



Double Vision

Management of Twinning in Dairy Cows

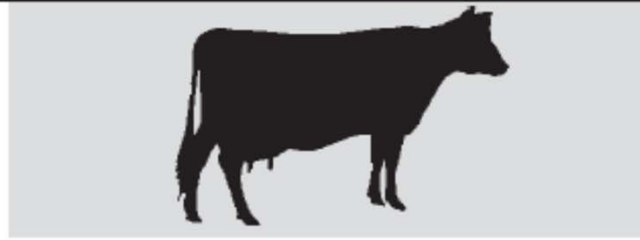
Paul M. Fricke, Ph.D.

Professor of Dairy Science
University of Wisconsin-Madison



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2001



*R*EVIEW: Twinning in Dairy Cattle

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Risk Factors

Genetics

Breed

Season

Ovarian cysts

Parity

Milk production

Management Strategies



Outline

- **Twinning Trend across Time**
- **Mechanism of Twinning**
- **Endocrinology of Twinning**
- **Identification of Cows Carrying Twins**
- **Strategies for Managing Twinning**

Outline

- **Twinning Trend across Time**

J. Dairy Sci. 90:1255–1264

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An Observational Analysis of Twin Births, Calf Sex Ratio, and Calf Mortality in Holstein Dairy Cattle

N. Silva del Río,* S. Stewart,† P. Rapnicki,† Y. M. Chang,* and P. M. Fricke*¹

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†Department of Veterinary Population Medicine, University of Minnesota, St. Paul 55108



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Data set description

Calving records	Herds	Cows
2,318,601	4,123	1,088,926

85% of herds had <100 calving events per year

Range = 11 to 1,877

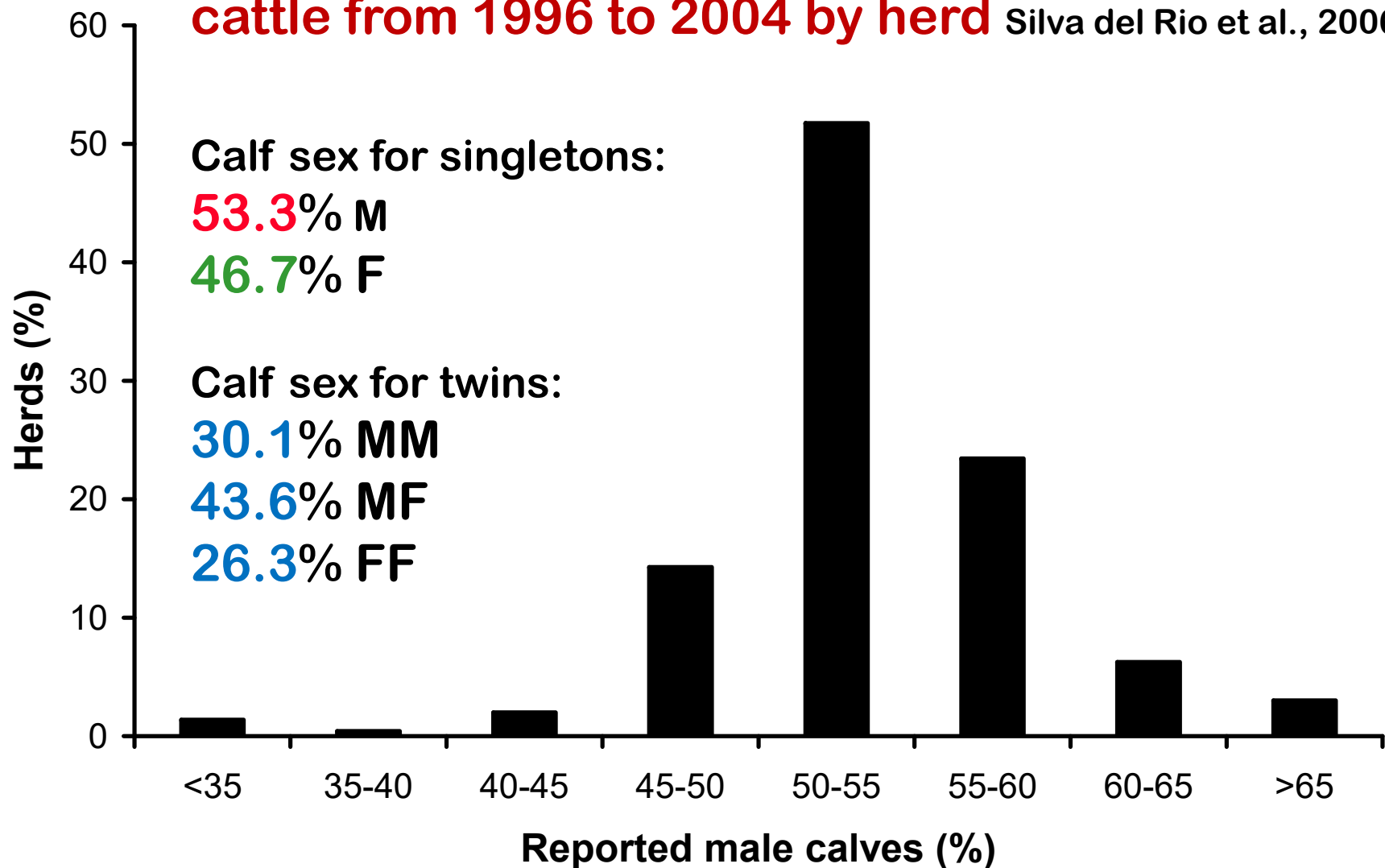
Twin calvings:

96,222

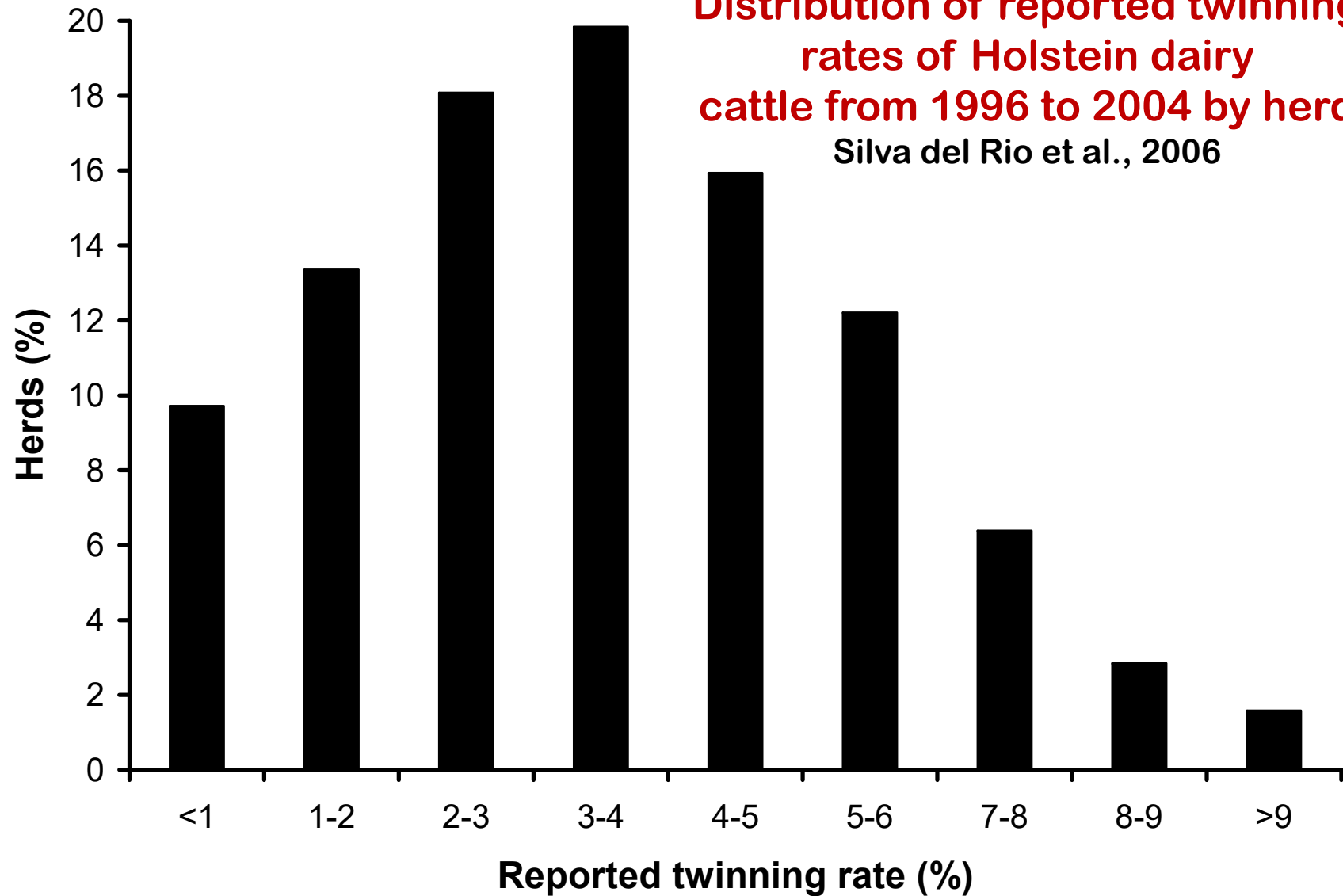
4.1% twining rate

Distribution of male calves born from Holstein dairy cattle from 1996 to 2004 by herd

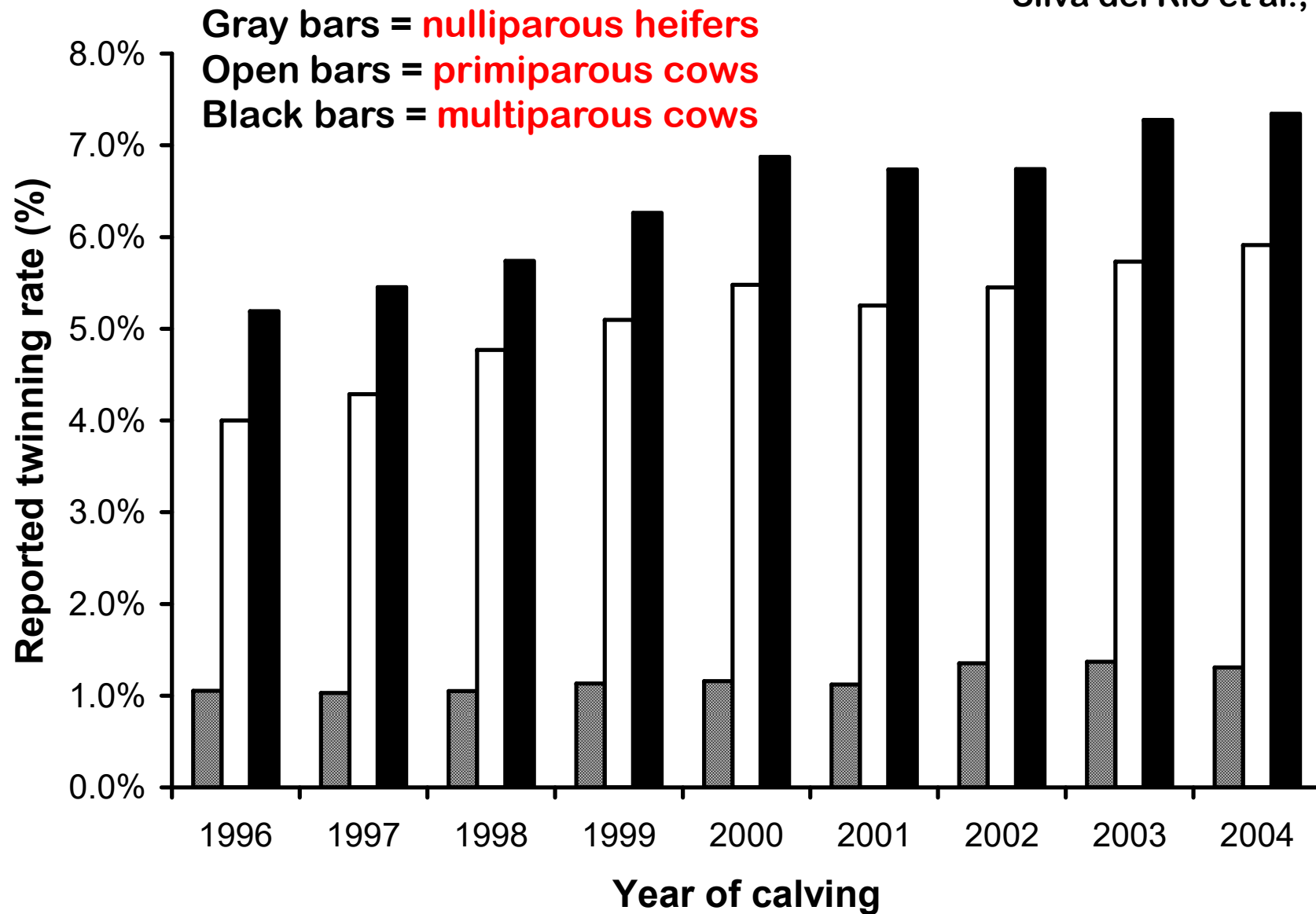
Silva del Rio et al., 2006



**Distribution of reported twinning
rates of Holstein dairy
cattle from 1996 to 2004 by herd**
Silva del Rio et al., 2006



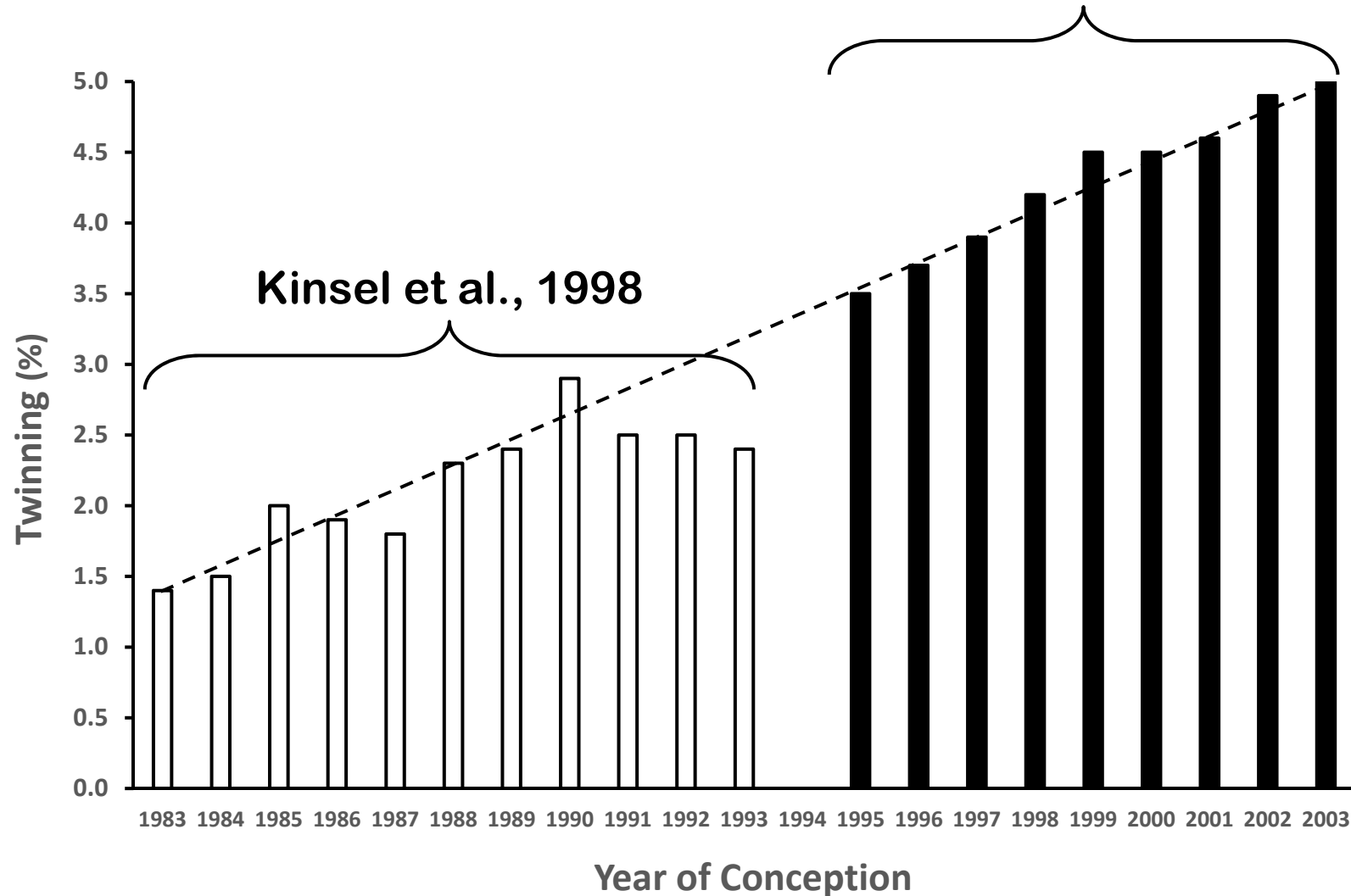
Silva del Rio et al., 2006



Twining by time in Holsteins

(1983 to 2004)

Silva del Rio et al., 2006



Negative Impacts of Twinning



Increased average days open and services per conception during the subsequent lactation

Increased risk for retained placenta, dystocia, metritis displaced abomasum, and ketosis

Increased risk of culling



Abortion, stillbirth, neonatal calf mortality, and reduced birth weight are greater for calves born as twins than calves born as singletons

Reduced gestation length

Increased incidence of dystocia

Twinning & Replacement Heifers

Reference	Replacement heifers per calving event (%)	
	Singleton	Twin birth
Silva del Rio et al., 2007	43.3	42.9
Nielen et al., 1989	48.0	42.0
Day et al., 1995	42.2	29.2

The effect of twinning on available replacement heifers is **NOT** a consequence of Freemartinism

Freemartinism



- The earliest developmental abnormalities of the female reproductive tract resulting in freemartinism occur between **49** to **52** d after fertilization.
- About **92**% of heifer calves born co-twin with bull calves are freemartins; thus, about **8**% of heifers born co-twin with bull calves are fertile.

Freemartinism & Replacements



100 Singleton Births

50 Bull calves

50 Heifer calves

50 fertile Heifers



100 Twin Births

50 Bull – Heifer pairs

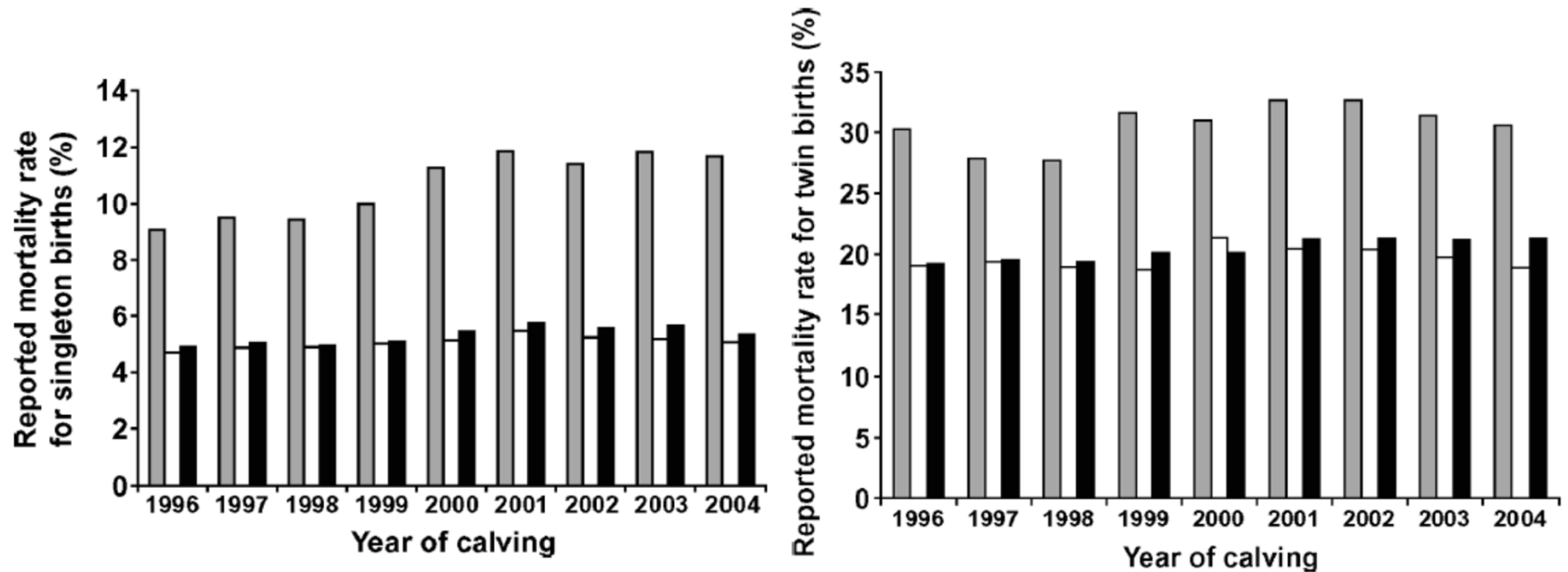
25 Bull – Bull pairs

25 Heifer – Heifer pairs

50 fertile Heifers

Calf mortality of Holstein dairy cattle calving singletons vs. twins from 1996 to 2004

Silva del Rio et al., 2006; J. Dairy Sci. 90:1255-1264.

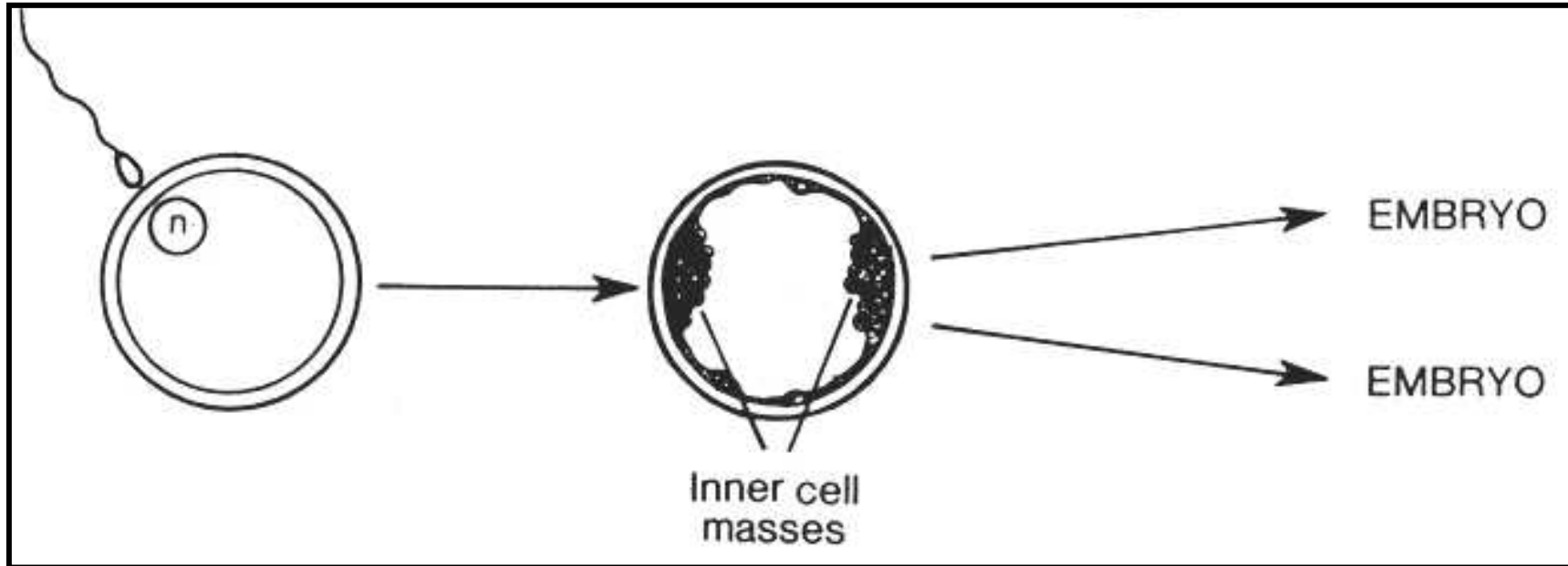


Gray bars = nulliparous heifers; Open bars = primiparous cows; Black bars = multiparous cows

Outline

- **Twinning Trend across Time**
- **Mechanism of Twinning**

Monozygous (identical) Twinning



Mathematical estimates of monozygotic twinning using Bonnier's equation:

7.4⁰% of all twin births (Erb & Morrison, 1959; J. Dairy Sci. 42:512)

13.6⁰% of all twin births (Ryan & Boland, 1991; Theriogenology 36:1)

Less than **0.3⁰**% of all births



Available online at www.sciencedirect.com



Theriogenology

Theriogenology 66 (2006) 1292–1299

www.journals.elsevierhealth.com/periodicals/the

Observed frequency of monozygotic twinning in Holstein dairy cattle

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Received 27 January 2006; accepted 6 April 2006

Ear biopsies were collected from **107** sets of Holstein twins from 6 Wisconsin dairies.

40 MF twins; **29 MM** twins; **38 FF** twins

DNA from ear biopsies from the **67** same-sex twins was PCR amplified for 5 polymorphic microsatellite DNA markers.



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Frequency of monozygotic (MZ) twinning determined empirically or estimated mathematically

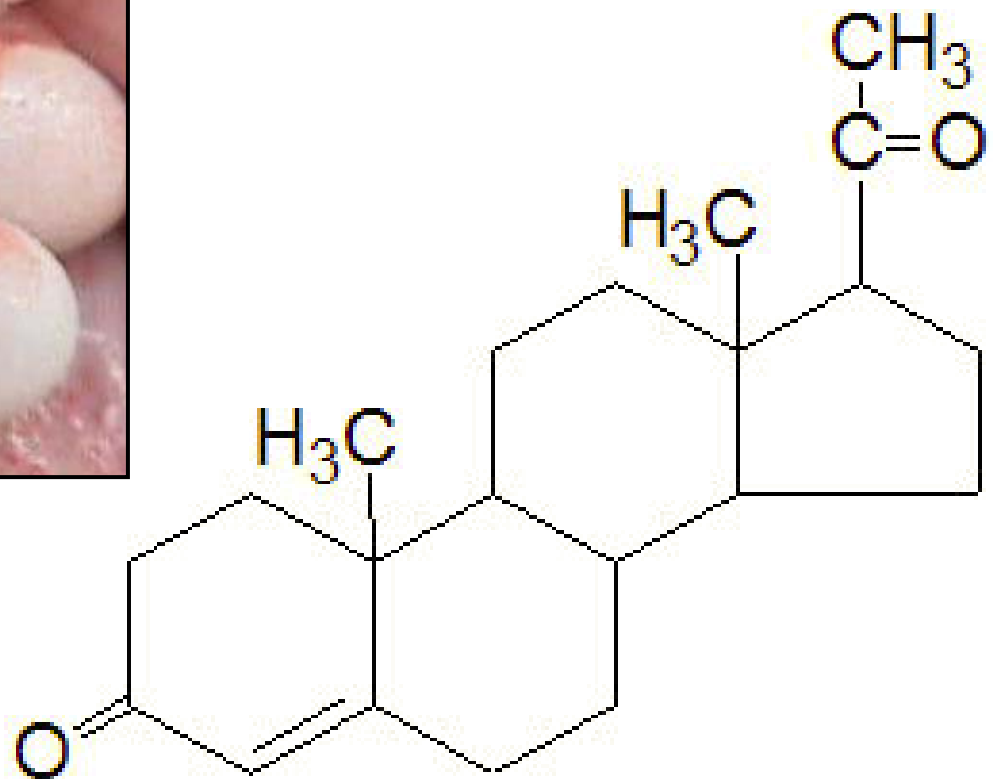
Silva Del Rio et al., 2006; Theriogenology 66:1292

Classification		Empirical		Mathematical
		DZ	MZ	MZ
	n	% (n)	% (n)	%
MM twins	29	86 (25)	3 (1)	-
FF twins	38	97 (37)	14 (4)	-
All same-sex	67	93 (62)	8 (5)	39.5
Opposite-sex	40	100 (40)	-	-
All twins	107	95 (102)	5 (5)	24.7

Outline

- Twinning Trend across Time
- Mechanism of Twinning
- Endocrinology of Twinning

Progesterone



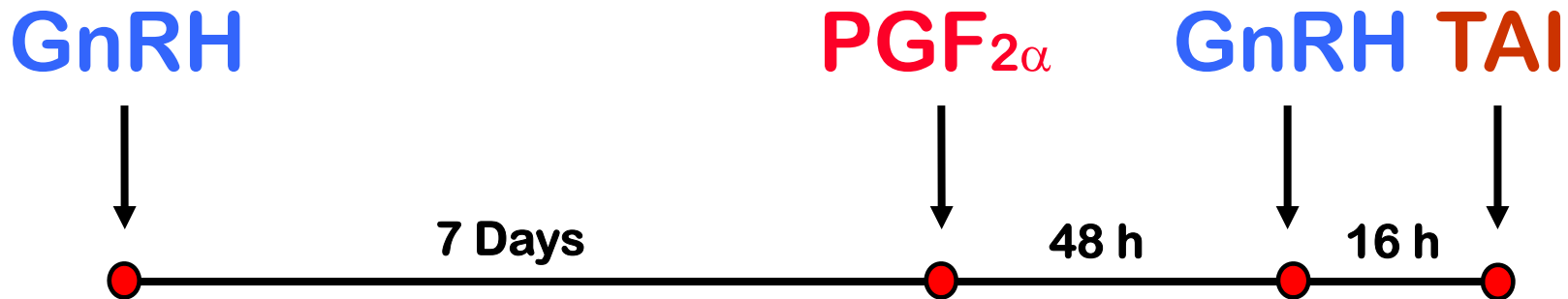
Response of cycling and anovular cows to 21 d estrus detection

Gumen et al., 2003. Dairy Sci. 86:3184

Item	Cycling	Anovular	<i>P</i>
AI during 21 d, % (n)	72 (135)	29 (31)	0.0001
Cows with no ov, % (n)	11 (135)	58 (31)	0.0001
Cows with a Dbl Ov, % (n)	16 (120)	38 (13)	0.044
Ovulation but no estrus, % (n)	19 (120)	85 (13)	0.0001

Ovsynch

Pursley, Mee, & Wiltbank, 1995
Theriogenology 44:915



Incidence of Anovulation at First AI

19 Different studies summarized
in Bamber et al., 2009

23.3% (n = 5,818)

6 Different studies summarized
in Wiltbank et al., 2006

26.1% (n = 2,783)

8 Different studies summarized
in Bisinotto et al., 2010

22.6% (n = 5,607)

Stevenson et al., 2008

25.5% (n = 1060)

Veira-Neto et al., 2014

22.2% (n = 1,569)

Colazo et al., 2013

17.6% (n = 608)

Herlihy et al., 2012

24.7% (n = 373)

Total

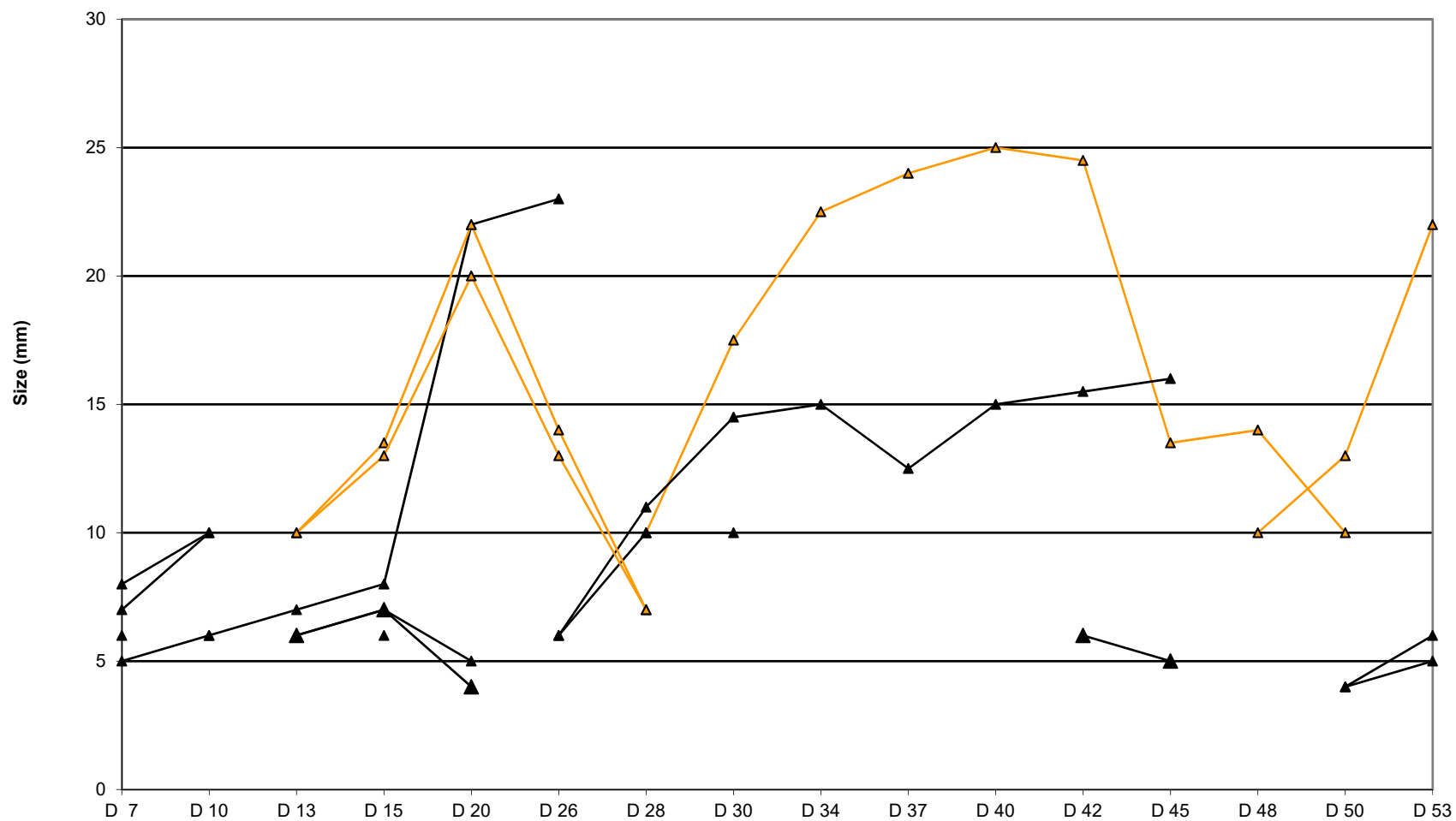
23.2% (n = 16,651)

Ovulatory responses of cycling and anovular cows to Ovsynch

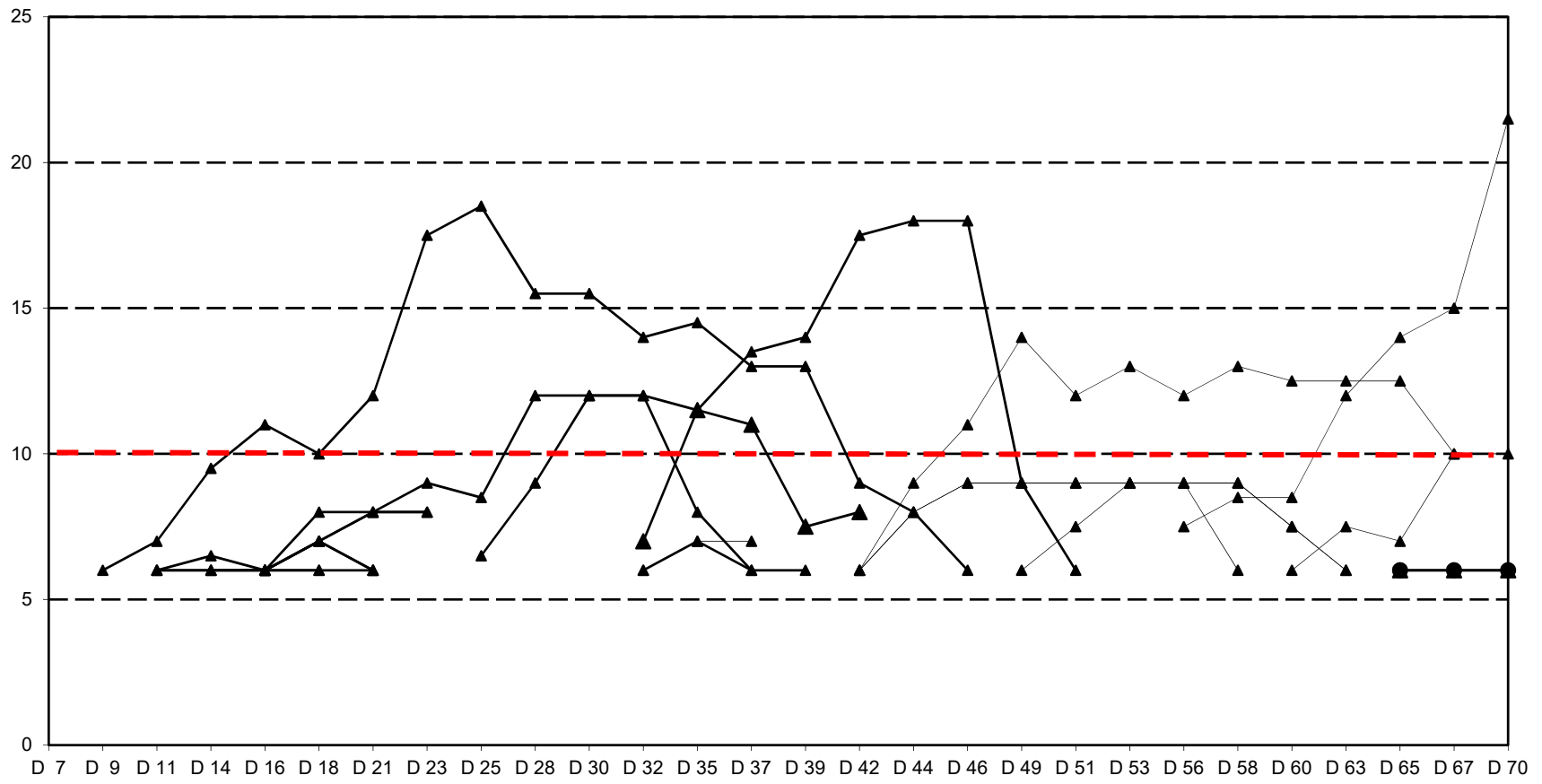
Gumen et al., 2003; J. Dairy Sci. 86:3184.

Item	Cycling	Anovular	<i>P</i>
Ovulation to G1, % (n)	62 (117)	88 (33)	0.004
No CL regression to PGF, % (n)	5 (117)	3 (33)	0.614
Ovulation to G2, % (n)	97 (117)	94 (33)	0.323
Dbl Ov to G1, % (n)	4 (72)	41 (29)	0.0001
Dbl Ov to G2, % (n)	12 (114)	13 (31)	0.926
Short luteal phase, % (n)	6 (114)	23 (31)	0.006

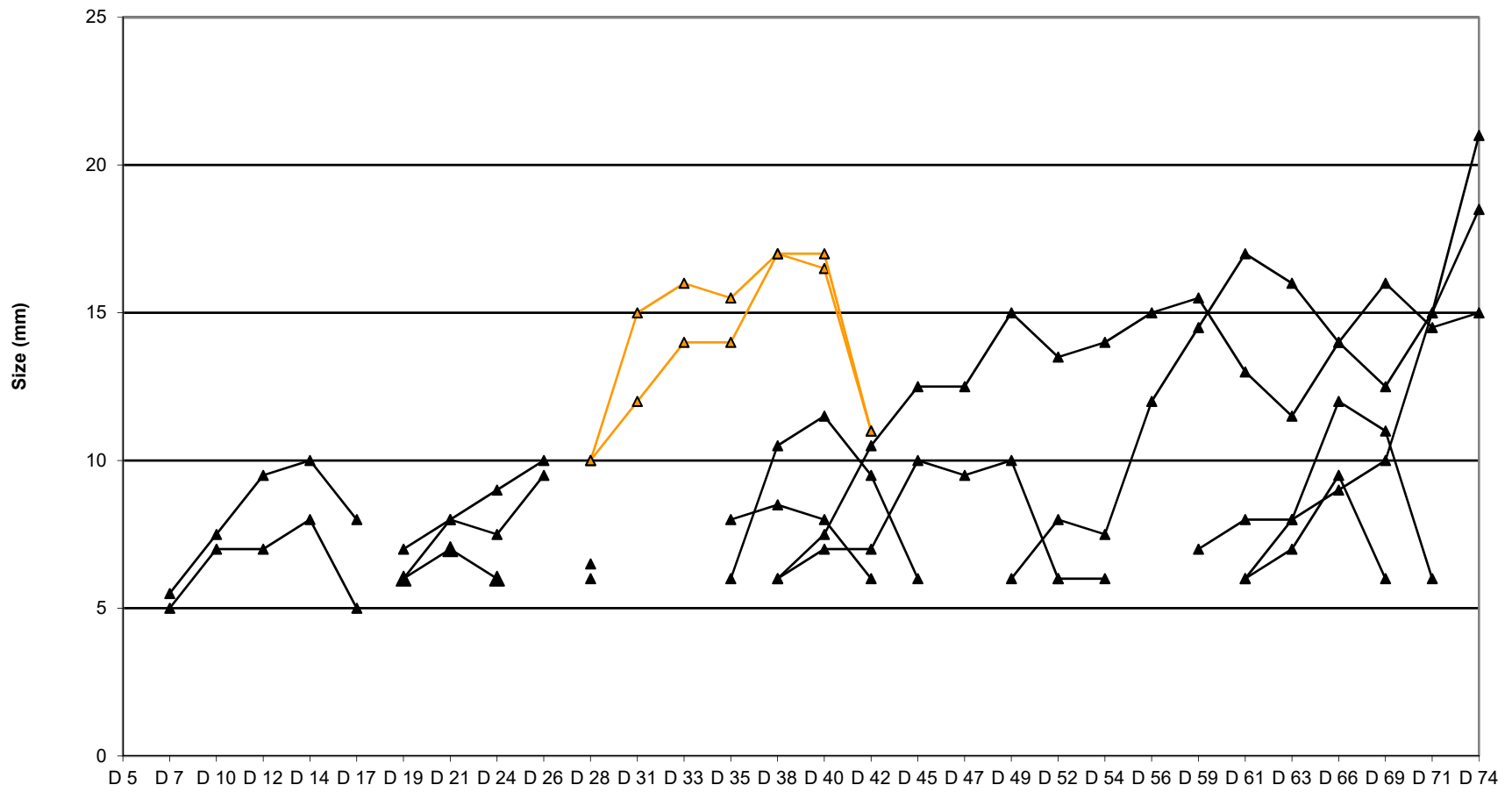
5193 Short Luteal Phase



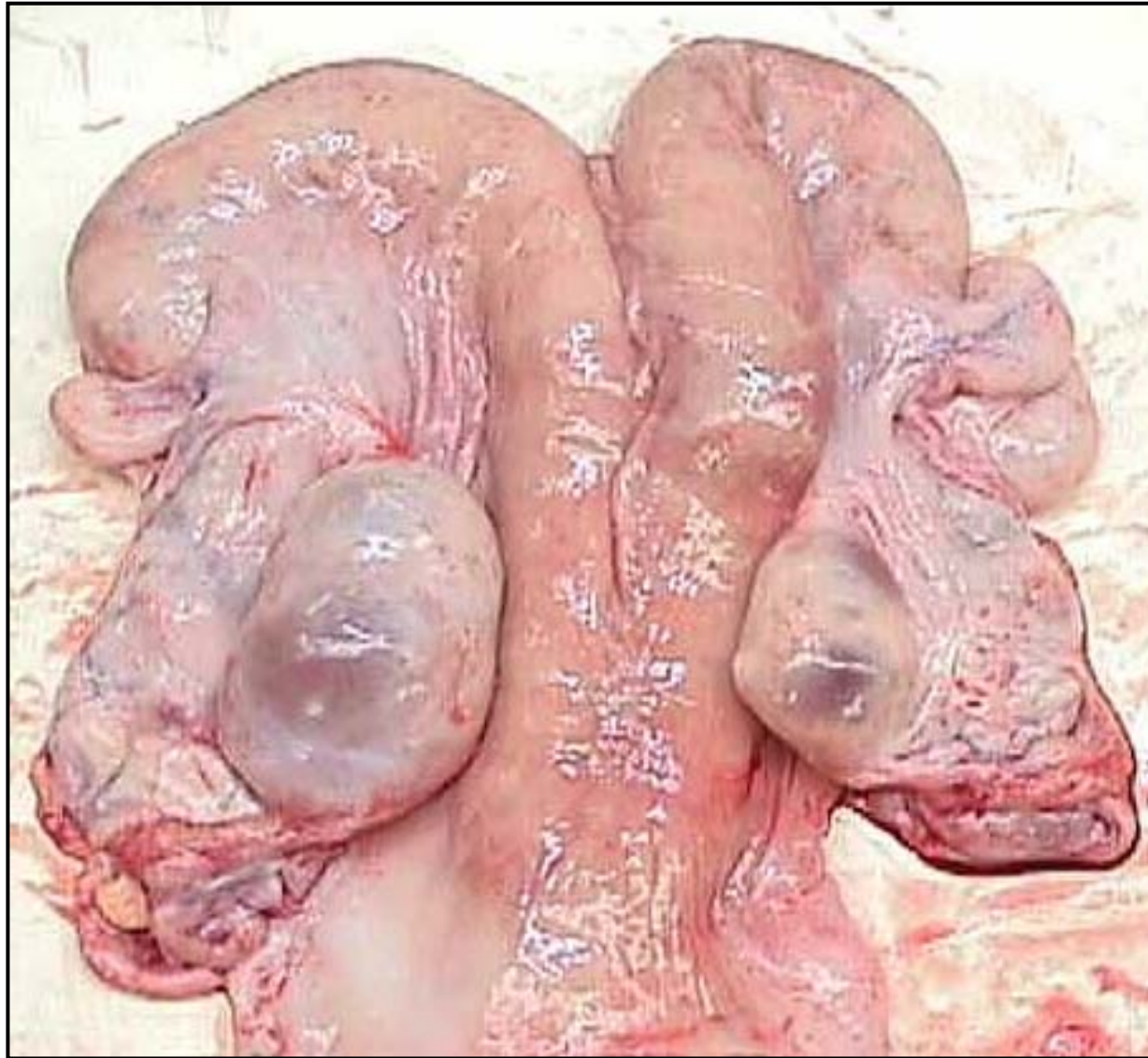
4689 Anovular



4779 Anovular after 1st ovulation



Ovarian Cysts



Effect of breeding method on incidence of double ovulation in Holstein dairy cows

Silva del Rio et al., 2009; Theriogenology 71:1462-1471.

Variable	n	%	Odds ratio	95% CI	P-value
Ovsynch	58/250	23	0.298	0.169 - 0.523	0.003
Estrus	62/293	21	0.307	0.173 – 0.545	0.007
Cystic Ovsynch	36/73	49	-	-	-

Breeding method did NOT affect pregnancy loss in this analysis

Effect of Milk Production on Incidence (%) of Double-Ovulation

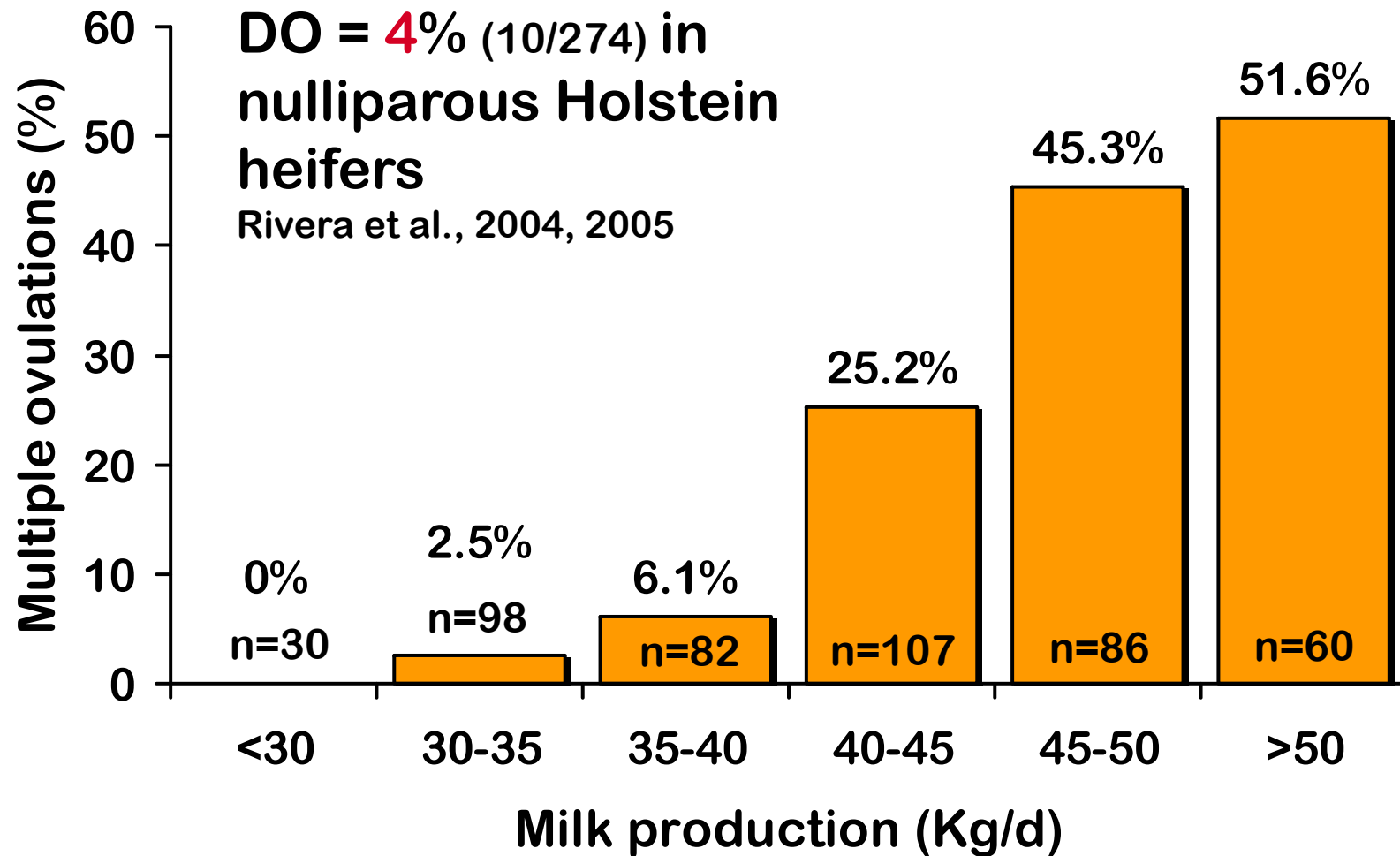
Fricke & Wiltbank, 1999; Theriogenology 52:1133

Item	≤ 40 kg/d	> 40 kg/d	Overall
Milk (kg/d)	31.1 ± 0.7	50.7 ± 0.7	40.5 ± 0.8
Parity 1	7	22	10 ^x
Parity 2	4	14	11 ^y
Parity 3	9	28	20 ^z
Overall	7 ^a	20 ^b	13
	(7/102)	(19/94)	(26/196)

^{a,b}Differ ($P < 0.01$) ^{x,y,z}Linear increase ($P = 0.09$)

Effect of Milk production on Multiple Ovulation Rate

Lopez et al., J. Dairy Sci. 88:2783; 2005

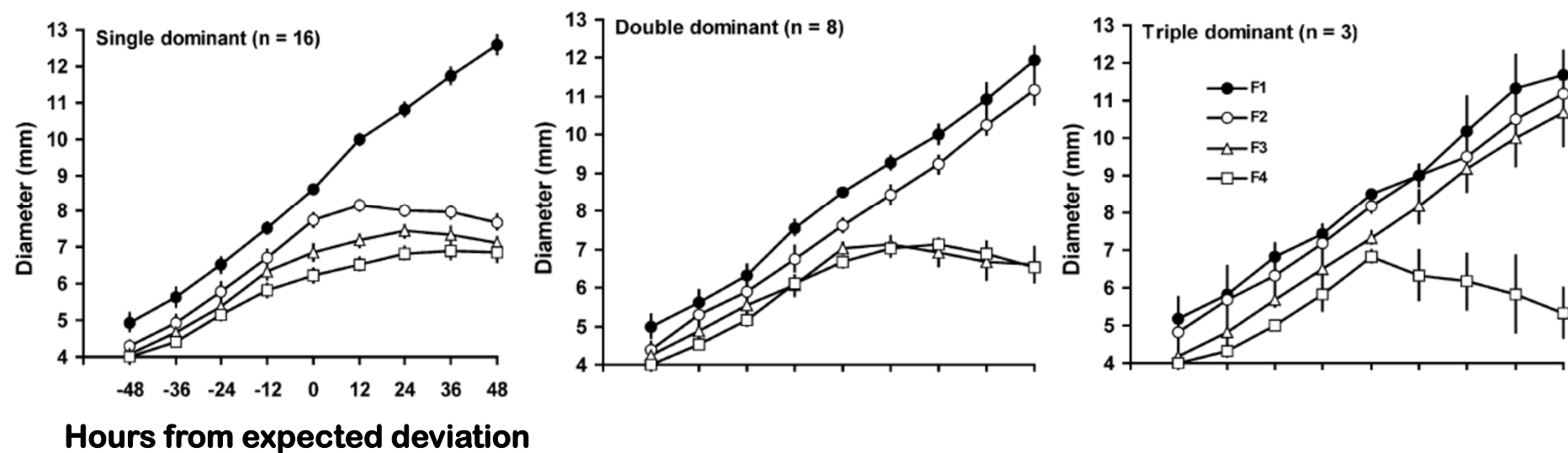


Reproductive Hormones and Follicular Growth During Development of One or Multiple Dominant Follicles in Cattle¹

Hernando Lopez,³ Roberto Sartori,^{3,5} and Milo C. Wiltbank^{2,3,4}

Department of Dairy Science³ and Endocrinology-Reproductive Physiology Program,⁴ University of Wisconsin, Madison, Wisconsin 53706

Embrapa Genetic Resources and Biotechnology,⁵ Brasília, DF 70770-900, Brazil

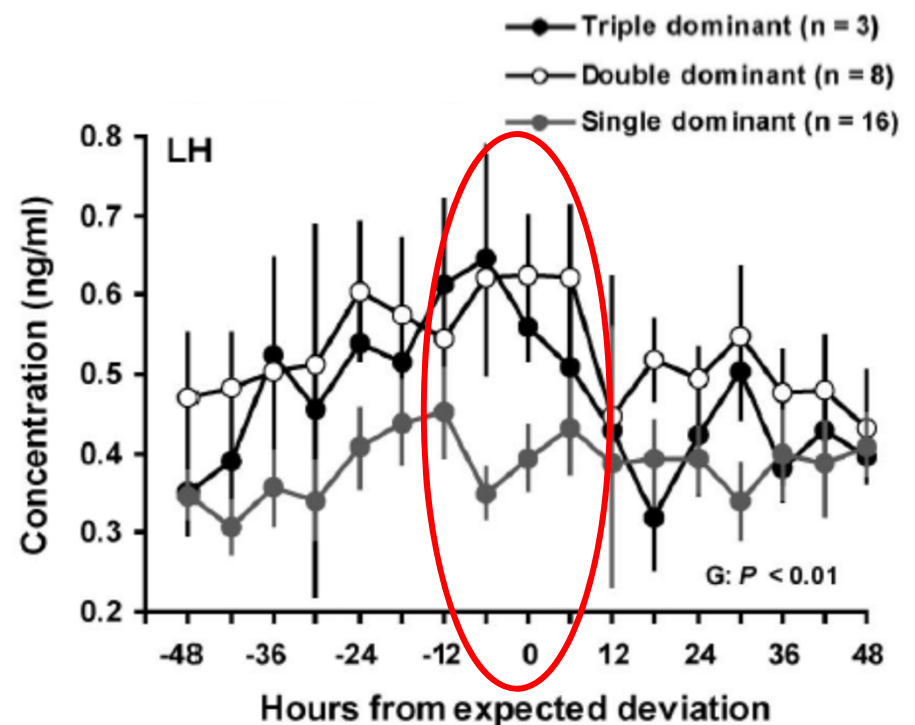
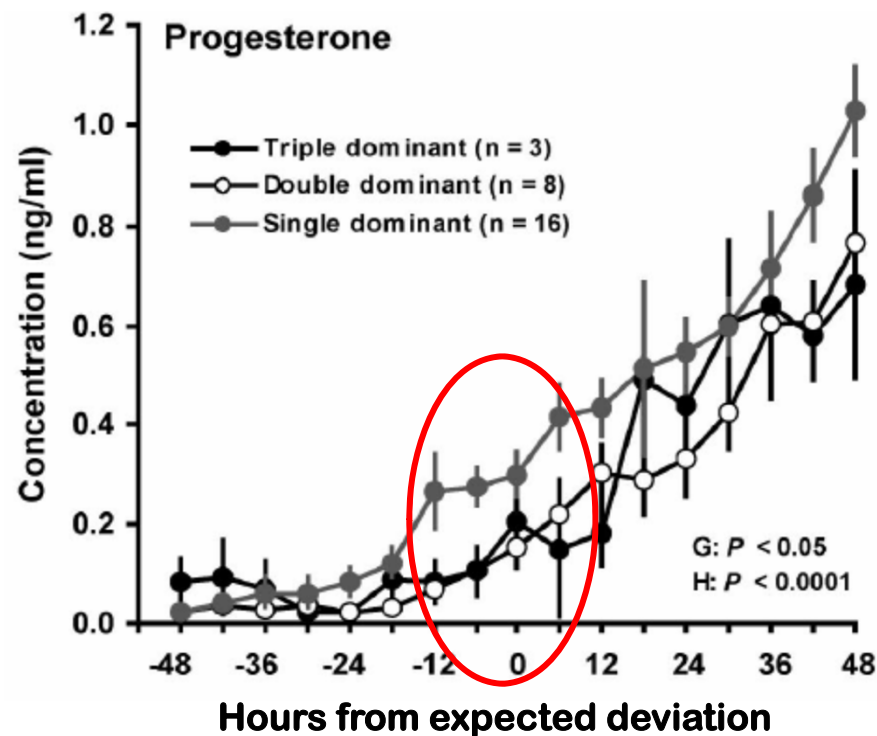


Reproductive Hormones and Follicular Growth During Development of One or Multiple Dominant Follicles in Cattle¹

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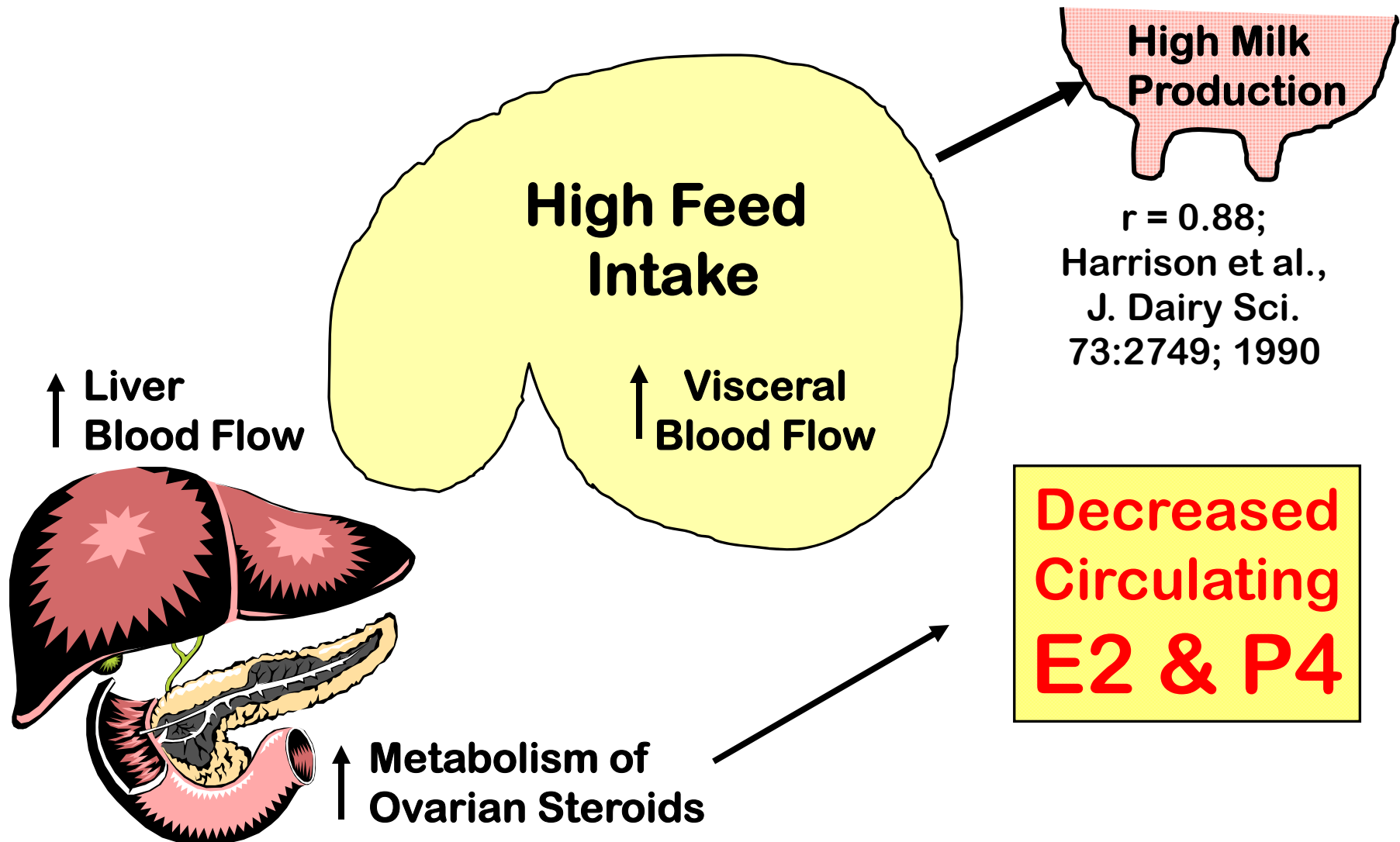
Department of Dairy Science³ and Endocrinology-Reproductive Physiology Program,⁴ University of Wisconsin, Madison, Wisconsin 53706

Embrapa Genetic Resources and Biotechnology,⁵ Brasília, DF 70770-900, Brazil

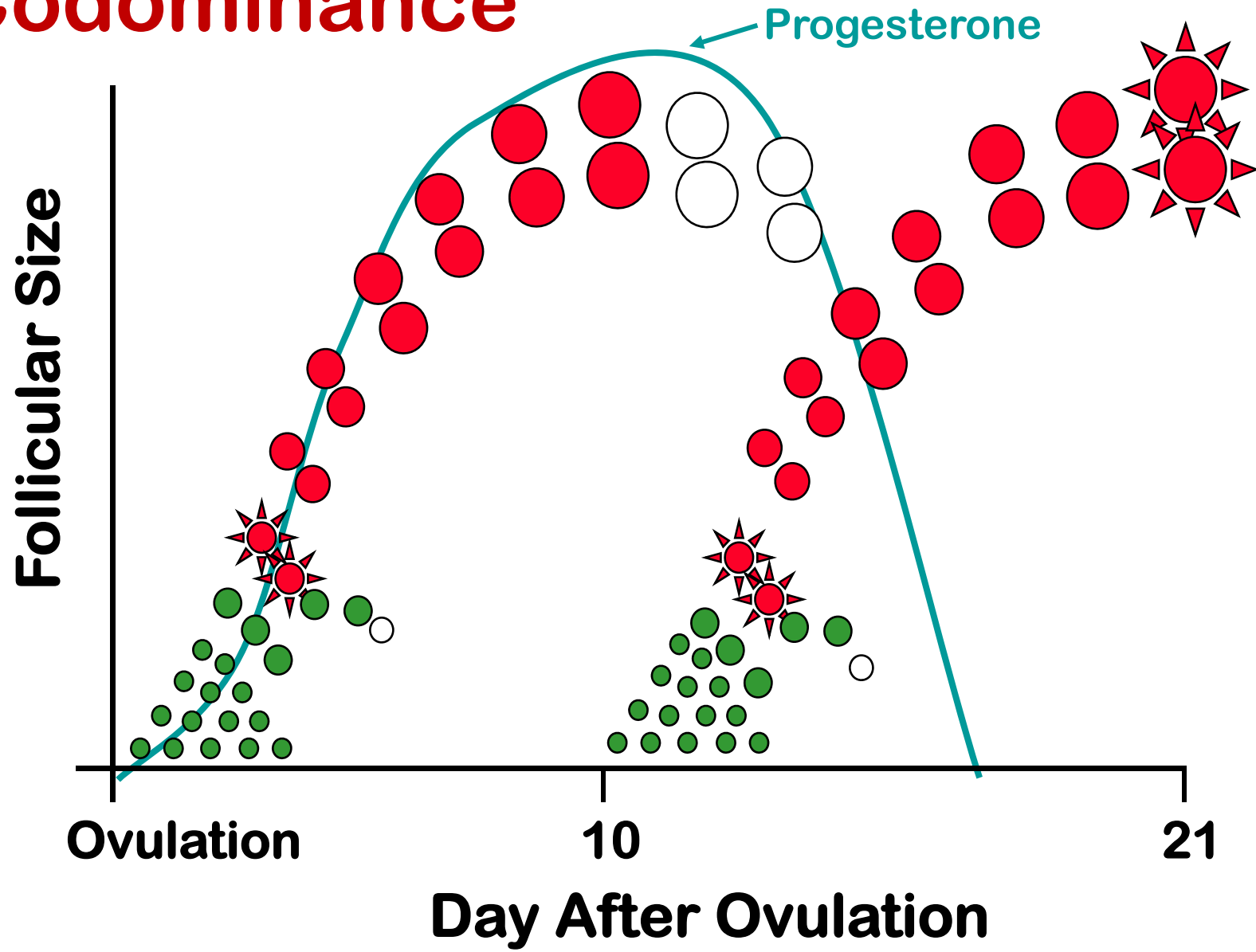


Hepatic Steroid Metabolism

Milo Wiltbank, UW-Madison



Codominance

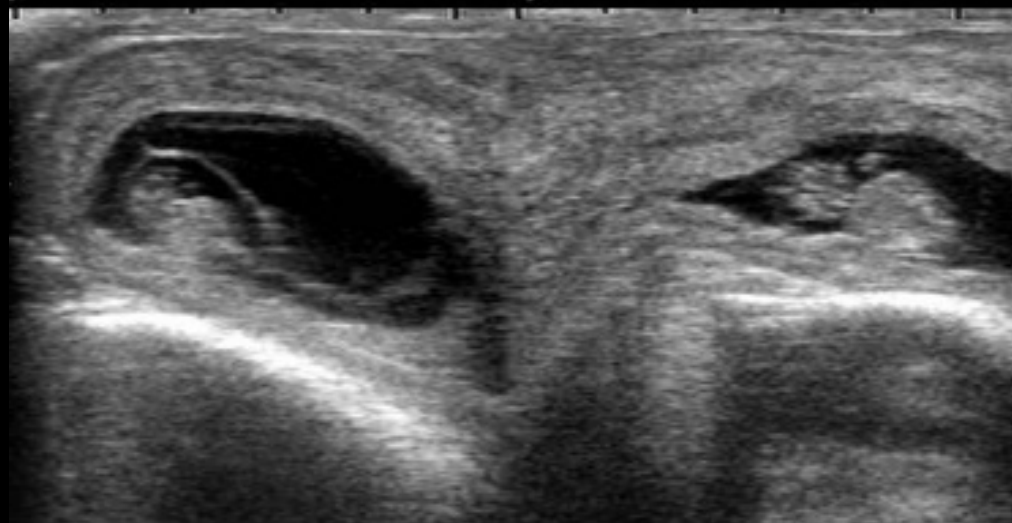


Outline

- **Twinning Trend across Time**
- **Mechanism of Twinning**
- **Endocrinology of Twinning**
- **Identification of Cows Carrying Twins**



BOVINE SERVICES, LLC NAME: AUG.13.01
ID: 6:40:39
V60mm 5MHz



GAIN:68dB
BD:60dB BE:2
PP:4 PER:3
P:5.0-7.0
x2.0
REPRO

WINFIELD MEDICAL NAME:4436
SHIMADZU ULTRASOUND ID:OPEN DAY 12

APR.24.00
AM 8:03:01
V60mm 5MHz

1
2
3
4



GAIN:68dB
BD:63dB BE:2
PP:1 PER:3
P:5.0-7.0
x2.0
CATTLE REPRO

FOCUS
Y POS.

CATTLE CATTLE CATTLE CATTLE OB VTR FOCUS PRE-
HEAD-0 HEAD-1 FBI BODY 1 REPO. ON/OFF POS. QUENCY

Embryo viability, pregnancy loss, and single embryo reduction

Silva del Rio et al., 2009; Theriogenology 71:1462-1471.

Item	Pregnancy type	
	Single	Twin
Cows with embryos at 1 st exam (n)	518	98
Cows with non-viable embryos at 1 st exam, % (n)	4 (19)	-
Cows with viable embryos at 1 st exam (n)	499	98
Cows with pregnancy loss by 2 nd exam, % (n)	5 (23)	13 (13)
Cows with twins undergoing single reduction, % (n)	-	11 (11)
Cows maintaining pregnancy by 2 nd exam, % (n)	92 (476)	76 (74)

1st exam: 25-40 d after AI; 2nd exam: 48-82 d after AI.

Reproductive Events Before Day 90 of Gestation in Cows With Twin Fetuses

Lopez-Gatius and Hunter, 2004; Theriogenology 63:118-125.

	Bilateral	Uni-Right	Uni-Left	Total
	----- n (%) -----			
No. of cows	86 (41)	74 (35)	51 (24)	211
Preg Loss	7 (8)	24 (32)	20 (39)	51 (24)
Single EED ¹	8 (9)	16 (22)	11 (22)	35 (17)
Reduction ²	6 (75)	4 (25)	3 (27)	13 (37)

¹Presence of one dead of the two embryos.

²Embryo reduction without compromising embryo maintenance as a % of total cows with single embryo death.

BOVINE SERVICES, LLC NAME:
ID :

JUL.31.01

6:39:55

V60mm 5MHz

GAIN:67dB

BD:60dB BE:2

PP:4 PER:3

F:5.0-7.0

x2.0

REPRO



SEARCH
PLAY
MOVE
WINDOW



EQUINE CATTLE BOVINE CATTLE CATTLE VTR FRE- FOCUS
SAC TRUNK BODY L HEAD-D HEAD-L ON/OFF QUENCY POS.

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The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Increased calf production in cattle selected for twin ovulations

S. E. Echternkamp, R. M. Thallman, R. A. Cushman, M. F. Allan and K. E. Gregory

J Anim Sci 2007.85:3239-3248.

doi: 10.2527/jas.2007-0210 originally published online Aug 8, 2007;



Effect of twinning on gestation length and calf survival in cows genetically selected for multiple ovulations

Echternkamp et al., 2007; Theriogenology 71:1462-1471.

Pregnancy type	n	Gestation length (d)	No. of calves	Calf survival (%)	
				Birth	Weaning
1 Left	300	284.5 ± 0.2 ^a	711	97.3 ± 1.1 ^a	87.6 ± 1.5 ^a
1 Right	360	284.2 ± 0.2 ^a	876	97.0 ± 1.0 ^a	88.3 ± 1.3 ^a
2 Left	96	277.2 ± 0.2 ^b	446	83.6 ± 1.4 ^b	70.7 ± 1.9 ^b
2 Right	167	277.0 ± 0.1 ^b	838	82.7 ± 1.0 ^b	73.2 ± 1.4 ^b
2 Bilateral	259	278.2 ± 0.1 ^c	1,158	94.0 ± 0.9 ^a	85.4 ± 1.2 ^a

Within a column, means with different superscripts differ ($P < 0.01$).

Effect of fetal number and location in utero on incidence of dystocia

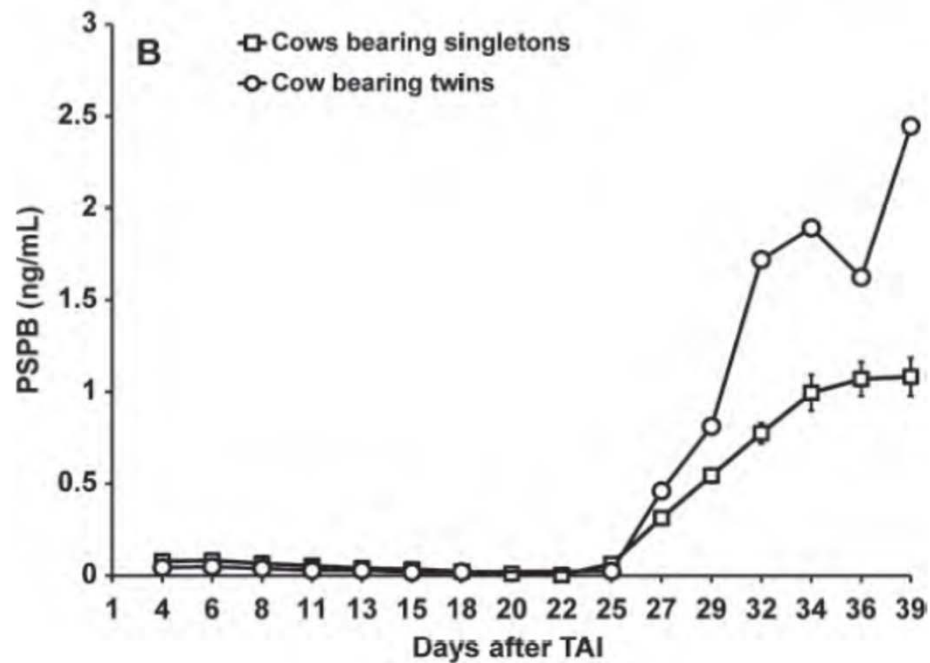
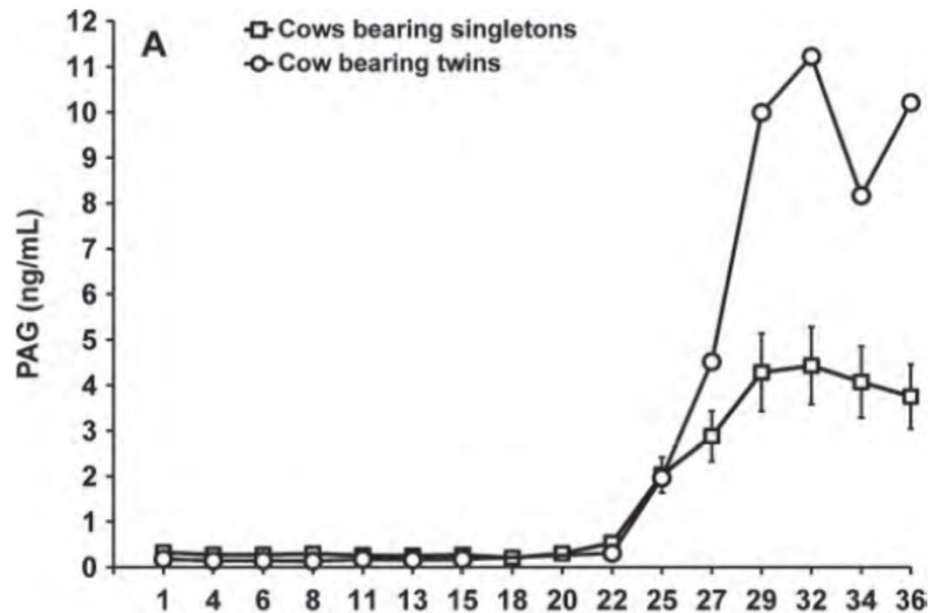
Echternkamp et al., 2007; Theriogenology 71:1462-1471.

Pregnancy type	Uterine horn location	n	Total (%)	Malpresentations (%)
Singleton	Left	711	25.1	4.8 ^a
	Right	876	20.3	6.0 ^a
Twin	Left	446	58.1	50.8 ^b
	Right	838	57.6	45.6 ^b
	Bilateral	1,158	49.8	33.6 ^c
Triplet	Unilateral	27	62.7	62.7 ^b
	Bilateral	120	70.4	70.4 ^d

Within a column, proportions with different superscripts differ ($P < 0.01$).

PAGs

Giordano et al., 2012
J. Dairy Sci. 95:683-697



Pregnancy loss is **3-fold** greater for cows with twins vs. singletons

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Strategies for Managing Twinning

- **Pregnancy termination**
- **Selective reduction**
- **Nutritional management during the transition period**
- **Hormonal manipulation before AI**

Time from treatment to cessation of the embryonic heartbeat and conceptus expulsion

Giordano et al., J. Dairy Sci. 95:683-697.

	Treatment			<i>P</i> -value
	Control	PGF _{2α}	Infusion	
n	10	10	9	
Time to cessation of embryonic heart beat (h)	-	36.0 ± 1.3	0.3 ± 1.4	<0.001
Time to conceptus expulsion (d)	-	1.9 ± 0.7 (1.5 - 2.5)	7.1 ± 0.8 (2.5 -12.0)	<0.001

Effect of treatment on time to cessation of embryonic heart beat (mean ± SE) and conceptus expulsion from the uterus (mean ± SE, range) in cows treated with saline solution IM (Control), PGF_{2α}, and intrauterine infusion of hypertonic saline 25% (Infusion).

Arguments Against Pregnancy Termination

- The economic loss incurred due to pregnancy loss has been estimated to range from **\$46** (Ferguson and Galligan, 2011) to **\$300** (Galligan et al., 2009).
 - Because the incidence of twinning increases with increasing milk production, cows diagnosed with twins often are the highest producing cows in the herd that incur the greatest economic loss associated with pregnancy loss.
- Although heritability and repeatability estimates for twinning in dairy cows are low (0.08 and 0.09, respectively; Van Vleck et al., 1991; Gregory et al., 1997), a prior incidence of twinning is a risk factor for subsequent twin births (Bendixen et al., 1989; Nielen et al., 1989).
 - Abort the twin pregnancy and end up conceiving twins again.

Selective Reduction

Department of Animal Production, University of Lleida, Lleida, Spain

The Effect on Pregnancy Rate of Progesterone Administration after Manual Reduction of Twin Embryos in Dairy Cattle

F. LÓPEZ-GATIUS

- 33 Holstein cows diagnosed with **unilateral** twin pregnancies using ultrasound at **34 d** in gestation.
- P4 treatment = PRID for 28 d after amnion rupture.

Treatment	n	Preg. loss % (n/n)	Abortion	Singleton calves	Twin calves
Control	11	27 (3/11)	1	0	7
Amnion rupture	11	100 (11/11)	-	-	-
Amnion rupture + P4	11	55 (6/11)	-	4	1



Clinical implications of induced twin reduction in dairy cattle

C. Andreu-Vázquez^a, I. Garcia-Ispuerto^b, M. López-Béjar^a, N.M. de Sousa^c,
J.F. Beckers^c, F. López-Gatius^{b,*}

^a *Department of Animal Health and Anatomy, Autonomous University of Barcelona, Barcelona, Spain*

^b *Department of Animal Production, University of Lleida, Lleida, Spain*

^c *Physiology of Reproduction, Faculty of Veterinary Medicine, University of Liège, Belgium*

Received 31 October 2010; received in revised form 5 March 2011; accepted 7 March 2011

- Holstein cows with **unilateral** and **bilateral** twin pregnancies were identified using ultrasound at **35 to 41 d** in gestation.
- Cows subjected to embryo reduction received a PRID for **21 d** after treatment.

Pregnancy loss for cows subjected to twin embryo reduction

Andrieu-Vazquez et al., 2011; Theriogenology 76:512-521.

Treatment	n	Pregnancy loss before 90 d of gestation
		% (n/n)
Unilateral twin pregnancy	27	
Control	14	64 (9/14)
Manual twin reduction	13	54 (7/13)
Bilateral twin pregnancy	28	
Control	14	0 (0/14)
Manual twin reduction	14	29 (4/14)

Pregnancy loss for cows subjected to twin embryo reduction

Andrieu-Vazquez et al., 2011; Theriogenology 76:512-521.

Conclusions:

- Laterality was the only variable significantly affecting pregnancy loss for cows carrying twins.
- Embryo reduction did not carry an additional risk of pregnancy loss for unilateral twin pregnancies, whereas it increased risk of loss in bilateral twin pregnancies.
- **44%** (12/27) of cows undergoing twin embryo reduction calved a singleton, whereas **54%** (15/28) of control cows calved twins.
- Benefits of preventing cows from delivering twins might also be considered for cows with bilateral twins.

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Effects of twin pregnancy and dry period feeding strategy on milk production, energy balance, and metabolic profiles in dairy cows

N. Silva-del-Río, P. M. Fricke and R. R. Grummer

J Anim Sci 2010.88:1048-1060.

doi: 10.2527/jas.2009-2206 originally published online Oct 23, 2009;

Hypothesis:

Feeding a close-up diet throughout the entire dry period will benefit dams bearing twins but will NOT benefit cows bearing singletons.



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2 x 2 Factorial Design

	Singletons	Twins	Total
3W diet	14	10	24
8W diet	12	11	23
Total	26	21	47

Twin pregnant dams:

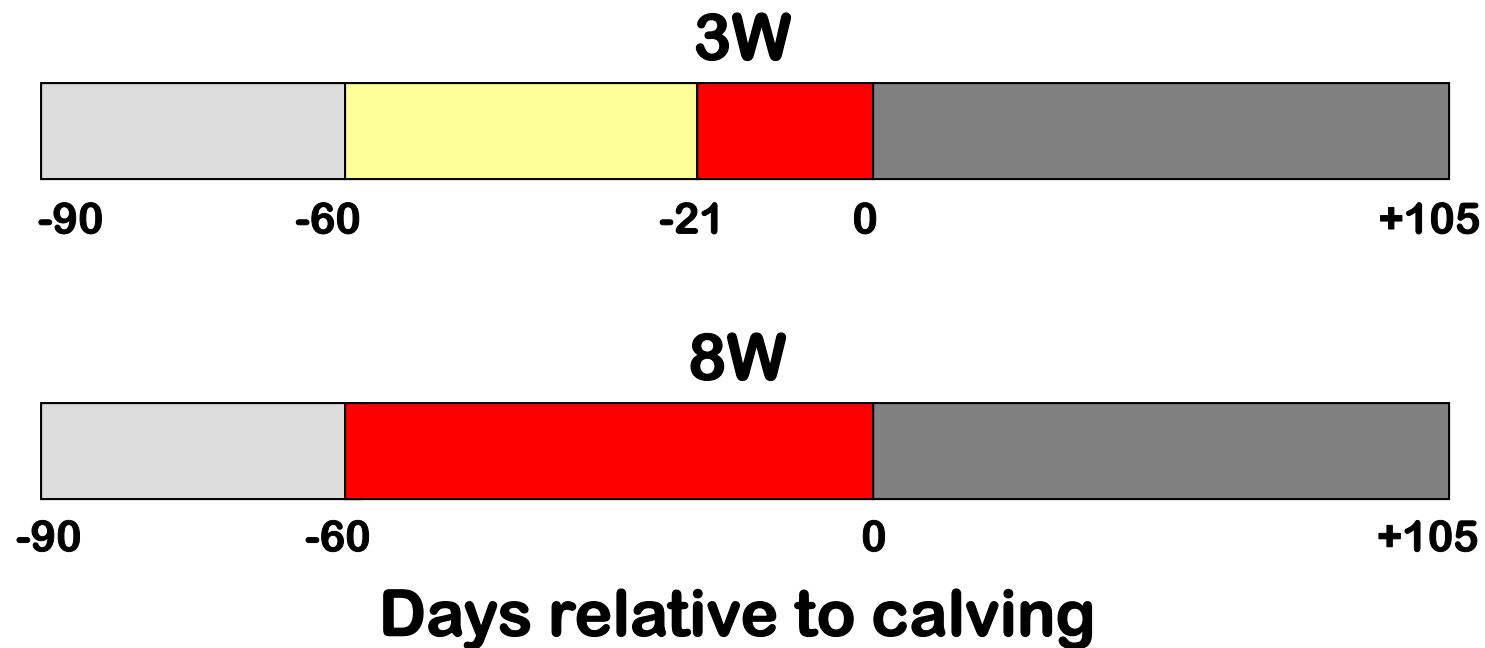
19 cows conceived twins naturally


2 cows induced to twin using multiple ET


Parity:


39 multiparous cows


8 primiparous cows



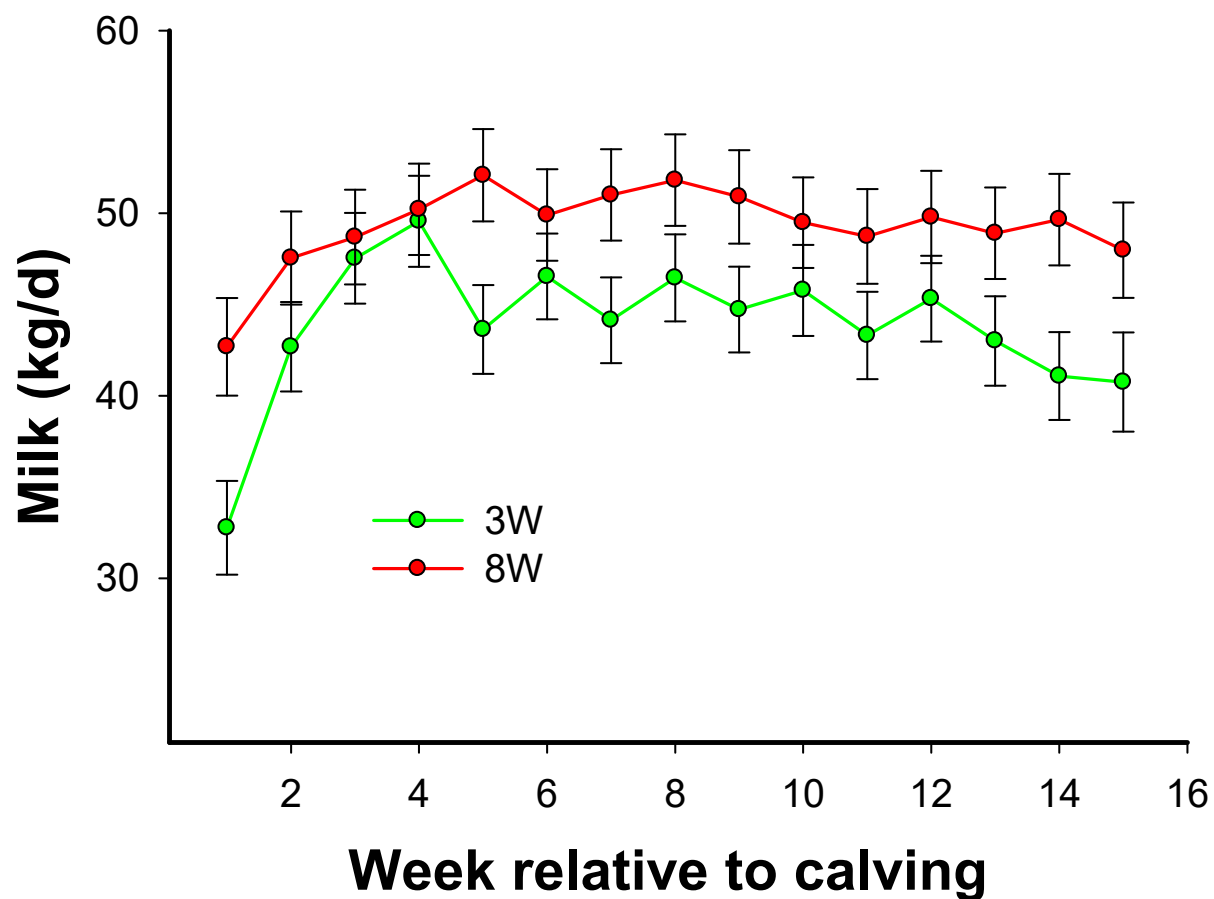
 Late Lactation (LL) diet
= 1.58 NE_L Mcal/kg
= 16.7 CP % of DM
= 32.4 NDF % of DM

 Far-off (FO) diet
= 1.32 NE_L Mcal/kg
= 13.5 CP % of DM
= 54.4 NDF % of DM

 Close-up (CU) diet
= 1.54 NE_L Mcal/kg
= 12.8 CP % of DM
= 42.4 NDF % of DM

 Early Lactation (EL) diet
= 1.71 NE_L Mcal/kg
= 17.0 CP % of DM
= 29.0 NDF % of DM

Milk Production by 3W vs. 8W



Least Squares Means

3W

43.3

8W

48.5

P-Value

D

0.04

D*Time

NS

Conclusions

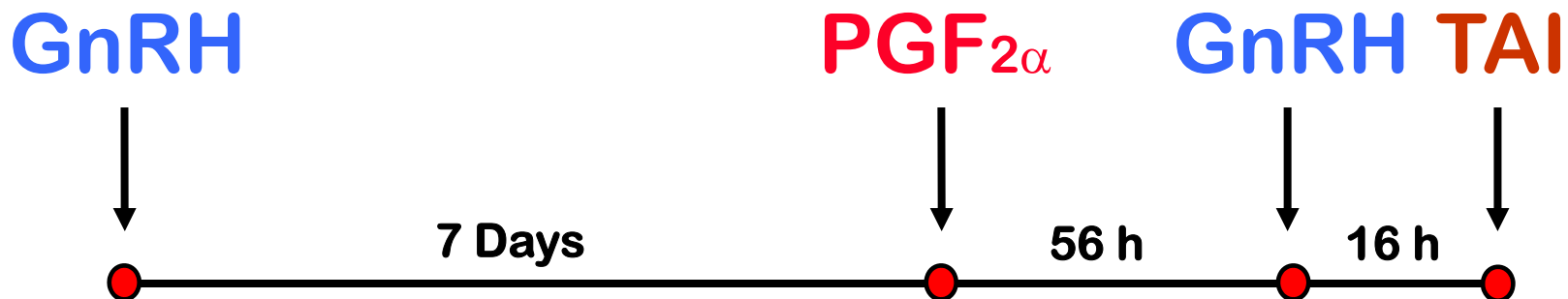
Contrary to our hypothesis, **no interactions** were found between pregnancy type and dry cow feeding strategy for most variables.

Milk production data supported **8W as a superior feeding strategy** compared to 3W, independent of pregnancy type.

Hormonal Manipulation Before AI

Ovsynch

Pursley, Mee, & Wiltbank, 1995
Theriogenology 44:915



Question:

Does Ovsynch cause twinning?





Double Ovsynch

Souza et al., 2008; Theriogenology 70:208–215

Sun	Mon	Tue	Wed	Thu	Fri	Sat
					GnRH	
					PGF	
	GnRH					
	GnRH		High Progesterone			
	PGF		GnRH	TAI		



Short Double Ovsynch

Cunha et al., 2008 J. Dairy Sci. 91(E-Suppl. 1):246 (Abstr.)

Sun	Mon	Tue	Wed	Thu	Fri	Sat
					GnRH	
					PGF	
	GnRH		Low Progesterone			
	PGF		GnRH	TAI		

High vs. Low P₄ during Ovsynch on double ovulation rate and fertility in lactating cows

Cunha et al., 2008 J. Dairy Sci. 91(E-Suppl. 1):246 (Abstr.)

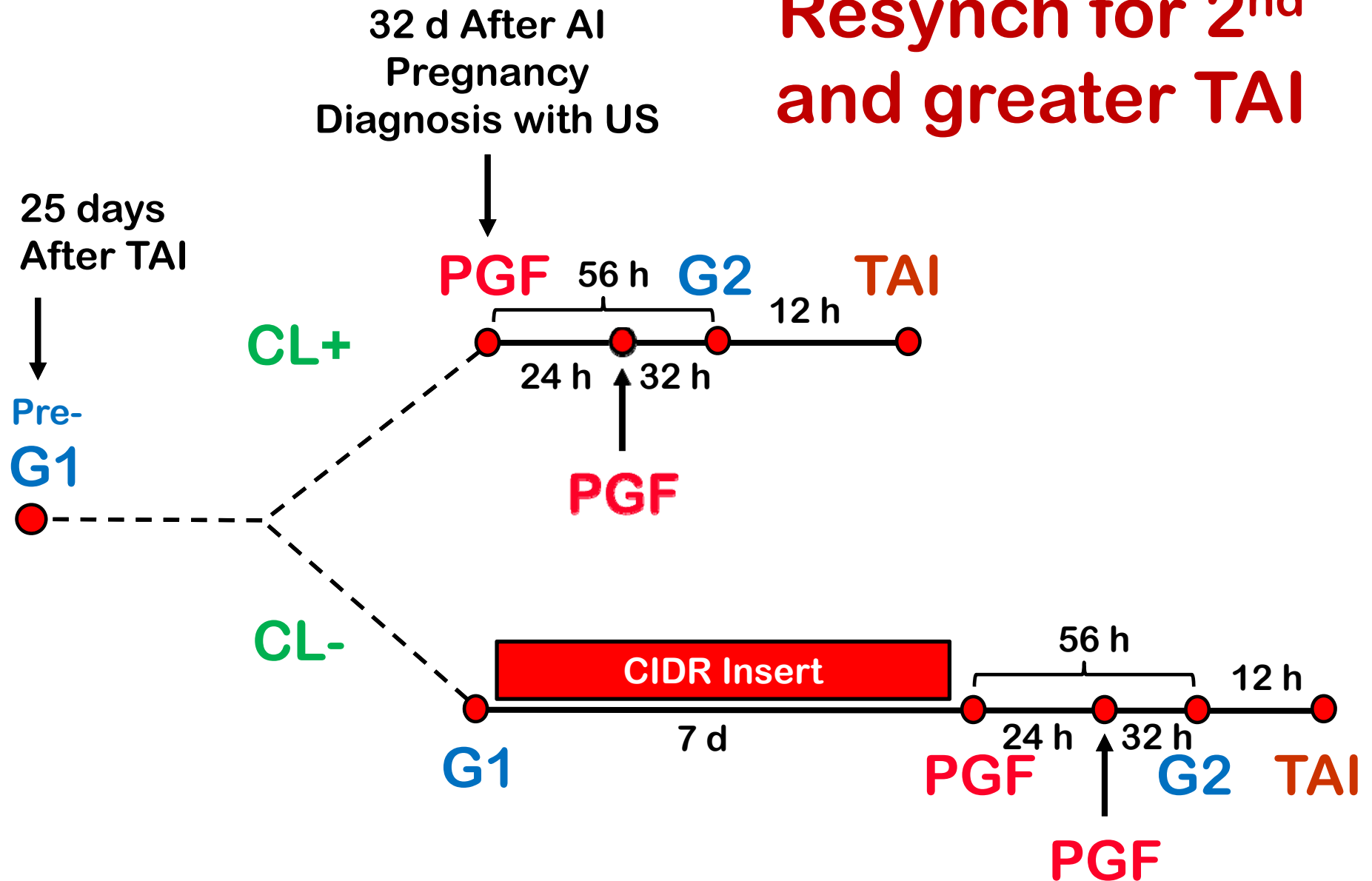
Item	Low P4 (n=259)	High P4 (n=255)	P-value
P4 at 1 st GnRH (ng/ml)	0.28	1.84	-
P4 at PGF (ng/ml)	2.23	4.40	-
Ovulation to 2 nd GnRH (%)	94.5	95.1	NS
Double Ovulation (%)	21.0	7.1	<0.05
P/AI at 29 d (%)	33.2	48.2	<0.01
Pregnancy Loss 29 to 57 d (%)	15.6	4.3	<0.05



Double Ovsynch for First TAI

Sun	Mon	Tue	Wed	Thu	Fri	Sat
					GnRH	
					PGF	
	GnRH					
	GnRH					
	PGF	PGF	GnRH	TAI		

Resynch for 2nd and greater TAI




We can now formulate a two-pronged approach to mitigate the negative impacts of twinning in dairy cows:

- **First, the incidence of double ovulation and dizygotic twinning can be decreased in high-producing Holstein cows by hormonally manipulating ovarian function to increase progesterone during growth of the preovulatory follicle before AI.**
 - **Submit cows to first timed AI after a Double Ovsynch protocol including a second PGF treatment.**
 - **Treat with GnRH 7 d before pregnancy diagnosis and classify nonpregnant cows based on the presence or absence of a CL. Cows with a CL continue the Ovsynch protocol including a second PGF treatment, whereas cows without a CL restart Ovsynch with a CIDR and a second PGF treatment.**

We can now formulate a two-pronged approach to mitigate the negative impacts of twinning in dairy cows:

- **Second, cows identified with bilateral twins using transrectal ultrasonography 32 to 39 d after AI should be allowed to continue gestation with extra assistance provided at calving, whereas selective reduction can be attempted for cows diagnosed with unilateral twins.**

Stronge David:-

 16°F

DR. FRICKE'S SPEAKING SCHEDULE

Wednesday, February 18th, 2015

The overall goal of my extension program is to improve 21-day pregnancy rates in dairy herds by applying knowledge gained through scientific research to develop practical management strategies and assess new reproductive technologies, and to disseminate that information throughout Wisconsin, the ...

Date/Time	Event
03/05/2015 All Day	Western Dairy Management Conference
03/19/2015 10:15 am - 12:20 pm	PDPW Business Conference
03/24/2015 12:00 pm - 1:30 pm	Central Plains Dairy Expo