Fatores que afetam a perda gestacional em bovinos de corte e de leite: compreendendo sua origem para desenvolver estratégias de mitigação

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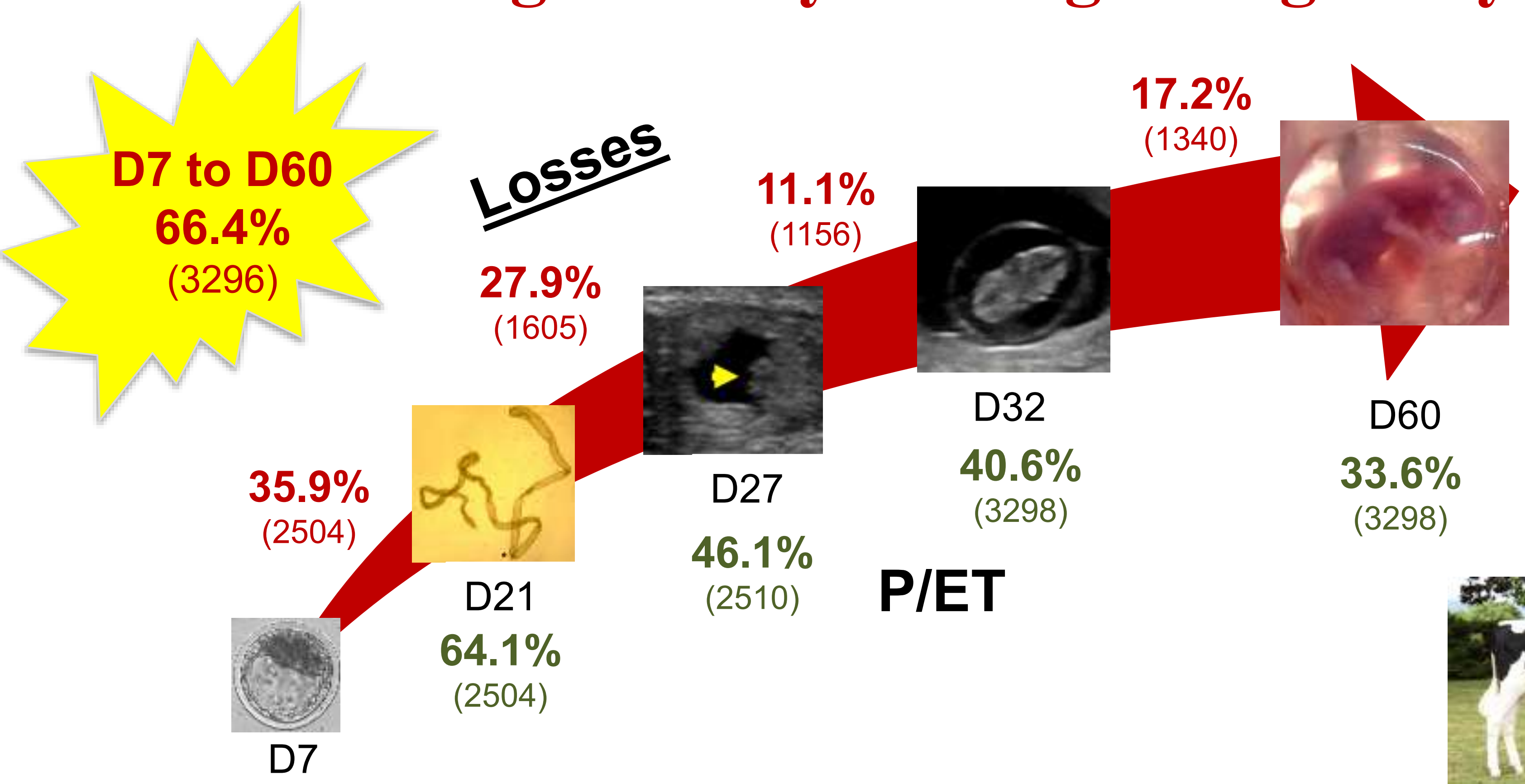
11º SIRAA
SIMPÓSIO INTERNACIONAL DE REPRODUÇÃO ANIMAL APLICADA

DEPARTMENT OF DAIRY SCIENCE
University of Wisconsin-Madison

The Reproductive Physiology Lab

Understanding Fertility Through Pregnancy Loss



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Review article

Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows

Milo C. Wiltbank[a,*], Giovanni M. Baez[a], Alvaro Garcia-Guerra[a], Mateus Z. Toledo[a], Pedro L.J. Monteiro[a,b], Leonardo F. Melo[a,b], Julian C. Ochoa[a], José E.P. Santos[c], Roberto Sartori[a,b]

Proceedings of the 10th International Ruminant Reproduction Symposium (IRRS 2018); Foz do Iguaçu, PR, Brazil, September 16th to 20th, 2018.

Physiological mechanisms involved in maintaining the corpus luteum during the first two months of pregnancy

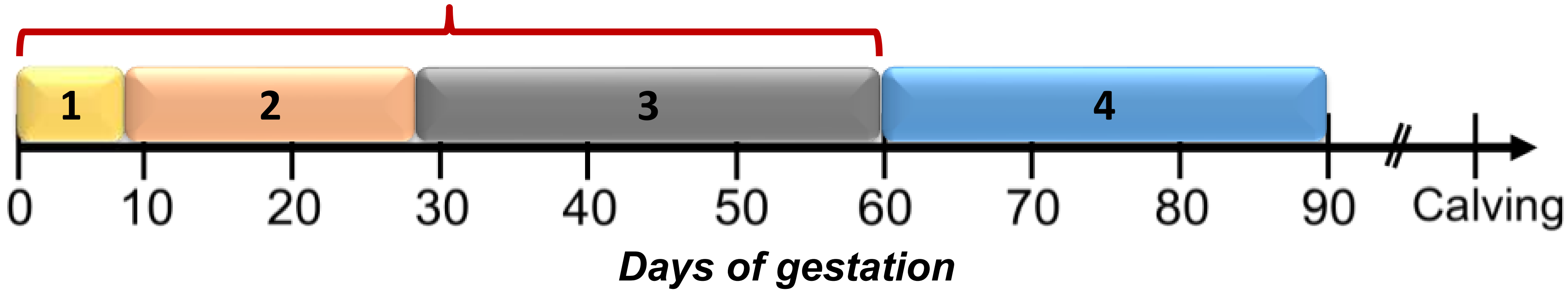
Milo C. Wiltbank[1,2,*], Megan A. Mezera[1,2], Mateus Z. Toledo[1], Jessica N. Drum[1,3], Giovanni M. Baez[4], Alvaro García-Guerra[5], Roberto Sartori[3]

✓ Incidence

✓ Risk factors

✓ Physiology

✓ Potential mitigating strategies



Period 1: The first week of pregnancy

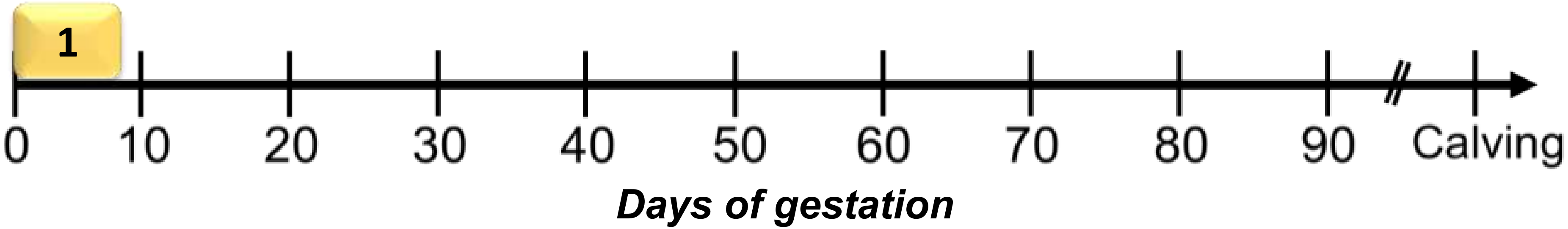
Failure during this period due either lack of fertilization or death of the early embryo.

Loss during this period results in reinitiation of the estrous cycle as if pregnancy had not been initiated.

Events

- Fertilization*
- Oviductal transport
- <u>Early embryo development</u>
- Embryonic genome activation

AI: Lactating Dairy cows	**15-50%**
AI: Beef cows/heifers	**28%**



Period 1: What is the problem?

- **Fertilization failure**
  a) Improper AI technique
  b) Lack of detection of estrus
  c) Improper timing of AI
  d) Inflammatory diseases
  e) High P4 near AI (gamete transport)
  f) Reduced sperm quality
  g) Reduced oocyte quality (e.g. heat stress)

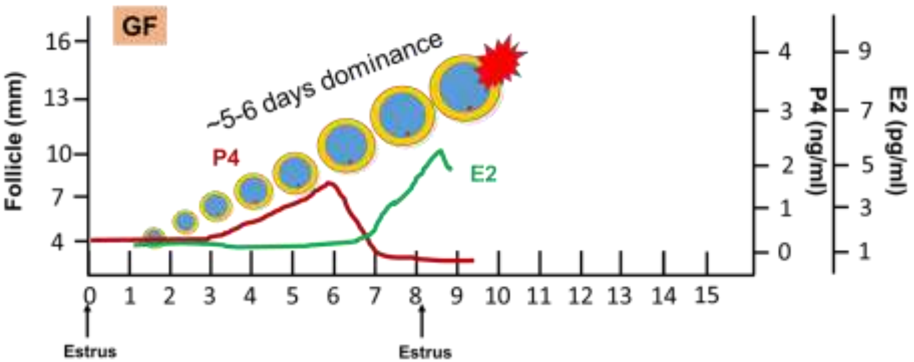
- **Impaired early embryo development**
  a) Prolonged follicular dominance
  b) Low P4 during follicle development
  c) Inflammatory diseases
  d) Genetic (e.g. aneuploidy)
  e) Nutritional (e.g. NEB – BCS loss)
  f) Age

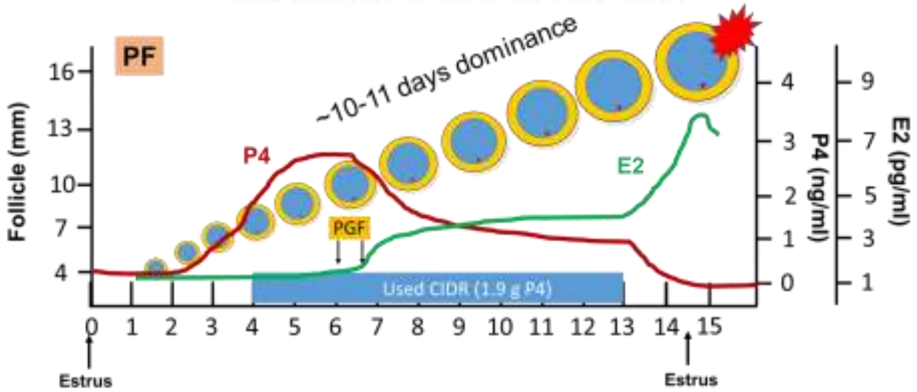
Impact of follicle development on fertility

Relationship of Fertility to Patterns of Ovarian Follicular Development and Associated Hormonal Profiles in Dairy Cows and Heifers[1]

Cooperative Regional Research Project, NE-161[2]

J. Anim. Sci. 1996. 74:1943–1952





- Low P4 short period
- Short exposure to high LH pulse

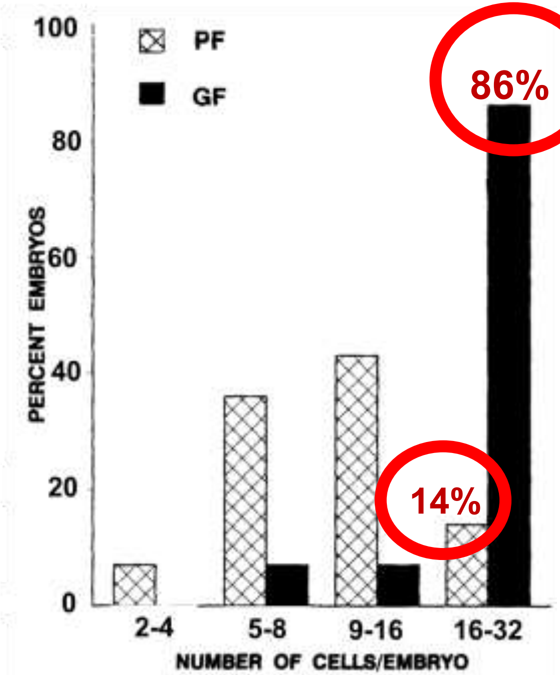
GF

PF

- Low P4 prolonged period
- Prolonged exposure to high LH pulse frequency

Impact of follicle development on fertility

Category	Growing Follicle (GF)	Persistent Follicle (GF)
N	22	20
Fertilization rate	94	100
Accessory sperm (n)	38 ± 12 (NS)	



Category	Growing Follicle (GF)		Persistent Follicle (GF)
Cows	54.2% (26/48) [a]	3.8 -fold	14.0% (7/50) [b]
Heifers	62.0% (31/50) [a]	1.7 -fold	35.9% (14/39) [c]
Total	58.2% (57/98) [d]	2.5 -fold	23.6% (21/89) [e]

Chi-square analyses: [a] vs [b] or [d] vs [e] $P < .001$; [a] or [b] vs [c] $P < .05$.

Ahmad et al., 1995

High vs. Low P4 during Preovulatory Follicular Wave

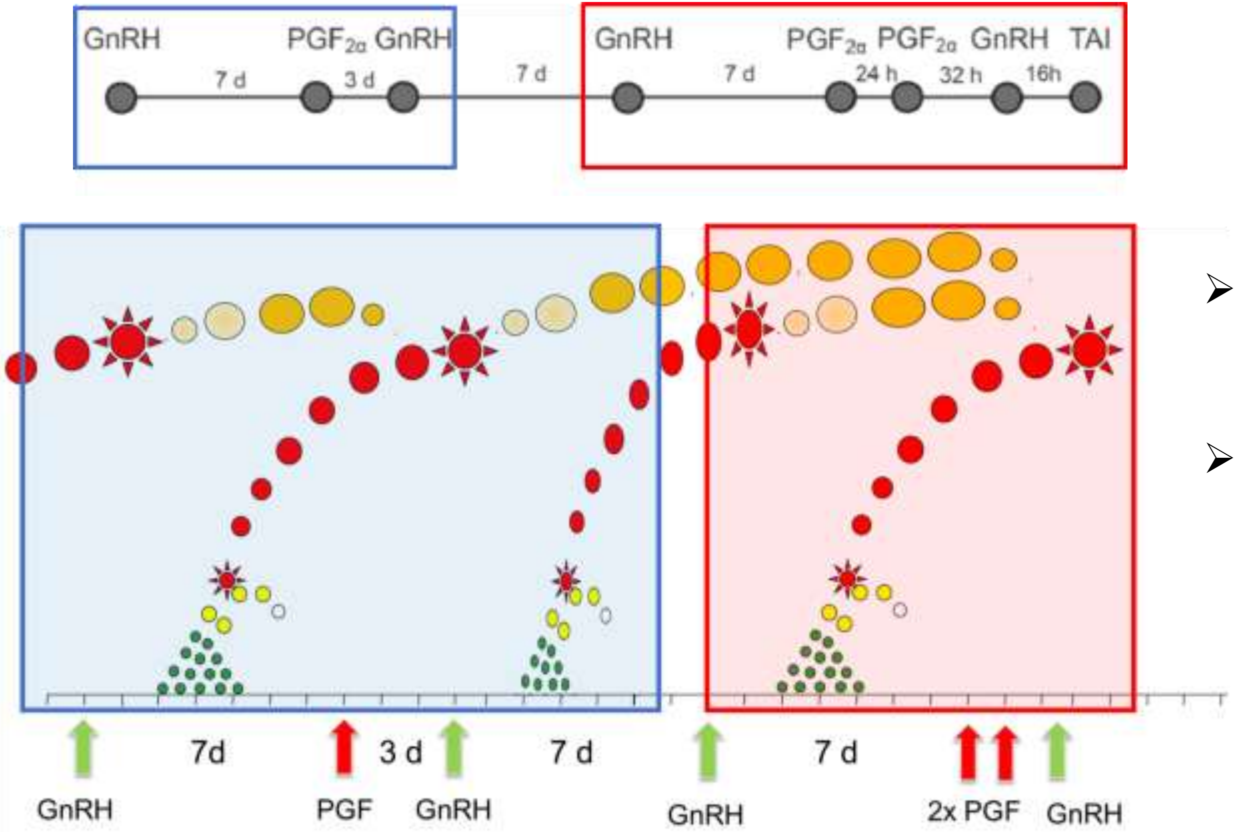
Sun	Mon	Tue	Wed	Thu	Fri	Sat
					GnRH	
					PGF	
	GnRH					
	GnRH	Higher Progesterone				
	PGF	PGF	GnRH	TAI		
				Flush Embryos		

Sun	Mon	Tue	Wed	Thu	Fri	Sat
					GnRH	
					PGF	
	GnRH					
	GnRH	+PGF	Lower Progesterone			
	PGF	PGF	GnRH	TAI		
				Flush Embryos		

Embryos	Low P4	High P4	P-value
Grade 1	34.6% (9/26)	67.6% (25/37)	0.01
Grade 1 + 2	61.5% (16/26)	86.5% (32/37)	0.02

Wiltbank et al., unpublished

How we control follicular development and P4?



Double Ovsynch

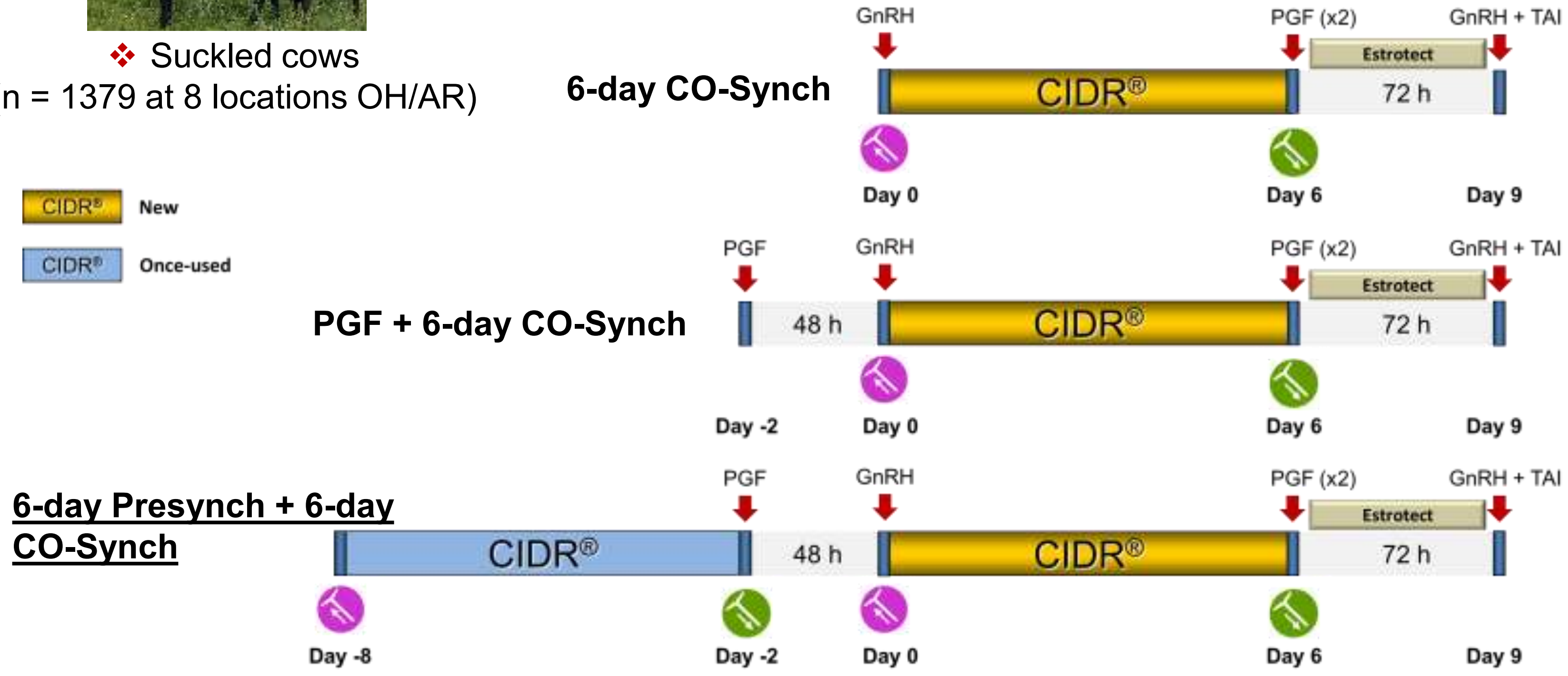
- Follicle with ovulatory capacity at GnRH-1 of breeding Ovsynch (length of dominance)
- Circulating P4 during follicle development (LH stimulation)

How we control follicular development and P4?

Presynchronization

- Suckled cows

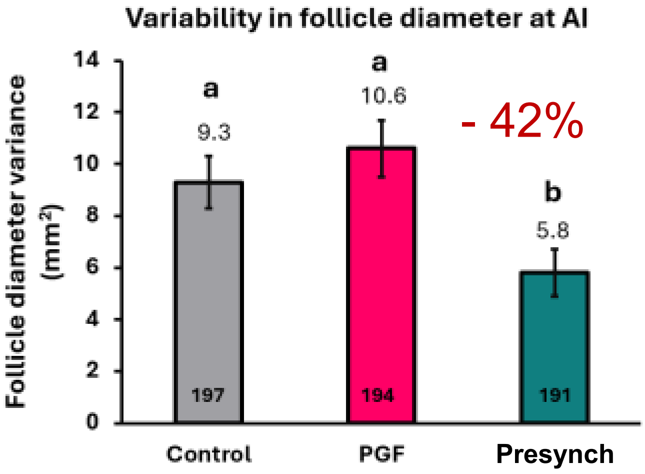
(n = 1379 at 8 locations OH/AR)



Crist et al., 2022

Effect of different presynchronization strategies on synchronization efficiency

Treatment	Control	PGF	Presynch	P-value
N	197	194	191	
Day 0 (GnRH-1)				
Follicle size (mm)	12.2 ± 0.3[b]	12.8 ± 0.3[b]	15.1 ± 0.3[a]	<0.001
P4 (ng/mL)	2.5 ± 0.2[a]	0.8 ± 0.1[b]	0.4 ± 0.1[c]	<0.001
Ovulation to GnRH-1	**65.8%[b]**	**76.2%[b]**	**93.4%[a]**	<0.001
Day 9 (GnRH-2)				
Dominant follicle (%)	90.7 ± 2.4[ab]	89.0 ± 2.7[b]	95.9 ± 1.5[a]	0.04
Follicle size (mm)	13.3 ± 0.4	13.1 ± 0.4	13.7 ± 0.3	0.11
Follicle growth rate (mm/d)	1.0 ± 0.07[b]	1.1 ± 0.07[ab]	1.2 ± 0.07[a]	0.002



Effect of presynchronization strategies on fertility

Treatment	Control	PGF	Presynch	P-value
N	466	460	453	
Estrus (%)	**71.8%**	**77.8%**	**73.4%**	0.29
Time to Estrus (h)	**67.5 ± 1.0**	**66.8 ± 0.9**	**68.7 ± 0.9**	0.31
P/AI D35	**58.4%^{B}**	**61.3%^{B}**	**69.9%^{A}**	0.002
P/AI D90	**56.3%^{B}**	**57.2%^{B}**	**66.1%^{A}**	0.007
Pregnancy loss	**2.7%**	**5.2%**	**3.8%**	0.31

Crist et al., 2022

Control of follicular development and the endocrine environment is critical to reducing losses and maximizing fertility

Period 2: Days 8 to 27/32

The second period encompasses the time of normal luteolysis or “maternal recognition of pregnancy” (day ~16)

Events

- Conceptus elongation
- Nutritional transition (histotroph to choriovitelline)
- Apposition of trophoblast and maternal uterine microvilli
- Maternal recognition of pregnancy – Maintenance of the CL
- Binucleate giant cells (PAG production)
- Immunomodulation

AI: Lactating Dairy cows

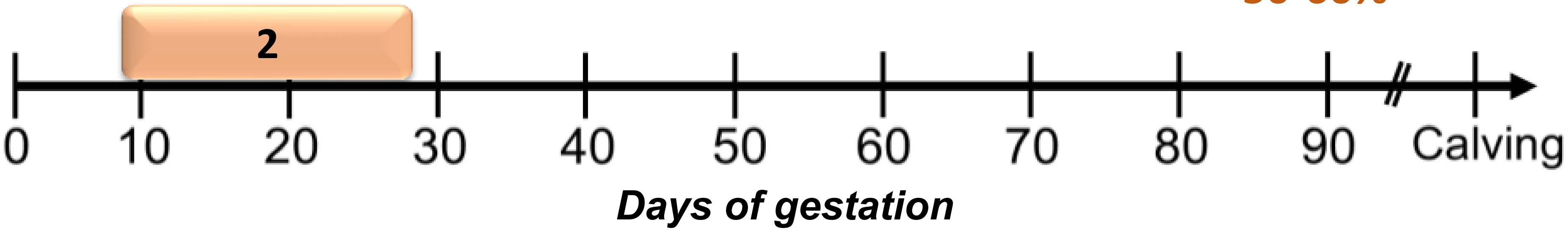
30%

AI: Beef cows/heifers

27%

ET: Dairy heifers (IVP)

50-60%

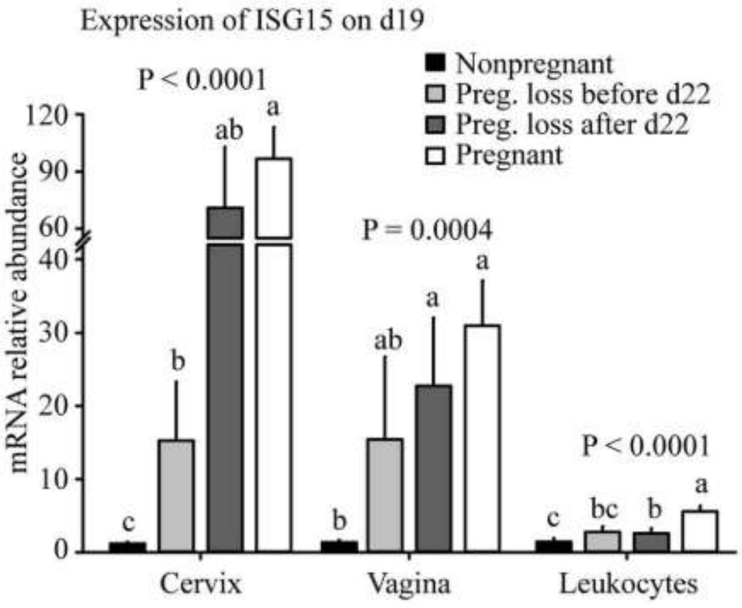


Period 2: What is the problem?

- Estradiol during the preovulatory period
- Attachment (trophoblast differentiation)
- P4 during the postovulatory period
- Breeding method (AI, ET-IVD, ET-IVP, ET-SCNT)
- Inflammatory diseases
- Embryo-uterine asynchrony
- Embryo quality and developmental stage
- Age
- Parity
- Sire

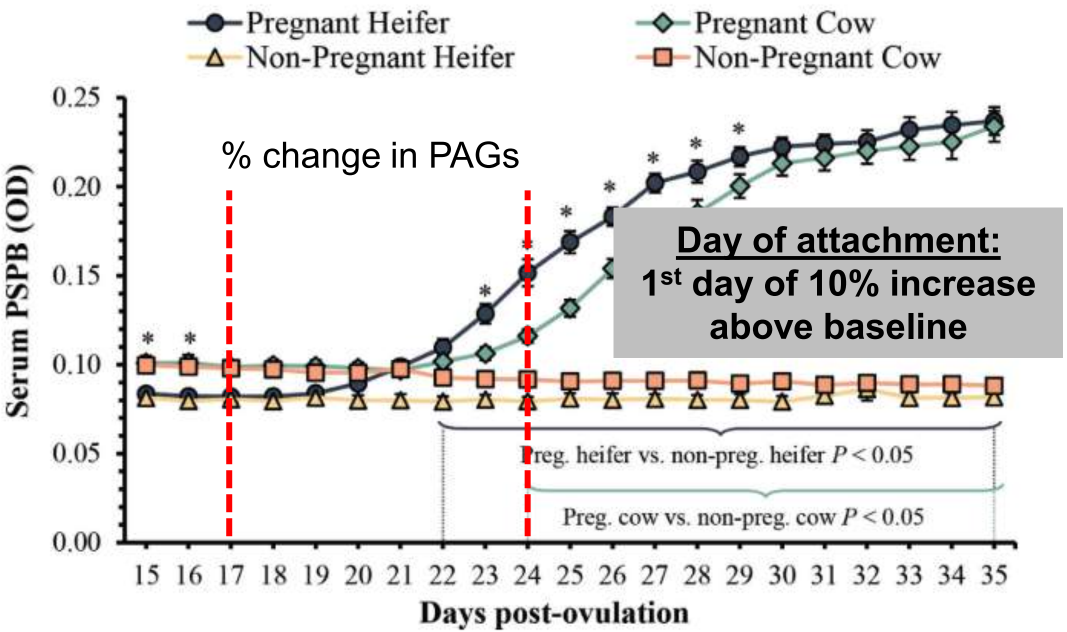
New tools to investigate early pregnancy loss (Period 2)

Interferon-stimulated genes (ISG)



Domingues et al., 2023

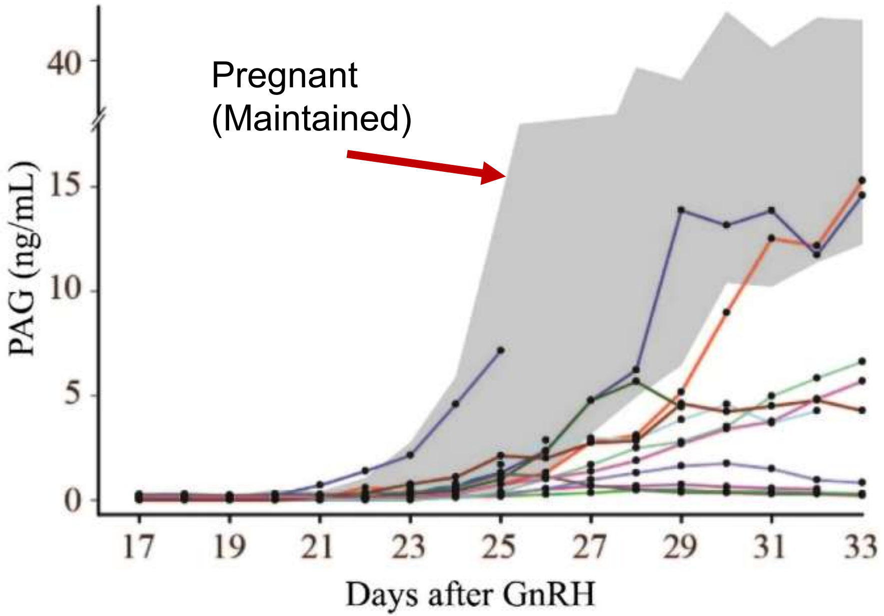
Pregnancy-associated glycoproteins (PAG)



Day of attachment:
1st day of 10% increase above baseline

Middleton et al., 2022

Cows that lose their pregnancy have delayed attachment



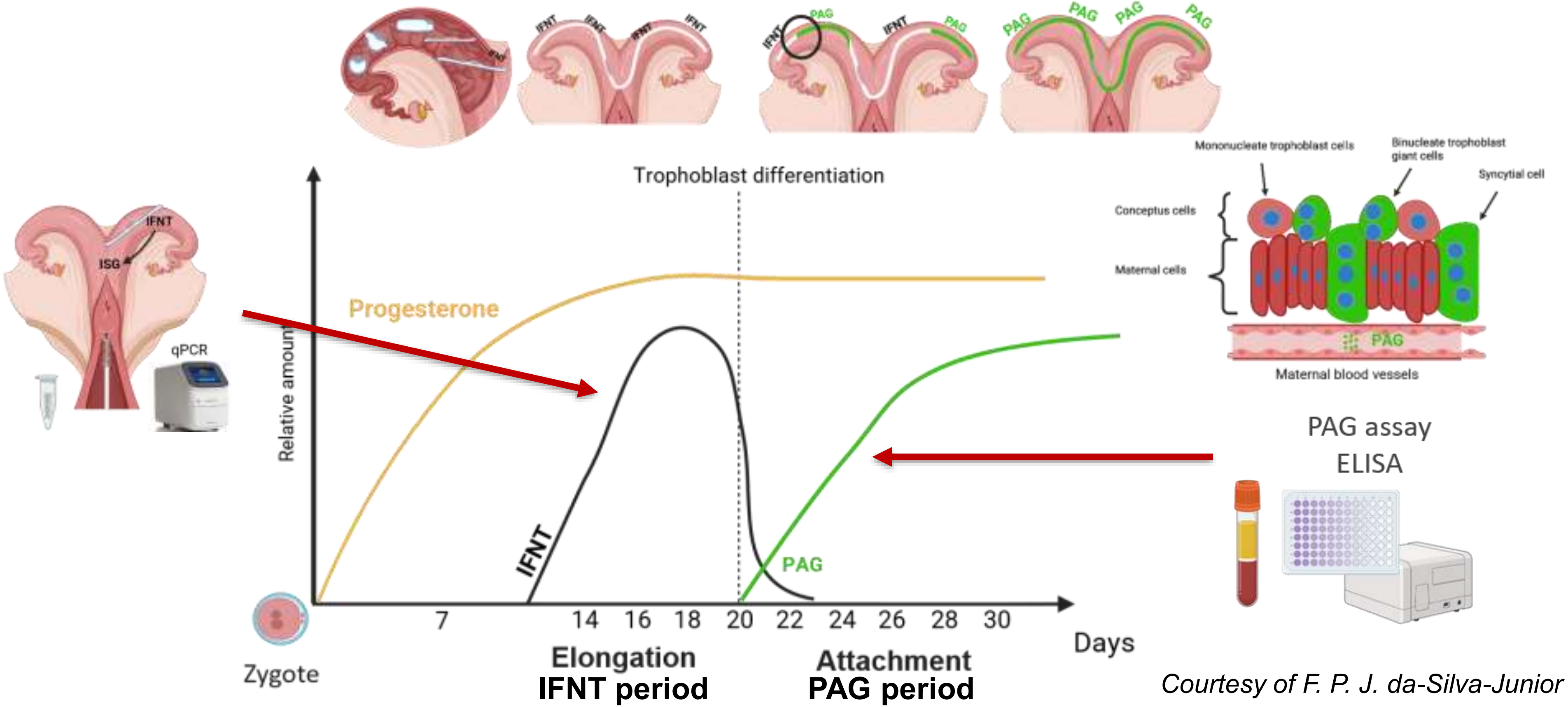
Pregnancy loss (d20 to d33)

24.6% (15/61)

Outcome	Maintained	Pregnancy Loss
Day of attachment	21.3 ± 0.1[a]	22.8 ± 0.6[b]

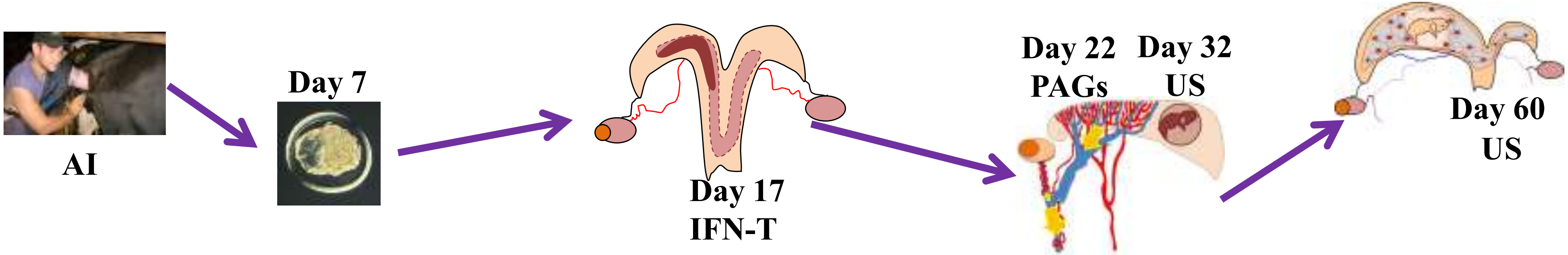
Domingues et al., 2023

How do we assess embryonic development in vivo?



Courtesy of F. P. J. da-Silva-Junior

Revisiting pregnancy loss during Period 2



Double Ovsynch

N = 86

	AI	Day 14-17 ISGs	Day 22-24 PAGs	Day 31-32 US
Double Ovsynch		81.4%	70.9%	53.5%
5-day CO-Synch		89.3%	68.6%	56.6%

Losses between time points:

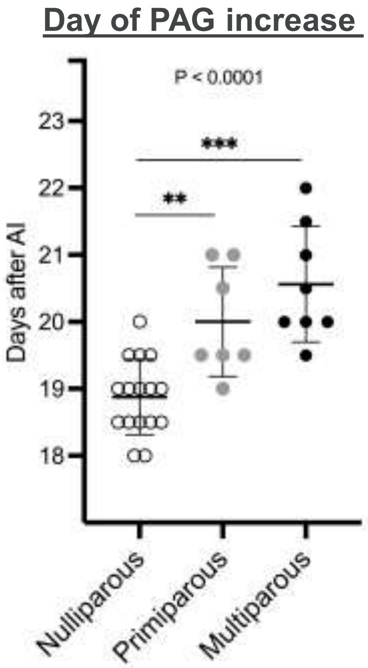
	AI to Day 14-17	Day 14-17 to Day 22-24	Day 22-24 to Day 31-32
Double Ovsynch	18.6%	12.8%	24.6%
5-day CO-Synch	10.7%	23.2%	17.4%

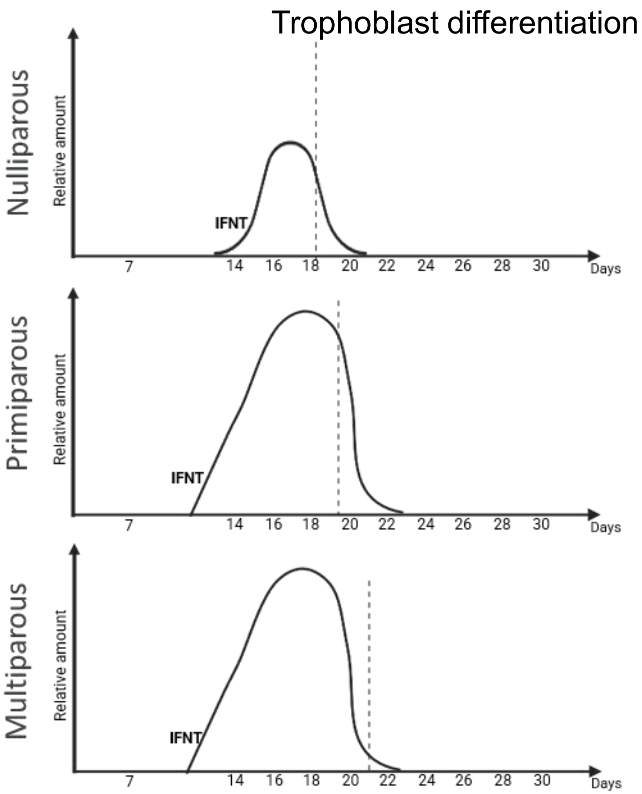
5-day CO-Synch

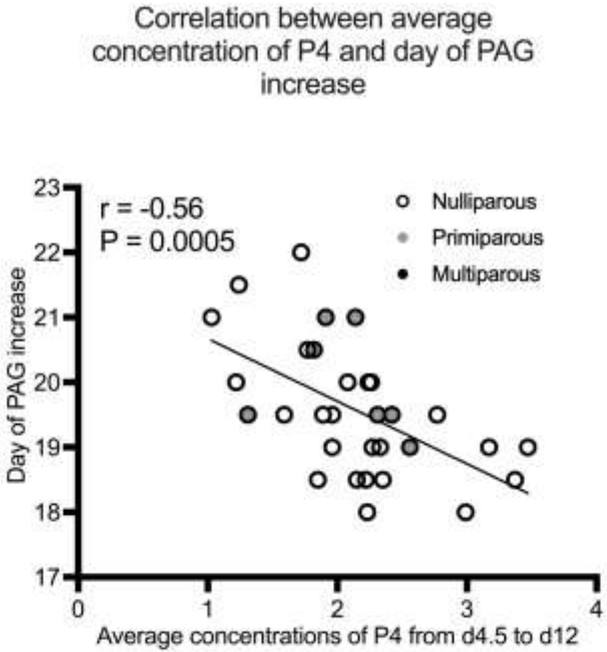
N = 159

Adapted from Domingues et al., 2023 and F. P. J. da-Silva-Junior et. al., 2026

Parity influence timing of trophoblast differentiation



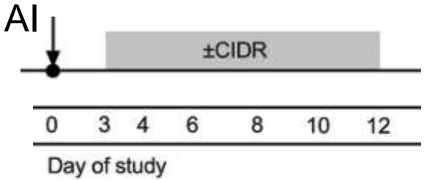




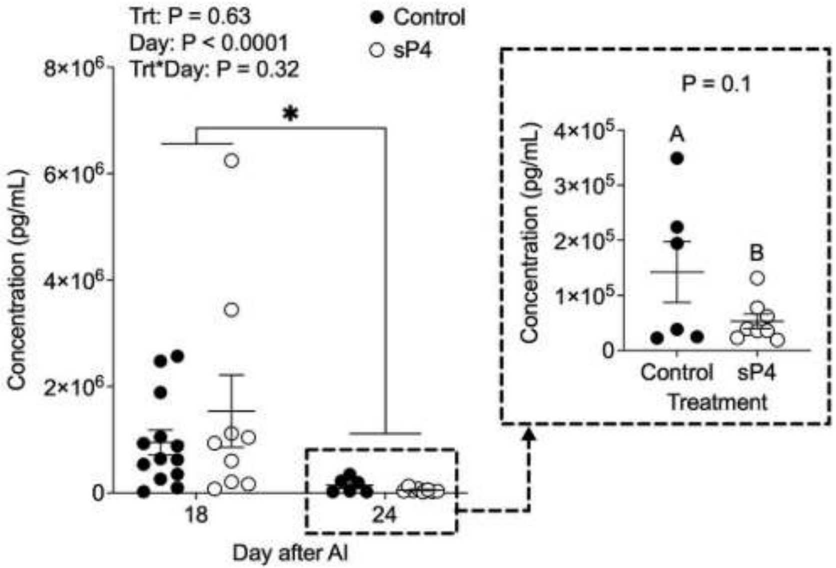
F. P. J. da-Silva-Junior et. al., 2026

[ab]Different superscript letters indicate significant difference among parity within each day

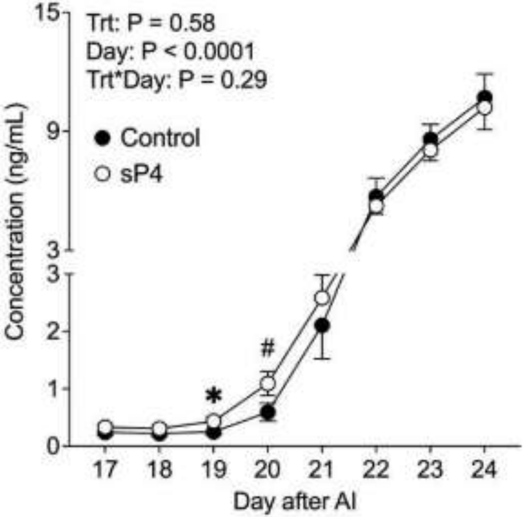
P4 supplementation hastens trophoblast differentiation



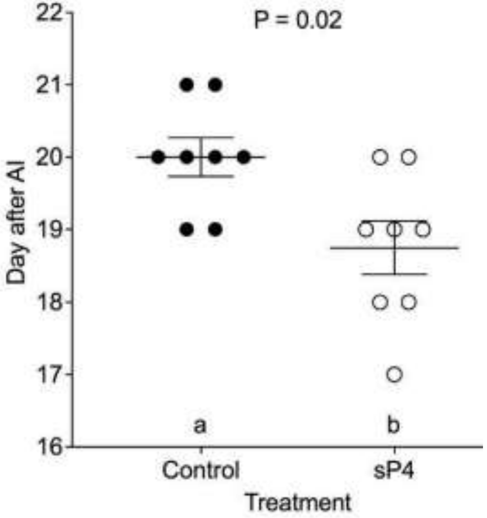
IFNT uterine fluid



PAGs

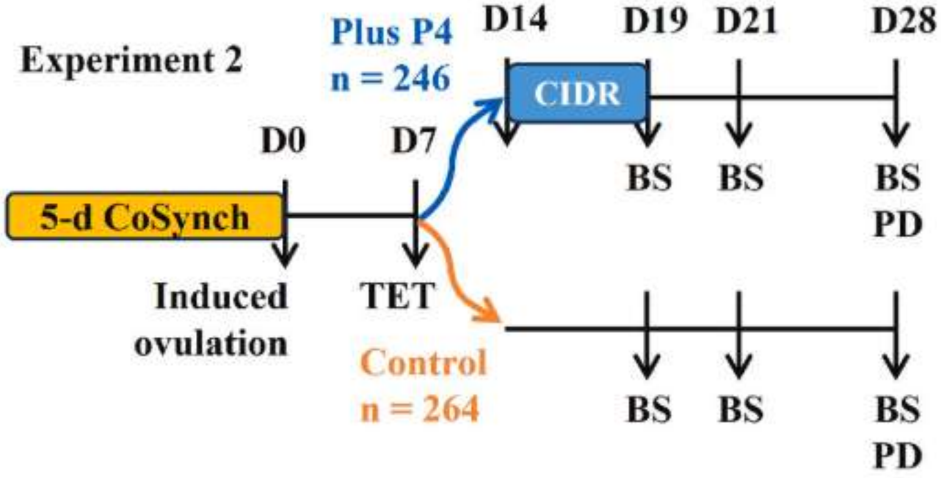


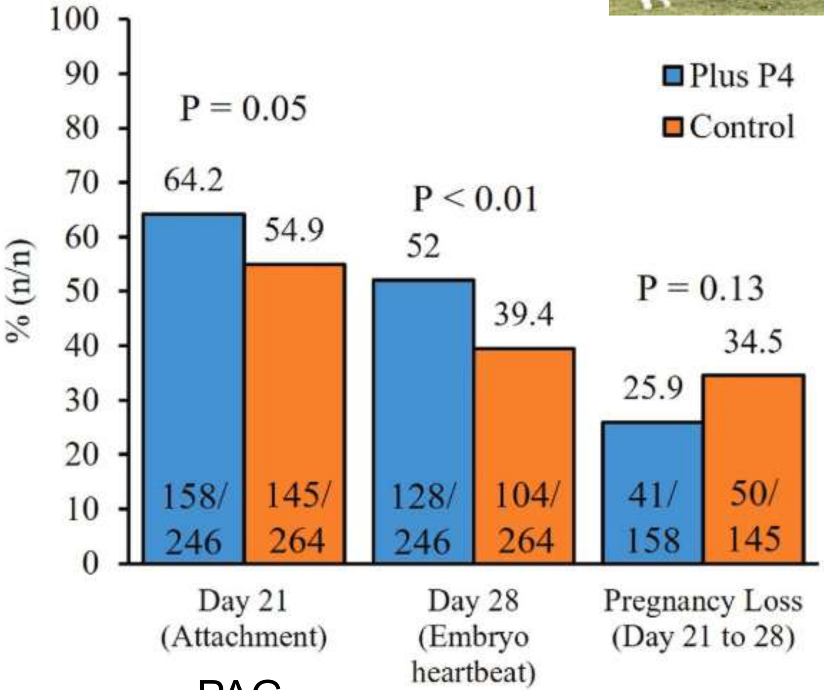
Day of PAG increase



Ehresmann et al., 2026

P4 supplementation in ET recipient heifers





PAG

Andrade et al., 2025

P4 supplementation in ET recipient heifers

Pregnancy outcomes in Holstein embryo recipient heifers in response to P4 supplementation from d14-19 (PlusP4) based on the tercile of circulating PAG on d21.

Item	PlusP4	Control	P-Value
Low tercile			
d21 Attachment	24.4 % (20/82)	25 % (22/88)	0.92
d28 Embryo heartbeat	12.2 % (10/82)	2.3 % (2/88)	0.02
Pregnancy Loss	65 % (13/20)	100 % (22/22)	0.03
Medium tercile			
d21 Attachment	78.1 % (64/82)	56.8 % (50/88)	<0.01
d28 Embryo heartbeat	58.5 % (48/82)	38.6 % (34/88)	0.01
Pregnancy Loss	32.8 % (21/64)	40 % (20/50)	0.42
High tercile			
d21 Attachment	90.2 % (74/82)	83 % (73/88)	0.17
d28 Embryo heartbeat	85.4 % (70/82)	77.3 % (68/88)	0.18
Pregnancy Loss	9.5 % (7/74)	11 % (8/73)	0.76

Andrade et al., 2025

Progesterone supplementation may allow us to rescue pregnancies with suboptimal/delayed attachment

Period 3: Days 28/32 to 60

The third period encompasses the time of development of a chorioallantoic cotyledonary placenta.

Events

- Continued increase in the size of the conceptus
- Strengthening of the conceptus-maternal adhesion
- Development of cotyledons – placentomes
- Disappearance of the yolk sack
- Nutritional transition (choriovitelline to chorioallantoic)
- Vascular changes (increase in uterine blood flow)
- Increase in binucleate giant cells

AI: Lactating Dairy cows

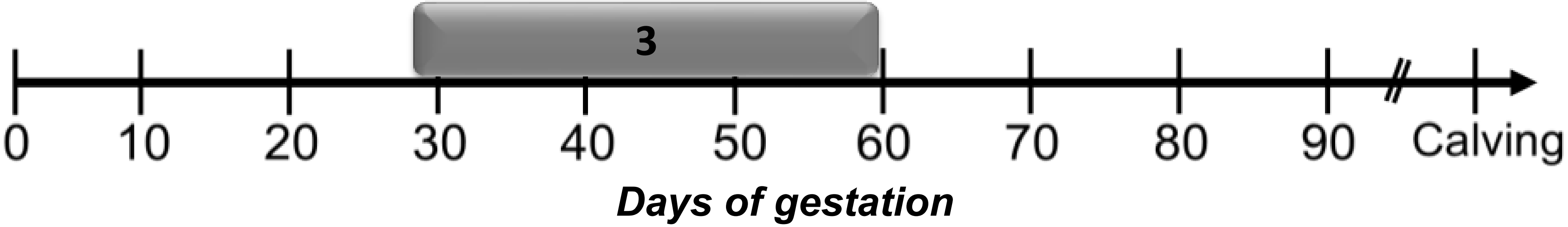
12%

AI: Beef cows/heifers

6%

ET: Dairy heifers (IVP)

17-20%

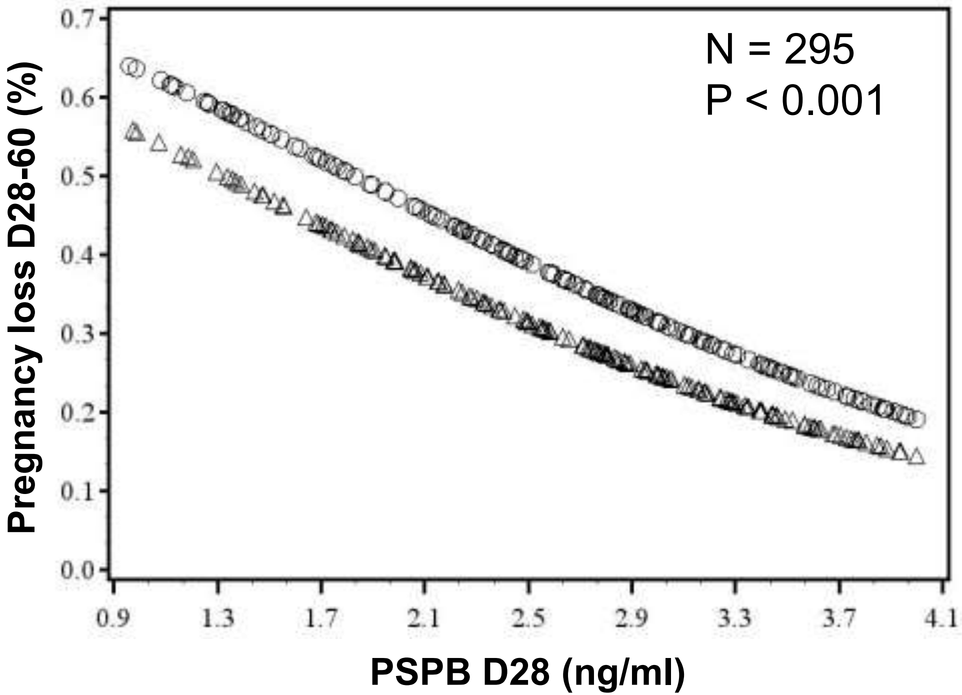


Period 3: What is the problem?

- Breeding method (AI, ET-IVD, ET-IVP, ET-SCNT)
- Developmental deficiencies (e.g. inadequate placentation)
- Anovulation
- P4 during follicle development
- P4 during the postovulatory period
- Estrus expression
- Parity
- Nutritional
- Inflammatory diseases
- Twinning
- Embryo-uterine asynchrony
- Embryo quality and developmental stage
- Age
- Sire

PAGs – Pregnancy loss D28-60

FTET
IVP



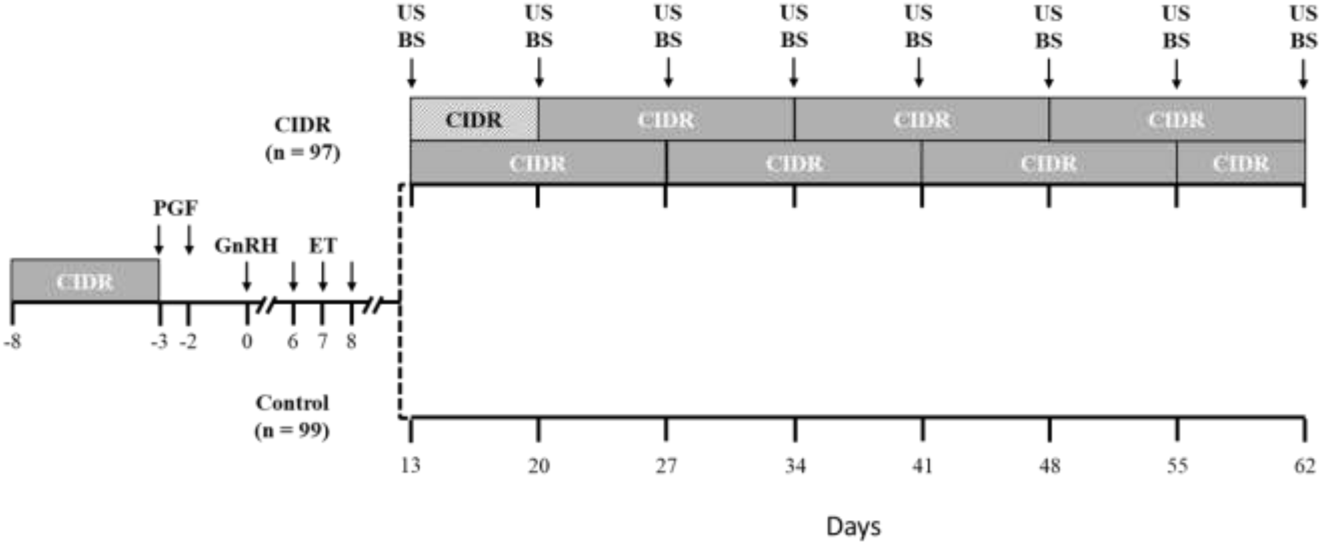
Suboptimal placentation

Garcia-Guerra et al., 2020

Effect of P4 supplementation on pregnancy loss in ET recipients

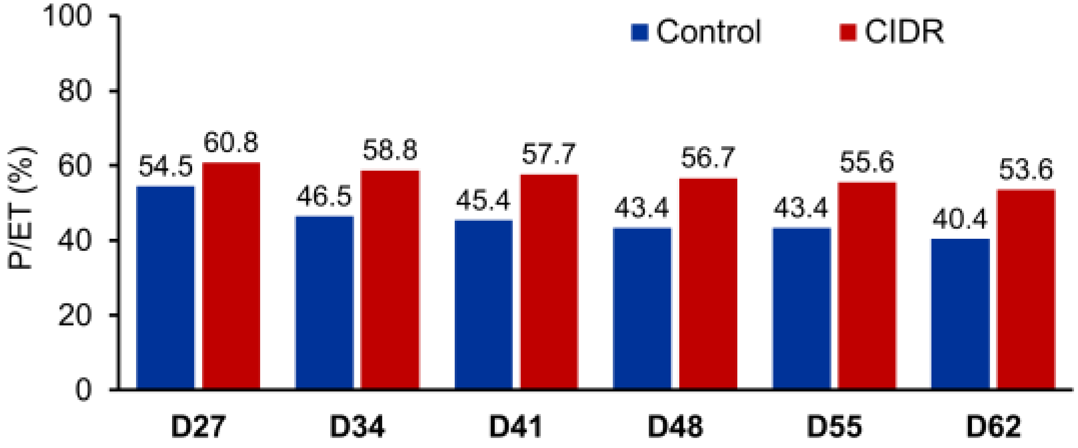
Holstein heifers (N=200)

Fresh IVP embryos

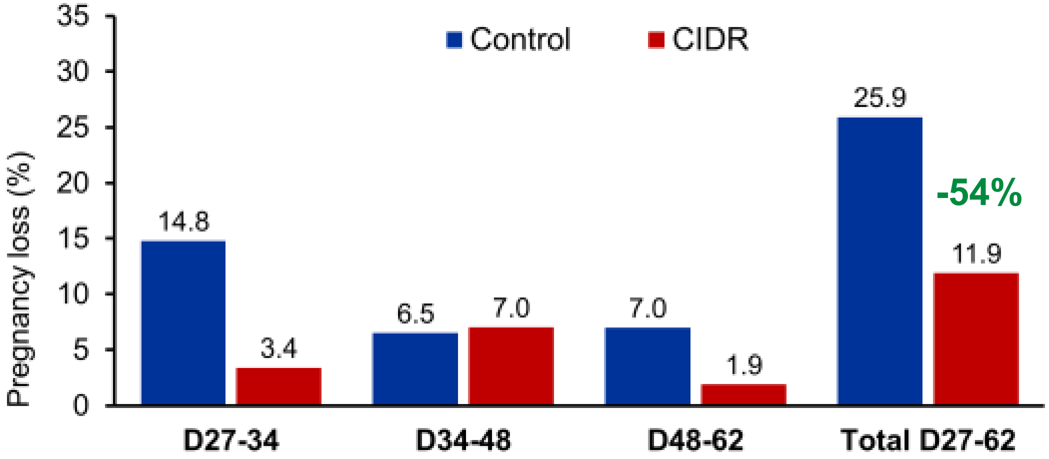


Garcia-Guerra et al., 2016

Effect of P4 supplementation on pregnancy loss in ET recipients

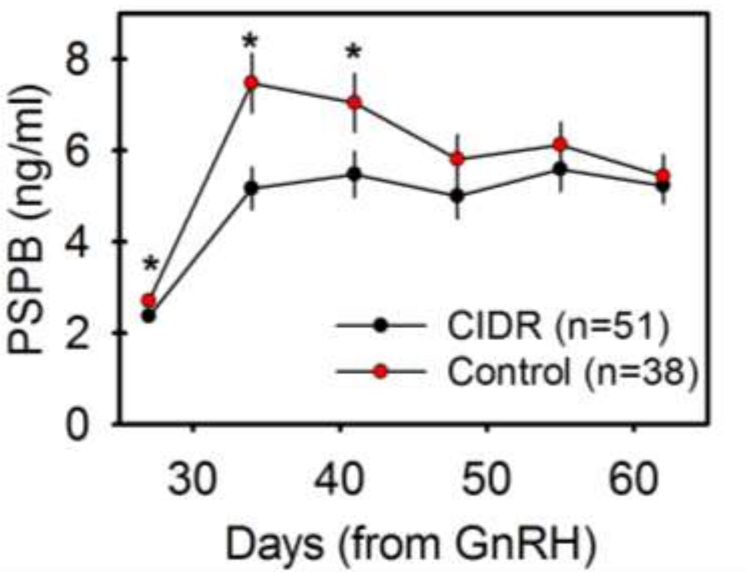


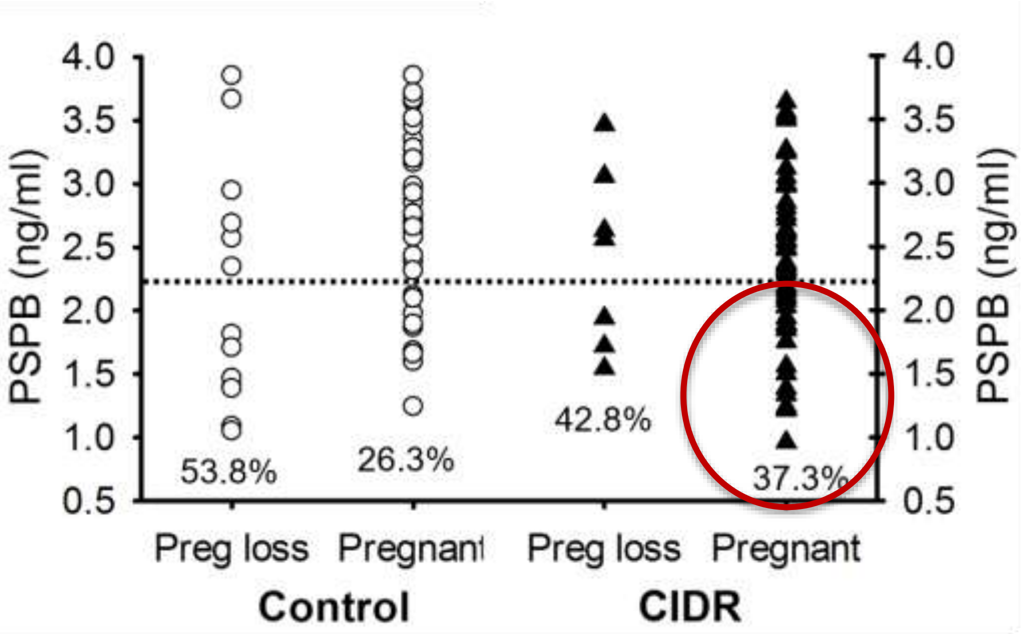
Holstein heifers (N=200)



Garcia-Guerra et al., 2016

Effect of P4 supplementation on pregnancy loss in ET recipients

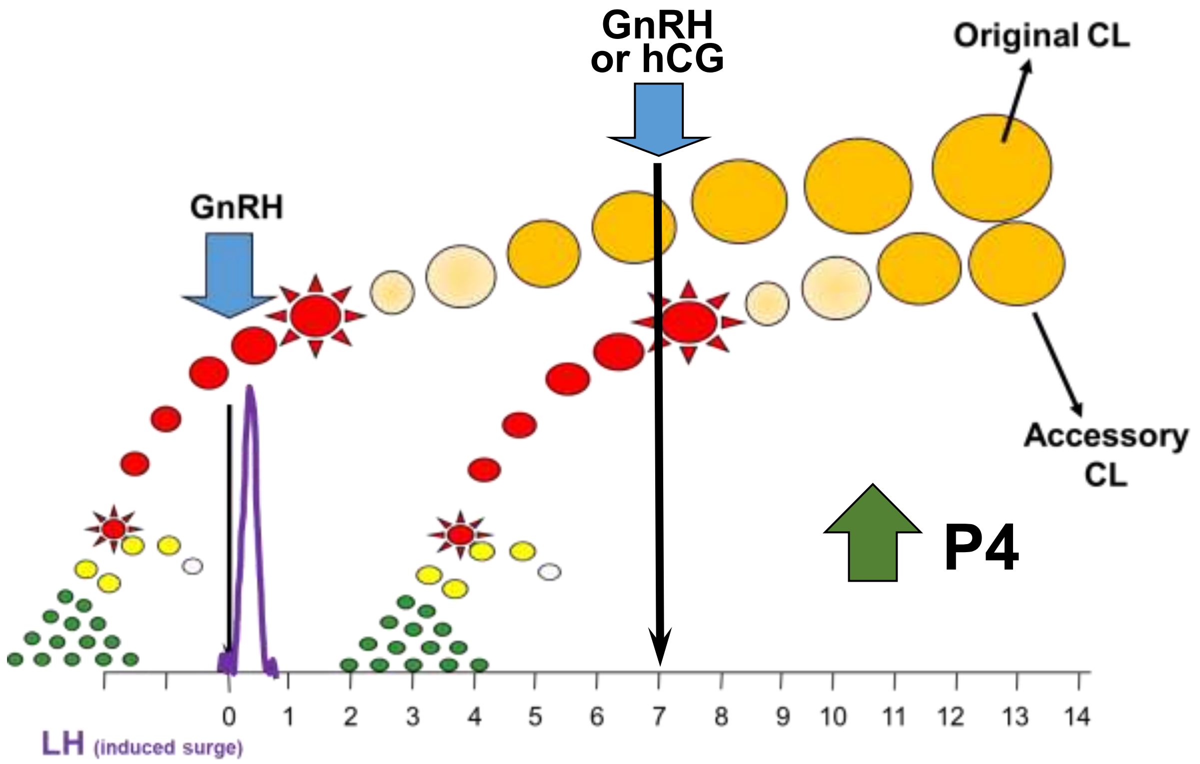




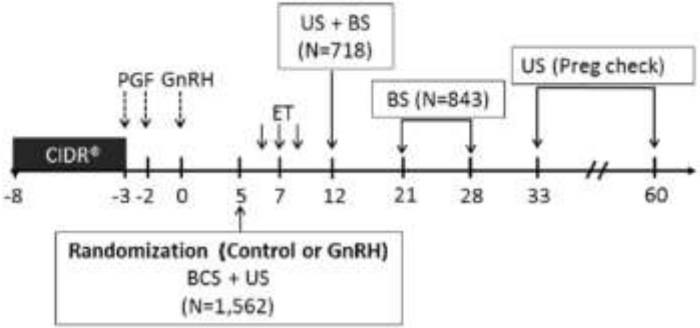
Percentages shown indicate the proportion of heifers in each category with PSPB concentrations below 2.15 ng/ml (lowest quartile).

Garcia-Guerra et al., 2016

Practical approaches for P4 supplementation



Effect of GnRH on D5 on accessory CL and serum P4



Fresh IVP embryos

GnRH = 200 ug gonadorelin acetate

Parameter	N	Control	GnRH	P-value
CL Volume D5 (mm^3)	1562	3648 ± 62	3638 ± 64	0.97
Ovulation rate D5	718	**3.3 %**	**83.9%**	<0.01
CL Volume D12 (mm^3)	718	5639 ± 120	7995 ± 151	<0.01
P4 D12 (ng/ml)	467	6.0 ± 0.1	7.2 ± 0.1	<0.01
P4 D21 (ng/ml)	504	**7.4 ± 0.2**	**9.5 ± 0.2**	<0.01

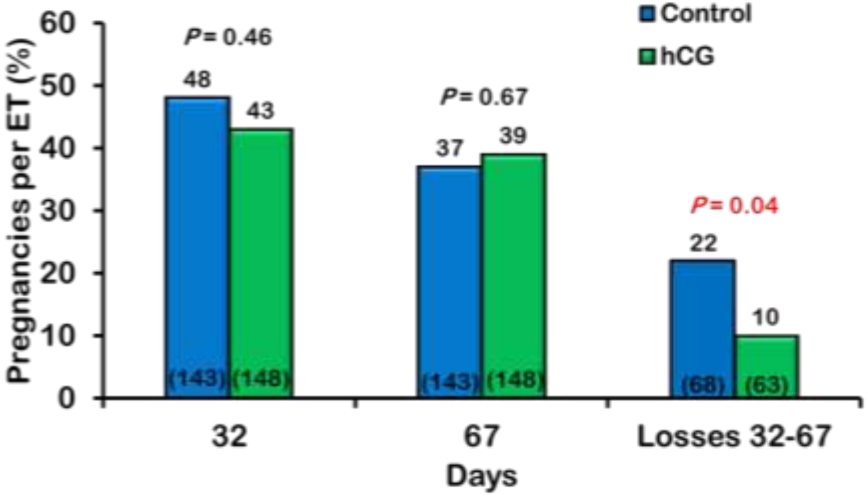
Parameter	Control	GnRH	P-value
P/ET D33	37.5% (768)	37.0% (794)	0.45
P/ET D60	28.3% (768)	30.2% (794)	0.17
Pregnancy loss (D33-60)	**24.7%** (288)	**18.4%** (294)	0.02

Garcia-Guerra et al., 2020

Effect of GnRH/hCG at ET on embryo recipients (IVP)

Parameter	Control	GnRH	P-Value
P/ET D30	52.8% (477/903)	52.3% (473/904)	0.83
P/ET D66	42.3% (382/903)	44.03% (398/904)	0.46
Pregnancy loss (D30-66)	**19.9%** (95/477)	**15.9%** (75/473)	**0.10**

E2+CIDR+ECP

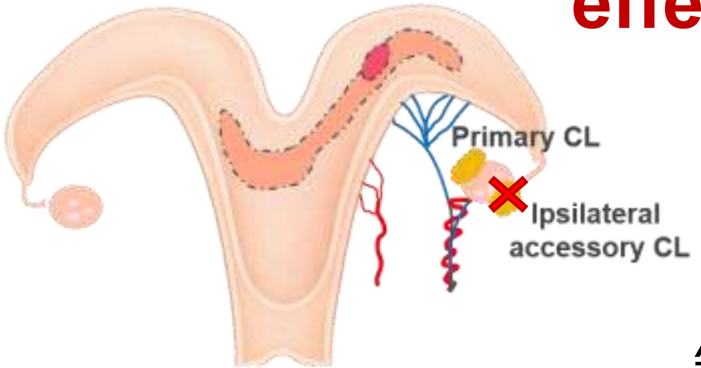


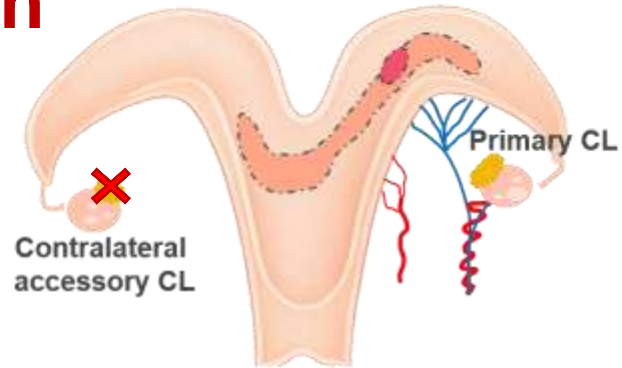
5-day CO-Synch

Munhoz et al., 2014

Niles et al., 2019

Accessory CL location: effect on CL lifespan





Accessory CL regression

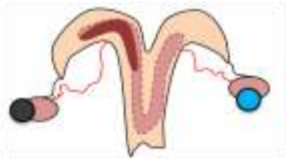
~71% of contralateral accessory CL regress between day 30 and 60

Contralateral
66.2% (130)
52.7% (148)
58.9% (158)
50.0% (20)

Effect of accessory CL location on fertility

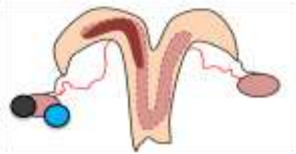
Lactating dairy cows (FTAI) – GnRH D5

Parameter	Single	Contralateral	Ipsilateral	P-value
P/AI D26	57.3% (289)	62.8% (241)	55.2% (239)	0.10
P/AI D61	49.4% (289)	53.8% (241)	51.5% (239)	0.31
Pregnancy loss (D26-61)	**13.7%[A]** (164)	**14.0%[A]** (148)	**6.6%[B]** (130)	0.06



Contralateral

Vs.



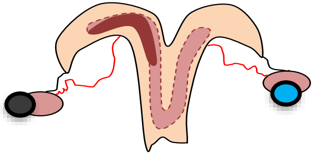
Ipsilateral

Dairy heifers (FTET) – GnRH D5

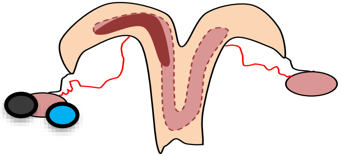
Parameter	Single	Contralateral	Ipsilateral	P-value
P/ET D33	40.3% (340)	42.5% (139)	37.6% (173)	0.67
P/ET D60	28.8% (340)	30.9% (139)	31.2% (173)	0.83
Pregnancy loss (D33-60)	**28.5%** (137)	**27.1%** (59)	**16.9%** (65)	0.21

Monteiro et al., 2021 / Garcia-Guerra and Wiltbank, unpublished

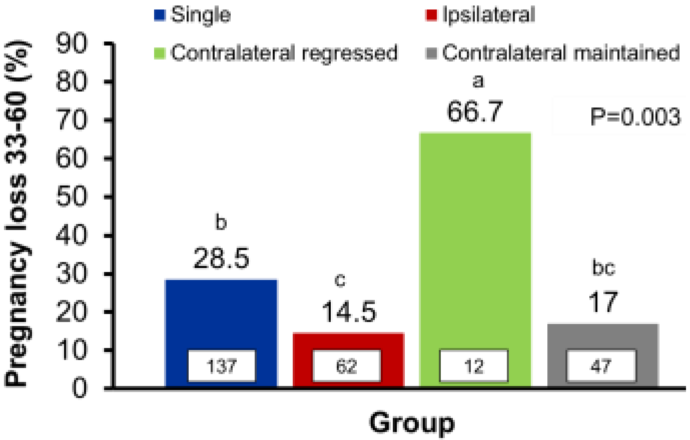
Effect of accessory CL location on fertility



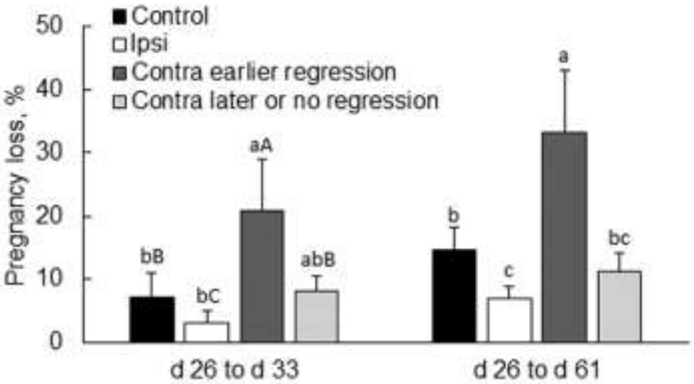
	Early accessory CL regression	
Group	FTET	FTAI
Contralateral	20.3%^{A} (59)	16.2%^{A} (148)
Ipsilateral	4.6%^{B} (65)	0%^{B} (130)



Holstein heifers (FTET)



Lactating Holstein cows (FTAI)

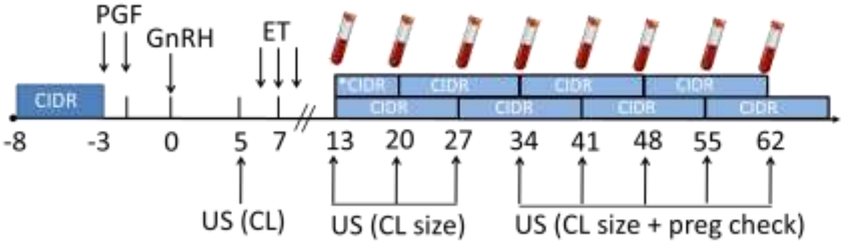


Garcia-Guerra and Wiltbank, unpublished

Monteiro et al., 2021

Progesterone supplementation may allow us to rescue pregnancies with suboptimal placentation

Pregnancy loss: embryo/fetal death or CL regression?



Embryonic death = No embryo or Embryo w/o heartbeat

CL regression = CL absence + Embryo with heartbeat

Wiltbank et al., 1956

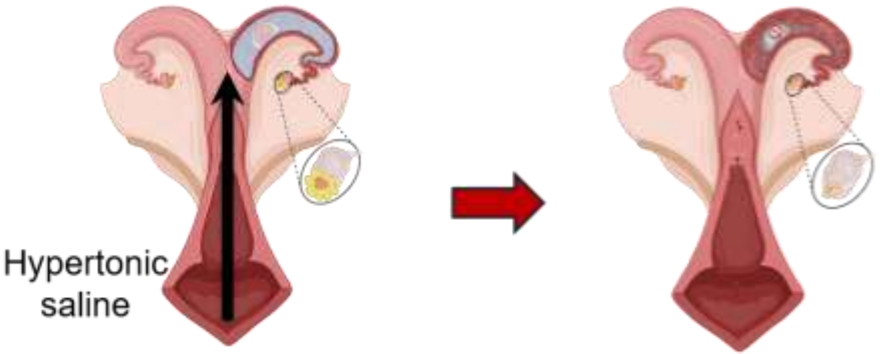
Why is the CL regressing?

How is the CL maintained after the classical MRP (IFNT) period ?

Induced pregnancy loss model (2nd month)

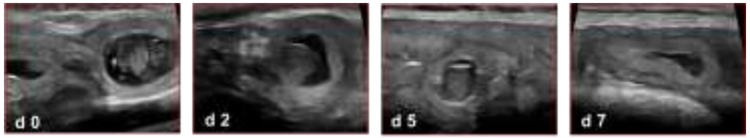
Day 35 of pregnancy

Conceptus demise (< 30 min)



Luteolysis
~ 7-8 days later

Induced conceptus demise

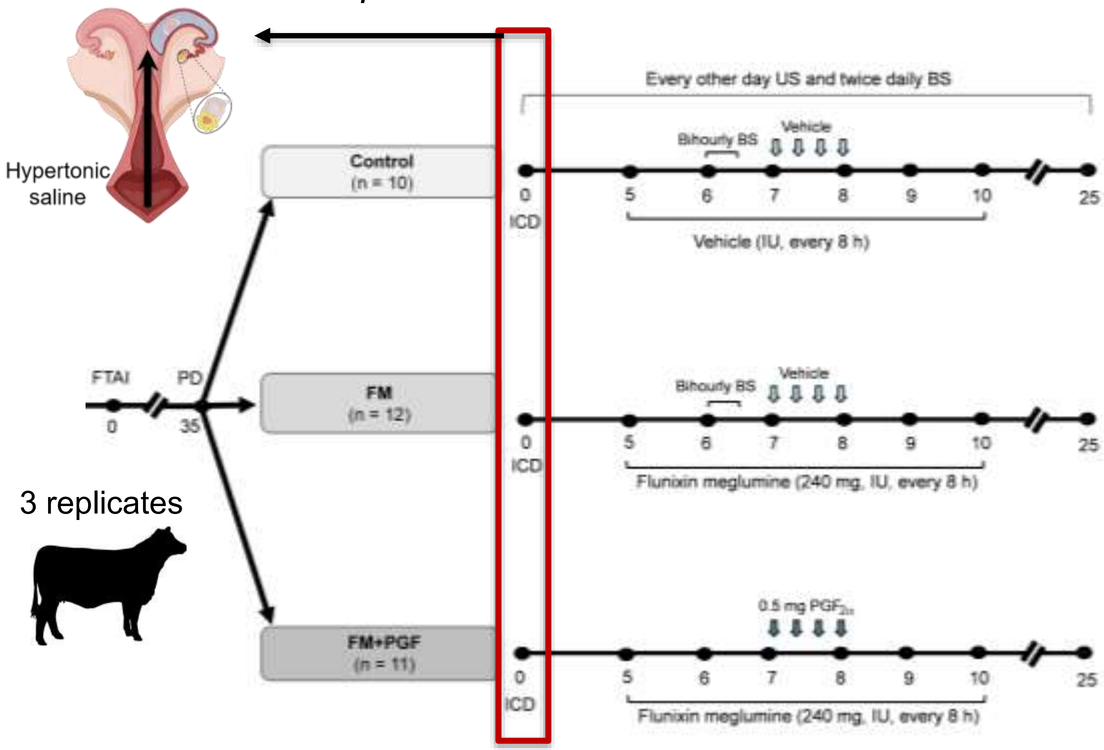


Is PGF2α responsible for CL regression following conceptus demise?

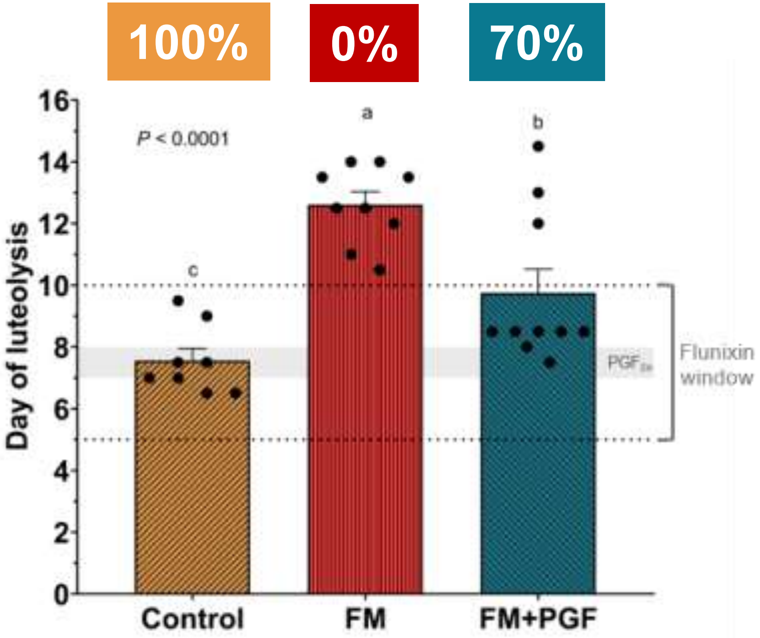
Duran et al., 2024

Is PGF2α responsible for luteolysis after ICD?

Induced conceptus demise



Luteolysis during treatment period

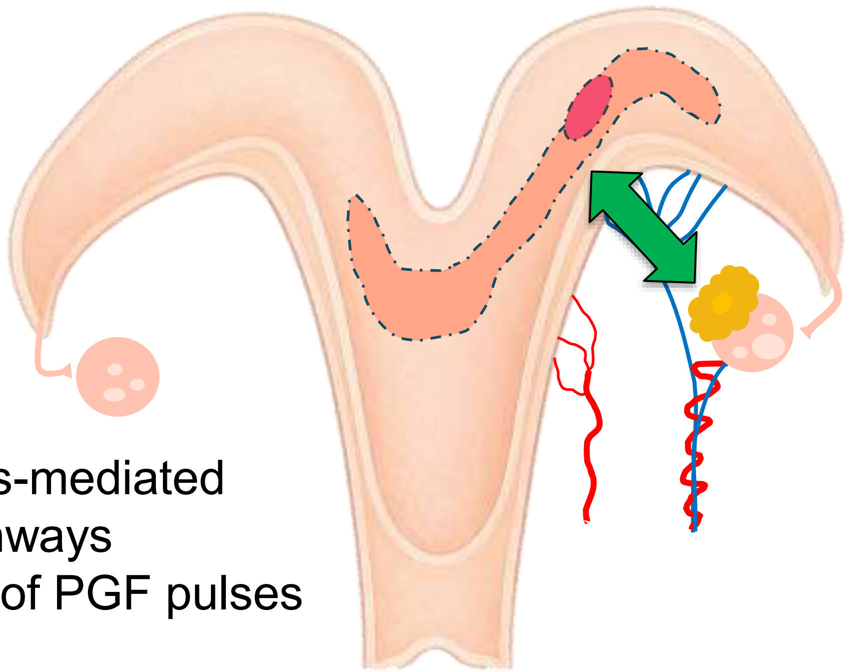


Yes!

FTAI = fixed-time artificial insemination, PD = pregnancy diagnosis, ICD = induced conceptus demise, US = ultrasonography, IU = intrauterine, BS = blood sampling

Folchini et al., 2026

Proposed mechanisms for CL maintenance after classical MRP (2nd month)



- Conceptus-mediated
- Local pathways
- Presence of PGF pulses
- ~~IFNT~~

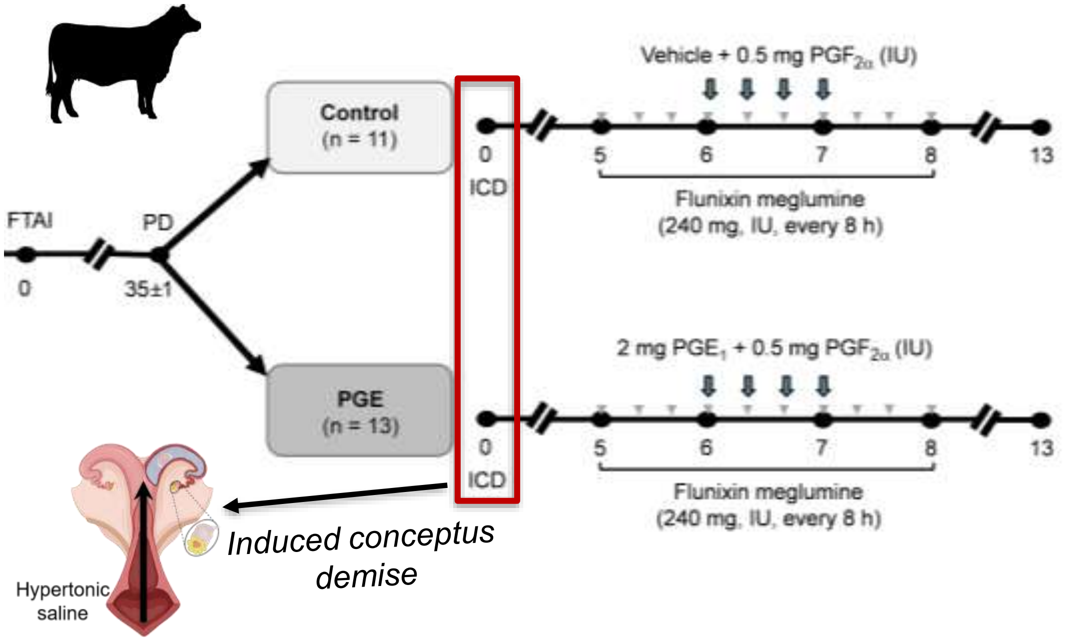
Luteoprotective factors

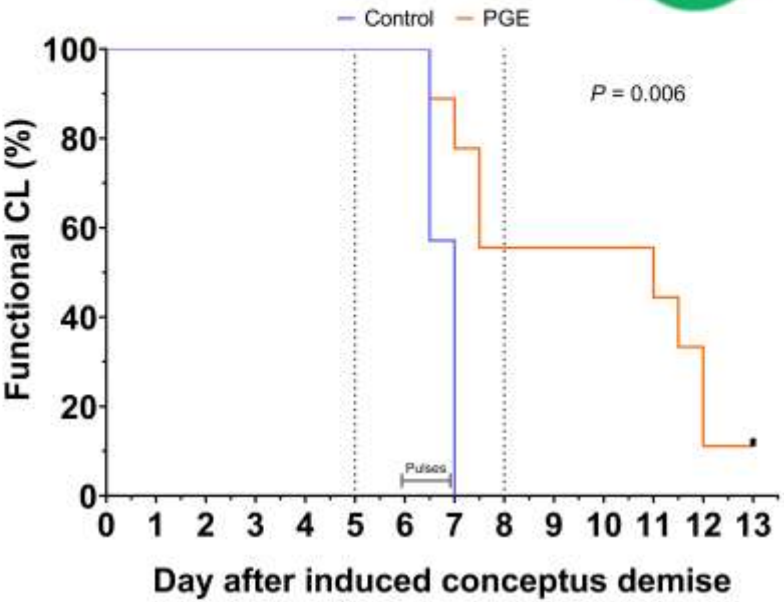
- Prostaglandin E (PGE)
- PAGs *(Martino-Duarte et al., 2026)*

Wiltbank et al., 2023

Can PGE extend luteal lifespan after ICD (2nd month)?

Yes!

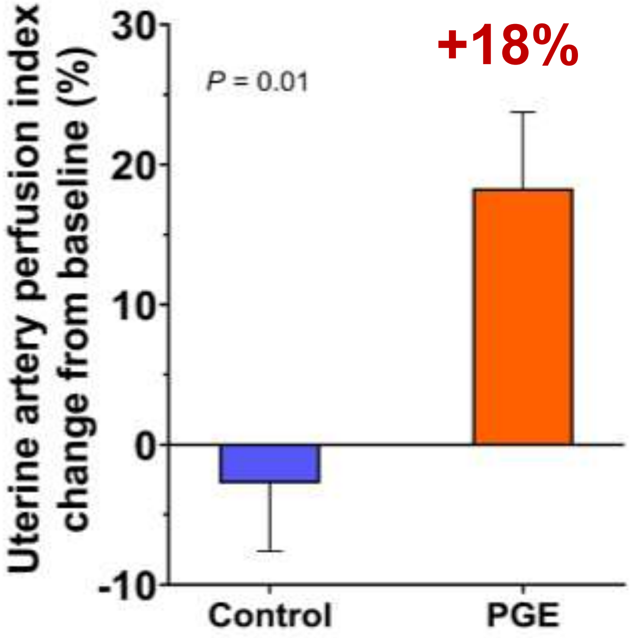


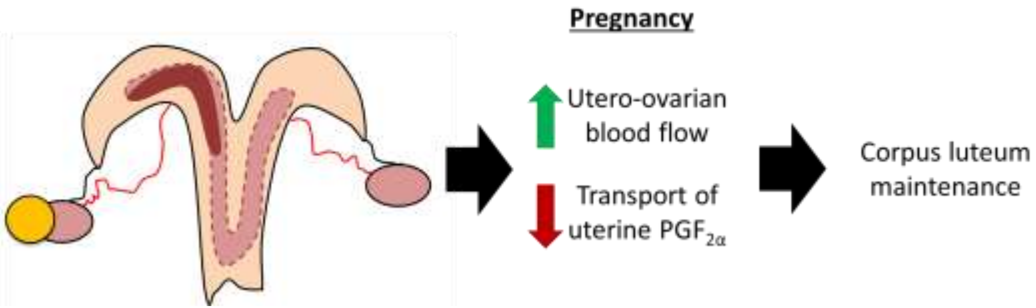


FTAI = fixed-time artificial insemination, PD = pregnancy diagnosis, ICD = induced conceptus demise, IU = intrauterine

Folchini et al., 2026

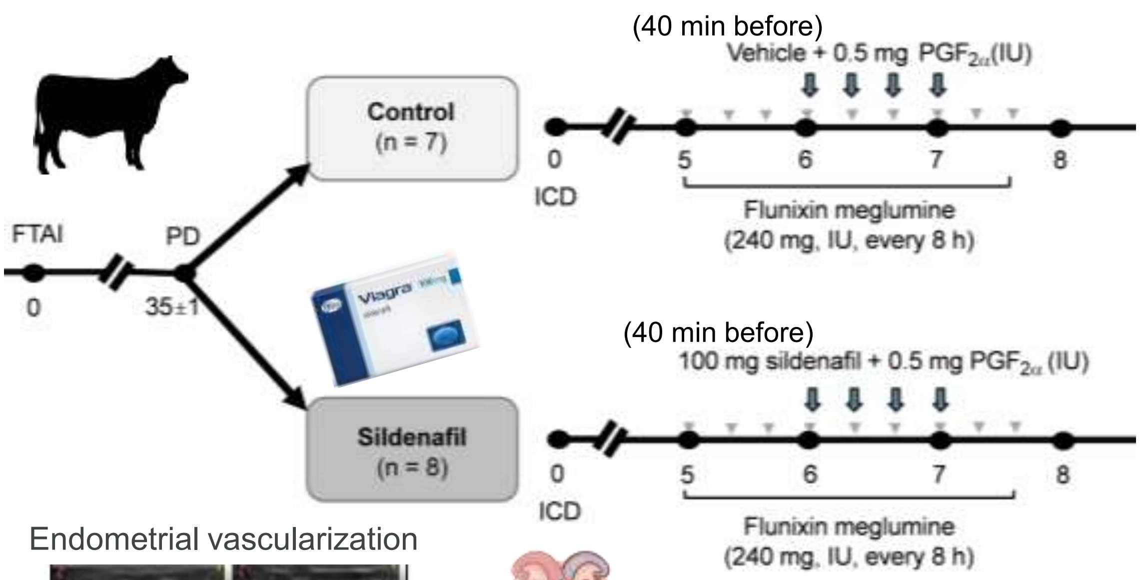
Uterine blood flow after PGE administration

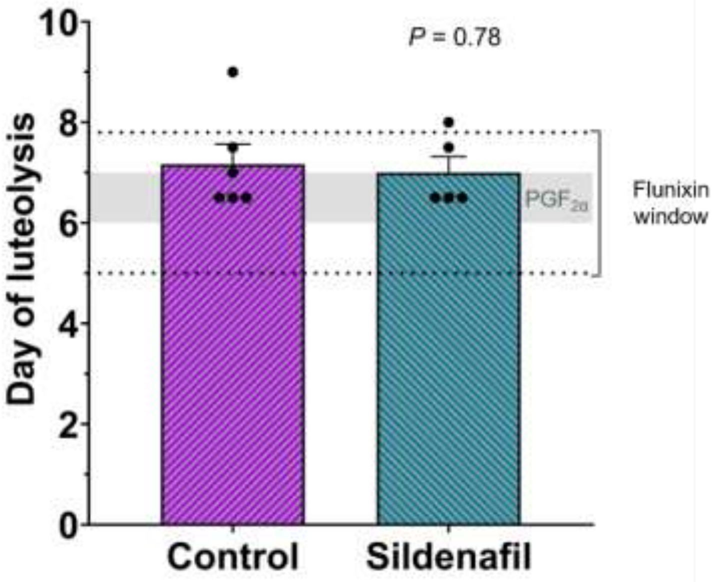




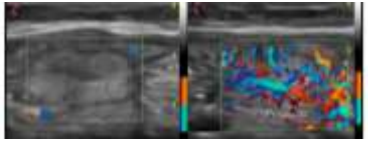
Is the luteoprotective effect of PGE mediated by enhanced uterine perfusion?

Can increased uterine blood flow extend luteal lifespan?





Endometrial vascularization

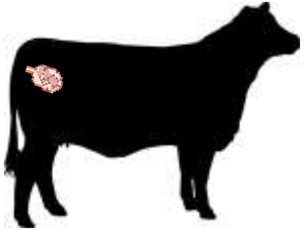


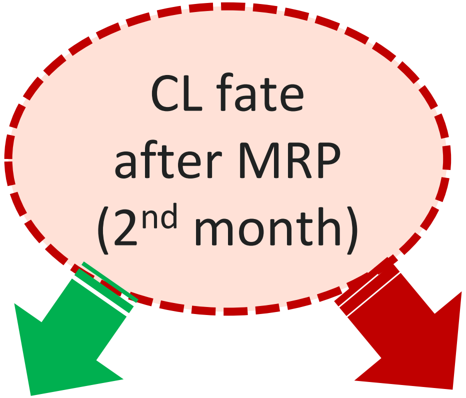
No!

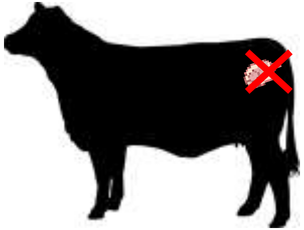
FTAI = fixed-time artificial insemination, PD = pregnancy diagnosis, ICD = induced conceptus demise, IU = intrauterine

Folchini et al., 2026

Conclusions







<u>Luteoprotective mechanism:</u>
Conceptus-mediated
Local (Ipsilateral)
Presence of $PGF_{2\alpha}$

Candidates: PGE – PAGs?

Pregnancy maintenance

Disruption of luteoprotective mechanism

Pulsatile PGF secretion

CL regression

Pregnancy loss

THE OHIO STATE UNIVERSITY

Understanding Fertility Through Pregnancy Loss

- Pregnancy losses are informative
- Timing matters
- Understanding physiology creates opportunities

Failure at any checkpoint = pregnancy loss

The key to reducing pregnancy loss is understanding which checkpoint failed, and why it failed.

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theriogenology foundation
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USDA National Institute of Food and Agriculture
U.S. DEPARTMENT OF AGRICULTURE
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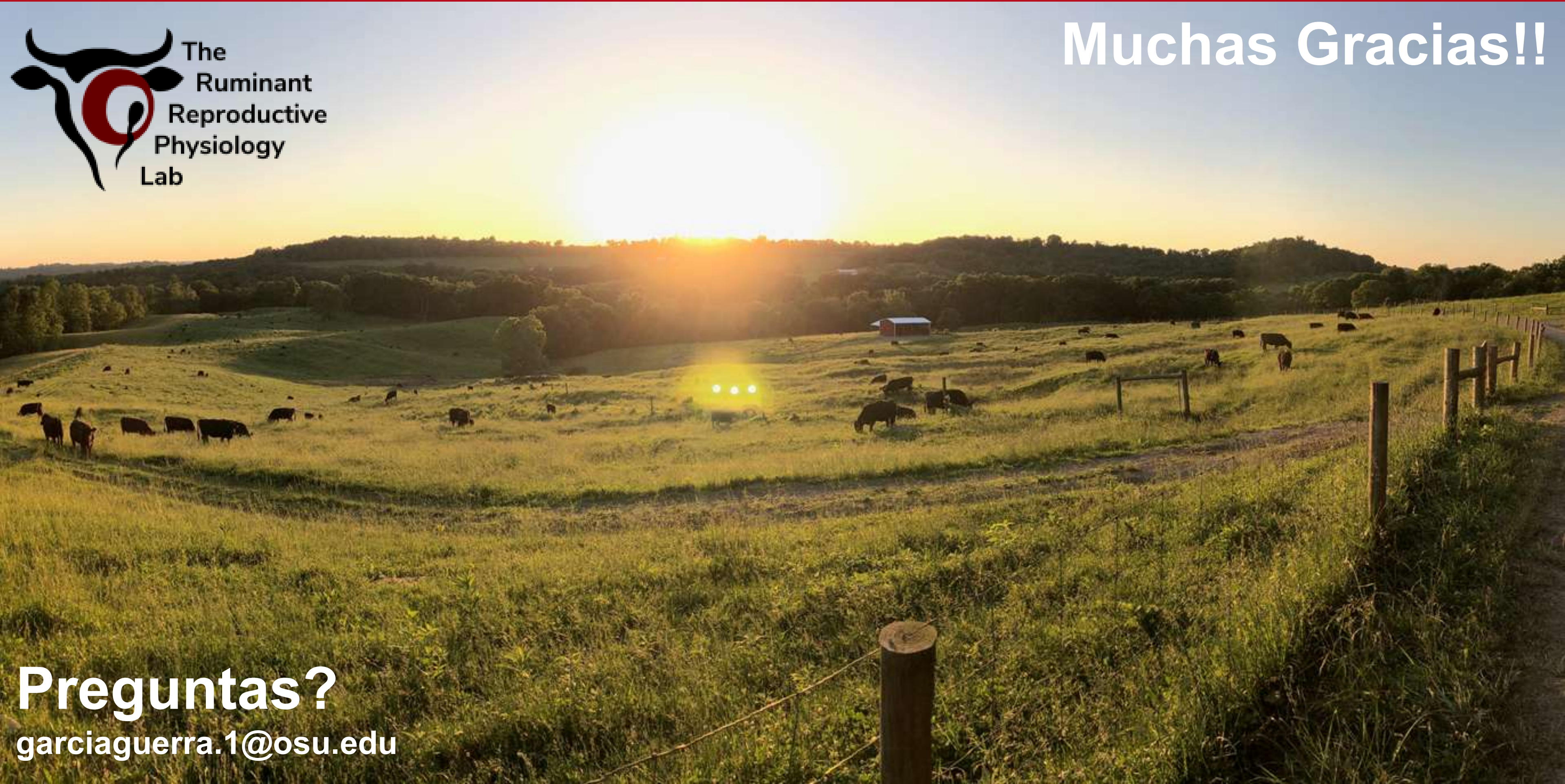
The Ruminant Reproductive Physiology Lab

Muchas Gracias!!



Preguntas?

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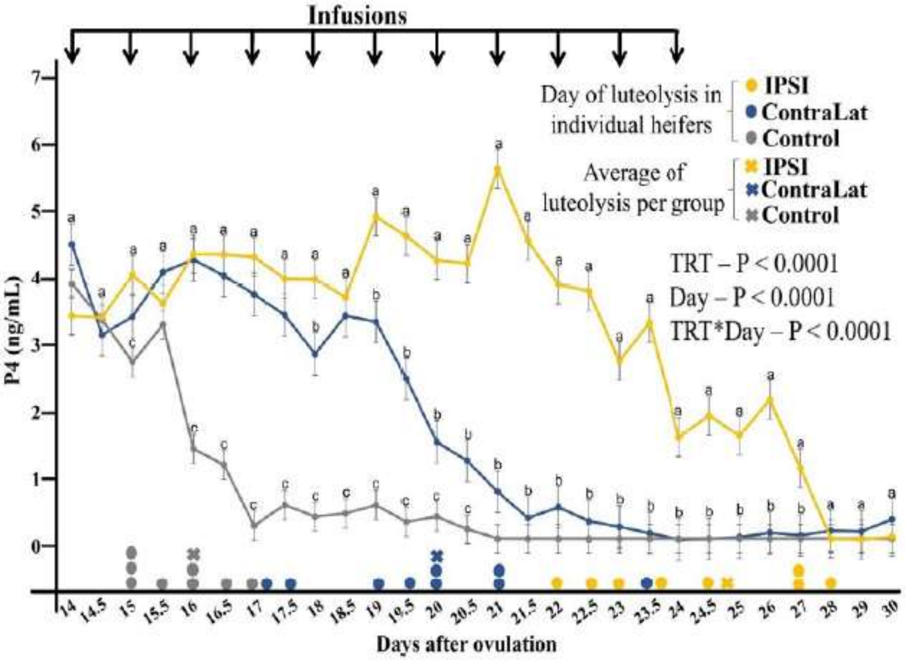
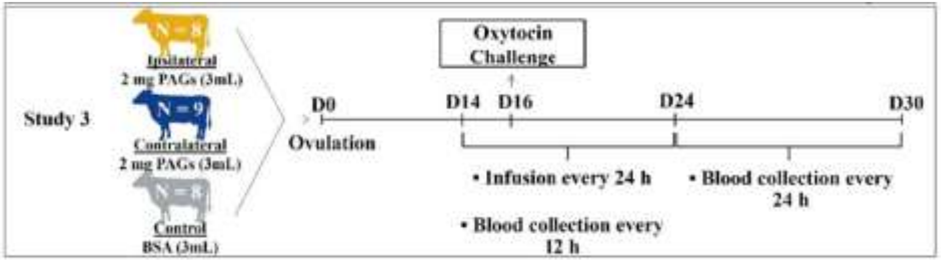
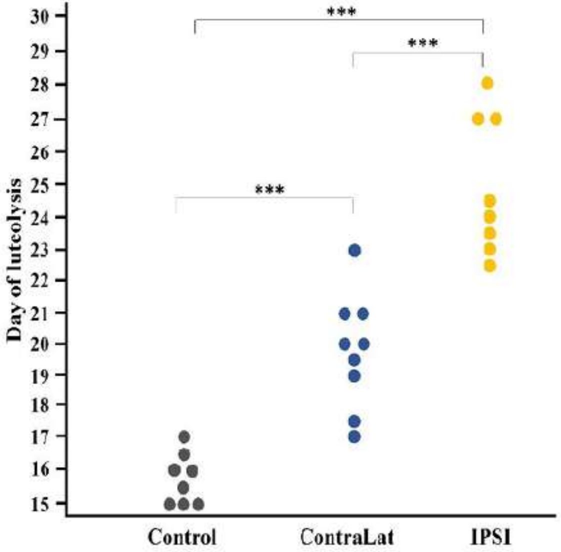
Evidence for a role of PAGs in CL maintenance







Martino-Duarte et al., 2026

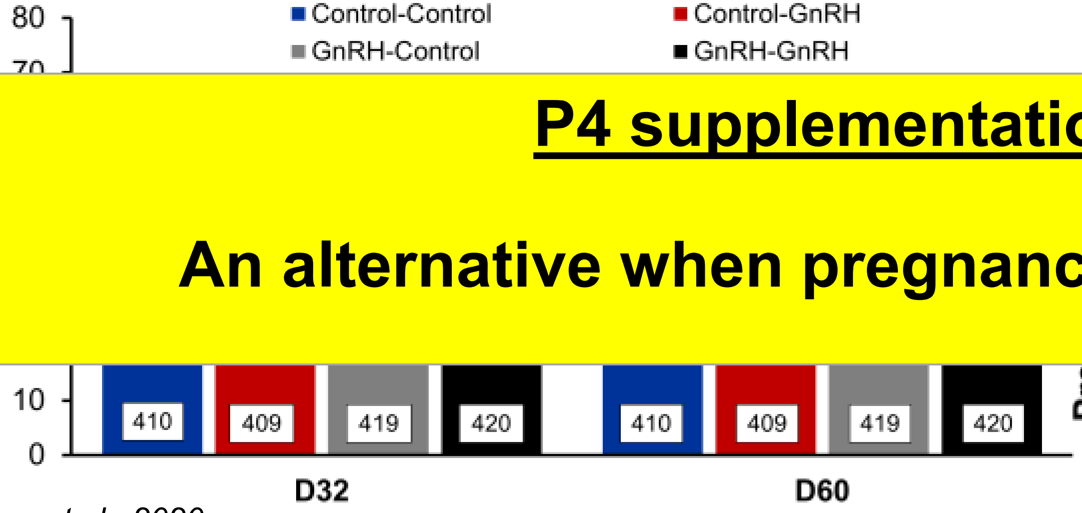
Effect of GnRH on D5 on Fertility

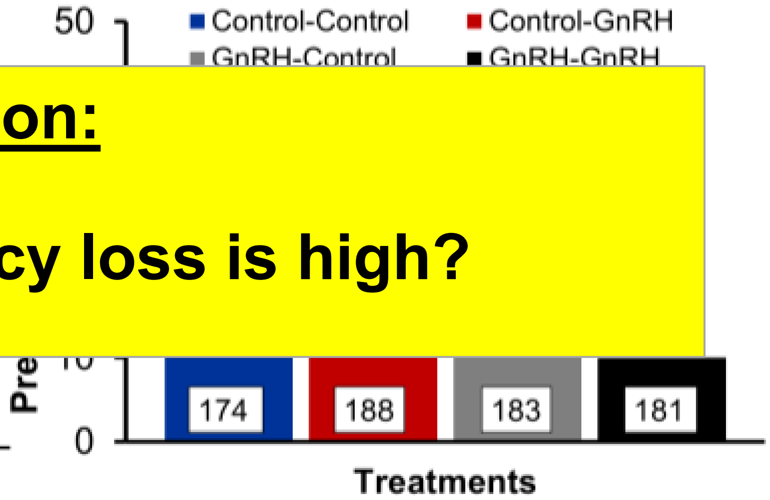
Embryo Stage	Group	P/ET d33, % (n)	P/ET d60, % (n)	Pregnancy loss, % (n)
6 (Blastocyst)	Control	**28.6%** (374)	**22.7%** (374)	**20.6%^{AC}** (107)
	GnRH	**30.8%** (357)	**23.5%** (357)	**23.6%^{AC}** (110)
7 (Expanded blastocyst)	Control	**45.9%** (394)	**33.5%** (394)	**27.1%^{BC}** (181)
	GnRH	**42.1%** (437)	**35.7%** (437)	**15.2%^{A}** **(184)**
P-value	Stage	<0.01	<0.01	0.23
	GnRH	0.45	0.18	0.08
	Interaction	0.30	0.67	**0.04**

Effect of GnRH treatment at Day 7 and/or 21 on P/ET

- Holstein Heifers (n=1,660)
- Fresh IVP embryos
- 2x2 factorial

Treatment Day 7	Treatment Day 21	Ovulation D7 (n)	Ovulation D21 (n)*	P4 D28 (ng/ml)
Control	Control	1.7%[A] (410)	0.5%[A] (188)	7.1 ± 0.5[C]
	GnRH	3.6%[A] (409)	**39.0%**[B] (205)	8.3 ± 0.5[BC]
GnRH	Control	**72.3%**[B] (417)	0.7%[A] (200)	9.0 ± 0.6[AB]
	GnRH	**67.1%**[B] (420)	**36.5%**[B] (200)	9.7 ± 0.5[A]





P4 supplementation:

An alternative when pregnancy loss is high?

Duran et al., 2020