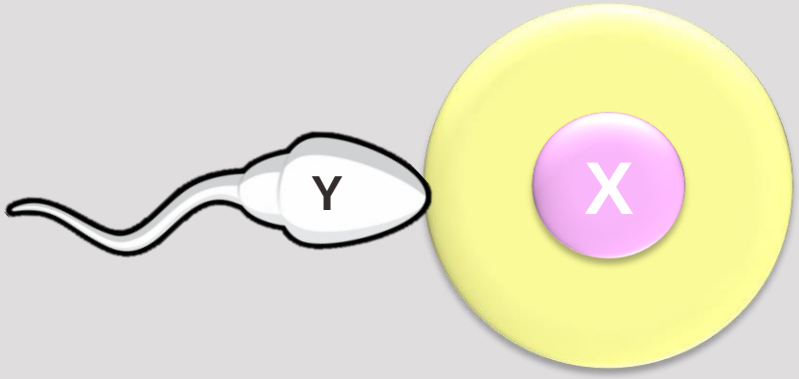
OPPORTUNITIES WITH SEXED SEMEN



= XX
= female



= XY
= male

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Sexed Semen

- Semen sorted via Flow Cytometry
- X-sperm contain approximately 4% more DNA
- Possible to measure DNA content (90% accuracy)
- DNA content determined using Hoechst fluorescing dye
- X-sperm end up with 4% more bound dye and give off more fluorescence
- Computer recognizes fluorescence and sperm separated based on electrical charge given to droplet
- Lower dose of sperm cells per straw
  - 2 or 4 million vs. 15 to 20 million cells

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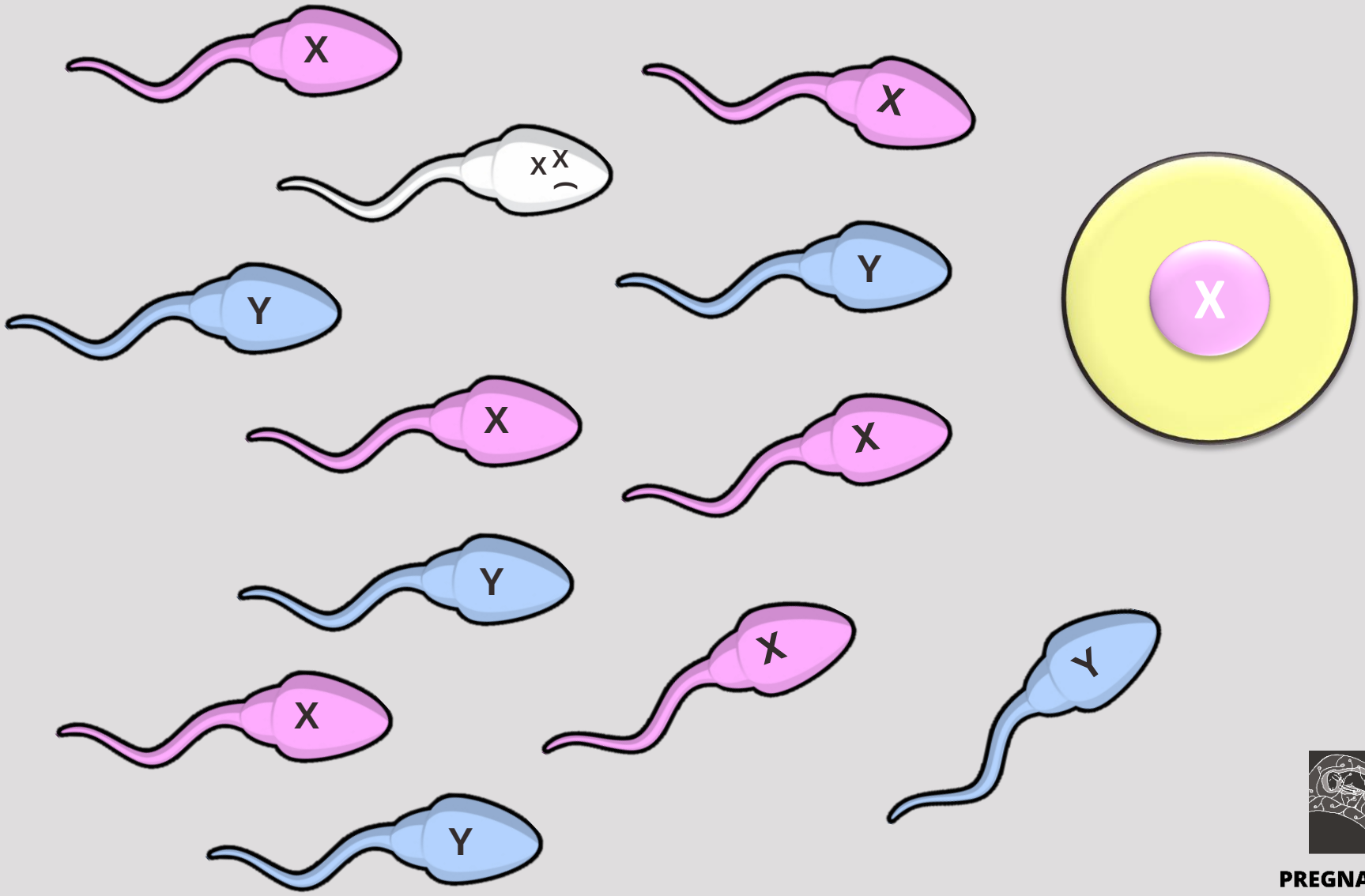
Conventional Semen



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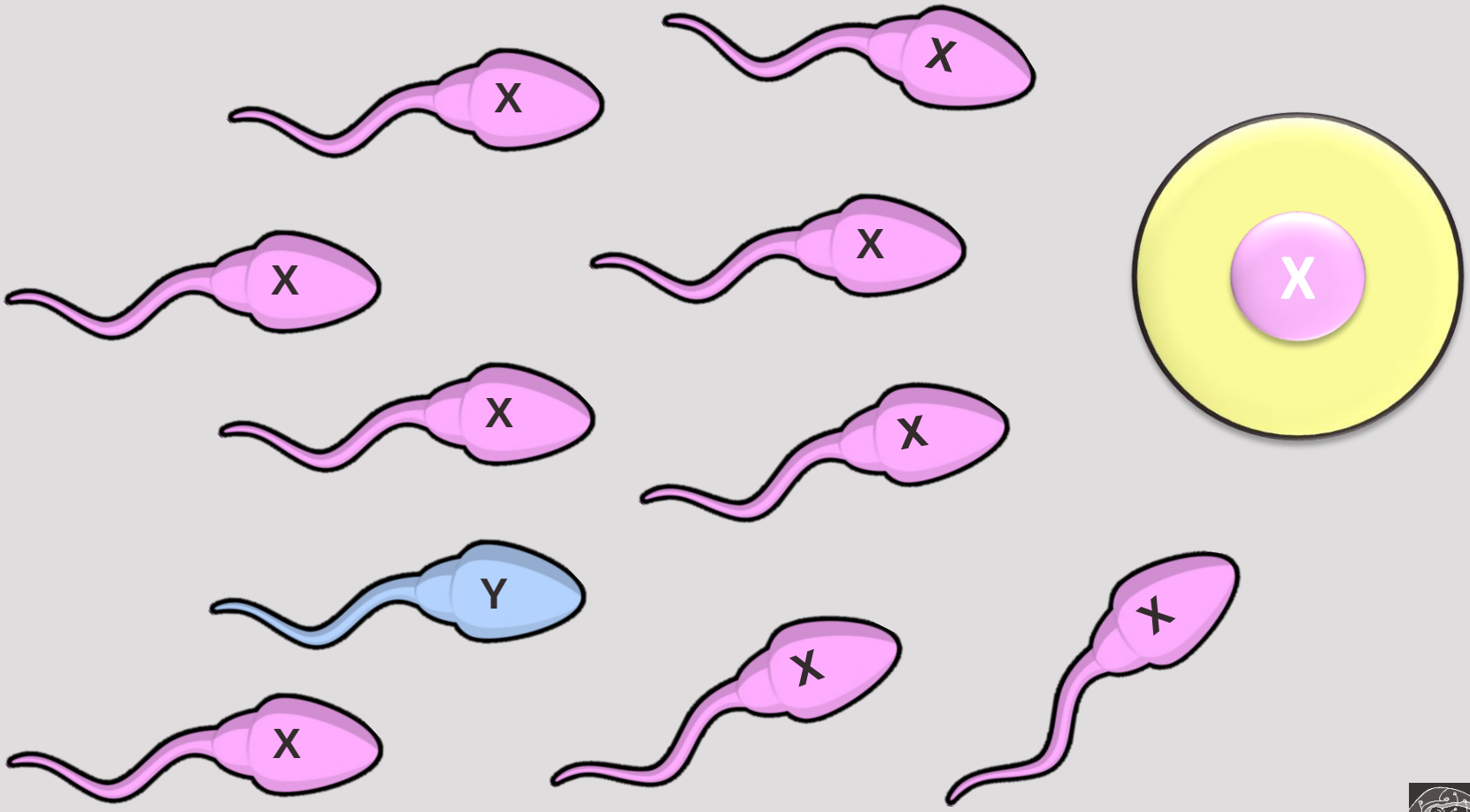
Sexed Semen



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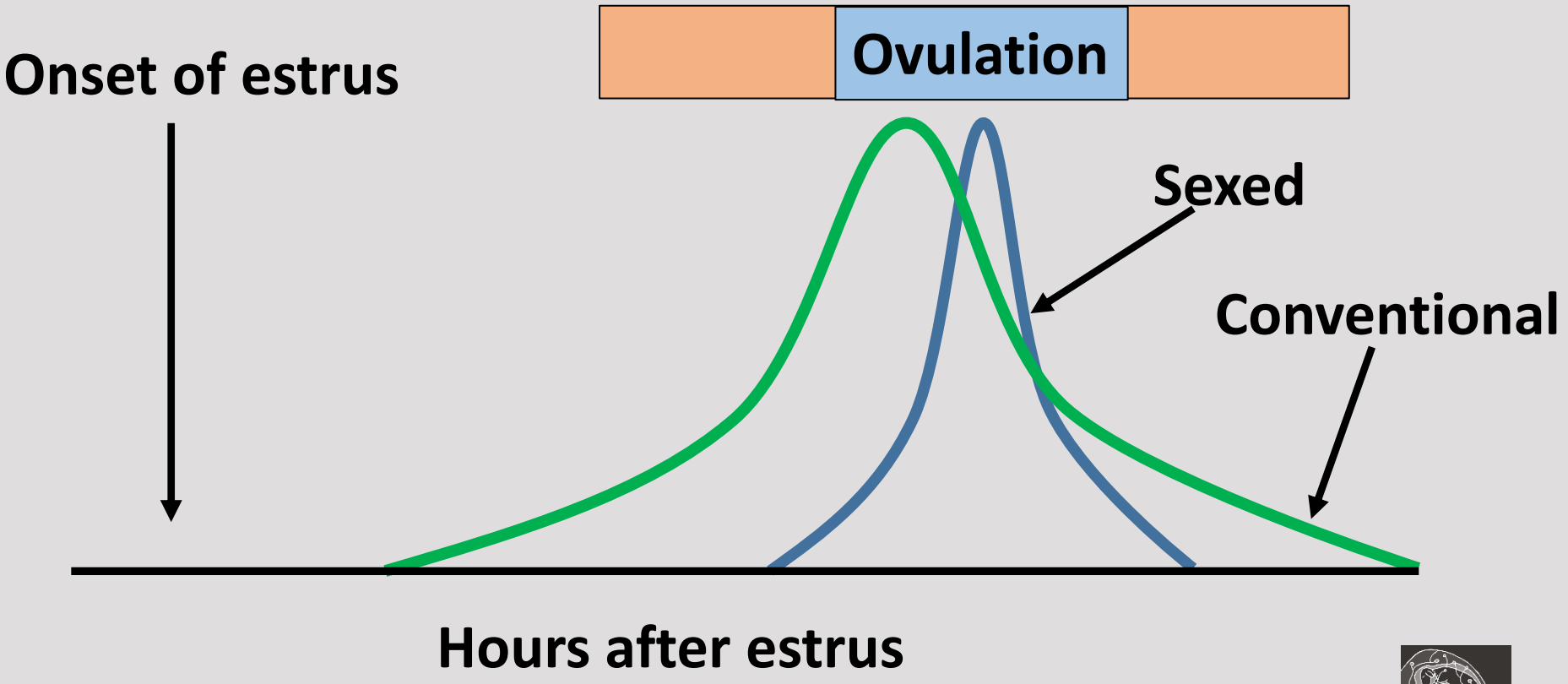
Conventional vs Sexed Semen



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Sexed Semen

- Slow adoption in beef industry
- Pregnancy rates reduced compared to conventional semen
  - PR/AI between 32 - 90% of conventional reported
  - Reduced post-thaw motility, reduced no. of sperm cells with intact membranes, potential premature capacitation, acrosomal alterations
- Large scale adoption of sexed semen
  - TAI protocols that result in acceptable pregnancy rates
  - Reduced difference in PR/AI vs. conventional

Carvalho et al., 2010; Sales et al., 2011; Thomas et al., 2014

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Delayed Insemination

- Delayed AI suggested to improve pregnancy rates
  - Rather than 12-18 h after estrus, 18-24 h after estrus detected
- Lactating dairy cows:
  - AI with sex-sorted semen closer to expected ovulation yielded greater PR/AI
  - Cows AI between 23 and 41 h after onset of estrus had the greatest PR/AI
- Dairy heifers:
  - PR/AI 15.2% greater after insemination with sex-sorted semen when TAI delayed from 54 to 60 h
  - PR/AI were still significantly lower than those of conventional semen (31.4 vs. 51.8%)

Seidel et al., 1999; Sales et al., 2011; Bombardelli et al., 2016

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Experimental Design

- 2,855 *Bos taurus* heifers
- 23 locations
- 11 states
- 24 different sires
  - Sexed semen and conventional semen from same bull(s) at each location
- 8 Treatment Groups
  - Presynchronized with PGF or not
  - TAI at 54 or 72 hours
  - Conventional vs. Sexed Semen

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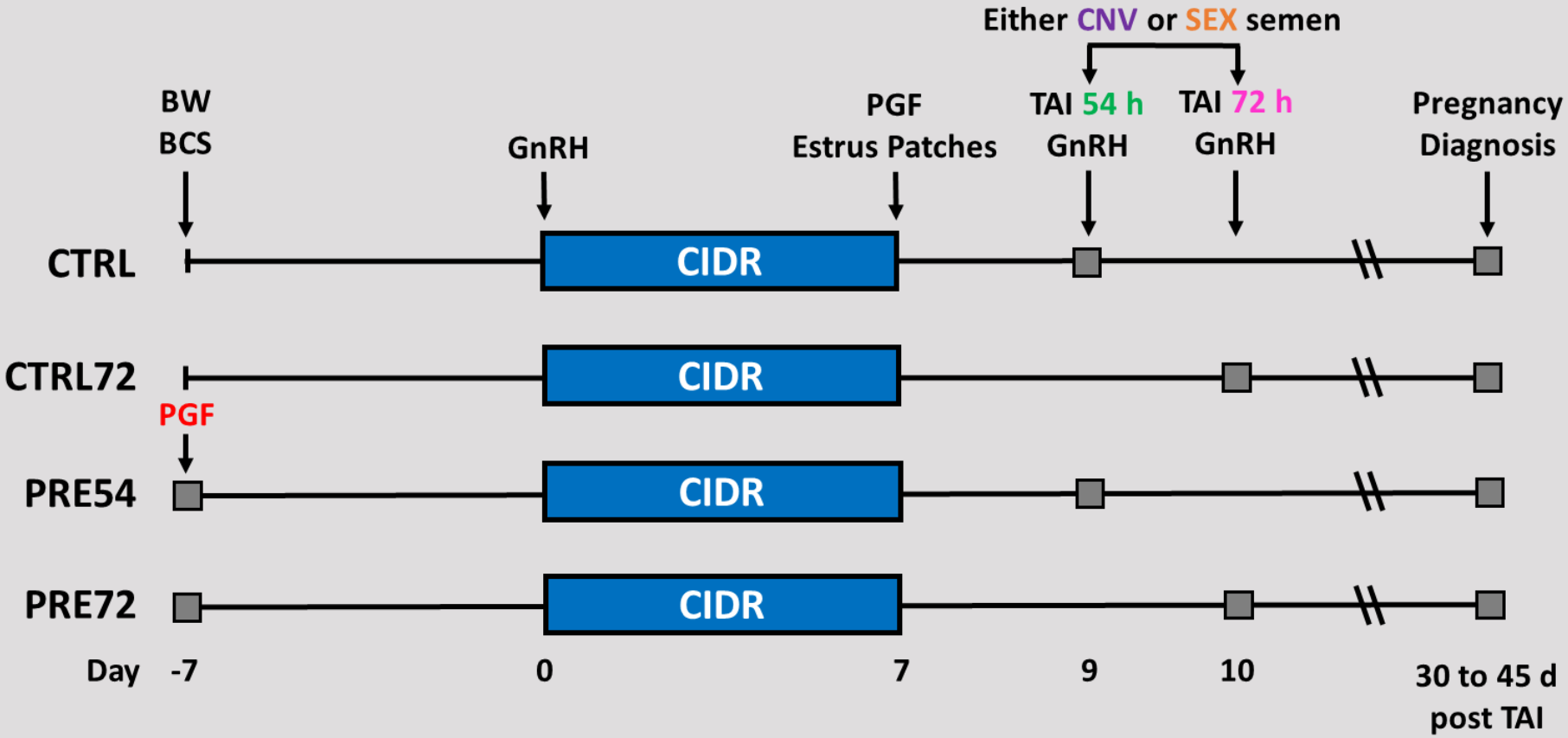
Experimental Design



- Estrus expression was evaluated at respective TAI based on estrus detection patches

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Treatment Groups

- Conventional 7-d CO-Synch + CIDR
  - CTRL54-CNV and CTRL54-SEX
- TAI delayed to 72 hours
  - CTRL72-CNV and CTRL72-SEX
- Presynchronized with PGF + TAI at 54 hrs
  - PRE54-CNV and PRE54-SEX
- Presynchronized with PGF + TAI at 72 hrs
  - PRE72-CNV and PRE72-SEX

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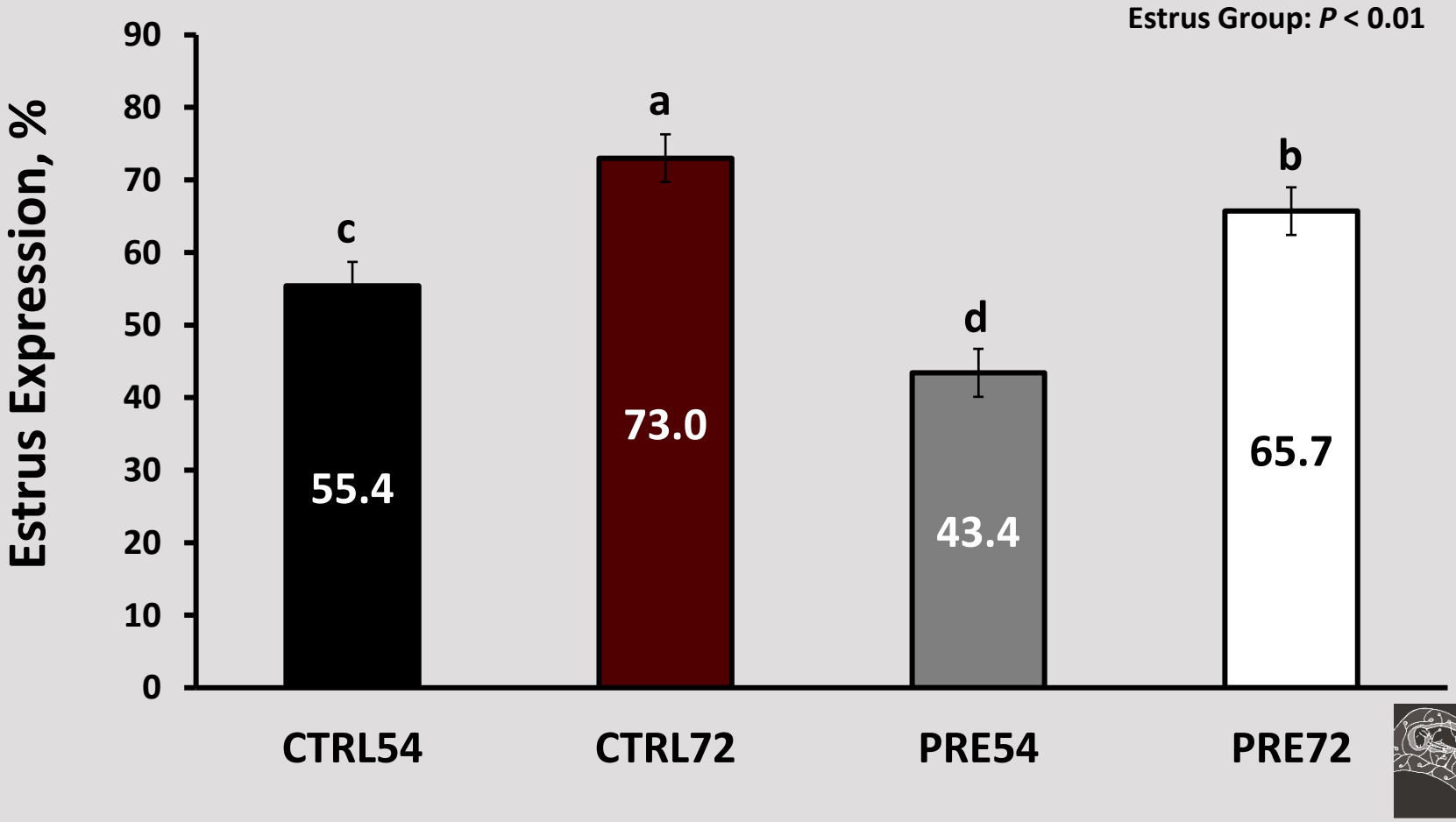
Overall Estrus Expression



[a,b,c] Bars with different superscripts differ ($P < 0.05$)

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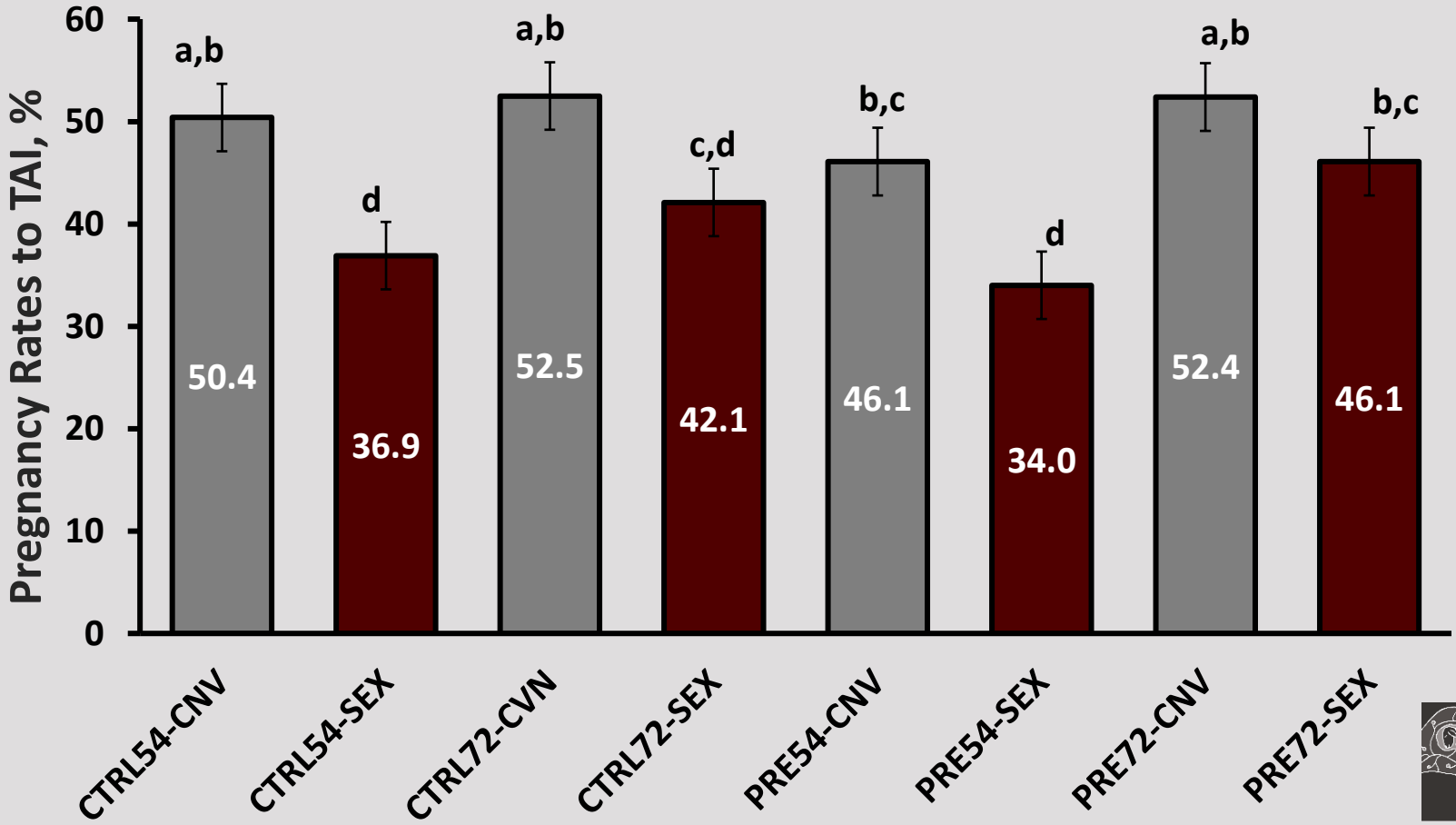
Pregnancy Rates to TAI

Treatment: $P < 0.01$
Estrus: $P < 0.01$
Treatment × Estrus: $P = 0.13$



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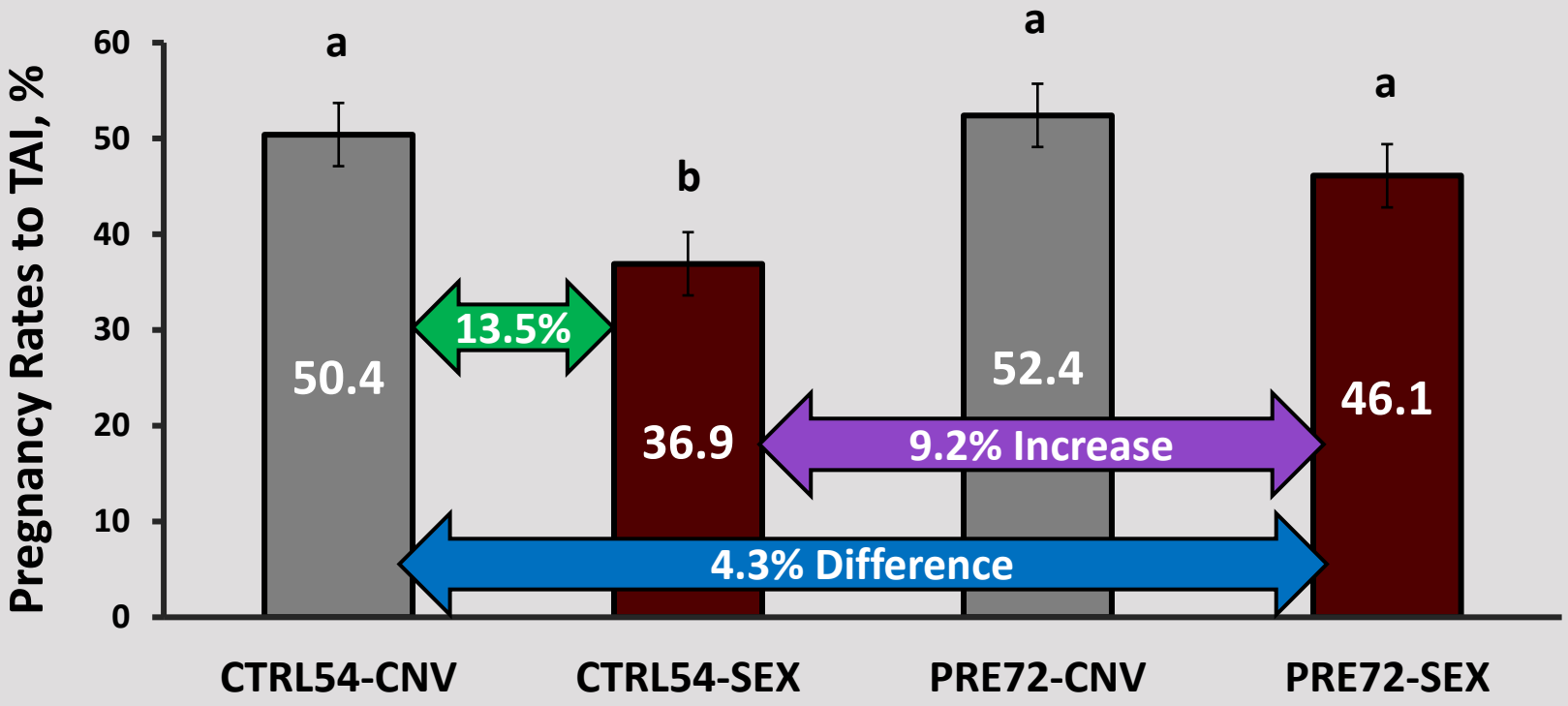
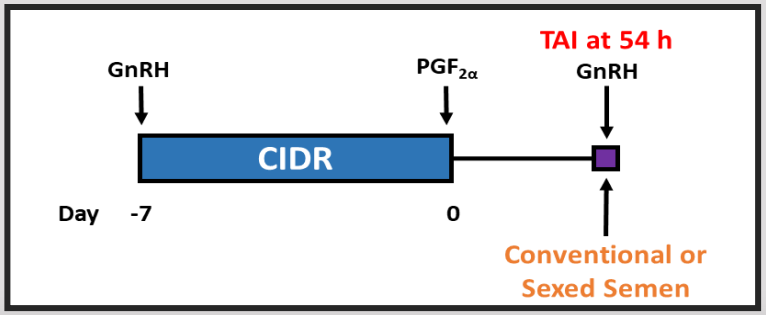
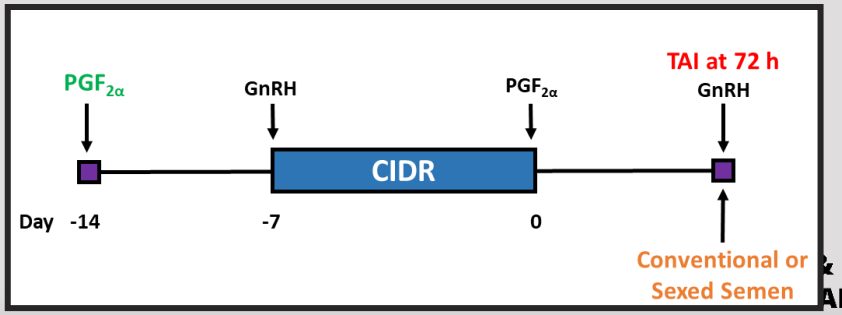
Pregnancy Rates to TAI



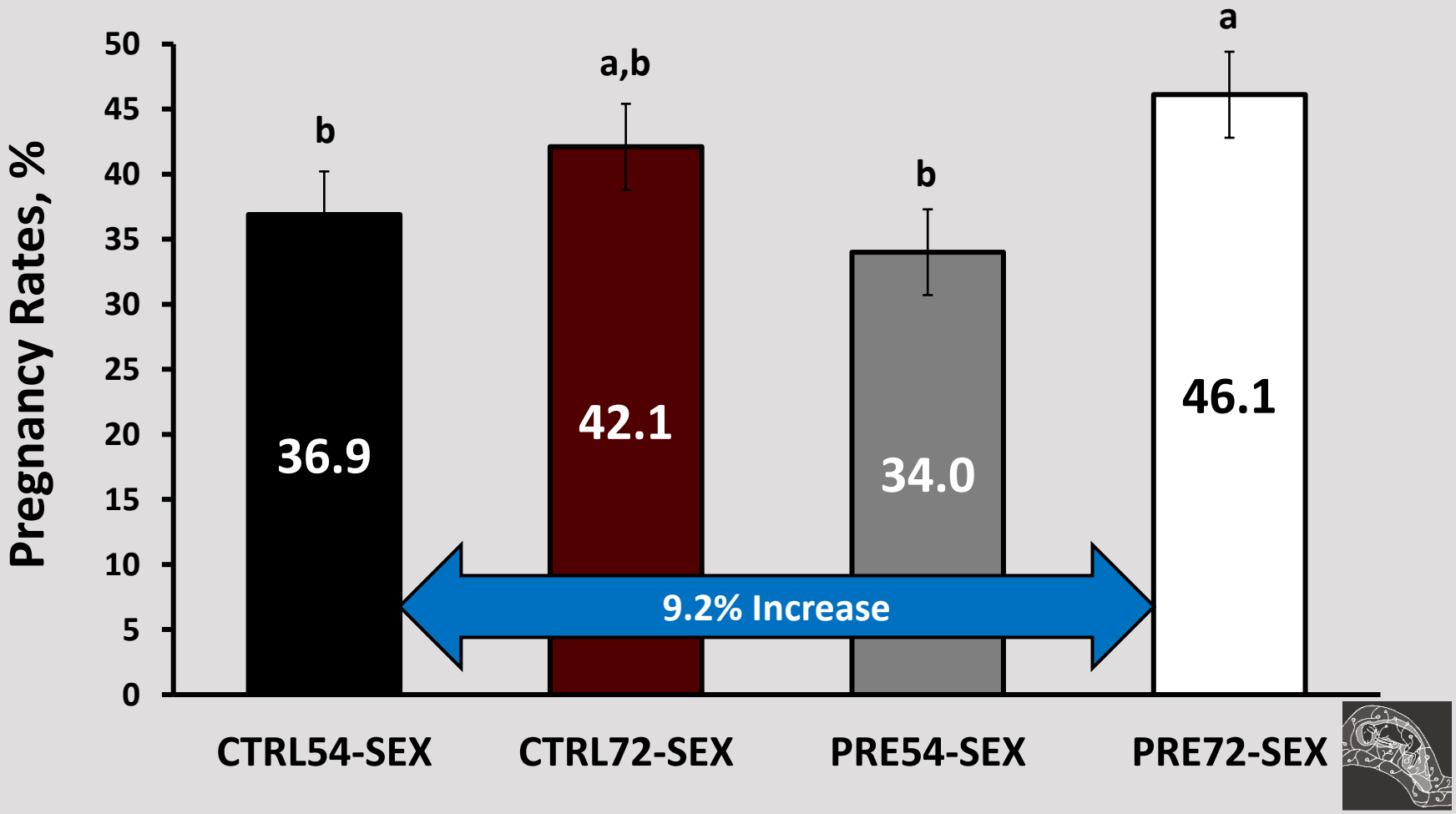




[a,b] Bars with different superscripts differ ($P < 0.05$)

PROGRAMMING

Treatments with Sexed Semen



[a,b,c] Bars with different superscripts differ ($P < 0.05$)

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Economic Analysis

- Partial budget analysis of results to create decision aid tool for beef cattle producers
- Economic feasibility of incorporating sex-sorted semen or combination into a heifer production system when compared to conventional semen
- Economic outcomes measured
  - Increased returns and decreased costs
  - Decreased returns and increased costs
- Gain/loss per heifer exposed to TAI
  - Conventional vs. sex-sorted semen
  - Conventional vs. combination
  - Sex-sorted semen vs. combination

PREGNANCY & DEVELOPMENTAL PROGRAMMING

Economic Analysis

Required inputs

- No. Heifers
- No. Clean-up bulls
- Desired sex
- Premium per head

Fixed Values

- No. of animal handlings for the different estrus synchronization protocols
- Required hormonal doses
- Expected sex-ratio per semen type

Changeable Values

- Expected PR/AI for conventional semen
- Mean calf weight gain per day
- Expected final pregnancy rates
- Clean-up bull values: purchase price, maintenance costs, useful life, salvage value, salvage weight
- Cost of labor and no. of employees required
- Cost of AI tech
- Cost of the estrus synchronization drugs
- Cost of the different types of semen
- Amount borrowed to finance the costs and interest rate
- Expected WW of male and female calves
- Expected price of male and female calves

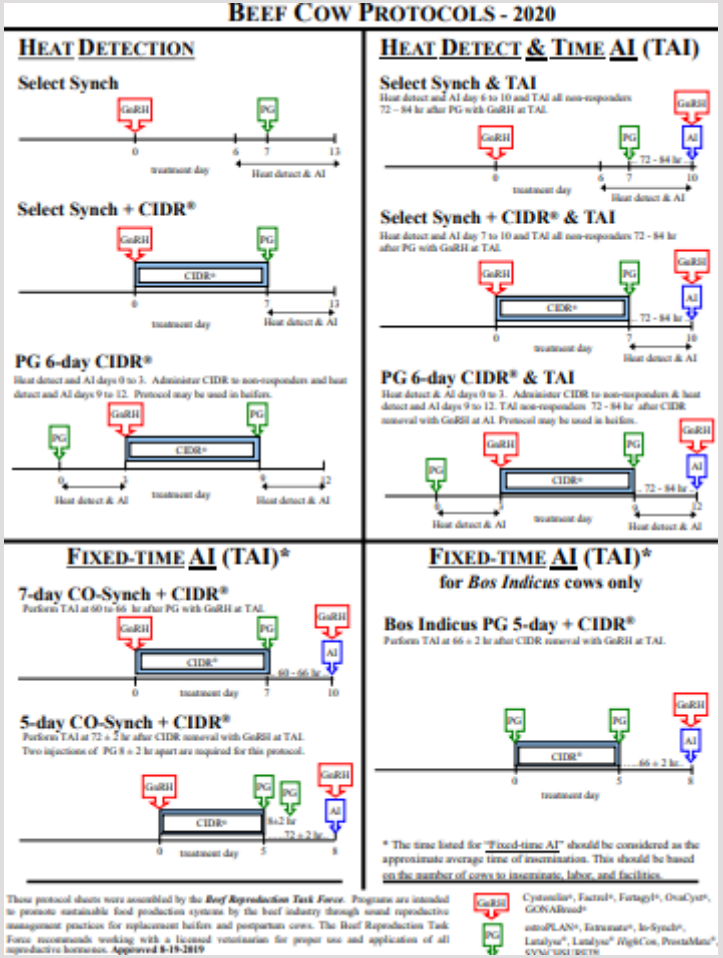
PREGNANCY & DEVELOPMENTAL PROGRAMMING

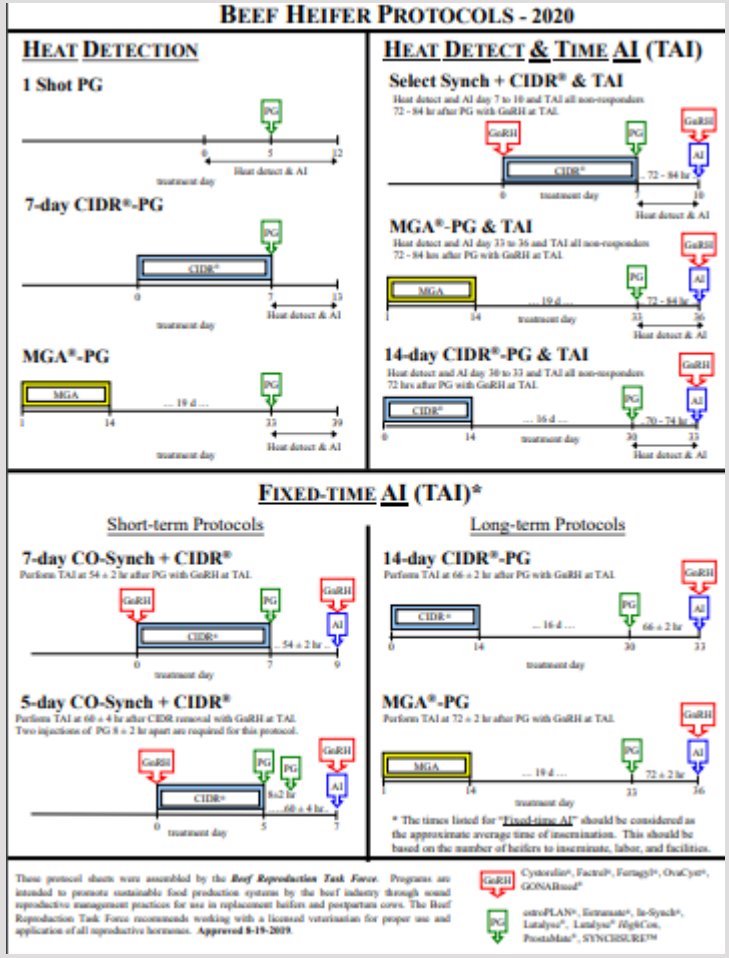
Economic Analysis

- Primary factors influencing gain or loss per heifer exposed to TAI
  - Expected premium for the desired sex
  - Cost of sex-sorted semen
  - Size of the herd
  - Weaning weights
  - Expected PR/AI of estrus synch protocol
- For X-sorted sperm to be more profitable, perceived premium of greater than $154 per head required

PREGNANCY & DEVELOPMENTAL PROGRAMMING

Sexed Semen Protocols





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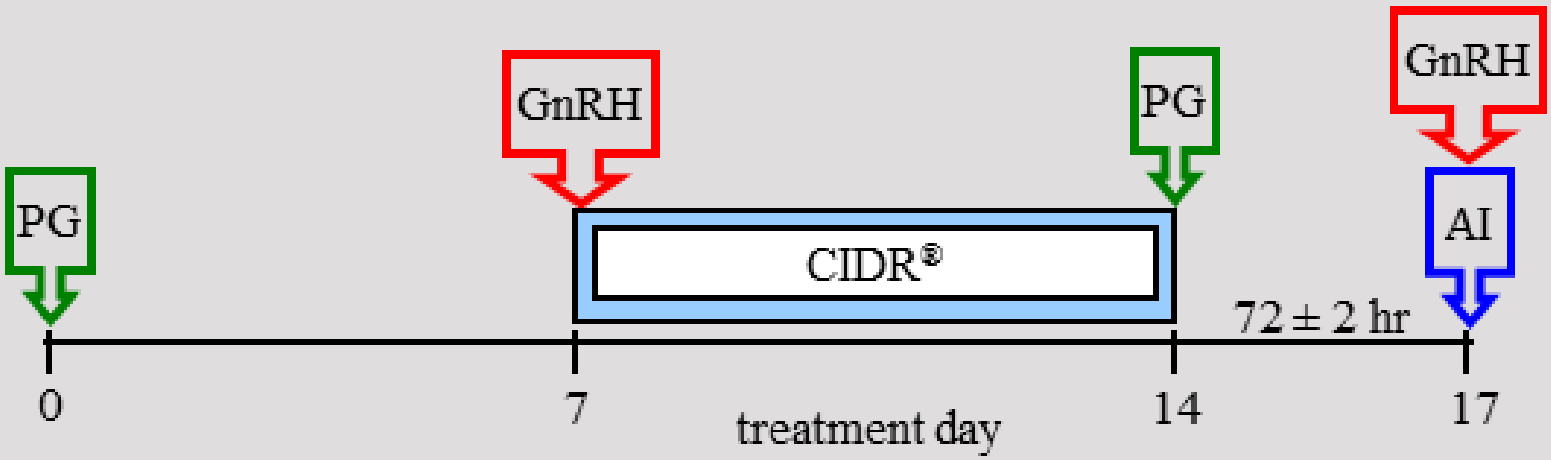
Sexed Semen Protocols

Heifers only



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Sexed Semen and Conventional Semen

Treatment: $P < 0.01$
Estrus: $P < 0.01$
Treatment × Estrus: $P = 0.13$

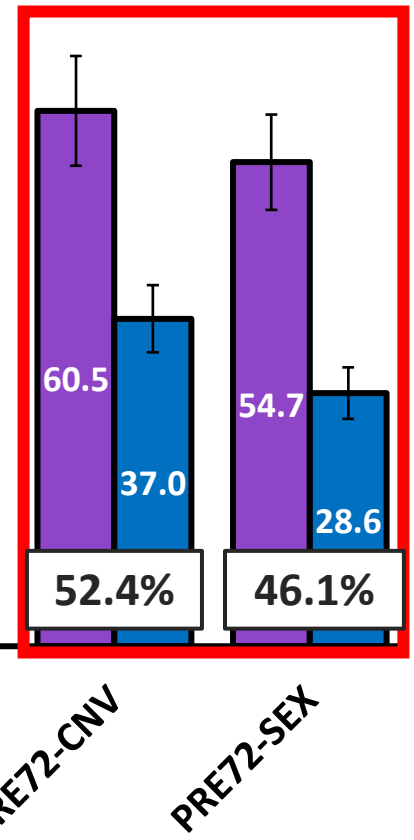
Could we potentially make use of estrus detection patches?

- **65.7%** of **PRE72** showed estrus
- **34.3%** of **PRE72** did not show estrus

- **54.7%** of **PRE72-SEX** expressing estrus became pregnant
- **37.0%** of **PRE72-CNV** that did not express estrus became pregnant

65.7% x **54.7%** = **35.9%** pregnancy from **PRE72-SEX** heifers
34.3% x **37.0%** = **12.7%** pregnancy from **PRE72-CNV** heifers

35.9% + **12.7%** = **48.6%**



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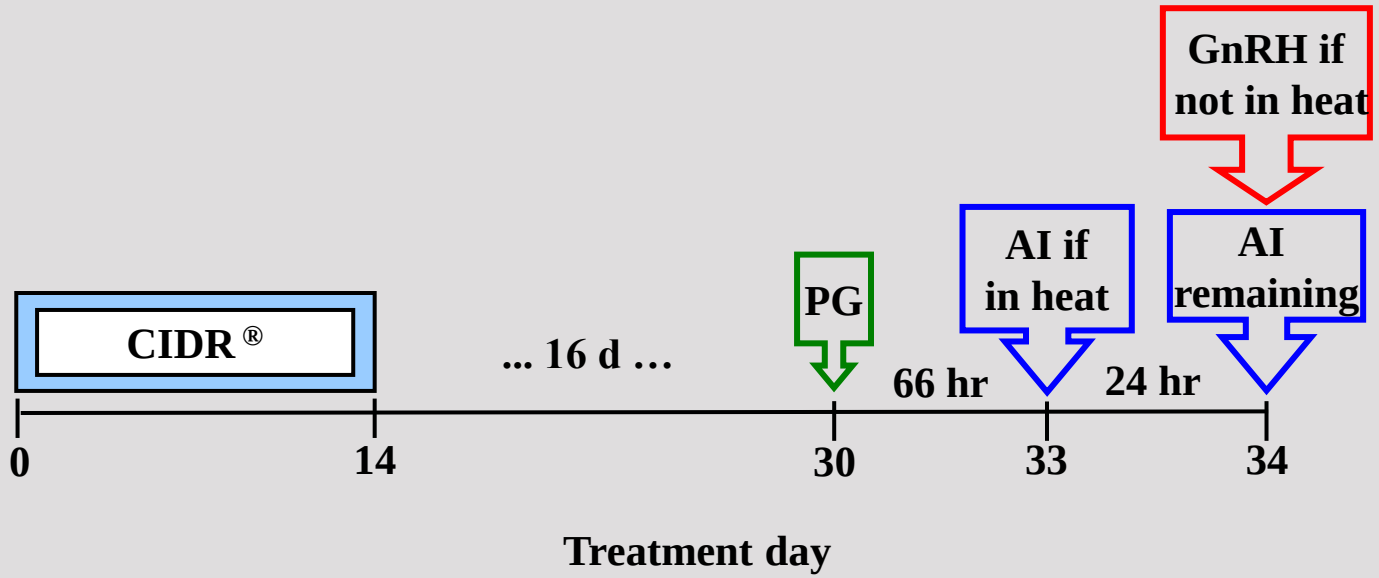
Sexed Semen Protocols

Heifers



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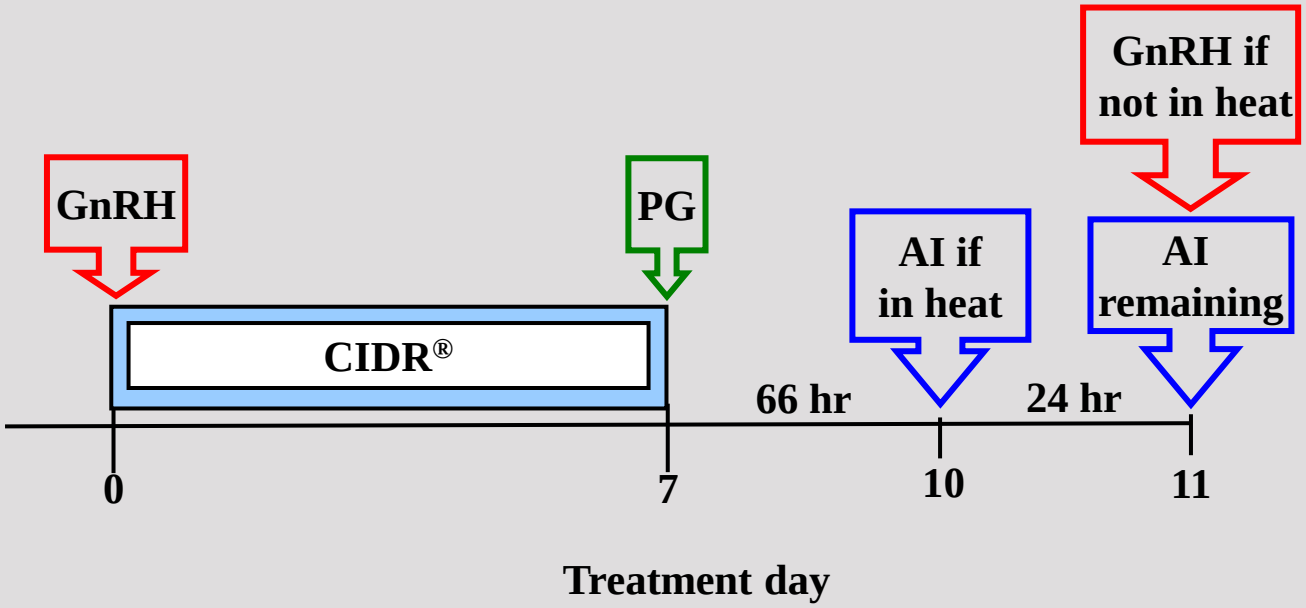
Sexed Semen Protocols

Cows and Heifers



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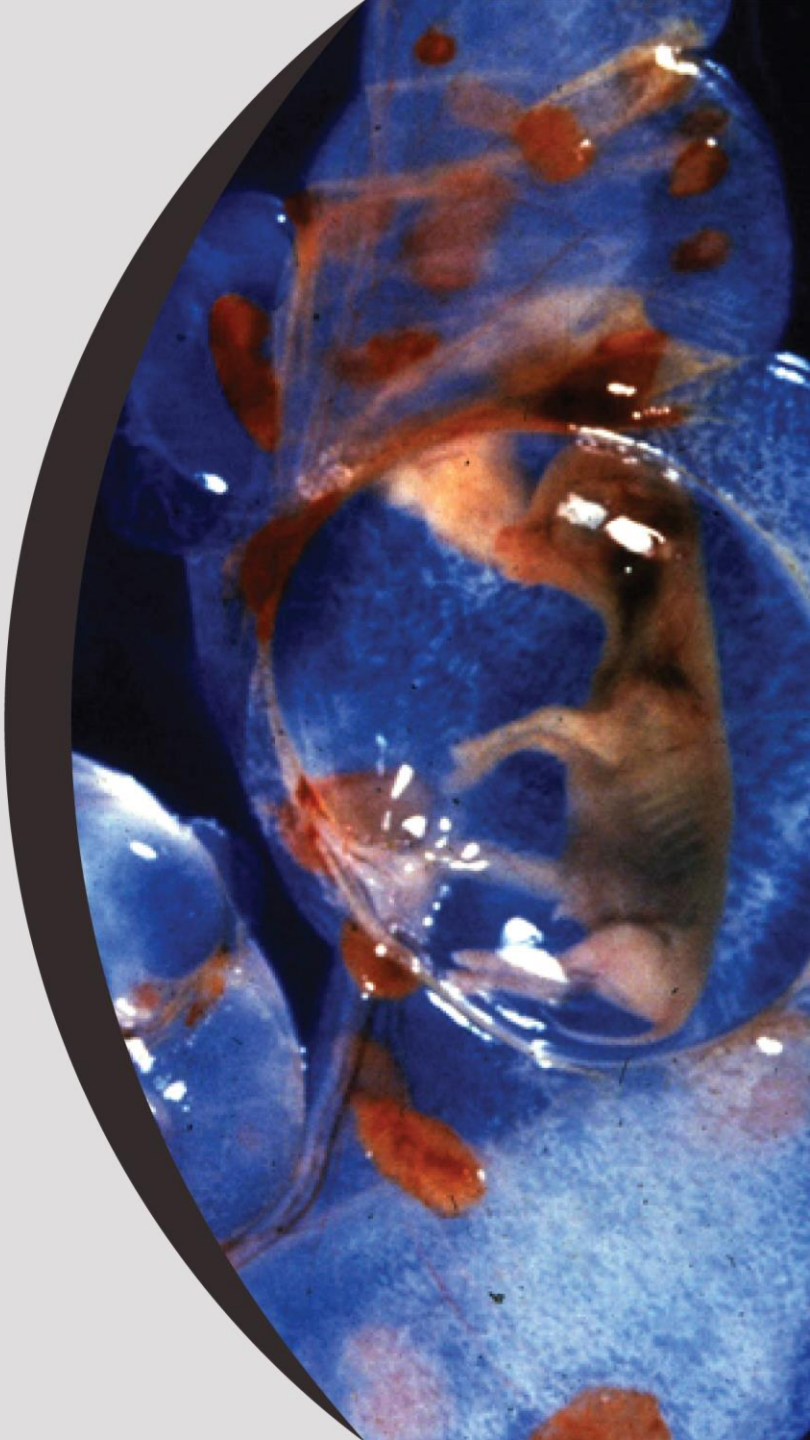
Sexed Semen for Embryo Transfer Programs



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Use of Sexed Semen in MOET

- To produce embryos of a desired sex after superovulation
- Differences exist between use of sexed and conventional semen in embryo production
- Superovulated *Bos taurus* dairy heifers and cows
  - Reduced number of viable embryos collected in the females that received sexed semen
  - Greater number of unfertilized ova and degenerate embryos produced in the sexed treatment
  - Embryo quality grades significantly lower for cows and heifers receiving sexed semen

(Mikkola and Taponen, 2017)

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Use of Sexed Semen in MOET

- 32 Angus cows were superstimulated and embryos were collected in a switch-back design
- Two treatments
  - Four inseminations of conventional semen (15-18 mil. sperm)
  - Four inseminations of sexed semen (2.1 mil. sperm)
- Cows were inseminated at 0 (1x), 12 (2x), and 24 (1x) hr after estrus
- Embryos were collected on day 7 after estrus and were evaluated for embryo stage and quality grade

(Larson et al., 2010)

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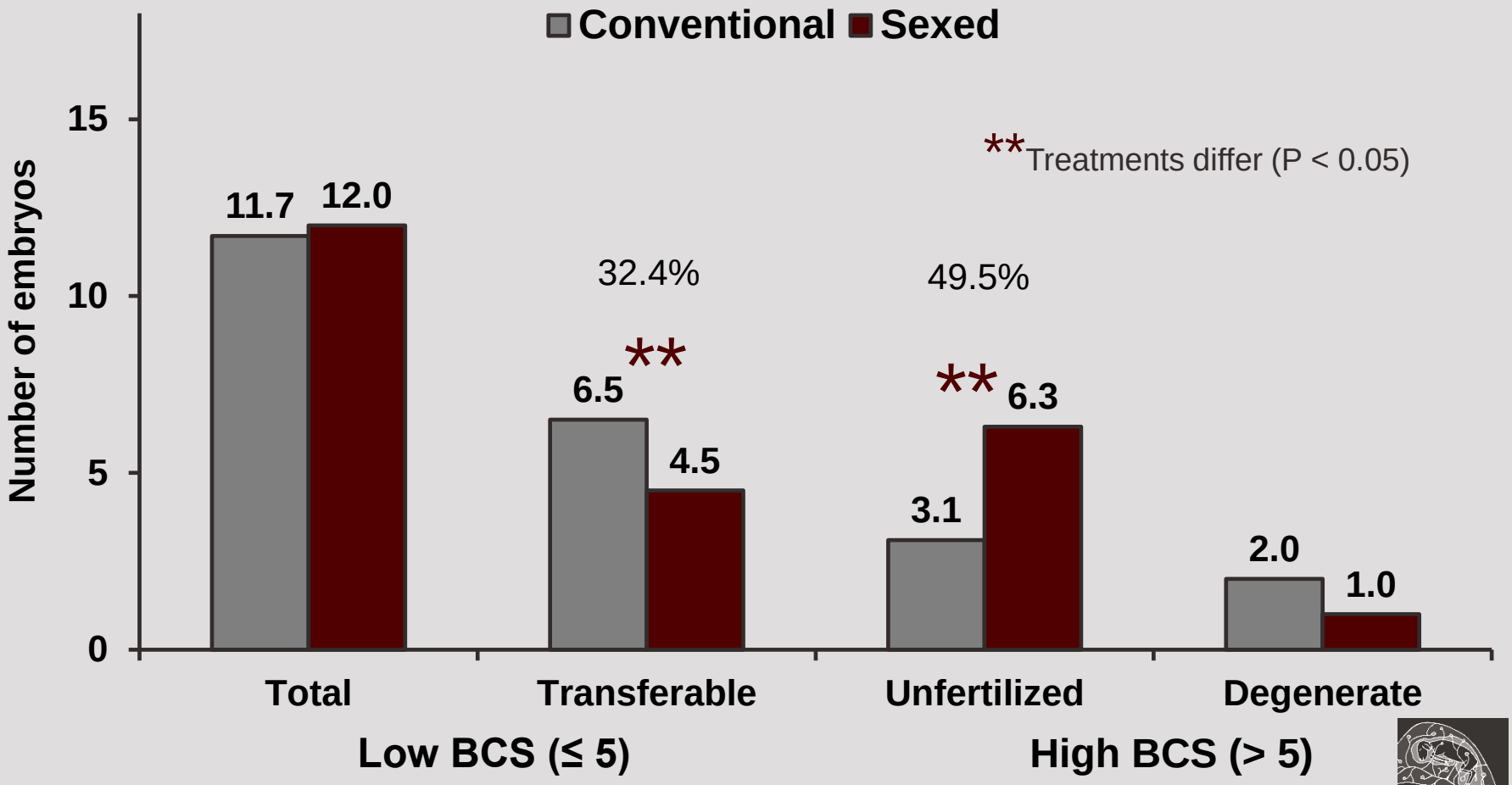
Embryo Production of Superovulated Cows



Larson et al., 2010

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Delayed AI with Sexed Semen for Superovulation

Superstimulated Nelore (*Bos indicus*) donors

	Treatment			*P*-Value
	NS 12/24 n = 17	SX 12/24 n = 18	SX 18/30 n = 19	
Transferable embryos	6.8 ± 2.6[a]	2.4 ± 1.8[c]	4.5 ± 3.0[b]	< 0.01
Freezable embryos	6.0 ± 2.4[a]	2.0 ± 1.4[c]	3.7 ± 2.8[b]	< 0.01
Unfertilized oocytes	0.5 ± 0.7[a]	3.7 ± 3.6[b]	2.9 ± 2.6[b]	< 0.01

Soares et al., 2011

PREGNANCY & DEVELOPMENTAL PROGRAMMING

Delayed AI with Sexed Semen for Superovulation

Superstimulated Lactating Holstein (*Bos taurus*) donors

	Treatment			*P*-Value
	NS 12/24 n = 11	SX 12/24 n = 11	SX 18/30 n = 11	
Transferable embryos	8.7 ± 2.8^{a}	4.6 ± 3.0^{b}	6.4 ± 3.1^{ab}	< 0.01
Freezable embryos	6.9 ± 1.8^{a}	3.2 ± 1.8^{b}	5.4 ± 3.4^{ab}	< 0.01
Unfertilized oocytes	0.9 ± 1.4^{a}	5.2 ± 3.1^{b}	4.6 ± 2.6^{b}	< 0.01

Soares et al., 2011

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Use of Sexed Semen in IVF

- Fewer units of semen are required in IVF embryo production
  - Sexed semen can be efficiently incorporated
- *Bos indicus* beef semen split into non-sexed, X-sperm, and Y-sperm fractions for IVF
  - Motility of sexed sperm lower, and fewer intact membranes and acrosomes in both X- and Y-sperm compared with non-sexed
  - No differences among groups for IVF fertilization, cleavage, or blastocyst rate 8 days after insemination
  - Embryo development not significantly affected by sorting

(Carvalho et al., 2010)

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Use of Sexed Semen in IVF

- Dairy semen split into sexed and non-sexed
  - Greater IVF rate determined for unsorted semen than sexed semen from most bulls in study
  - Greater percentage of embryos reached two-cell and blastocyst stages when unsorted semen utilized compared with sexed semen from most bulls.
- Ability of bull to undergo sexing plays large role in subsequent fertility outcomes
- Selection of bulls with high sexing ability and fertilization capacity should be emphasized for successful embryo development in both IVF and MOET

(Liu et al., 2015)

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ACKNOWLEDGMENTS

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Collaborators
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Technical staff

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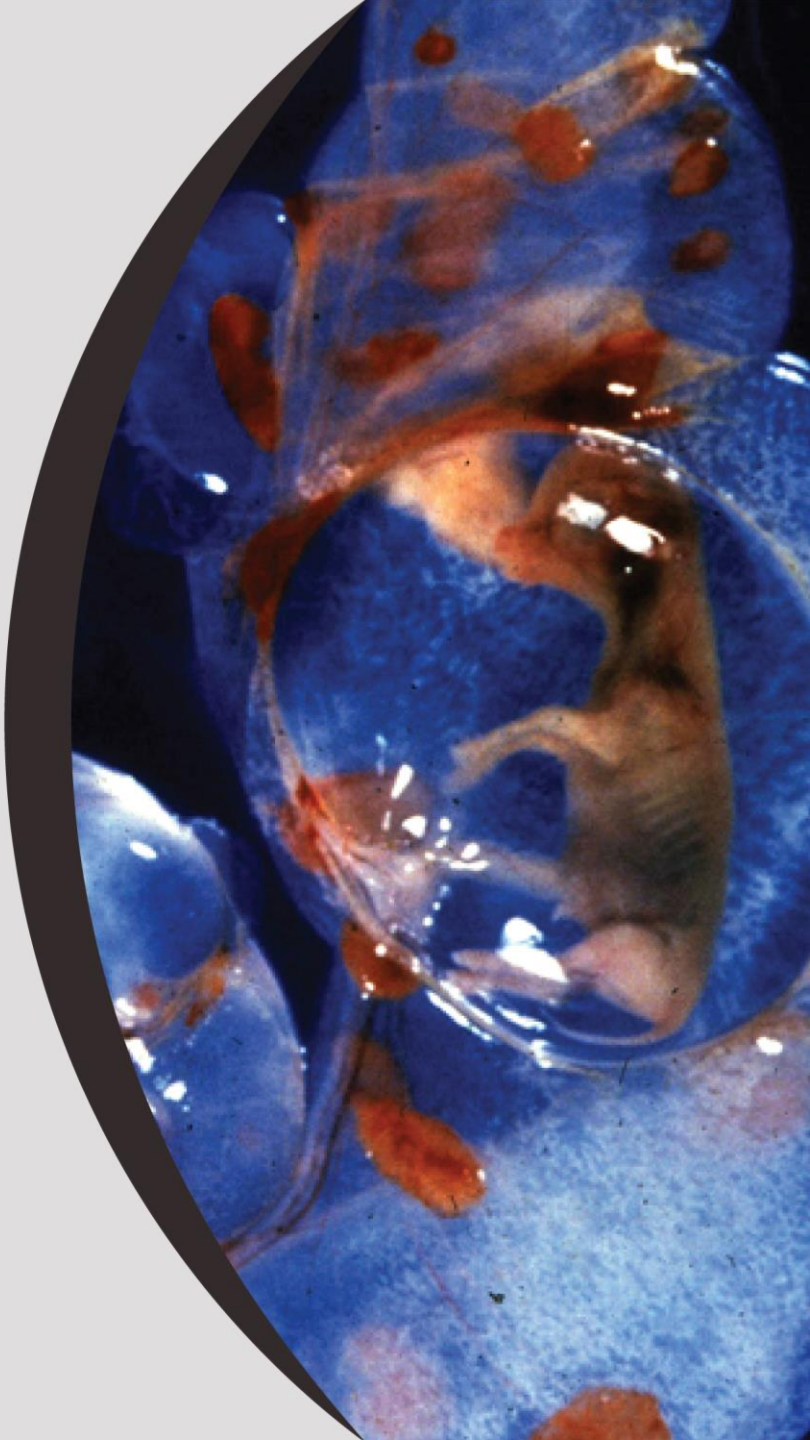

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