

# Essential Fatty Acids Improve Reproduction in Holstein Cows

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# Glossary

- EFA = essential fatty acid
- PUFA = poly-unsaturated fatty acid
- C18:2= linoleic acid = omega-6
- C18:3= linolenic acid= omega-3
- PGFM = prostaglandin F metabolite
- PMN= polymorphonuclear leukocytes  
= neutrophil

# Essential Fatty Acids (EFAs) = Polyunsaturated Fatty Acids (PUFAs)

- Cannot be made by cows. They must be obtained in the diet. When they enter the rumen, they are biohydrogenated and become saturated fatty acids. Calcium salts protect EFAs so they get to intestine.

# Essential Fatty Acids (EFAs)

Omega-6, Linoleic Acid, 18:2

Omega-3, Linolenic Acid, 18:3

# How Much Linoleic Acid (C18:2) Does a Cow Secrete Per Day in Milk?

In milk, 2-6% of milk fat is C18:2

100 lbs/d @ 3.5% fat and 4% 18:2 = 0.14 lbs/d  
**=64 g/d of C18:2**

Assuming no other need for 18:2, this would be the  
requirement

Since other cells in the body and other functions require 18:2,  
there is likely an additional requirement for 18:2 above 64  
g/d

# What do EFAs Do?

Aid in production of  
**steroid hormones**



**Eicosanoids**  
(biological signaling  
factors, including  
prostaglandins)

Fluidity and  
elasticity of all  
cell membranes

# EFA Amounts in Various Diets

Parameter	Basal	M	MR	EB	T	RS	WCS
<b>Fatty Acids*</b>							
Intake (g/d)	500	400	400	400	400	400	400
Rumen Escape (%)	15	54	54	0	2	16	1
Duodenum (g/d)	659	400	400	400	400	404	404
Absorbed (g/d)	479	327	337	291	293	298	300
Intest. Digestion (%)	73	82	84	73	73	74	74
<b>C18:1trans*</b>							
Intake (g/d)	0.1	0.0	0.0	1.6	5.2	0.0	0.0
Duodenum (g/d)	37.0	2.3	11.0	1.9	5.6	39.7	30.3
Absorbed (g/d)	29.0	1.8	9.1	1.5	4.4	31.2	23.8
<b>C18:2*</b>							
Intake (g/d)	225	28	127	7.2	18.8	230	157
Duodenum (g/d)	58	17	77	0.7	2.2	54	12
Absorbed (g/d)	48	17	<b>76</b>	0.6	1.8	43	10
<b>C18:3*</b>							
Intake (g/d)	23.9	0.80	18.81	0.0	33.9	13.2	1.60
Duodenum (g/d)	1.6	0.46	10.88	0.0	1.0	2.5	0.09
Absorbed (g/d)	1.1	0.40	<b>9.32</b>	0.0	0.8	1.9	0.06

\* From basal diet or supplement

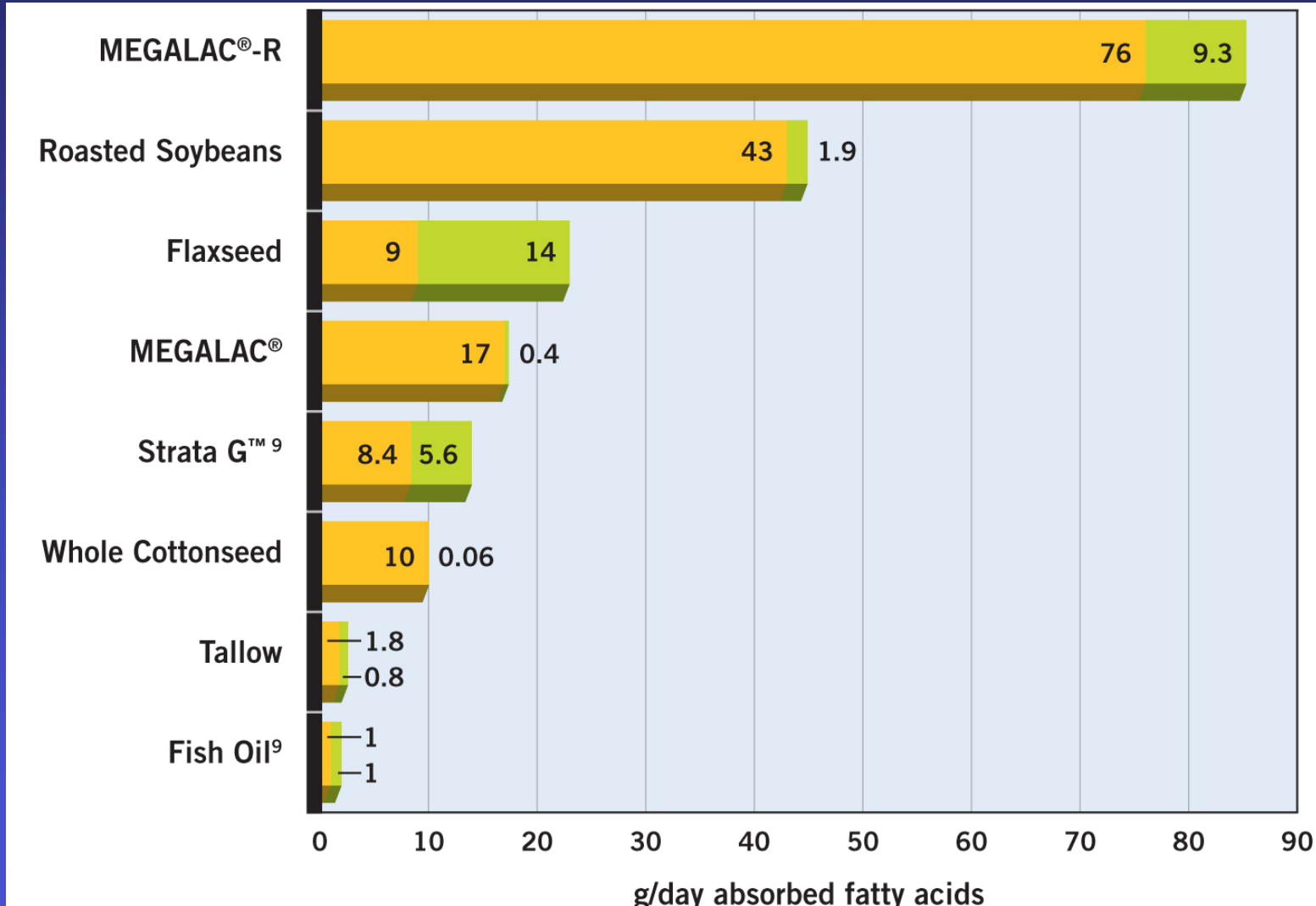
-Moate and Chalupa, ADSA Abstract (2002)

# EFA Metabolism by Reproductive Organs

- **Uterus**
  - Endometrial production of prostanoids
  - Linoleic acid → arachadonic acid → PGF
- **Ovaries**
  - Linoleic and linolenic acids for steroid precursors
- **Placenta**
  - Lack – retained fetal membranes
  - Abundance – increased growth and survivability
  - Linoleic – PGE<sub>2</sub> for cervical dilation and fetal organ maturation

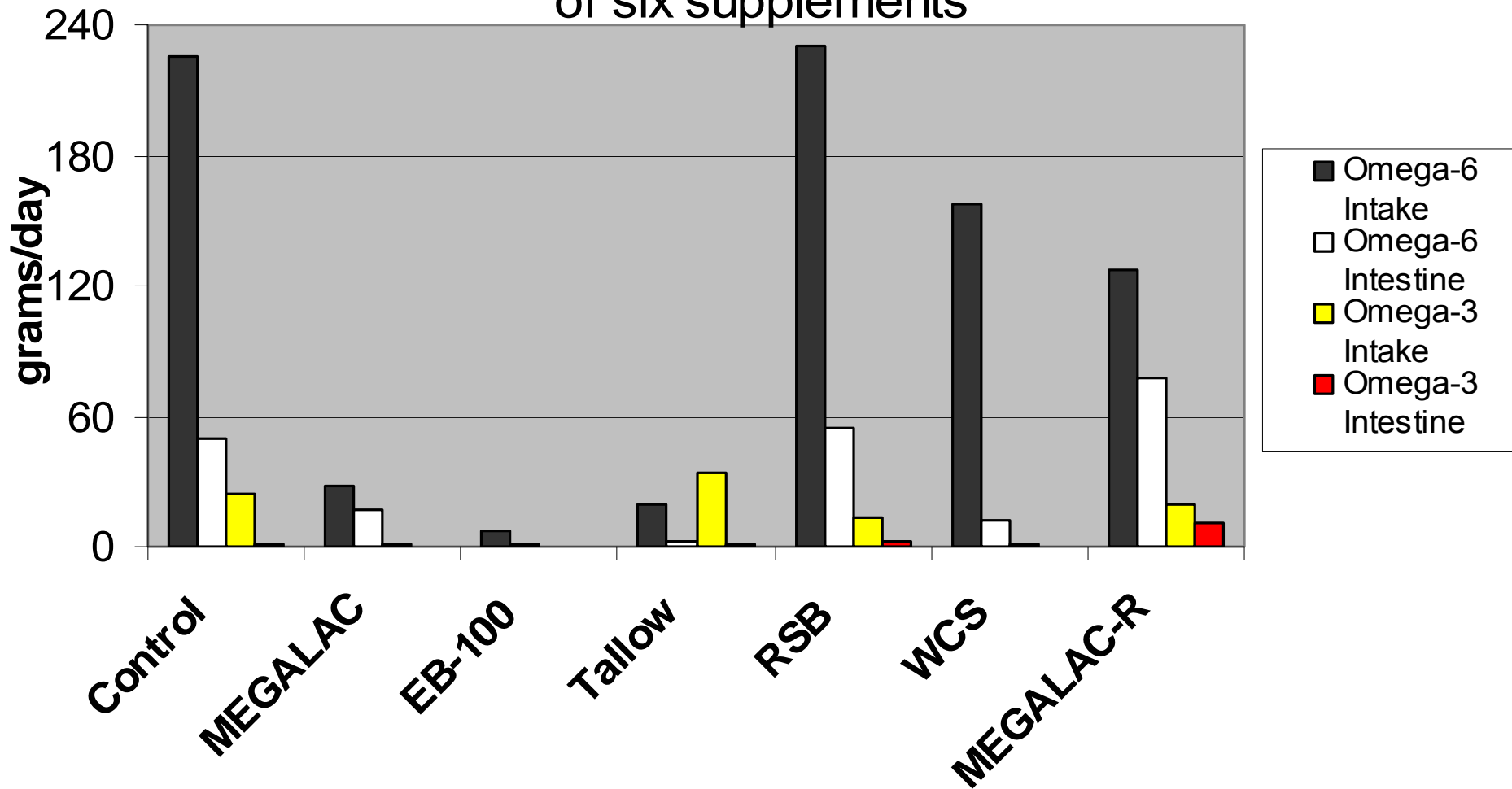
(Liggins & Thorburn, 1994; Wischeral et al., 2001)

# Sources of Omega-3 & -6

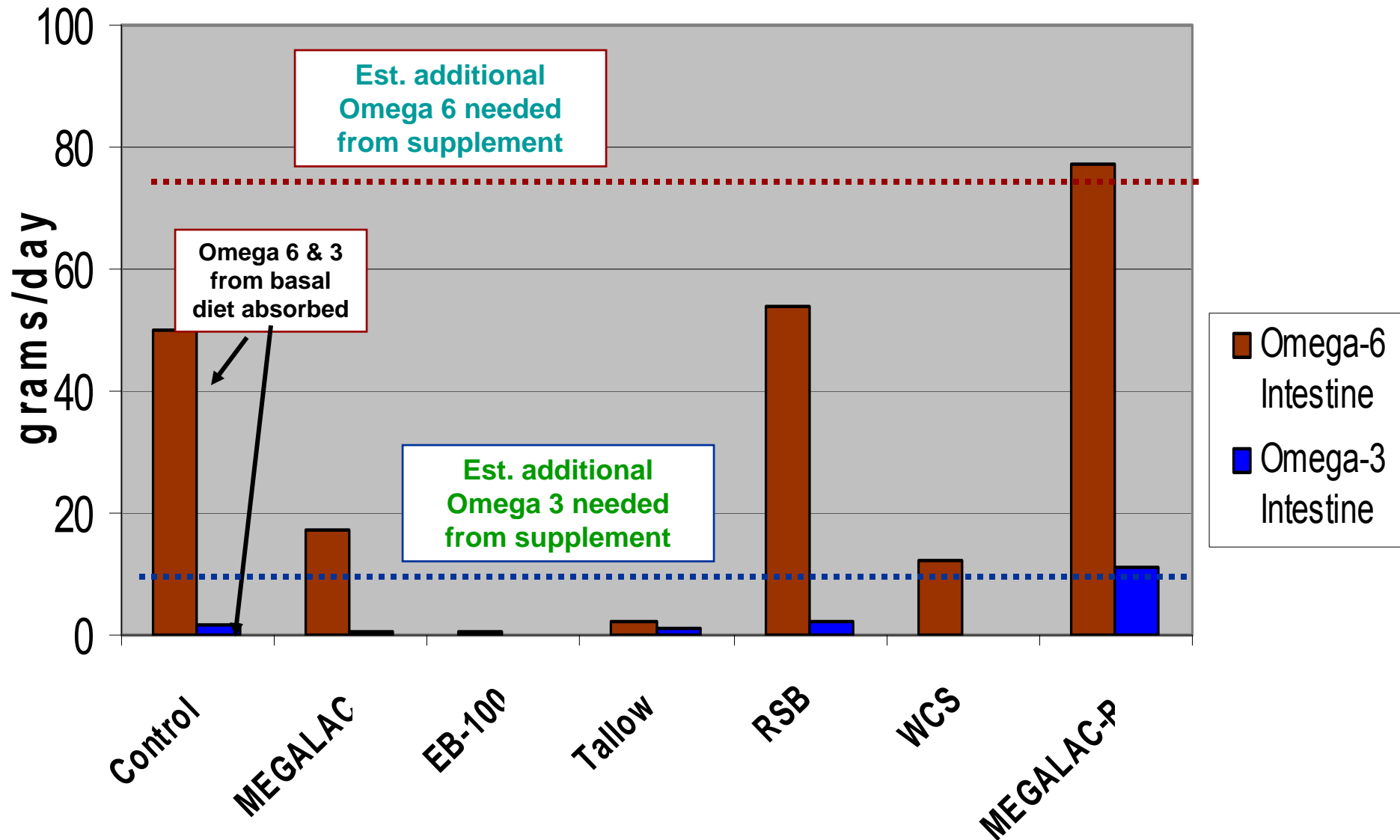


# How much Omega-6 and -3 actually get to the intestine?

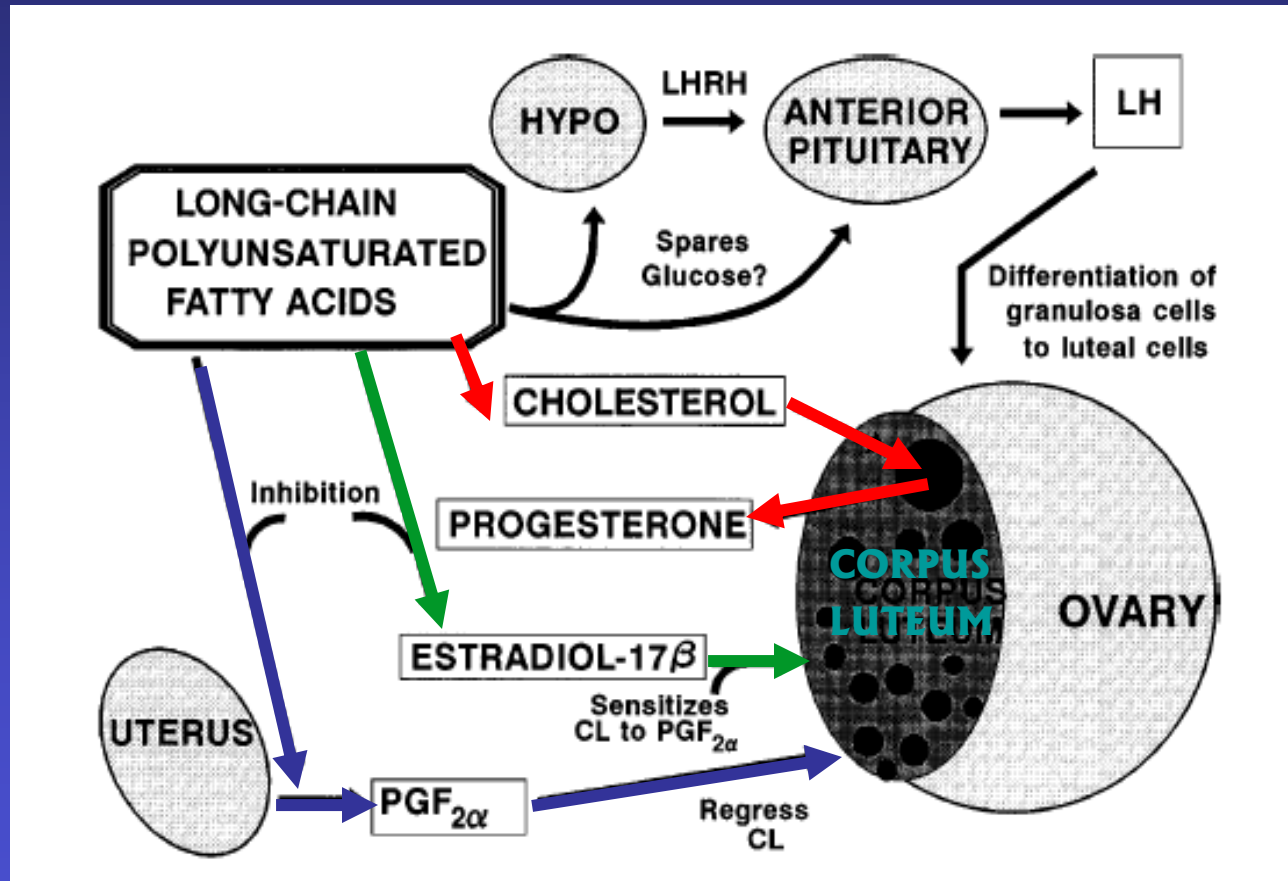
Basal diet (100 lb/d milk) and 400 g fatty acids from any of six supplements



# Do any of the supplements approach the estimated requirements?



# Mechanisms of EFA Action



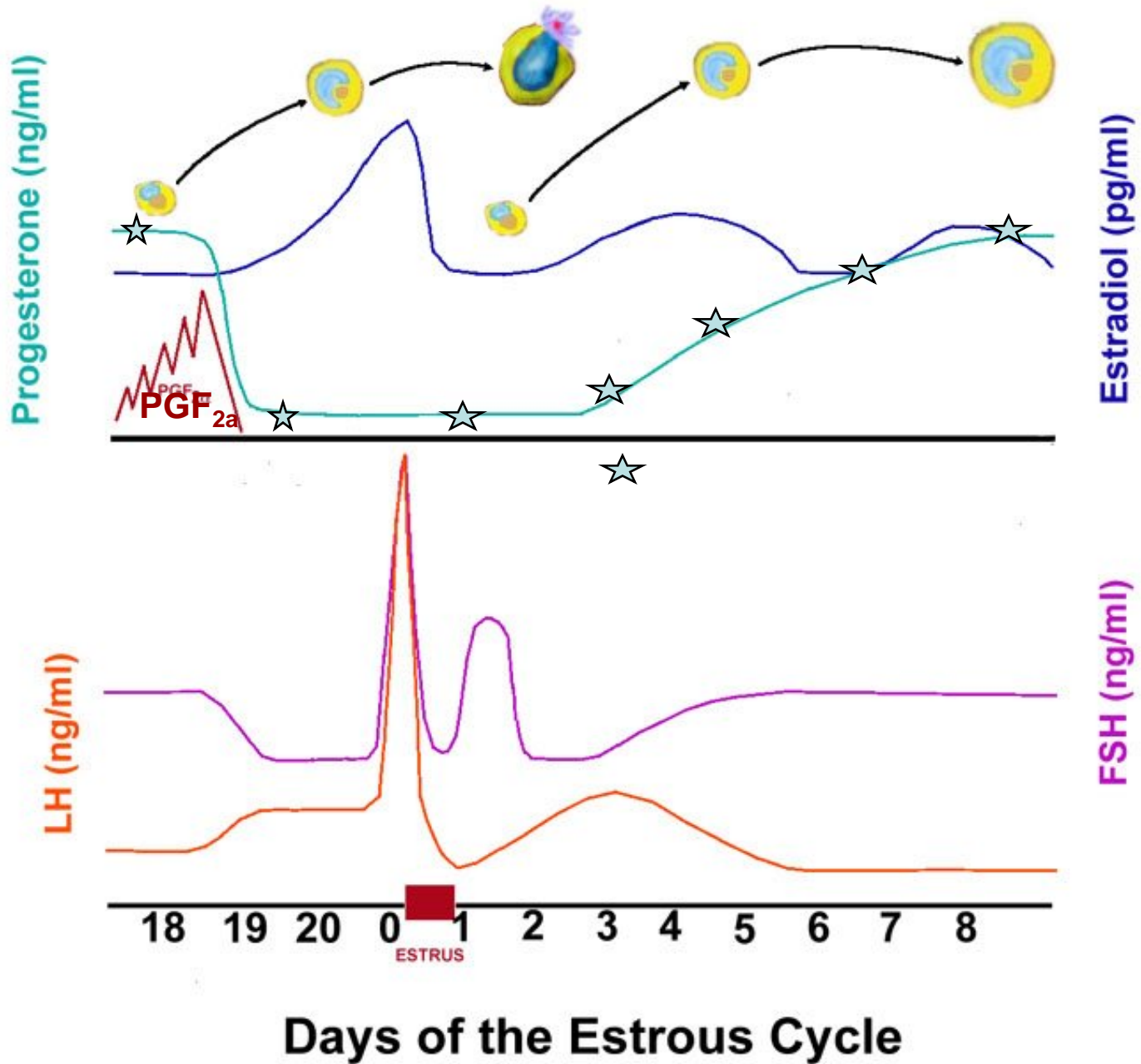
Staples, et al. (1998)

# Steroid Hormones

- Progesterone ( $P_4$ ): the Pregnancy hormone
  - Higher concentrations of  $P_4$  at end of heat cycle, show stronger heat signs
  - Stronger heat signs lead to more accurate heat detection and correctly timed breeding
  - Higher pre-breeding  $P_4$  concentrations = higher conception rates
  - Higher concentrations of  $P_4$  maintained through conception, more likely to remain pregnant

-Fonseca et al., 1983; Shemesh et al., 1983

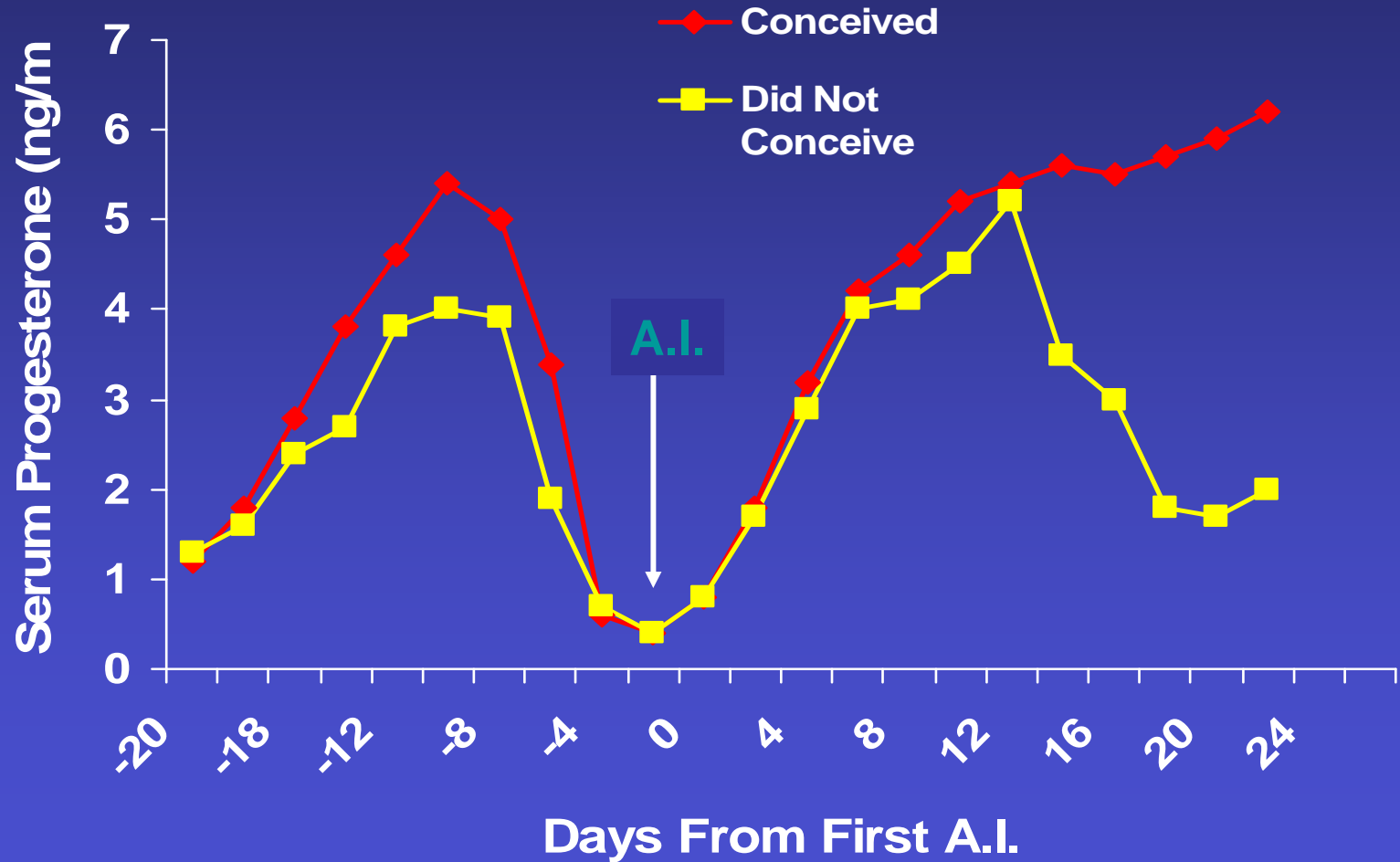
# Cow



# Exploratory Research

- Comparing calcium salts of Omega-3 & -6 to fish oil calcium salts and to non-essential fatty acids:
  - **Omega-3 & -6** and fish oils increased size of final ovulatory follicles
  - **Only Omega-3 & -6** increased prostaglandin receptors in uterus and on ovaries
  - **Only Omega-3 & -6** kept progesterone levels high through the end of the cycle

# Progesterone and Conception Rate

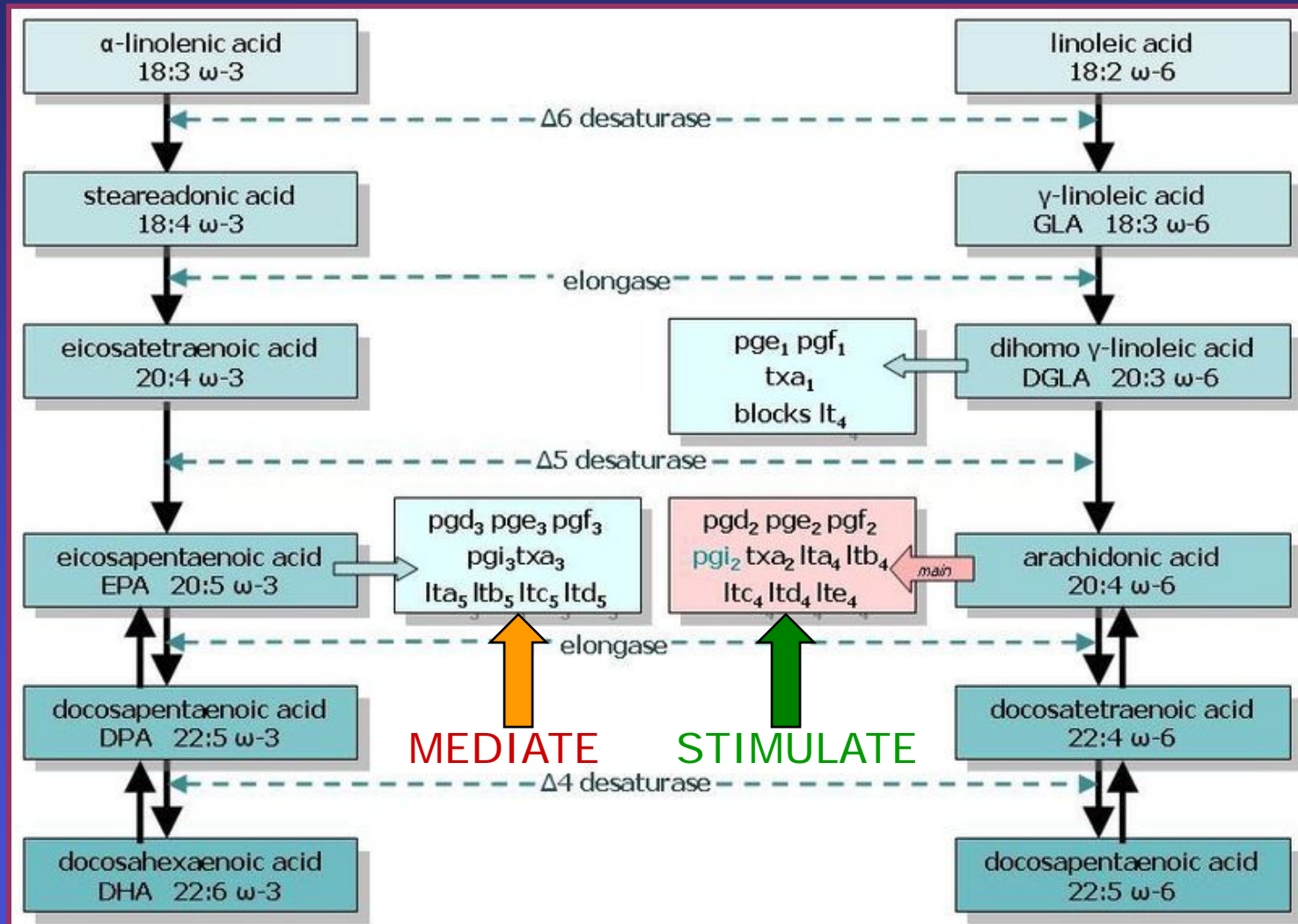


Fonseca et al. (1983)

# Synopsis of Staples et al. (1998)

- Found 11 studies reported an improvement either in first A.I. service conception rate or in the overall rate of conception or pregnancy
  - ( $P < 0.10$  or  $\geq 15$  percentage unit difference between means)
- A greater proportion of cows fed Ca-LCFA showed stronger signs of estrus, had more active ovaries, and required less exogenous  $\text{PGF}_{2\alpha}$  to induce estrus

# EFA, Eicosanoids, and Immune Factors



# Eicosanoids

- Prostaglandins:  $\text{PGF}_{2\alpha}$  most common, also PGE
- Used to treat reproductive problems and for synchronization
  - Indirectly cause ovulation and a subsequent estrous cycle
  - Enhance visible signs of heat
  - Increase blood flow to the ovaries to promote follicle growth

# Endometritis

- Uterine infection, generally bacterial
- Incidence ranges from 3-36% between herds across the U.S.
- Estimated loss to producer is \$106/case
  - Includes milk loss, reproductive loss, treatment cost, culling loss

(Fuhrmann, 2006)

# Immune Response

- 100% of cows have metritis at 14-21 days postpartum (Gilbert et al., 2005)
- Increased dietary EFAs contribute to more concentrated neutrophil response postpartum (Scheick, 1996)
- Increase prostaglandin synthesis to support uterine cleaning
  - Can measure immune response by analyzing serum concentrations of  $\text{PGF}_{2\alpha}$  metabolite (PGFM) (Risco et al., 1994; Mishra and Prakash, 2005)

# Polymorphonuclear Leukocytes (PMN)

- Phagocytic cells responsible for breakdown of placental attachment, bacterial and sperm clearance and promotion of inflammation
- Function can be altered by fatty acid composition
  - Chemical – decrease in production of peroxidase
  - Physical – inhibited responsiveness to and secretion of leukotrienes

# EFAs and Immunity

- Omega-6 (linoleic acid)
- $\text{PGF}_1$ ,  $\text{PGF}_2$ , and  $\text{PGE}_2$
- Stimulate inflammatory response through regulation of eicosanoids
- $\text{PGF}_2$  supports pre- and postpartum contractions for expulsion of fetus and lochia/placenta

# EFAs and Immunity

- Omega-3 (linolenic acid)
- Anti-autoimmune functions
  - Supplementation encouraged in many autoimmune disorders
- Mediates immune response
- Competitively inhibited in extreme ratios by omega-6

# What is Megalac<sup>®</sup>-R?

- Nutritional supplement containing Ca-LCFAs
- Maintains the energetic and rumen inert properties of regular Megalac<sup>®</sup>
- Devised to transport PUFAs (specifically linoleic and linolenic acids) to the small intestine for absorption



# Comparison of Megalac<sup>®</sup> and Megalac<sup>®</sup>-R

	Megalac <sup>®</sup>	Megalac <sup>®</sup> -R
<b>Linoleic acid</b>	7.00%	31.75%
<b>Linolenic acid</b>	0.20%	4.70%

Adapted from Arm & Hammer's Megalac<sup>®</sup>-R  
Research Summary (2002)

# UA Reproductive Trial I

- **Part 1:** Real-time ultrasonography to evaluate ovarian structures
- **Part 2:** P<sub>4</sub> analysis of milk samples for cyclic profiles
- **Part 3:** Tracking of uterine health through veterinarian's prescription of PGs
- **Part 4:** Ovsynch regimen to evaluate conception rates between treatments

# UA Reproductive Trial I

- Arizona commercial herd
- Number of cows = 2,003
  - Primiparous and multiparous cows included
- Milk = 9896 kg/cow/lactation
- Milk = 34.5 kg/d
- First service = 75 DIM
- Services per conception = 2.6
- Days open = 152

# UA Reproductive Trial II

- **Part 1:** Real-time ultrasonography to evaluate ovarian structures
- **Part 2:** PGFM analysis at DIM 10, 20, 30 from serum
- **Part 3:** Tracking of uterine health through veterinarian's prescription of PGs
- **Part 4:** Uterine cytology swabs for neutrophil counts

# UA Reproductive Trial II

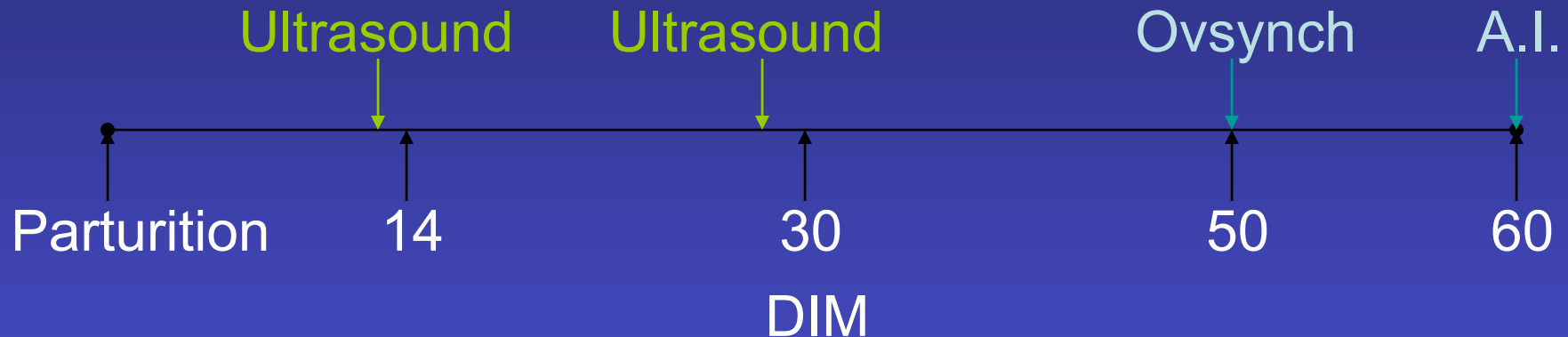
- Arizona commercial herd
- Number of cows = 8,032
  - Only multiparous cows used
- Milk = 10,703 kg/cow/lactation
- Milk = 35.5 kg/d
- First service = 65 DIM
- Services per conception = 2.6
- Days open = 156

# Trial Rations

- Prepartum
  - **Trial I:** All cows fed 0.114 kg/d Ca-LCFA for 21 d
  - **Trial II:** 0.114 kg/d of Ca-LCFA or Ca-LCFA+EFA for 14 d
- Parturition-Lactation
  - **Trial I:** 0.159 kg/d of Ca-LCFA or Ca-LCFA+EFA until 150 DIM or confirmed bred
  - **Trial II:** 0.227 kg/d of Ca-LCFA or Ca-LCFA+EFA until 60 DIM
- Rations balanced to equalize caloric intakes

# Trial I Timeline (April 2005- January 2006)

Cows fed Ca-LCFA 21d prepartum, Ca-LCFA or Ca-LCFA+EFA postpartum until 150DIM

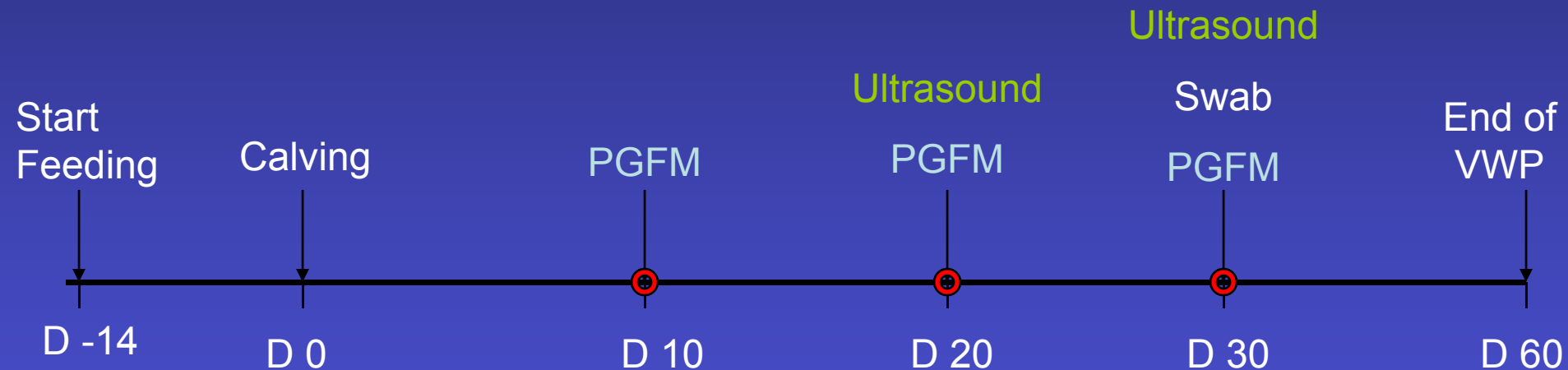


Milk samples collected twice weekly until ~100DIM

Uterine health data collected until ~90DIM

# Trial II Timeline

Cows fed either Ca-LCFA or Ca-LCFA+EFA 14d prepartum,  
Ca-LCFA or Ca-LCFA+EFA postpartum until 60 DIM



○ Blood Sample for Assay

At 60 DIM all trial animals were moved and combined into breeding pens with no supplementation

**Table 1.** Trials I and II combined ovarian status confirmed by ultrasound,  $\leq 20$  DIM.

	<b>Ca-LCFA (n=58 cows)</b>	<b>Ca-LCFA+EFA (n=57 cows)</b>	<b><i>p</i>-value</b>
Category 1	28 (24.1%)	24 (21.0%)	NS
Category 2	35 (30.2%)	28 (24.6%)	NS
Category 3	35 (30.2%)	49 (43.0%)	<b>&lt;0.05</b>
Inactive	18 (15.5%)	13 (11.4%)	NS

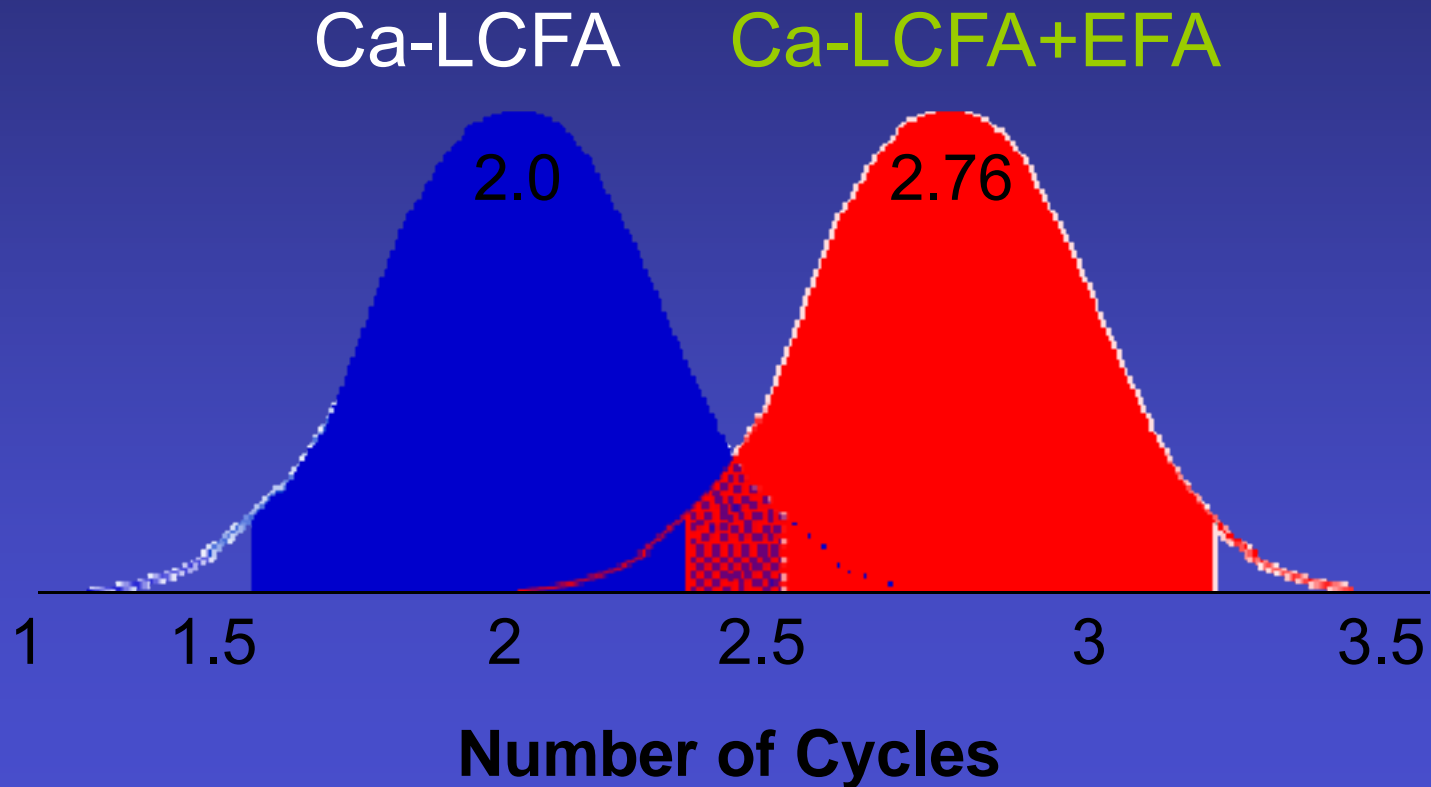
**Table 2.** Trials I and II combined ovarian status confirmed by ultrasound, 21-30 DIM.

	<b>Ca-LCFA (n=61 cows)</b>	<b>Ca-LCFA+EFA (n=55 cows)</b>	<b><i>p</i>-value</b>
Category 1	14 (11.5%)	3 (2.7%)	<0.001
Category 2	22 (18.0%)	23 (20.9%)	NS
Category 3	62 (50.8%)	74 (67.3%)	<0.02
Inactive	24 (19.7%)	10 (9.1%)	<0.001

**Table 3.** Effects of EFA supplementation on number of estrous cycles by 60 DIM in multiparous cows as determined by progesterone profiling.

	Ca-LCFA (n=18)	Ca-LCFA+EFA (n=17)	<i>p</i> -value
Overall [P] (ng/ml)	7.4	6.2	NS
Number cycles	<b>2.0</b>	<b>2.76</b>	<u>≤ 0.031</u>
Range in number cycles	0 – 3	1 - 5	
95 % C.I. for number cycles	1.52 - 2.48	2.27 - 3.26	

# Frequency Distribution of Cycles 60 DIM



**Table 4.** Effects of EFA supplementation on number of estrous cycles by 60DIM in primiparous cows as determined by progesterone profiling.

	Ca-LCFA n=5	Ca-LCFA+EFA n=5	<i>p</i> -value
Number cycles	1.8	1.8	<b>NS</b>
Range in number cycles	1 – 3	0 – 3	
95 % C.I. for number cycles	0.937 – 2.66	0.937 – 2.66	

-Jones et al. (2006)

**Table 5.** Effects of EFA supplementation on number of cows treated with PG for uterine health issues, combined results of Trials I and II.

<b>Parameter</b>	<b>Ca-LCFA treated/total</b>	<b>Ca-LCFA +EFA treated/total</b>	<b><i>p</i>-value</b>
Total treated	547/1405	252/784	<0.001
Total % treated	<b>38.9</b>	<b>32.1</b>	

-Jones et al. (2006) and Bowen (2008)

**21% improvement in PG  
injections**

**Table 6.** Subset of cows categorized with metritis determined by concentration of polymorphonuclear leukocytes with respect to somatic cells obtained in uteri.\*

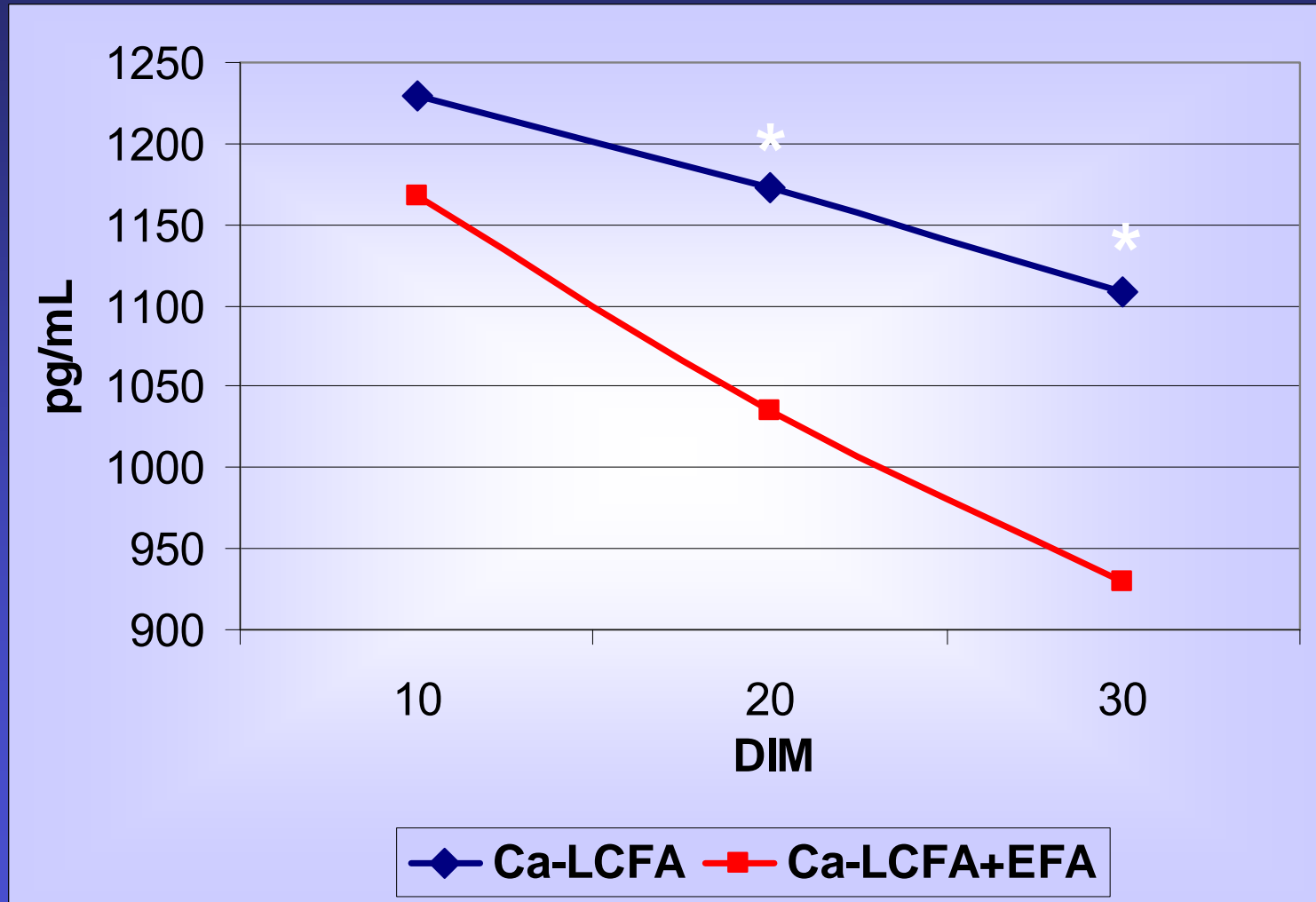
	Ca-LCFA n=25	Ca-LCFA +EFA n=27	<i>p</i> -value
Metritis <sup>a</sup>	14 (56%)	5 (18.5%)	<0.01
Normal	11 (44%)	22 (81.5%)	

-Bowen (2008)

<sup>a</sup> Slides with a ratio of  $\geq 15\%$  PMN to uterine somatic cells were predetermined to be indicative of uterine infection.

\* Samples obtained through transcervical uterine cytological swab, transferred to glass slides, fixed in methanol, prepared with Giemsa staining and read at 40X magnification by stereo microscopy

**Figure 1.** Serum concentrations of 13,14-dihydro-15-keto prostaglandin F2 metabolite determined by enzyme immunoassay for 10, 20 and 30 DIM



\* $p < 0.001$

-Bowen (2008)

## Table 7. Effects of metritis on days open and services per conception in Trial II.

	Metritis	Normal	<i>p</i> -value
Days open	119.6	109.1	<0.01
SPC	2.8	2.3	<0.01

-Bowen (2008)

Metritis really didn't help my postpartum depression.



TMI,  
Mabel,  
TMI.

**Table 8.** Effects of EFA supplementation on conception rates on cows time bred following Ovsynch.

<b>Trait</b>	<b>Control</b> % (n)	<b>Ca-LCFA+EFA</b> % (n)
Number cows confirmed pregnant by 200DIM	85.4 (111/130)	92.8 (103/111)
1 <sup>st</sup> service conception	29.2 (38/130)	24.3 (27/111)
2 <sup>nd</sup> service conception	46.7 (43/92)	39.2 (33/84)
Services/conception	2.00	2.00
DIM at 1 <sup>st</sup> service	63	63
Days open	101	103

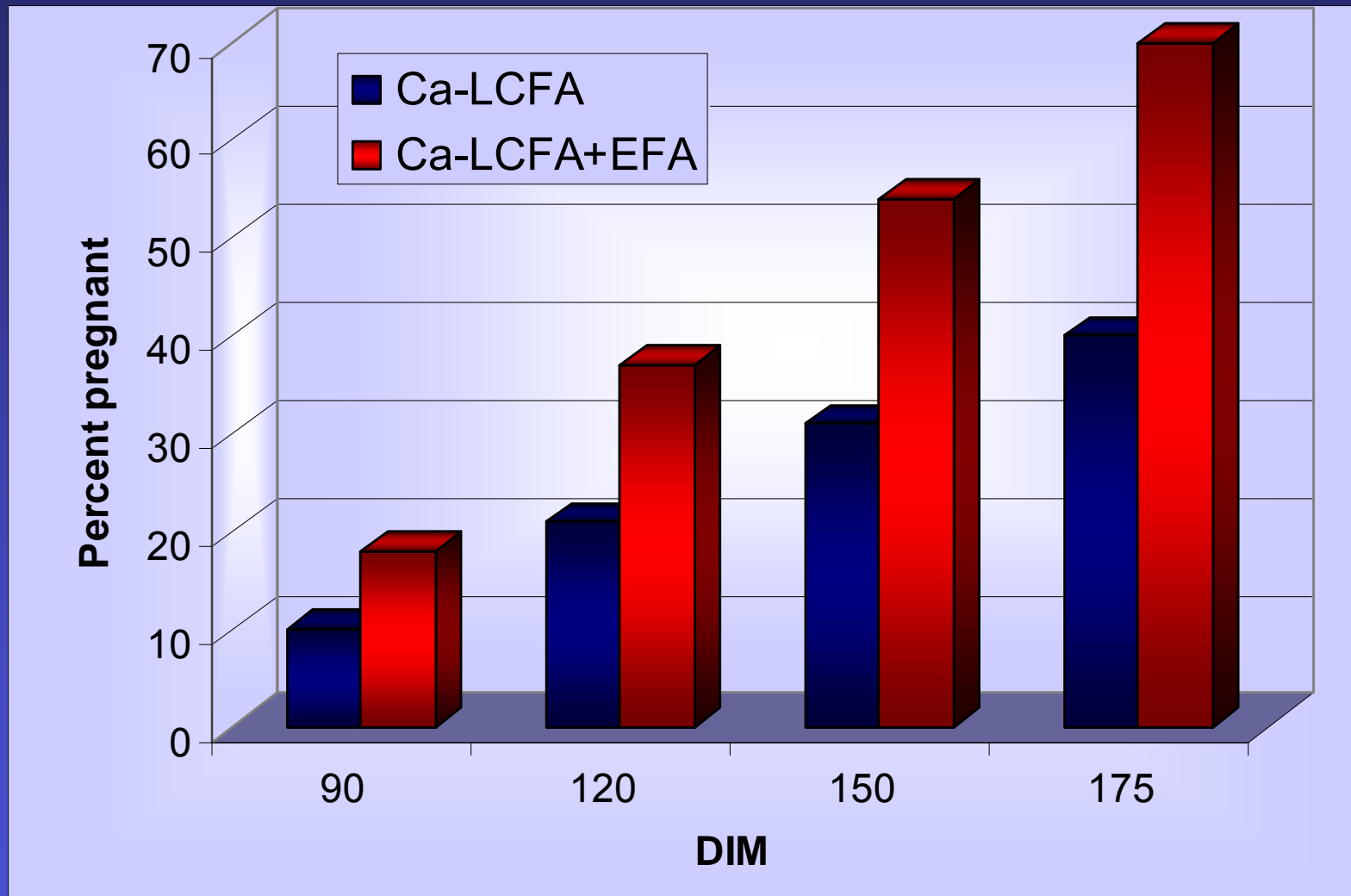
-Jones et al. (2006)

**Table 9.** Effects of EFA supplementation on days open, days to first service, services per conception and percent pregnant at 175 DIM in Trial II.

	<b>Ca-LCFA</b> n=76	<b>Ca-LCFA</b> <b>+EFA</b> n=93	<b>p-value</b>
Days open	117.2	100.7	<0.01
Days to 1st service	79.1	72.6	<0.01
SPC	2.8	2.1	<0.01
% pregnant at 175 DIM	40%	70%	<0.01

-Bowen (2008)

**Figure 2. Pregnancies confirmed by veterinarian via rectal palpation by DIM, Trial II.**



-Bowen (2008)

**Table 10.** Summary of effects of EFA supplementation on reproductive outcomes in Trial I.

<b>Trait</b>	<b>Ca-LCFA</b>	<b>Ca-LCFA +EFA</b>	<b><i>p</i>-value</b>
3.5%FCM (kg/d)	36.9	37.1	NS
Ovulations(%) by 30DIM	17/63 <b>(27%)</b>	28/57 <b>(49%)</b>	$\leq 0.01$
Number of cycles by 60DIM	<b>2.00</b>	<b>2.76</b>	$\leq 0.03$
Metritis (%) by 60DIM	511/1312 <b>(38.9%)</b>	205/708 <b>(29.0%)</b>	$\leq 0.01$

-Jones et al. (2006)

# Economic Benefits

<b>Expense</b>	<b>Per Cow</b>	<b>100 Cows</b>
14 d Pre-fresh (1/4 lb/d)	\$2.80	\$280
60 d Post-fresh (1/2 lb/d)	\$24.00	\$2,400
<b>Total</b>	<b>\$26.80</b>	<b>\$2,680</b>
<b>Income/cow</b>		
0.7 units semen/service @ \$15.00	\$10.50	\$1,050
Reduced Metritis by 10%	\$350	\$3,500
Increased Preg by 30%	\$300	\$9,000
<b>Total</b>	<b>\$108.70</b>	<b>\$13,550</b>

# Discussion

- EFA-supplemented cows had...
  - more ovulations early postpartum
  - more estrous cycles by VWP
  - fewer uterine health issues
  - more pregnant cows by 200 DIM

...than cows fed  
ordinary bypass fat.

# Discussion (con't.)

- Data agree
  - Ovulates sooner, more estrous cycles in a given period
  - More cycles, more opportunities to expel fluid and contaminants from uterus, i.e., fewer opportunities for uterine health problems

# Conclusion

- Feed EFAs not only for their energy, but because it **affects the overall reproductive health and efficiency of the cow!**

# Thanks to:

- 
- A photograph of a brown and white cow in a green field at sunset. The sky is a mix of orange, yellow, and purple. A bare tree stands in the background to the right.
- Goldman Dairy
  - Shamrock Farms
  - Dr. Tony Martin, Dairy Veterinary Services
  - Committee Members
    - Dr. Roy Ax
    - Dr. Glenn Duff
    - Dr. Lance Baumgard
    - Dr. Todd Bilby
  - Church & Dwight
    - Dr. Elliot Block
    - Dr. Dave Weber
  - Financial and product support
    - Arm & Hammer Animal Nutrition (MEGALAC<sup>®</sup> and MEGALAC-R<sup>®</sup>)
    - Pfizer (Lutalyse<sup>®</sup>)
    - Merial (Cystorelin<sup>®</sup>)
    - Arizona DHIA (collection vials)

# Questions?



# Suggested Reading

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