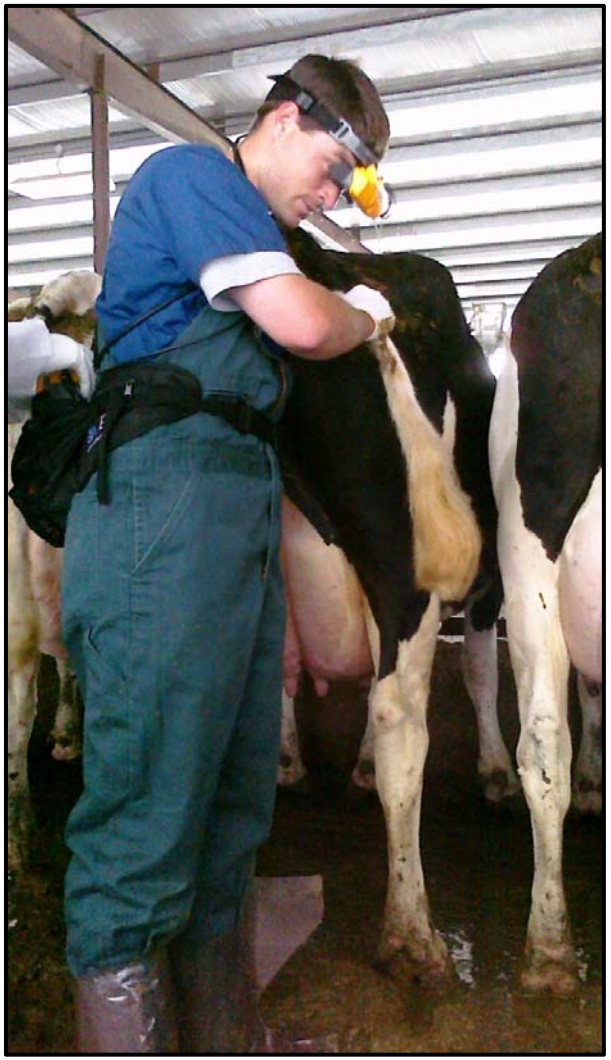
Milk vs. Blood

Which is best for PAG pregnancy diagnosis?

Paul M. Fricke, Ph.D.

Professor of Dairy Science
University of Wisconsin - Madison

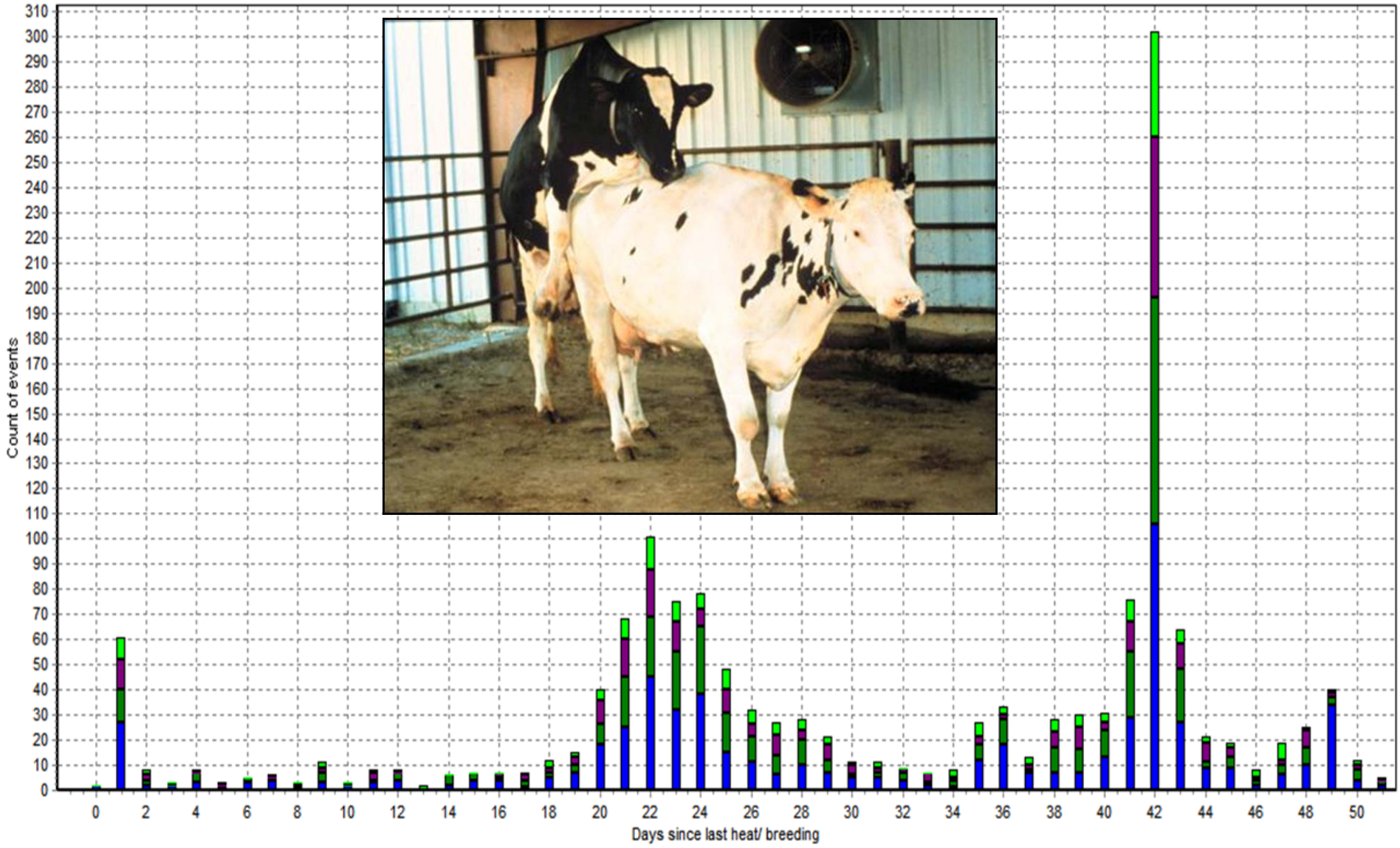
Why Nonpregnancy Diagnosis?



The key to early nonpregnancy diagnosis is to couple:

1) identification of **open** cows with
2) a strategy to **rapidly** return these cows to an AI service

What is the earliest method?



Methods of Nonpregnancy Diagnosis

Direct – direct detection of the tissues and/or associated fluids of the conceptus either manually or via electronic instrumentation

A brief history of direct methods for non-pregnancy diagnosis…

Wait and See

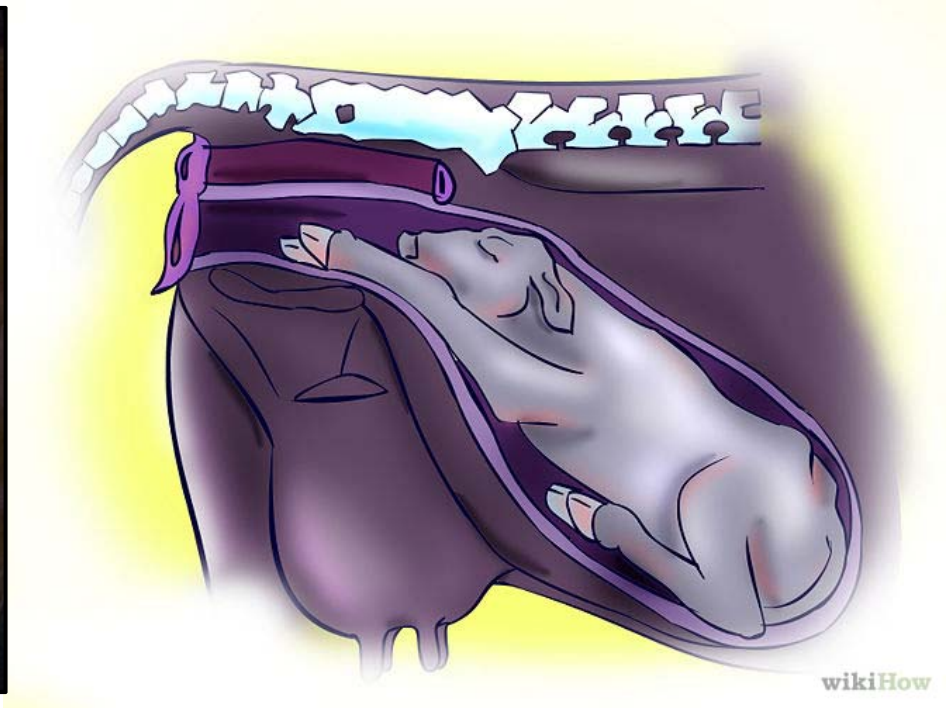
Gestation Length

Holsteins: ~**282** d; ~**9** mo

Women: ~**282** d; ~**9** mo

Calf Bumping

Accurate beginning
~180 to 210 d after AI
~6 to 7 mo



Transrectal Palpation

Accurate beginning ~35 d after AI

Palpation of the Amniotic Vesicle

Wisnicky W, Cassida LE. 1948. *A manual method for diagnosis of pregnancy in cattle*. J. Am. Vet. Med. Assoc. 113:451.

Transrectal Ultrasound

Accurate beginning ~30 d after AI

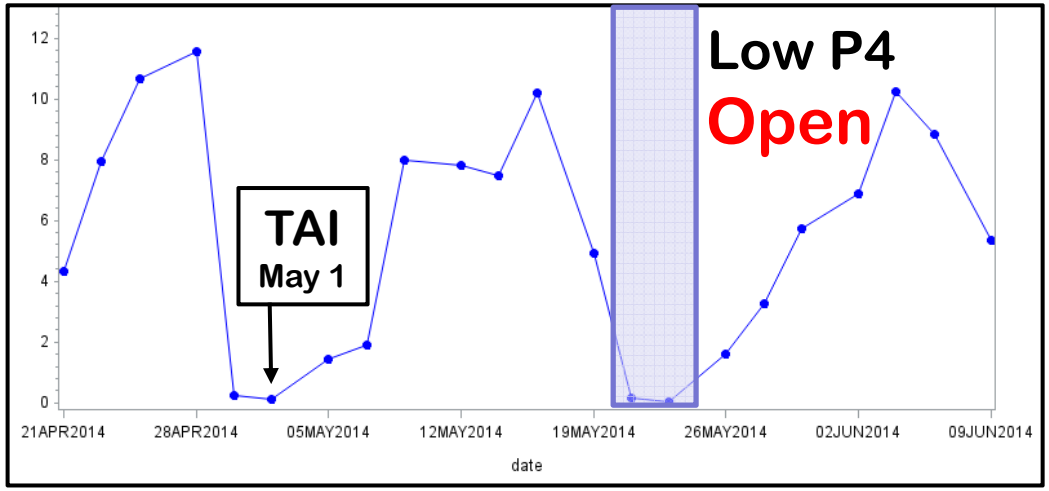
Cost per cow diagnosed using palpation or ultrasound

		Cows per hour			
	$/h	40	60	80	100
Palpation	120	$3.00	$2.00	$1.50	$1.20
	130	$3.25	$2.17	$1.63	$1.30
Ultrasound	150	$3.75	$2.50	$1.88	$1.50
	160	$4.00	$2.67	$2.00	$1.60

Methods of Non-pregnancy Diagnosis

Indirect

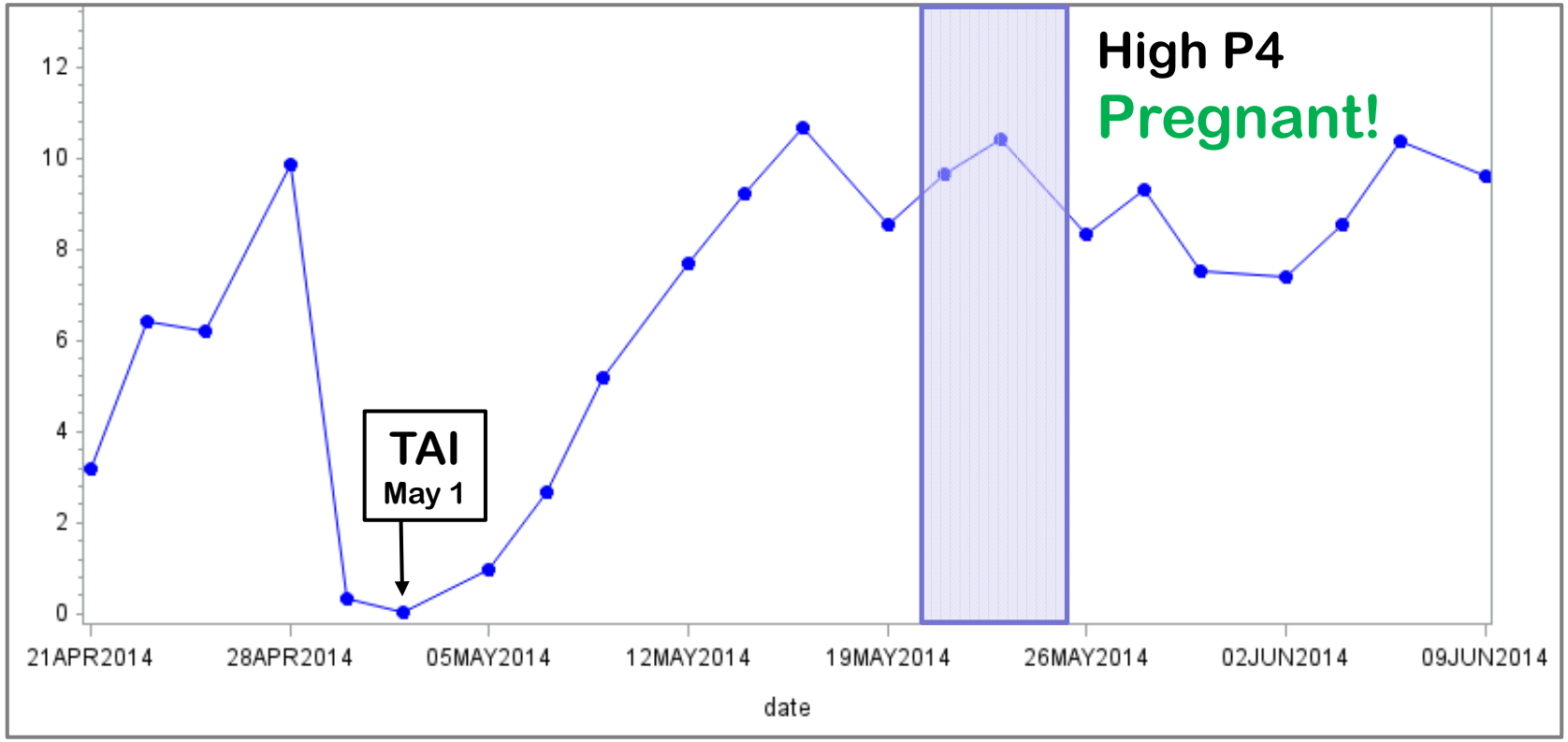
- qualitative or quantitative measures of reproductive hormones at specific stages after AI
- detection of conceptus specific substances in maternal body fluids



Progesterone

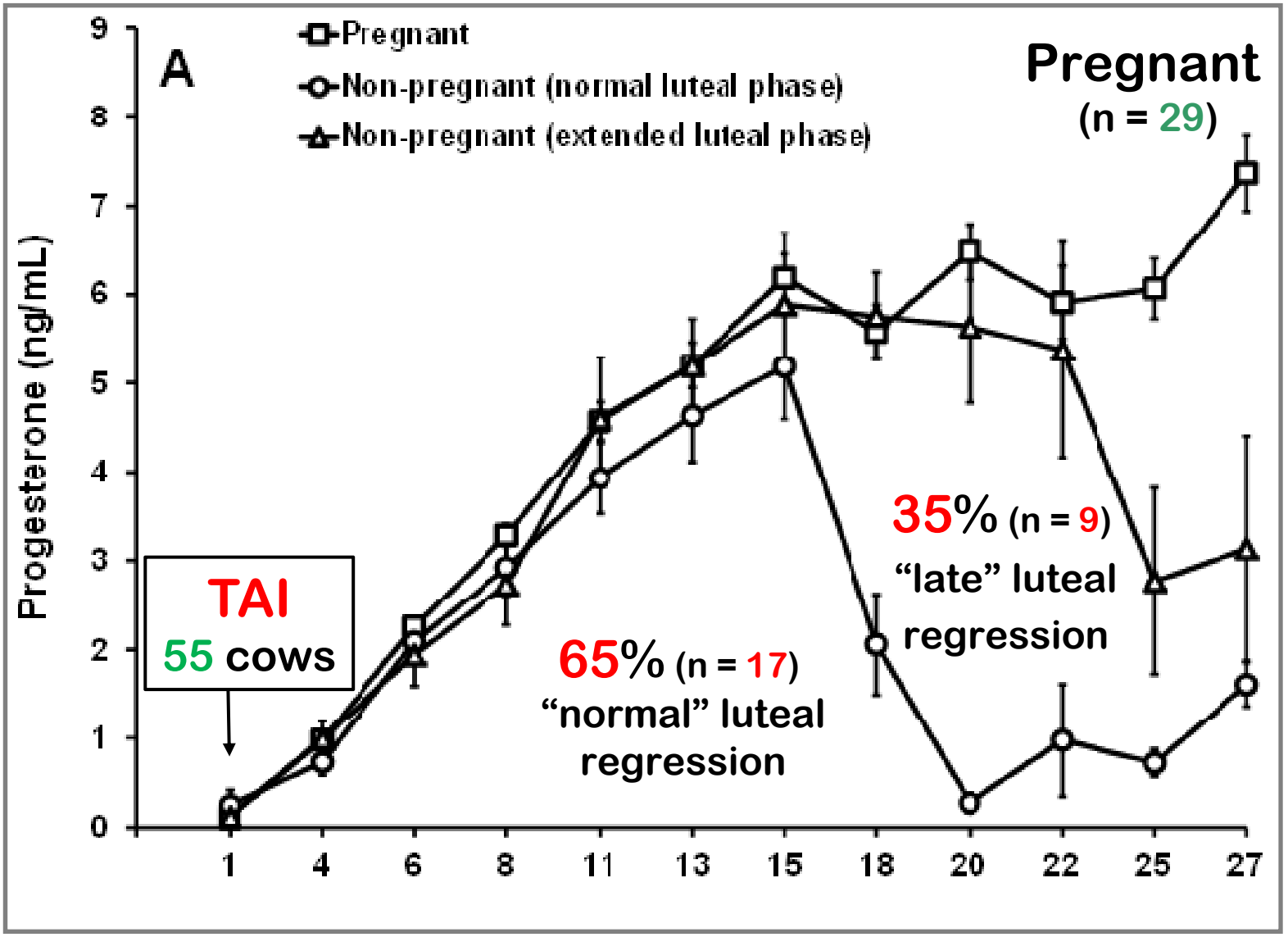
20 to 24 d after AI

<1 month in gestation

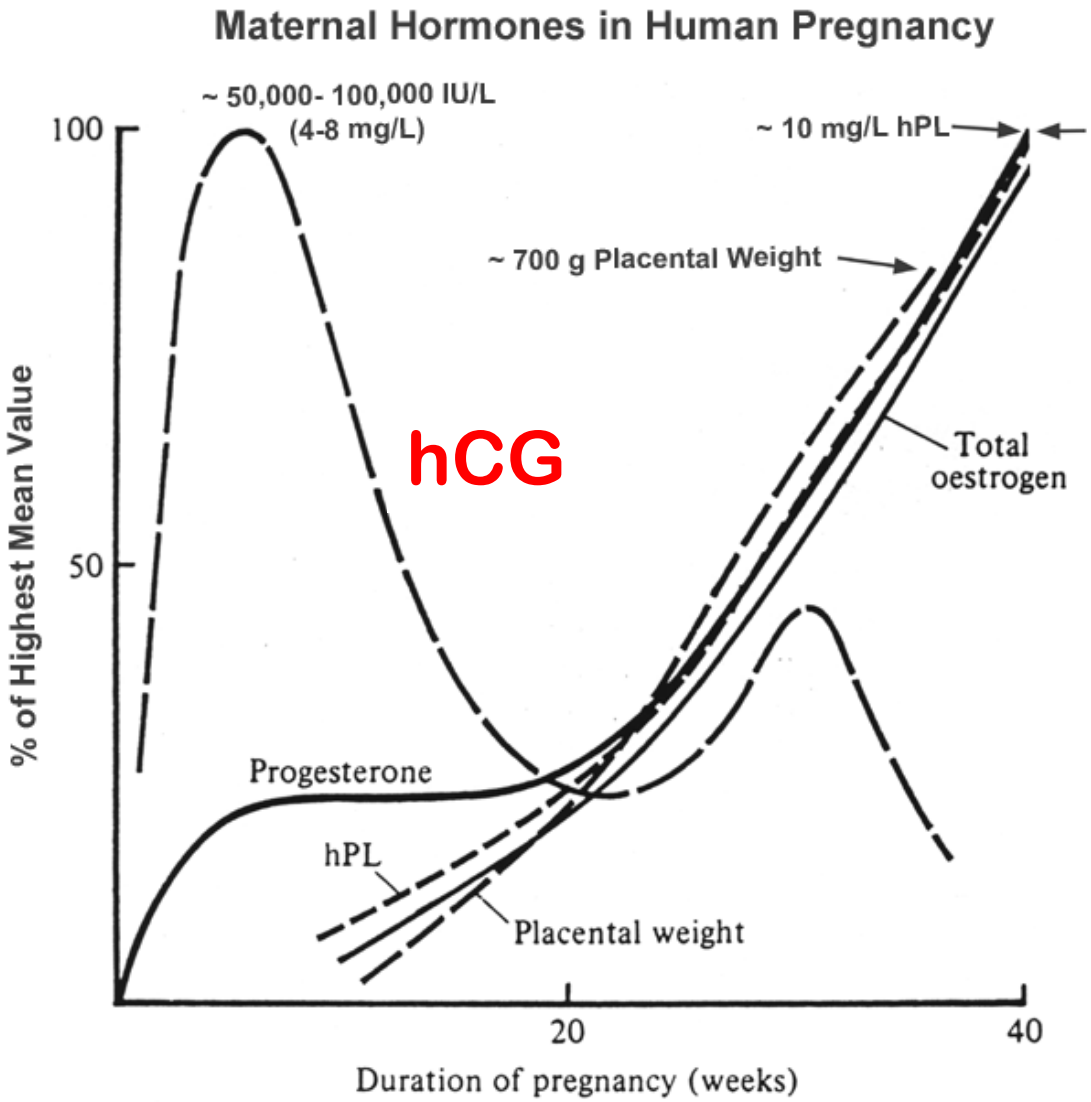


Giordano et al., 2012

J. Dairy Sci. 95:683-697



Chorionic Gonadotropins



(Modified from Austin & Short (ed) *Reproduction in Mammals, Book III: Hormonal Control of Reproduction,* Cambridge University Press: Cambridge, UK, 1984.)

Primates and equids are the only eutherians that produce chorionic gonadotropin:
Women: hCG
Mares: eCG (PMSG)

hCG-based Pregnancy Tests

- The most sensitive urine tests usually can detect hCG around implantation (~ 8 to 10 d after ovulation).
- Menstruation occurs ~14 d after ovulation.
- The likelihood of a false negative is low once a menstrual period is late.

BIOLOGY OF REPRODUCTION 26, 925–933 (1982)

Detection and Partial Characterization of Two Bovine Pregnancy-Specific Proteins[1,2]

J. E. BUTLER,[3] W. C. HAMILTON,[4] R. G. SASSER,[4]
C. A. RUDER,[4] G. M. HASS[5] and R. J. WILLIAMS[4]

Department of Animal Science[4]
and
Department of Bacteriology and Biochemistry[5]
University of Idaho
Moscow, Idaho 83843

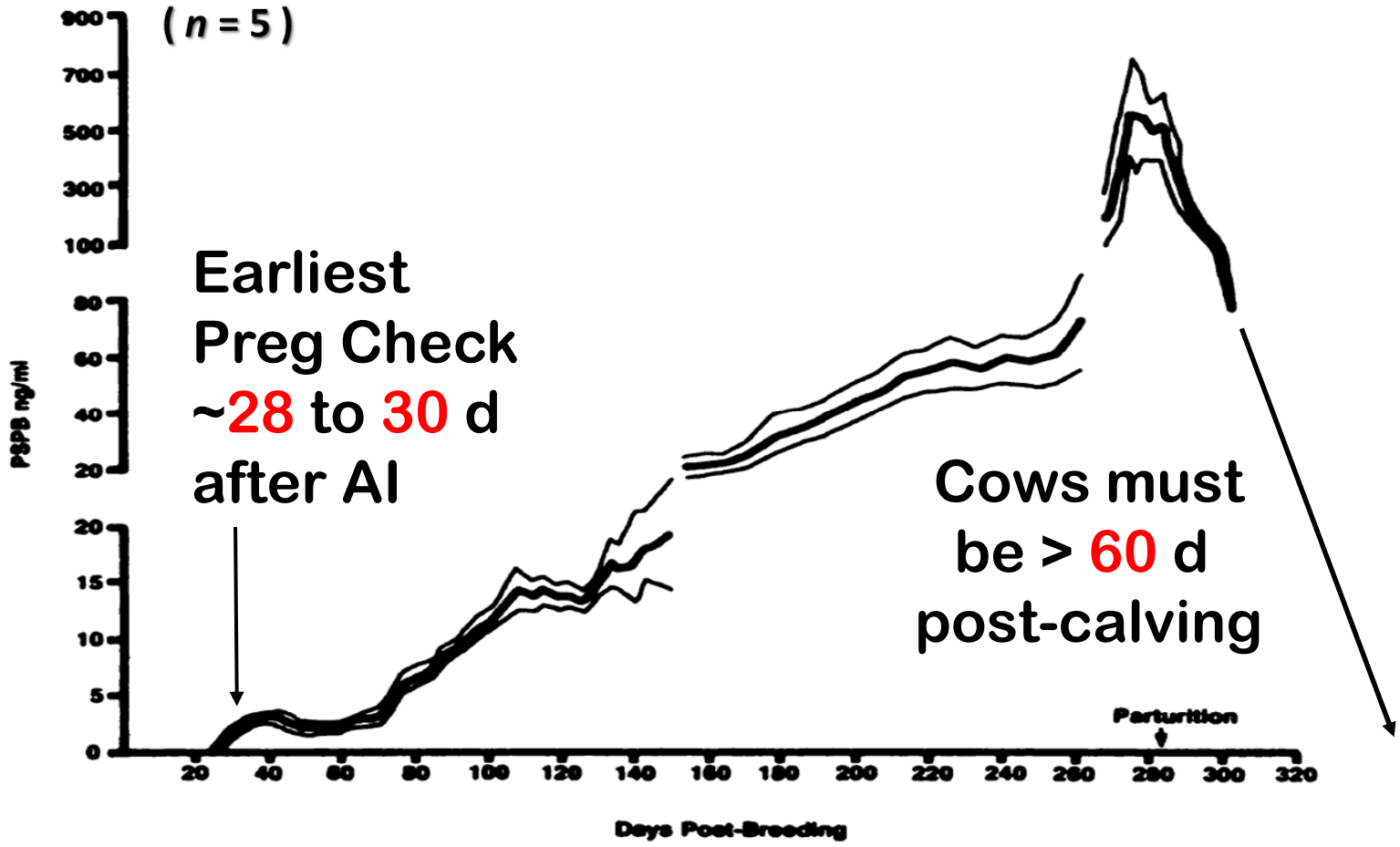
Pregnancy-specific protein A was identified as α-fetoprotein.

Pregnancy-specific protein B (PSPB) had no immunological cross-reactivity with the known proteins or organ extracts which were tested. **PSPB = a novel pregnancy marker produced by the bovine conceptus**.

BIOLOGY OF REPRODUCTION 35, 936–942 (1986)

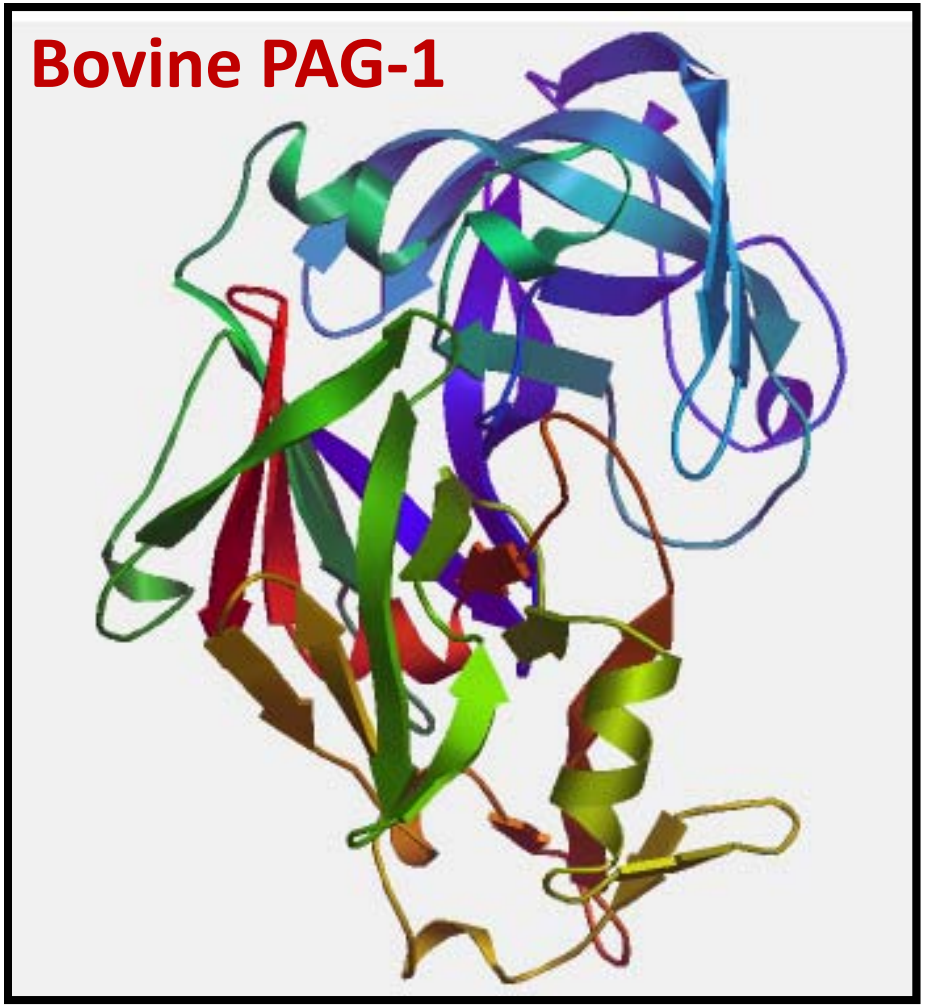
Detection of Pregnancy by Radioimmunoassay of a Novel Pregnancy-Specific Protein in Serum of Cows and a Profile of Serum Concentrations during Gestation[1]

R. GARTH SASSER,[2] CARLA A. RUDER, KRISTEN A. IVANI,[3]
JAMES E. BUTLER,[4] and WILLIAM C. HAMILTON



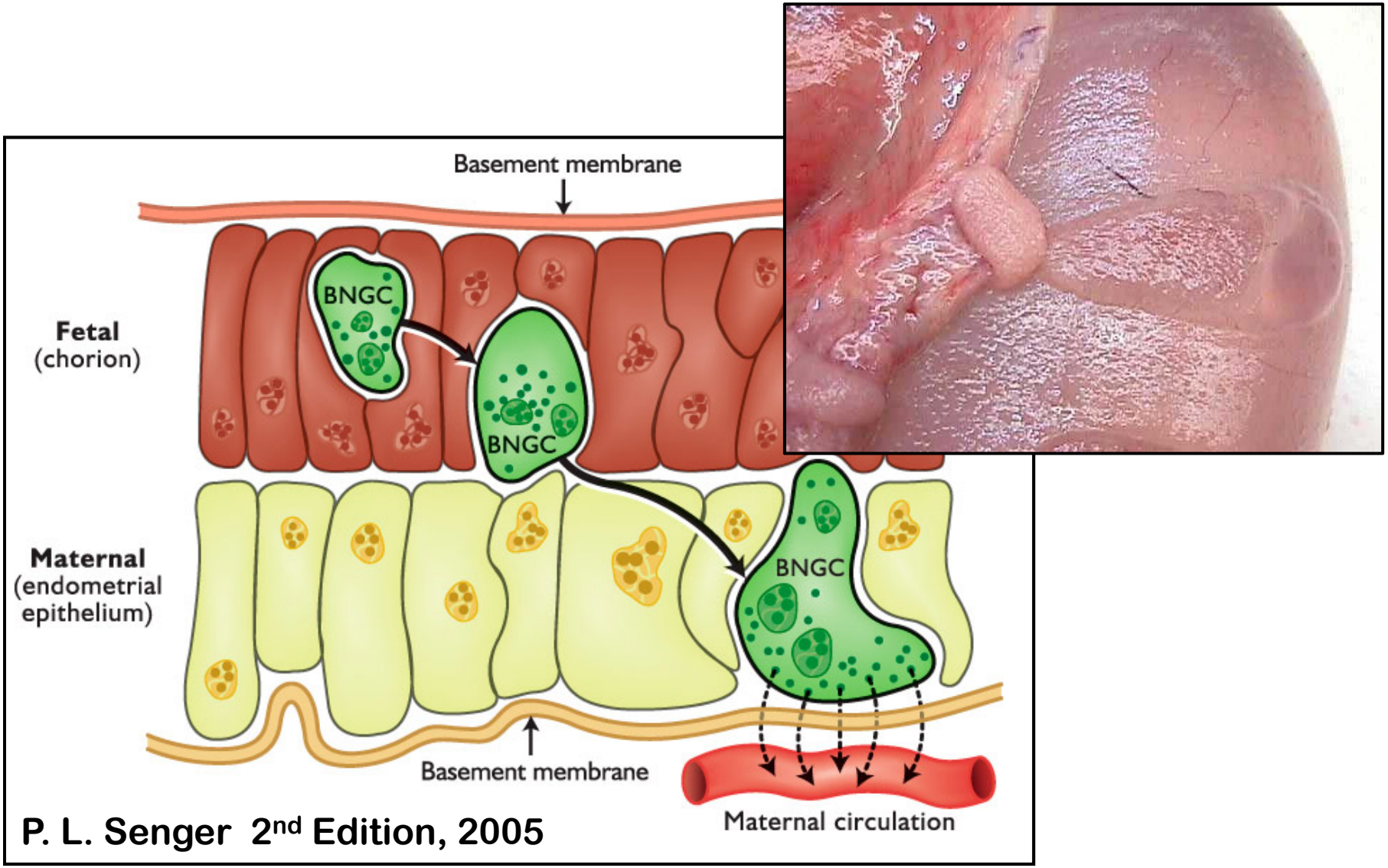
PSPB and PAGs

- Both PSPB and PAGs belong to a large family of >20 glycoproteins expressed during pregnancy
- Inactive aspartic proteinases
- Function of PSPB amd PAGs during pregnancy is unclear



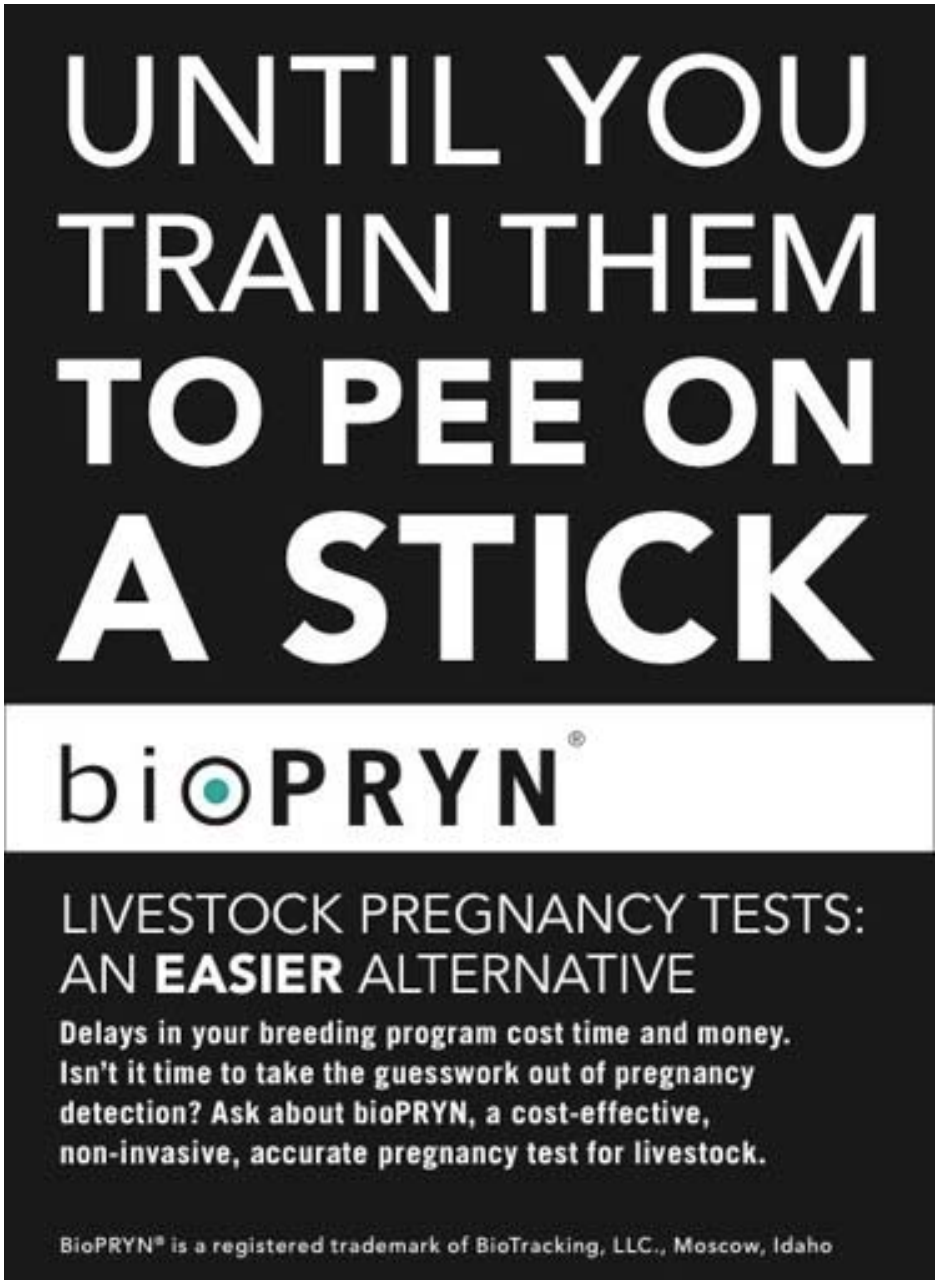
SWISS MODEL – Arnold et al., 2006
Bioinformatics., 22, 195-201

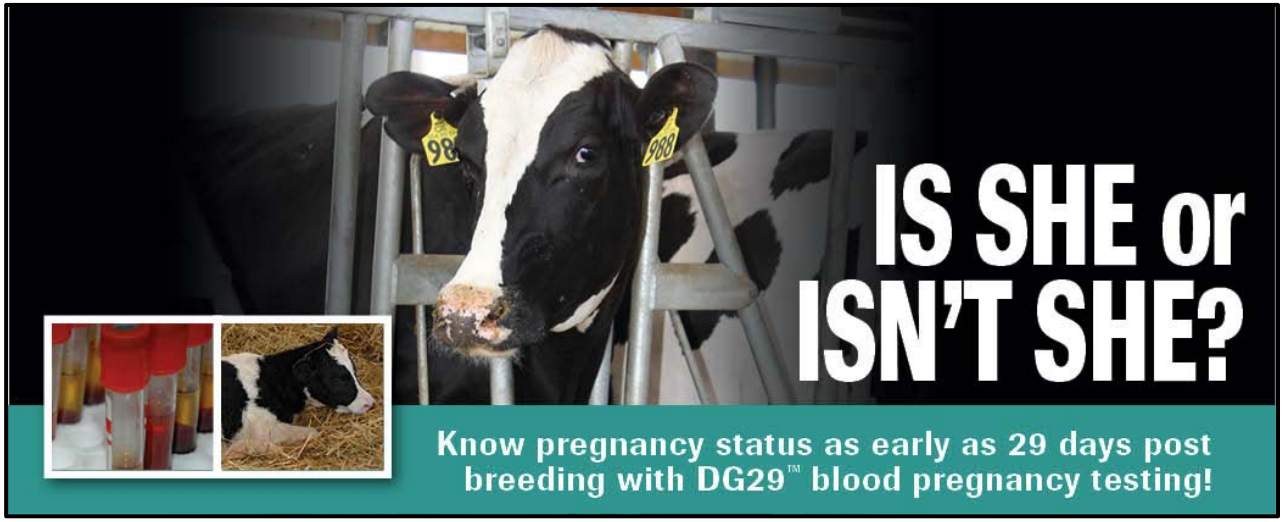
Pregnancy-Associated Glycoproteins



bio TRACKING

- Blood ELISA
- 40 affiliate labs in the US
- Independently owned
- 28 to 30 d after AI
- $2.40 to $2.75 per sample (cost of test only)
- Volume discounts





DG29

Blood ELISA

Test Only

Pricing

- 48 tests: $3.08/test
- 72 tests: $2.79/test
- 144 tests: $2.64/test
- 288 tests: $2.50/test

Complete Kit

- test analysis, blood tubes, needles, needle holder, shipping containers, shipping costs included

Pricing

- 24 tests: $3.54/test
- 48 tests: $3.44/test
- 72 tests: $3.38/test
- 144 tests: $3.30/test

IDEXX LABORATORIES

IDEXX Bovine Pregnancy Test

- **Blood ELISA**
- **$2.50 to $3.50 per test (cost of test only)**

Standard Curve

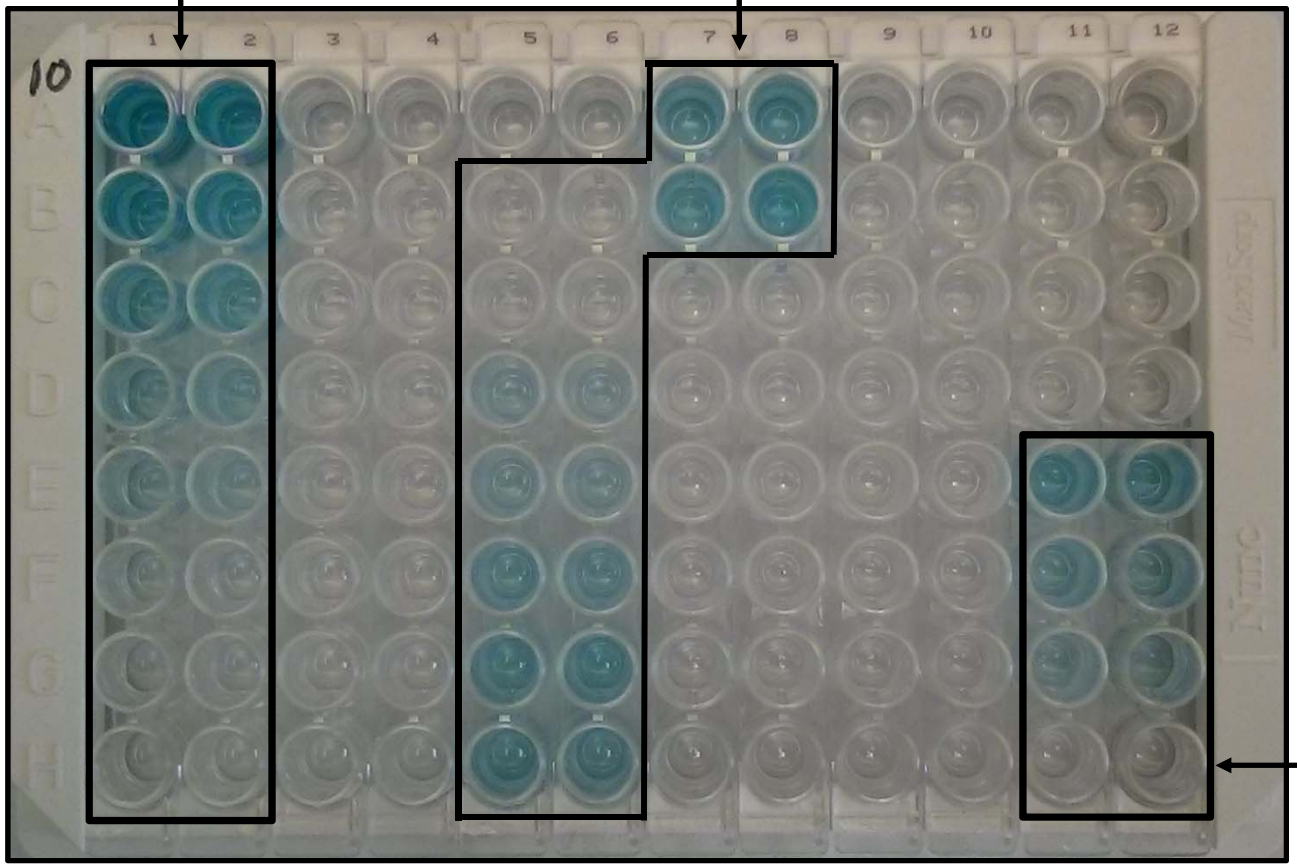
1:2 serial dilutions of serum from a pool of 5 cows > 150 d pregnant

Pregnant Cow

Serial samples from Day 20, 22, 25, 27, 29, 32, 34, 36, & 39 after TAI

IDEXX

Visual Read Pregnancy Test



Announced September, 2013

$336 for 192 wells

= $1.75/well

Positive and Negative Controls

J. Dairy Sci. 90:4612–4622
doi:10.3168/jds.2007-0276

Accuracy of a Pregnancy-Associated Glycoprotein ELISA to Determine Pregnancy Status of Lactating Dairy Cows Twenty-Seven Days After Timed Artificial Insemination

E. Silva,* R. A. Sterry,* D. Kolb,† N. Mathialagan,‡ M. F. McGrath,‡ J. M. Ballam,‡ and P. M. Fricke*[1]
*Department of Dairy Science, University of Wisconsin, Madison 53706
†Lodi Veterinary Clinic, Lodi, WI 53555
‡Monsanto Agricultural Company, St. Louis, MO 63167

DEPARTMENT OF
DAIRY SCIENCE
University of Wisconsin-Madison

PAG Resynch Schedule Silva et al., 2007

Sun	Mon	Tue	Wed	Thu	Fri	Sat
					TAI	
d 2						
d 9						
d 16				US d 27		
d 23		GnRH		PAG		
d 30		PGF		GnRH	TAI	
d 37						
d 44						
d 51						

6:30 a.m.

3:00 p.m.

Overnight Express

8:00 am

Arrive at Monsanto, St. Louis, MO

6:00 p.m

Time from sample collection to receive outcomes: ~36 h

Accuracy of PAG ELISA for determination of pregnancy status 27 d after timed AI

Silva et al., 2007; J. Dairy Sci. 90:4612-4622

Sensitivity[1] % (no./no.)	Specificity[2] % (no./no.)	PPV[3] % (no./no.)	NPV[4] % (no./no.)	Accuracy[5] % (no./no.)	Kappa
95 (596/625)	94 (987/1048)	91 (596/657)	97 (987/1016)	95 (1583/1673)	0.89

[1]Proportion of samples from pregnant cows with a positive PAG ELISA.
[2]Proportion of samples from not-pregnant cows with a negative PAG ELISA.
[3]Proportion of PAG ELISA with a pregnant outcome that truly were pregnant.
[4]Proportion of PAG ELISA with a not-pregnant outcome that truly were not-pregnant.
[5]Proportion of pregnancy status, pregnant and not-pregnant, that were correctly classified.

J. Dairy Sci. 96:949–961
http://dx.doi.org/10.3168/jds.2012-5704

Economics of resynchronization strategies including chemical tests to identify nonpregnant cows

J. O. Giordano, P. M. Fricke, and V. E. Cabrera[1]
Department of Dairy Science, University of Wisconsin, Madison 53706

To be economical, the sensitivity of the test had to be greater than:

94% when used 24 d after AI

96% when used 31 d after AI

DEPARTMENT OF
DAIRY SCIENCE
University of Wisconsin-Madison

IDEXX Introduces Milk-based Bovine Pregnancy Test That Simplifies and Streamlines Pregnancy Diagnosis in Dairy Cattle [Trade]

The first of its kind, the IDEXX milk-based test optimizes reproductive efficiency and helps milk recording laboratories expand their services

WESTBROOK, Maine, October 18, 2012—At the recent World Dairy Expo in Madison, Wisconsin, IDEXX announced the launch of the IDEXX Milk Pregnancy Test, the first milk-based test for detecting pregnancy as a means of optimizing reproductive efficiency in dairy herds. The ELISA-format test detects pregnancy-associated glycoproteins (PAGs) to determine pregnancy status while minimizing animal handling. With a high level of sensitivity and specificity from ≥35 days postbreeding and ≥ 60 days postcalving, producers can work with dairy herd improvement (DHI) organizations to get early, accurate confirmation of open cows in less than 3.5 hours.

"Expanding the use of DHI milk samples to include confirmation of pregnancy is a simple, cost-effective way for producers to increase the efficiency of their total operation," says Olivier te Boekhorst, VP General Manager, IDEXX Livestock and Poultry Diagnostics. "At the same time, the IDEXX Milk Pregnancy Test also helps recording laboratories make their herd health management programs more attractive to their customers."

- **DHIA laboratories**
- **MSRP: $3.50 per sample**
- **Range: $3.00 to $5.00**

J. Dairy Sci. 98:1–13
http://dx.doi.org/10.3168/jds.2014-8974

Factors associated with pregnancy-associated glycoprotein (PAG) levels in plasma and milk of Holstein cows during early pregnancy and their effect on the accuracy of pregnancy diagnosis

A. Ricci,*† P. D. Carvalho,* M. C. Amundson,* R. H. Fourdraine,‡ L. Vincenti,† and P. M. Fricke*[1]
*Department of Dairy Science, University of Wisconsin–Madison, Madison 53706
†Department of Veterinary Science, Università di Torino, Grugliasco 10090, Italy
‡AgSource Laboratories, Menomonie, WI 54751

Compared IDEXX blood and milk tests

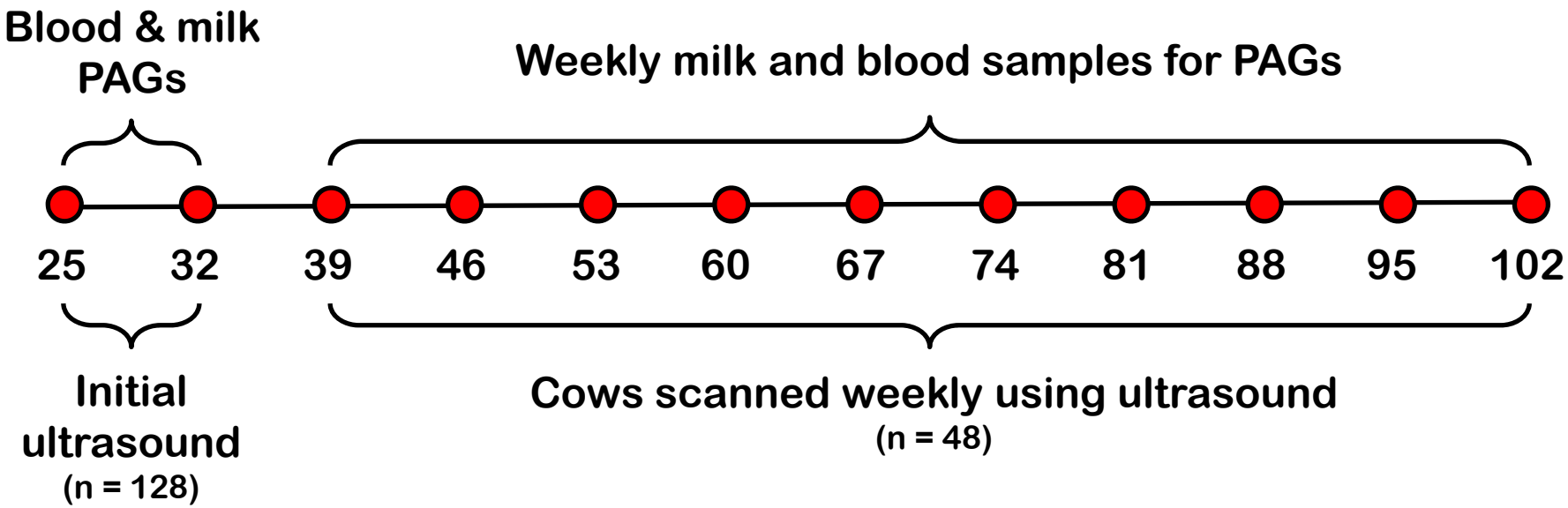
PAG levels in plasma and milk were associated with:

- stage of gestation
- parity (1st lactation vs. older cows)
- pregnancy loss
- milk production

DEPARTMENT OF
DAIRY SCIENCE
University of Wisconsin-Madison

Experimental Design

Ricci et al., 2015



Accuracy of PAG blood and milk ELISA for pregnancy status 32 d after timed AI

Ricci et al., 2015

	Sensitivity[1] % (no./no.)	Specificity[2] % (no./no.)	PPV[3] % (no./no.)	NPV[4] % (no./no.)	Accuracy[5] % (no./no.)
Plasma	100 (57/57)	87 (73/84)	84 (57/68)	100 (73/73)	92 (130/141)
Milk	98 (52/53)	83 (68/82)	79 (52/66)	99 (68/69)	89 (120/135)

[1]Proportion of samples from pregnant cows with a positive PAG ELISA.
[2]Proportion of samples from not-pregnant cows with a negative PAG ELISA.
[3]Proportion of PAG ELISA with a pregnant outcome that truly were pregnant.
[4]Proportion of PAG ELISA with a not-pregnant outcome that truly were not-pregnant.
[5]Proportion of pregnancy status, pregnant and not-pregnant, that were correctly classified.

Results

- Overall, 42% (57/137) of synchronized cows were diagnosed pregnant 32 d after TAI.
- 2 cows were diagnosed with twins 32 d after TAI, and these cows were removed from all subsequent analyses.
- The incidence of pregnancy loss for cows diagnosed with singleton pregnancies was 13% (7/55).

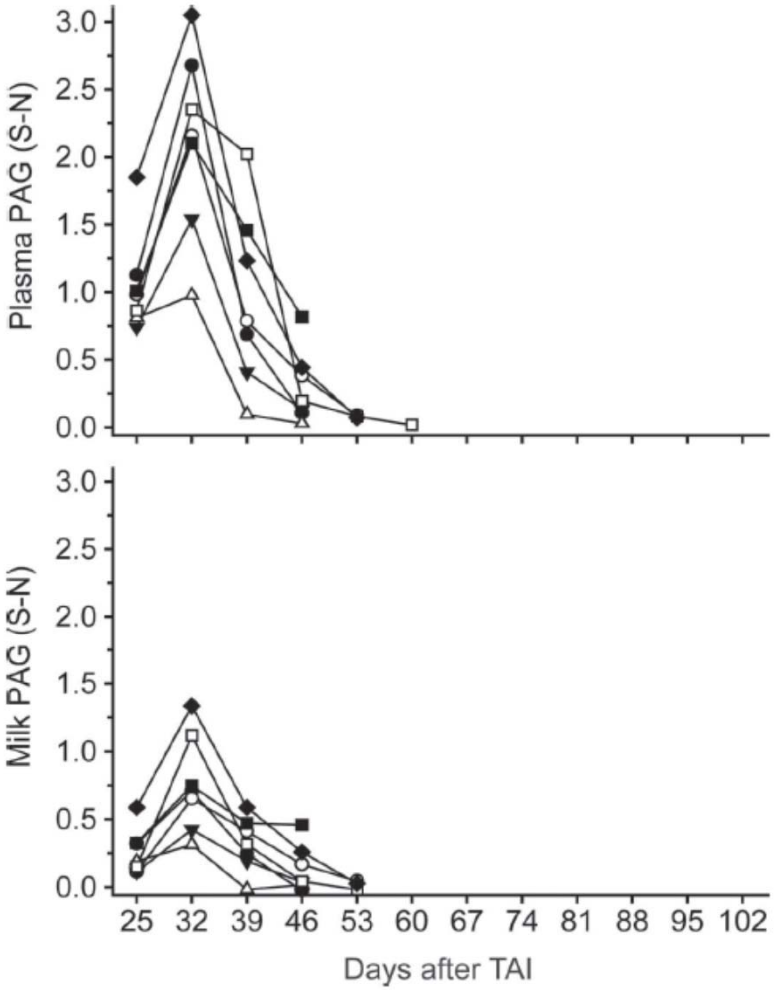
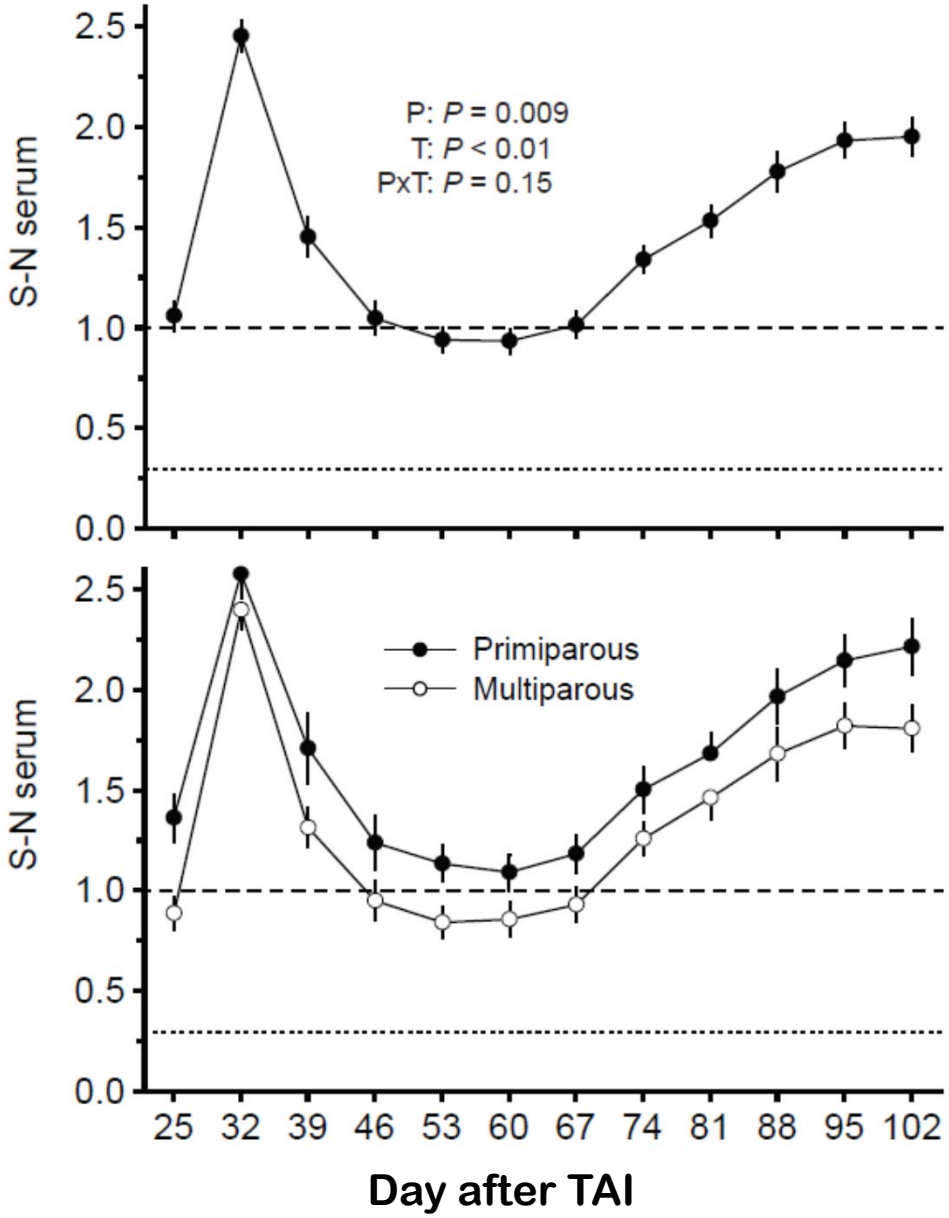
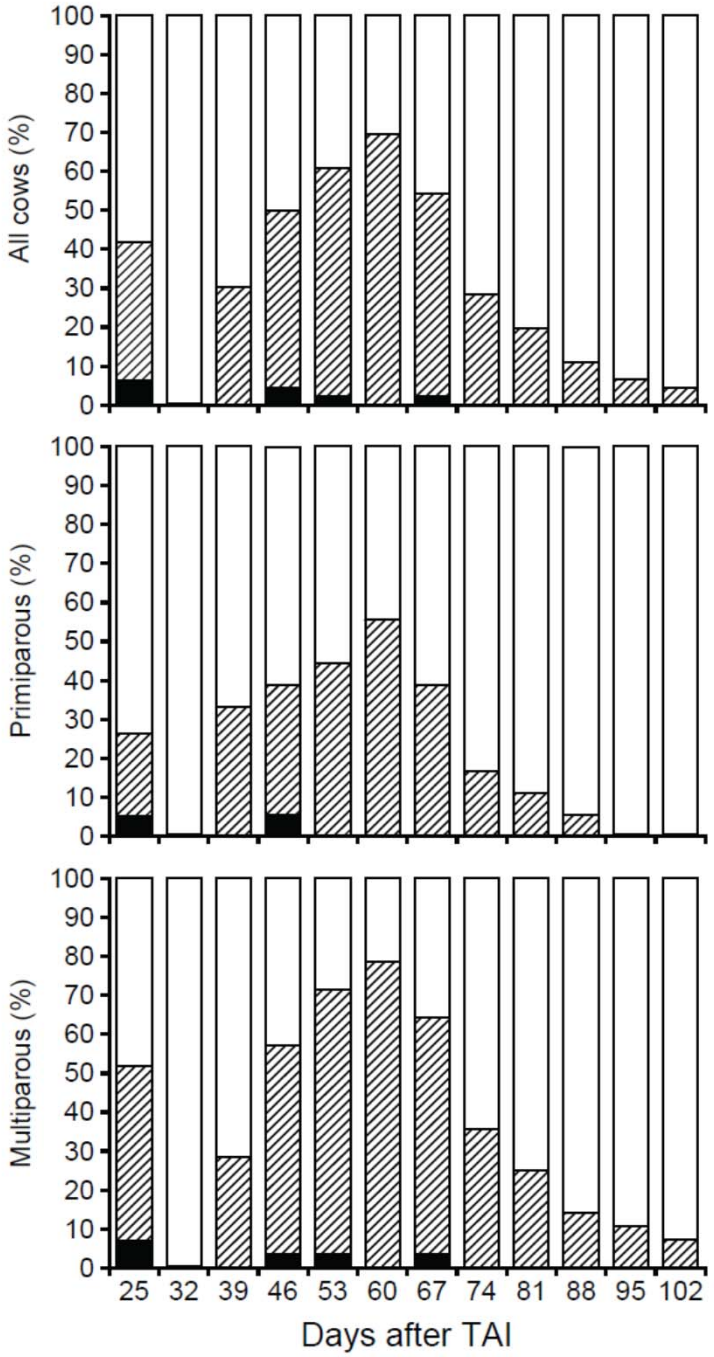


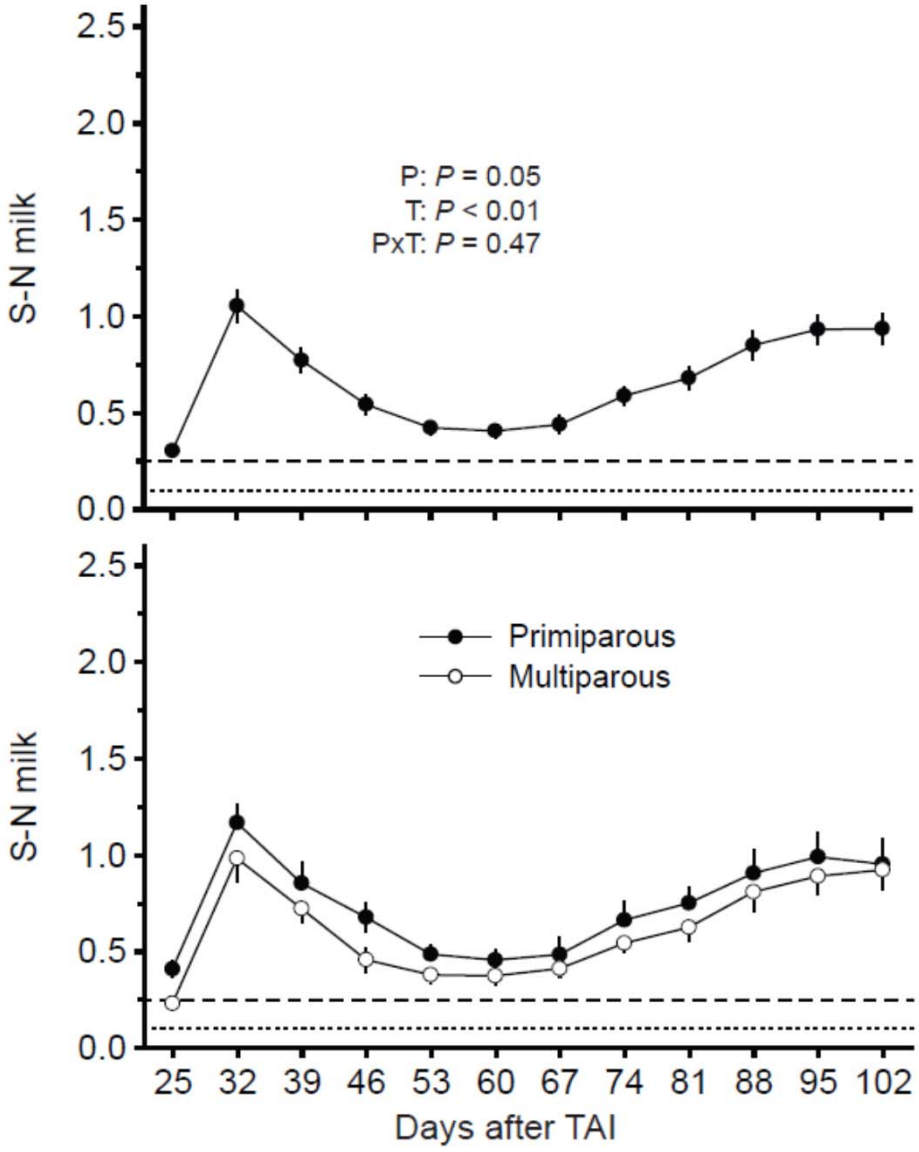
Figure 6. Profiles of pregnancy-associated glycoprotein (PAG) for individual Holstein cows (n = 7) diagnosed pregnant using transrectal ultrasonography 32 d after AI and subsequently undergoing pregnancy loss. (Upper panel) Individual plasma PAG profiles. (Lower panel) Individual milk PAG profiles. Plasma and milk PAG ELISA outcomes were calculated from the optical density (OD) of the sample [corrected by subtraction of the reference wavelength OD of the sample (S) minus the OD of the negative control (N) at 450 nm (with both values corrected by subtraction of the reference wavelength OD of the negative control)], which resulted in an S-N value. TAI = timed AI.

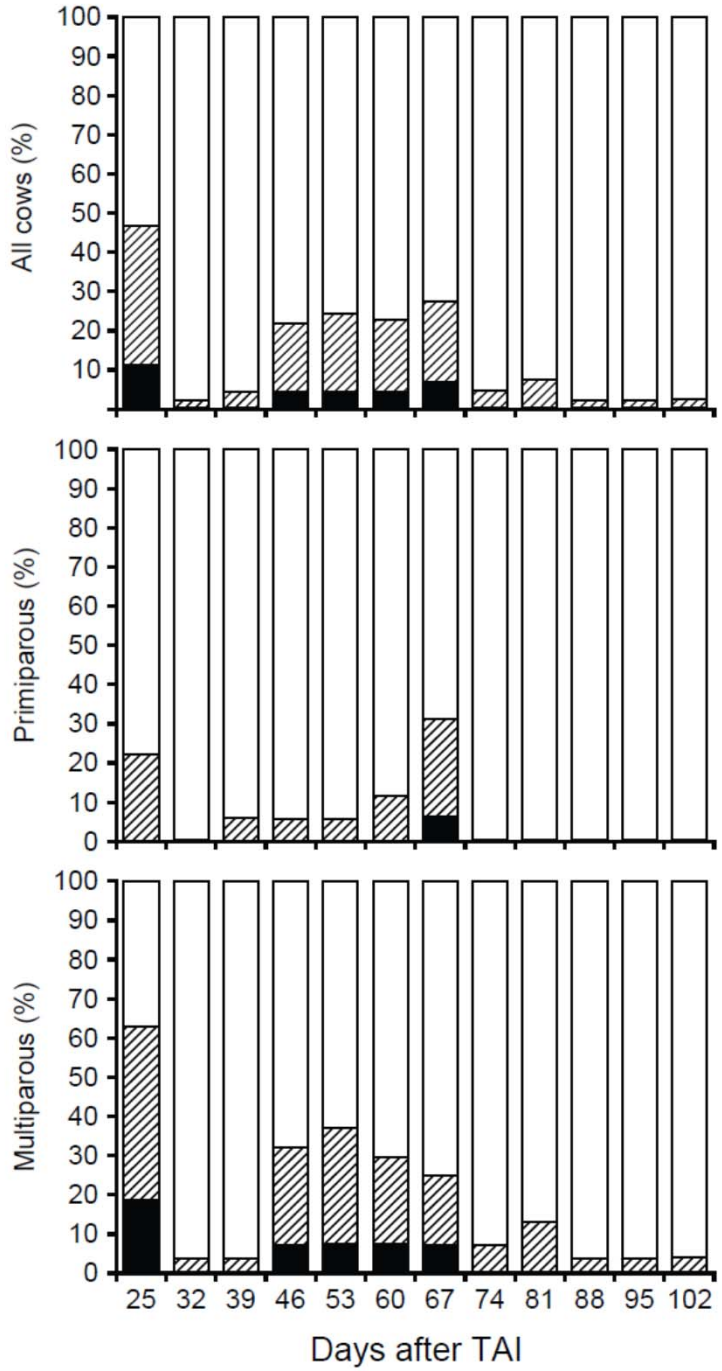
Plasma PAGs


S-N serum
P: P = 0.009
T: P < 0.01
PxT: P = 0.15
Primiparous
Multiparous
25 32 39 46 53 60 67 74 81 88 95 102
Day after TAI

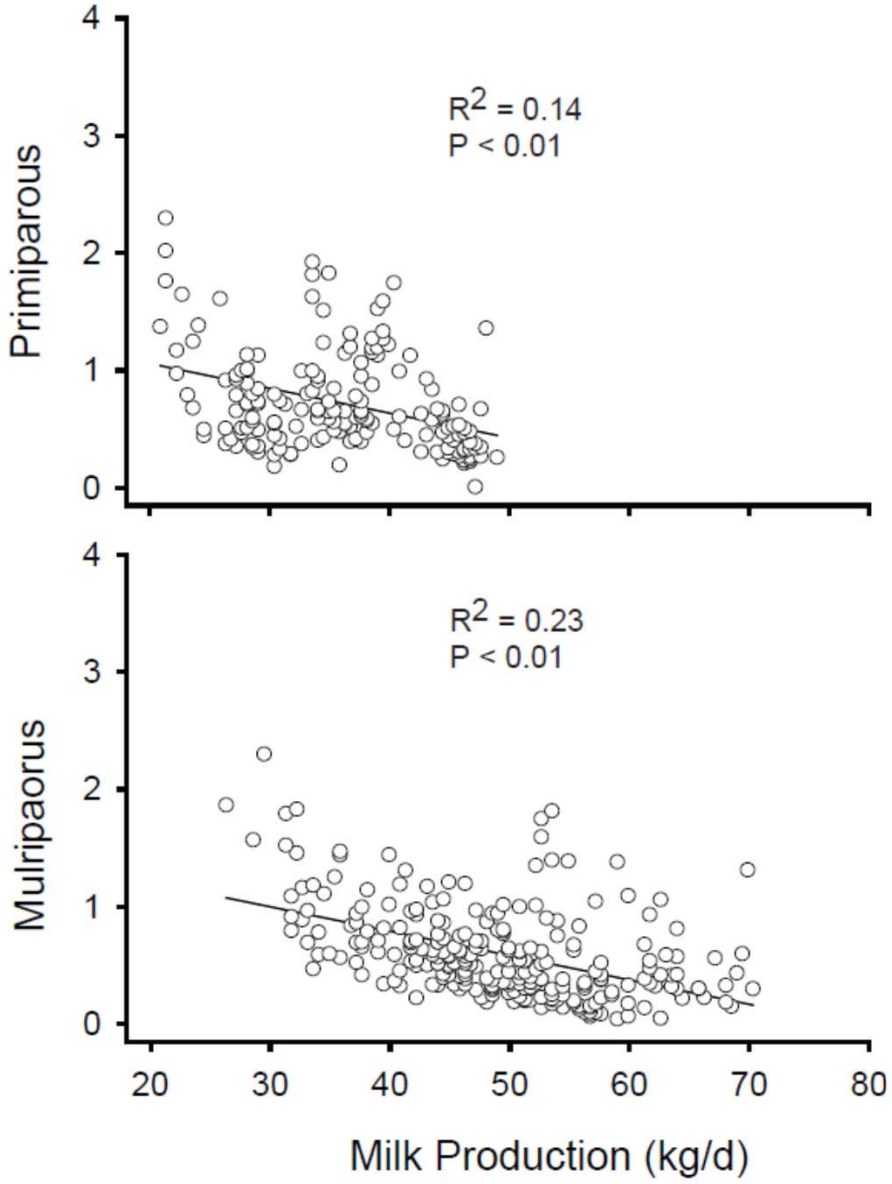

All cows (%)
Primiparous (%)
Multiparous (%)
25 32 39 46 53 60 67 74 81 88 95 102
Days after TAI

Milk PAGs

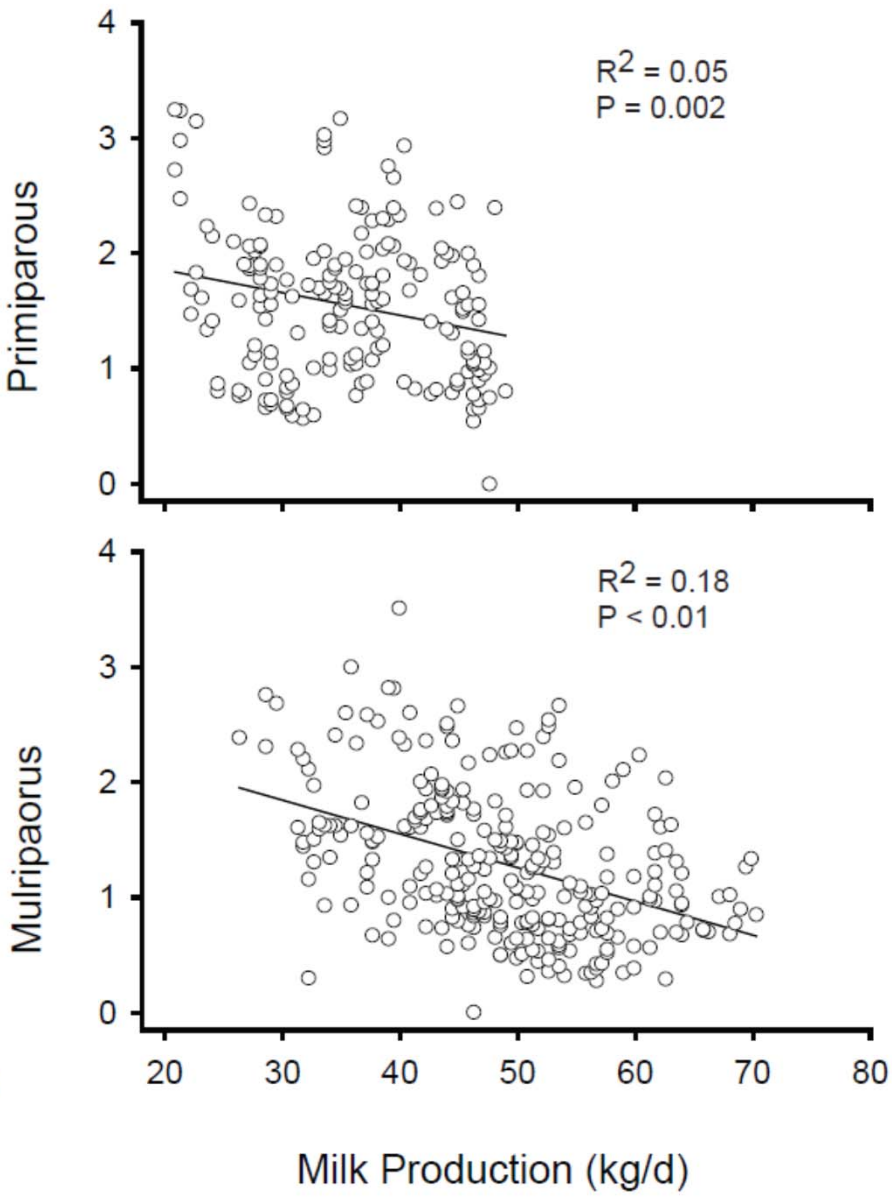

S-N milk
2.5
2.0
1.5
1.0
0.5
0.0
P: P = 0.05
T: P < 0.01
PxT: P = 0.47
Primiparous
Multiparous
25 32 39 46 53 60 67 74 81 88 95 102
Days after TAI

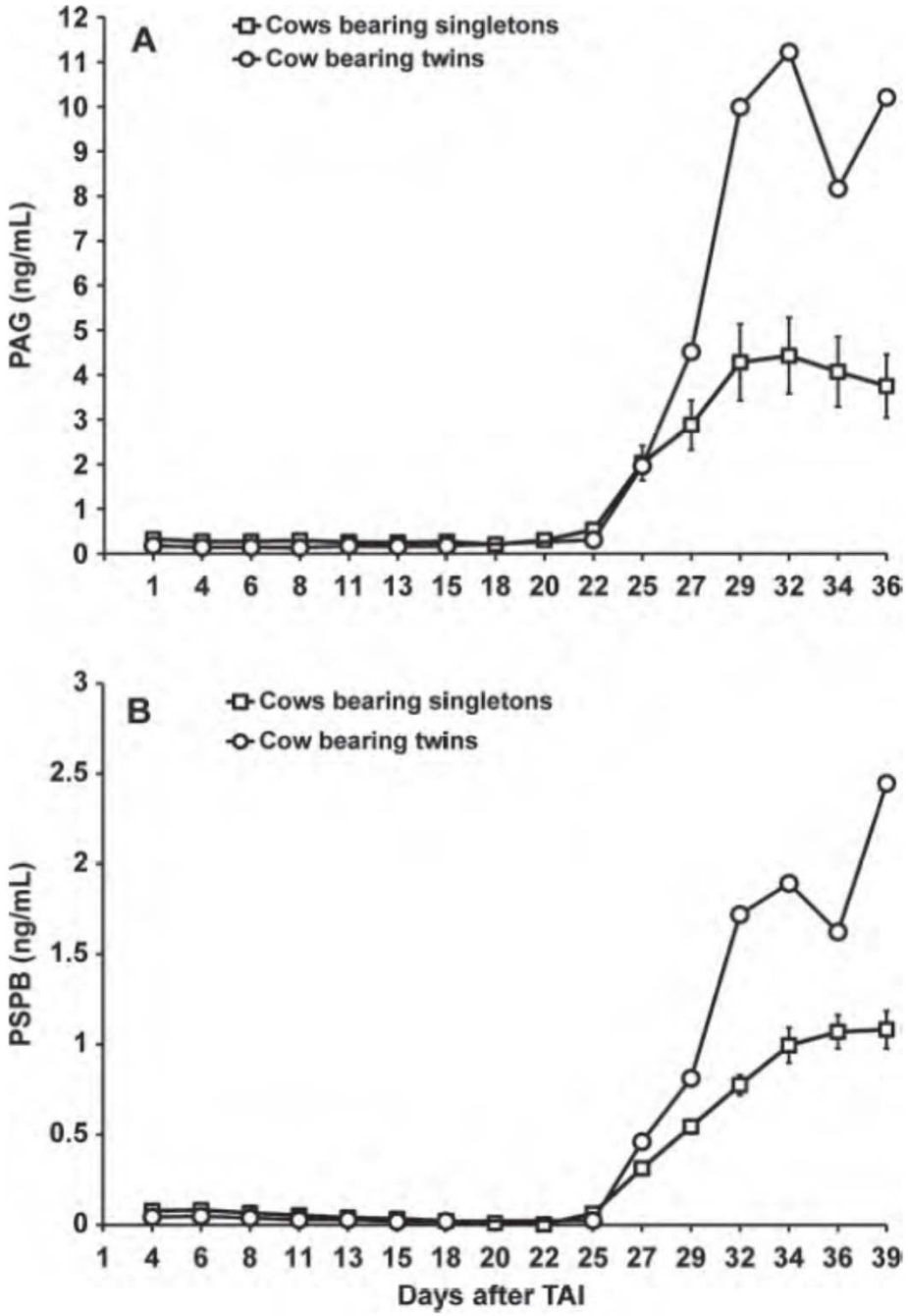

All cows (%)
Primiparous (%)
Multiparous (%)
100
90
80
70
60
50
40
30
20
10
0
25 32 39 46 53 60 67 74 81 88 95 102
Days after TAI

Milk PAGs



Plasma PAGs





#Twins

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

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

Conclusions

- Stage of gestation, parity, pregnancy loss, and milk production were associated with plasma and milk PAG levels after TAI similarly.
- Based on plasma and milk PAG profiles, the optimal time to conduct a first pregnancy diagnosis is ~32 d after AI coinciding with an early peak in PAG levels of pregnant cows.
- Because of the occurrence of pregnancy loss, all pregnant cows should be retested >74 d after AI.

Blood vs. Milk: Which is Better?

- The sensitivity of both the blood and the milk PAG tests are acceptable around 32 d and after ~74 d after AI, but not from 39 to 67 d.
- Costs of blood and milk PAG tests are competitive with palpation and ultrasound.
- Use of either of these tests to diagnose pregnancy status 32 d after AI would economically benefit a dairy farm.
- Choice comes down to management.
  - Preg check interval is the most important consideration

DEPARTMENT OF
DAIRY SCIENCE
University of Wisconsin-Madison