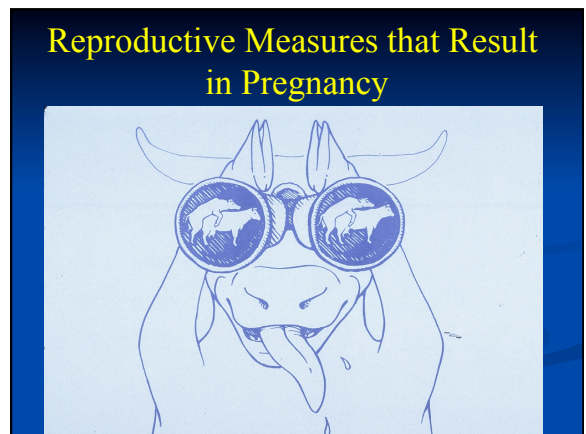
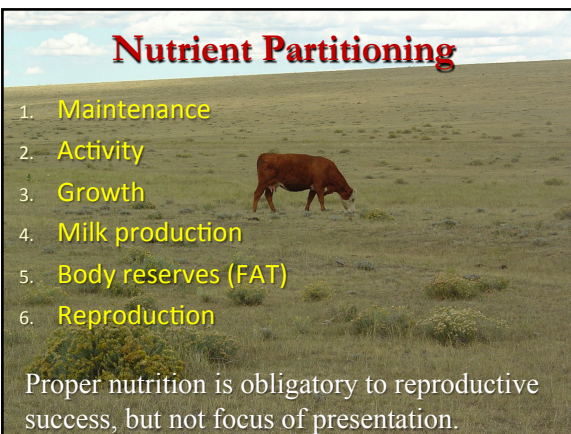
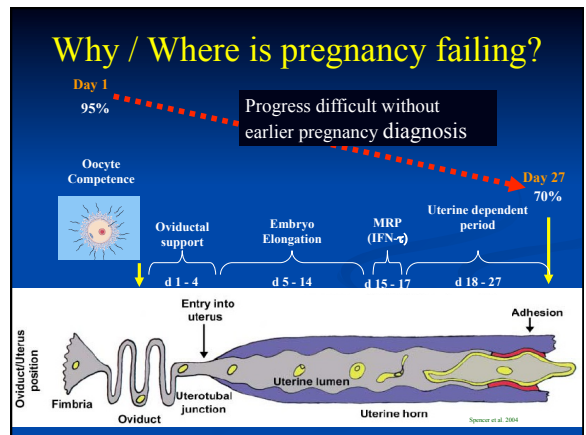


**Annual Cost of Early Embryonic Loss to U.S. Beef Industry**

40,000,000	Beef cows & heifers	
x 25%	20 d later pregnancy	
10,000,000	calve 20 d later	
x 3 lb/d	calf gain lost	
600,000,000	lbs lost gain	<b>Dairy Industry Loss: \$ 600,000,000</b>
6.25%	40 d later pregnancy	
150,000,000	lbs lost gain	<b>787,500,000 lbs</b>
1.5%	60 d later pregnancy	<b>x \$1.10/lb</b>
		<b>\$ 866,250,000</b>

**LOSS IS TO COW/CALF PRODUCER!**



## Single most important measure of fertility for cow/calf producer?

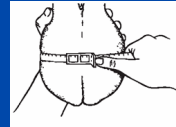


Each bull is expected to contribute to the production  
Limitations: Fertility measure on that given day  
Only about 1/3 of the "Fertility Picture"

## Importance of Bull Fertility on Breeding Season Pregnancy Rate

Year 1			Year 2		
	BSE + % Normal Sperm			BSE + % Normal Sperm	
	Control	> 80%		Control	> 80% > 70%
No. Cows	572	656	No. Cows	1,179	522 769
Pregnancy Rate	86%	93%	Pregnancy Rate	85%	90% 91%

Wiltbank et al., 1983



## Effect of Social Dominance on Percentage of Calves Sired

Bull	Year 1	Year 2	Year 3	Year 4	Year 5
<b>A</b> Age	10	11	12	13	14
Percent					
<b>B</b> Age	4	5	6	7	8
Percent					
<b>C</b> Age	3	4	5	6	7
Percent					
<b>D</b> Age	2	3	4	5	6
Percent					

## Effect of Social Dominance on Percentage of Calves Sired

Bull	Year 1	Year 2	Year 3	Year 4	Year 5
<b>A</b> Age	10	11	12	13	14
Percent	70				
<b>B</b> Age	4	5	6	7	8
Percent	17				
<b>C</b> Age	3	4	5	6	7
Percent	7				
<b>D</b> Age	2	3	4	5	6
Percent	6				

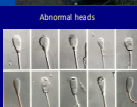
## Effect of Social Dominance on Percentage of Calves Sired

Bull	Year 1	Year 2	Year 3	Year 4	Year 5
<b>A</b> Age	10	11	12	13	14
Percent	70	76	12	0	0
<b>B</b> Age	4	5	6	7	8
Percent	17	18	63	73	25
<b>C</b> Age	3	4	5	6	7
Percent	7	6	12	13	63
<b>D</b> Age	2	3	4	5	6
Percent	6	0	12	15	12

✓ What if the dominant bull is sterile?

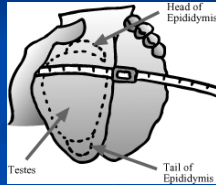
## Bull Fertility Measures – Fort Keogh

- Annual breeding soundness exams (BSE) on all bulls.
- Puberty exams at 10.5 mo of age (n = 1,100).
  - Phenotypes for genomics
  - Sperm morphology traits
    - Heritability
    - Puberty



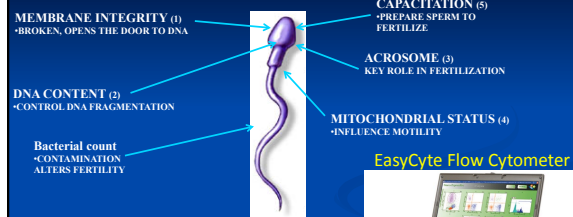
## Heritability of Bull Fertility Traits

Trait	$h^2$
Scrotal circumference, cm	$0.67 \pm 0.09$
% Normal	$0.18 \pm 0.07$
% Knobbed acrosome	$0.02 \pm 0.04$
% Head defects	$0.00 \pm 0.05$
% Distal midpiece reflex	$0.01 \pm 0.04$
% Dag defect	$0.50 \pm 0.10$
% Bowed midpiece	$0.19 \pm 0.07$
% Proximal droplets	$0.37 \pm 0.08$
% Distal droplets	$0.09 \pm 0.06$
% Coiled principle piece	$0.07 \pm 0.05$
% Bent principle piece	$0.18 \pm 0.08$
Gross motility score	$0.20 \pm 0.07$
% Progressive motility	$0.20 \pm 0.08$



**Selection for improved sperm morphology should increase fertility!**

## Key Parameters of Sperm Fertility



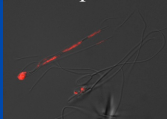
Measuring these key physiological functions provides insight into the fertilization potential of sperm.

**Currently: 2 studies on bull fertility with Guava EasyCyte.**

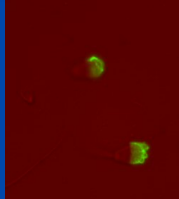


## Biological Markers Associated with Fertility

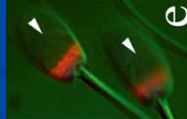
Ubiquitin



PNA

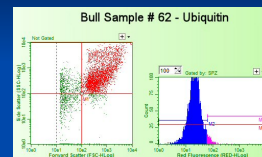


PAWP

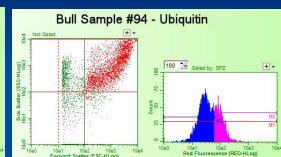


## Bull Fertility Biomarker Trial - UBI

Normal Histogram

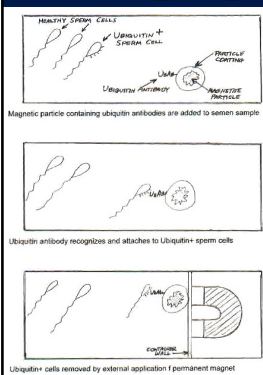


Abnormal Histogram



**Identify bulls of greater/lesser fertility???**  
**Selection for improved fertility in bulls???**

## IVF Using Magnetic Sperm Depletion



Sperm sorted with Ubiquitin antibody resulted in 2 to 4x higher fertilization rate.

## Future Research Bull Fertility Analysis

ASSAY	Microscope	CASA	Easy Cyte
Motility	++	+++	-
Concentration	-	+++	+++
Viability	+	+	+++
Acrosome	+	+	+++
Mitochondria	-	-	+++
Capacitation (Ca)	-	-	+++
DNA fragment	-	-	+++
Bacterial count	-	-	+++
Morphology/physiology	+/-	++/-	++/+++
Objectivity	+	++	+++

CASA = Computer Assisted Semen Analysis.

Easy Cyte = Flow cytometer with various assays built in.

**Additional "Chute Side" measures within 5 years**

“Grass always greener on the other side of the fence!” Cow Fertility?

Fertile Mating?



## Heritability Rates

■ Birth Weight	40%
■ Weaning Weight	30%
■ Yearling Weight	30%
■ Fleshing Ability (BCS)	40%
■ Frame Size	55%
■ Pelvic Area	40%
■ Fertility	10%

■ Source: “Scientific Farm Animal Production”

## Requirements for Reproductive Success

1. Puberty / Resume cycling ✓ / ✓
2. Fertile ovulation ✓
3. Conception (Ovum and Sperm) ✓
4. Pregnancy establishment ✓ d. 27
5. Pregnancy maintenance ✓

→ Successfully Reproduce

Fort Keogh Research:

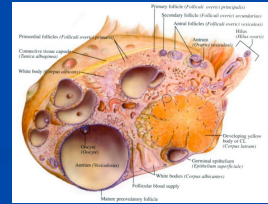
- Identify genes/DNA markers involved with **fertility** in beef cattle.

## Identification of New Phenotypic Traits involved in Fertility

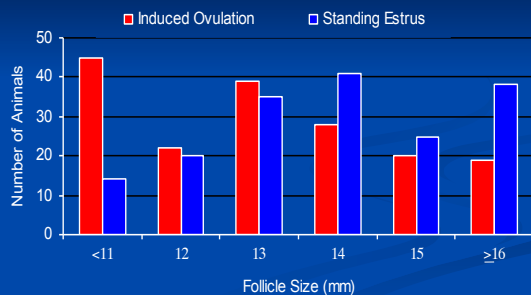
### ■ Beef Cow / Heifer

- Used AI to obtain numerous measures & keep bull fertility constant

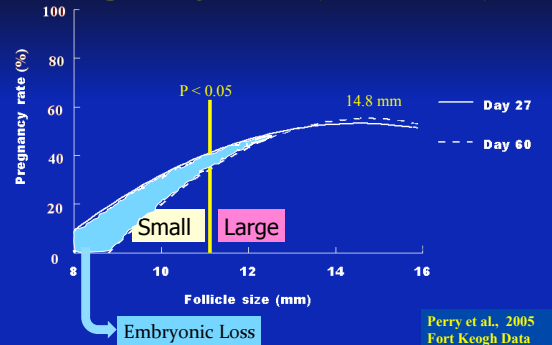
#### ■ Ovulatory follicle size



## Distribution of Ovulatory Follicle Size



## Effect of Follicle Size on Pregnancy Rate (Timed-AI)





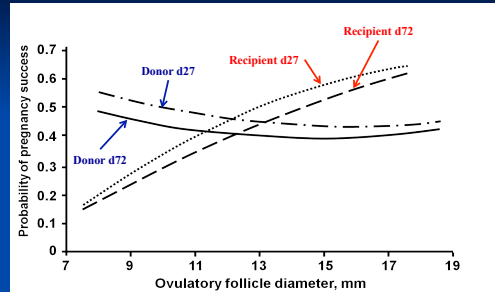
## Fort Keogh Reciprocal Embryo Transfer Study (2007-09)

**Goal:** Determine how size of the ovulatory follicle affects pregnancy establishment and maintenance.  
Does it affect oocyte maturation and/or viability?  
Does it affect the maternal reproductive tract in assisting pregnancy maintenance/establishment?

Treatment groups			
GnRH-induced follicle size			
No. of transfers	Donor	Recipient	Purpose
71	Small	Small	Negative control
111	Small	Large	Effects 1 <sup>o</sup> of oocyte origin*
122	Large	Small	Effects 1 <sup>o</sup> of maternal origin*
50	Large	Large	Positive control

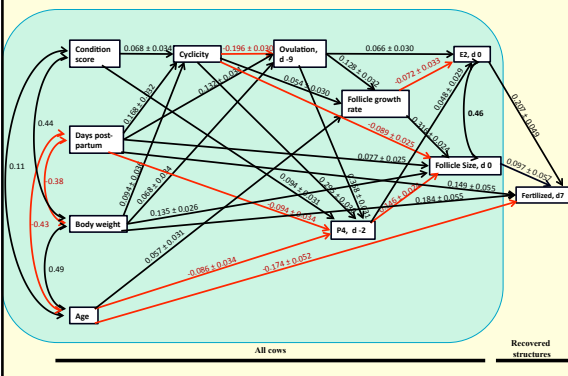
n = 1,164 suckled beef cows; 810 = donor and 354 = recipient

## Results – Reciprocal Embryo Transfer Study

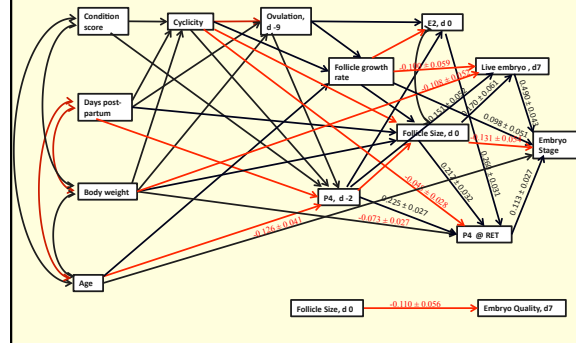


Suggests that ovulatory follicle size of the recipient is the only one that matters so effects of follicle size acting through maternal environment prepared for embryo.

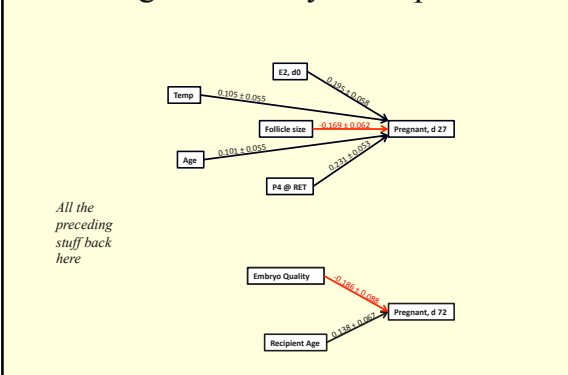
## Path Diagram: Embryo Donor Cow



## Path Diagram: Additional Fertility Measures



## Path Diagram: Embryo Recipient Cow



## Reciprocal Embryo Transfer Study: Fertility

Fertility of individual breeding affected by:

1. Estradiol at time of breeding 18%
2. Progesterone on d 7 after breeding 15%
3. Progesterone before breeding 8%
4. Ovulatory follicle size 7%
5. Embryo quality 3%
6. Days postpartum at breeding 2%

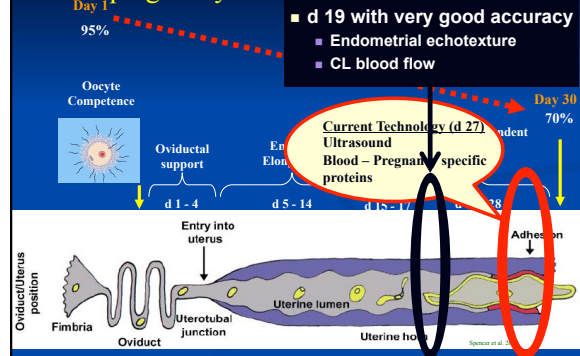
**NOTE:** Cows were not used if they exhibited estrus.

## Take Home Message – RET Study

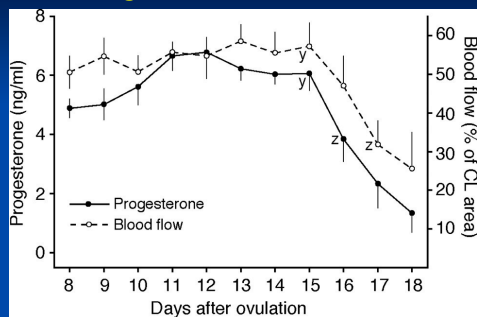
- Fertility is much more complicated than we hope.
- The most important variable related to pregnancy success was estradiol concentration at the time of breeding.

If using synchronization and timed AI, insemination must coincide with expression of estrus.

## Early pregnancy diagnosis to identify causes of pregnancy failure at Fort Keogh.

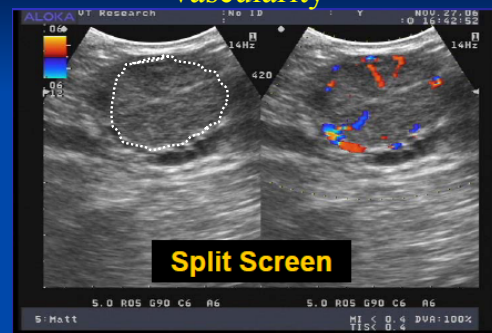


## Serum Progesterone vs CL Blood Flow

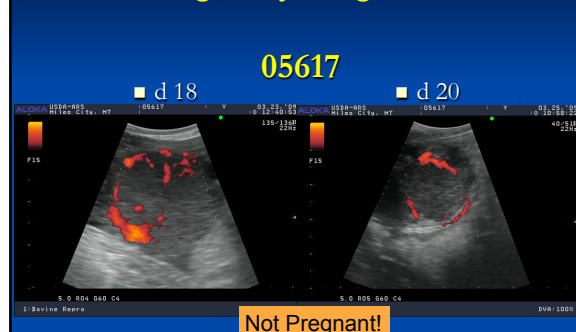


Ginther, O.J., et al. Biol Reprod 2007;76:506-513

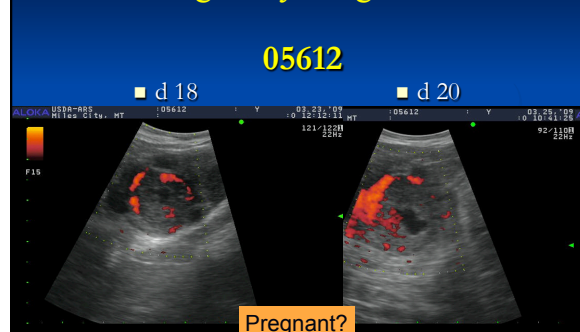
## Doppler Ultrasound Imaging of CL Vascularity



## Sequential CL Vascularity for Pregnancy Diagnosis



## Sequential CL Vascularity for Pregnancy Diagnosis



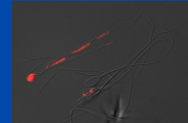
Thank You! Questions?



ROY WALLACE, SELECT SIRES

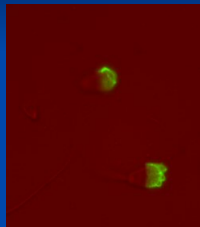
## Ubiquitin (UBI)

- Proteolytic marker peptide that binds to the surface of defective sperm.
- Ubiquitination causes immobilization and/or resorption of these defective sperm during epididymal passage.
- increased binding of anti-ubiquitin antibodies to the sperm surface reflect the occurrence of abnormalities



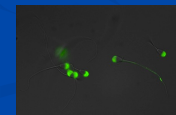
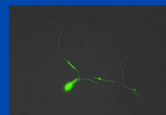
## Peanut Agglutinin (PNA)

- High affinity/strong specificity for disaccharides with terminal galactose, especially the D Gal  $\alpha$  (1,3) D GalNAc disaccharide
- Binds to the outer acrosomal membrane (OAM), exposed during the acrosome reaction



## Lentil Lectin (LCA)

- Shows a strong specificity to d-glucosyl and d-mannosyl residues
- Binds to the acrosome in normal sperm, whole surface in defective sperm



## Postacrosomal Sheath WW-Domain-Binding Protein (PAWP)

- Resides exclusively in the post-acrosomal sheath (PAS) region of the sperm head perinuclear theca (PT) and is expressed and assembled in elongating spermatids
- Promotes meiotic resumption and pronuclear development during fertilization
- Abnormal sperm may have unusually high levels of this protein



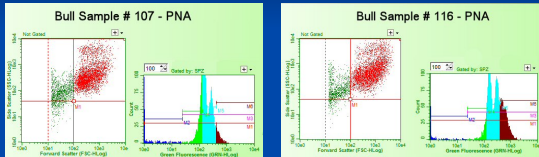
## Bull Fertility Biomarker Trial

- n = 162 samples
- Samples analyzed via UBI, PNA, LCA, PAWP
- Flow cytometry via GUAVA
- Analysis performed via Excel and SAS

## Bull Fertility Biomarker Trial - PNA

Normal Histogram

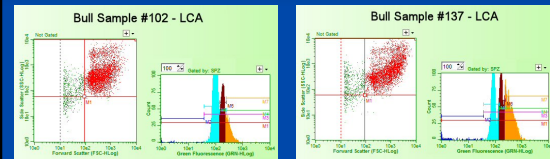
Abnormal Histogram



## Bull Fertility Biomarker Trial - LCA

Normal Histogram

Abnormal Histogram



## Bull Fertility Biomarker Trial - PAWP

Normal Histogram

Abnormal Histogram

