DEPARTMENT OF
DAIRY SCIENCE
University of Wisconsin-Madison

UW-Madison Dairy Science/USDA Project

"An Integrated Approach to Improving Dairy Cow Fertility"

Victor Cabrera, Paul Fricke, Pam Ruegg,
Randy Shaver, Kent Weigel, and Milo Wiltbank

THE UNIVERSITY of WISCONSIN MADISON

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

Specific Aim #2

The impact of nutrition on reproductive performance

Milo Wiltbank & Randy Shaver

THE UNIVERSITY of WISCONSIN MADISON

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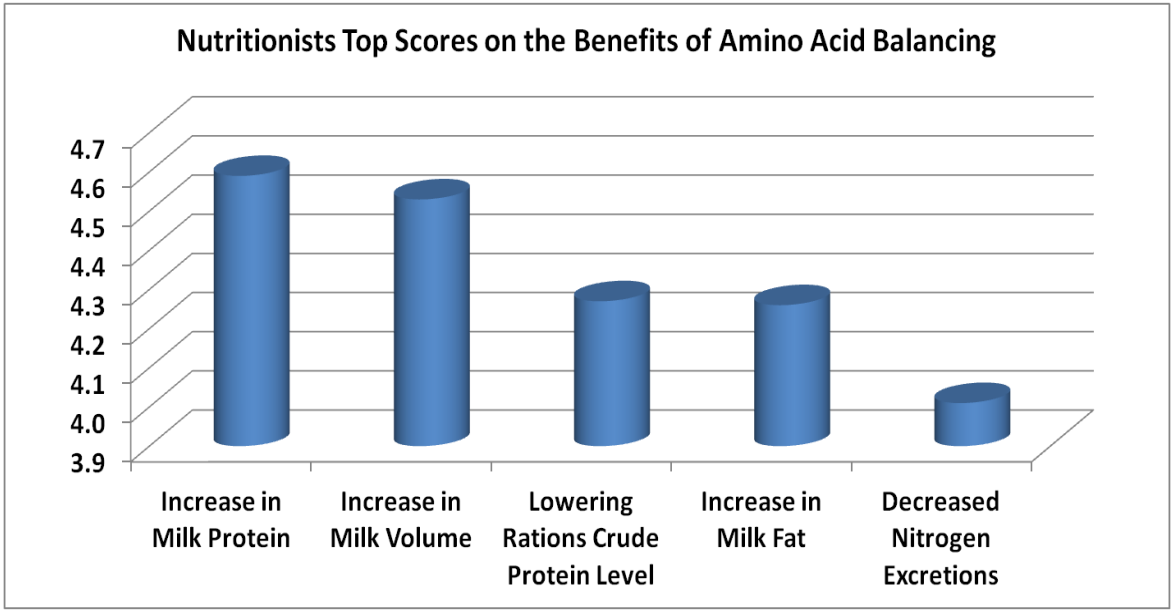
Nutrition Sub-Projects

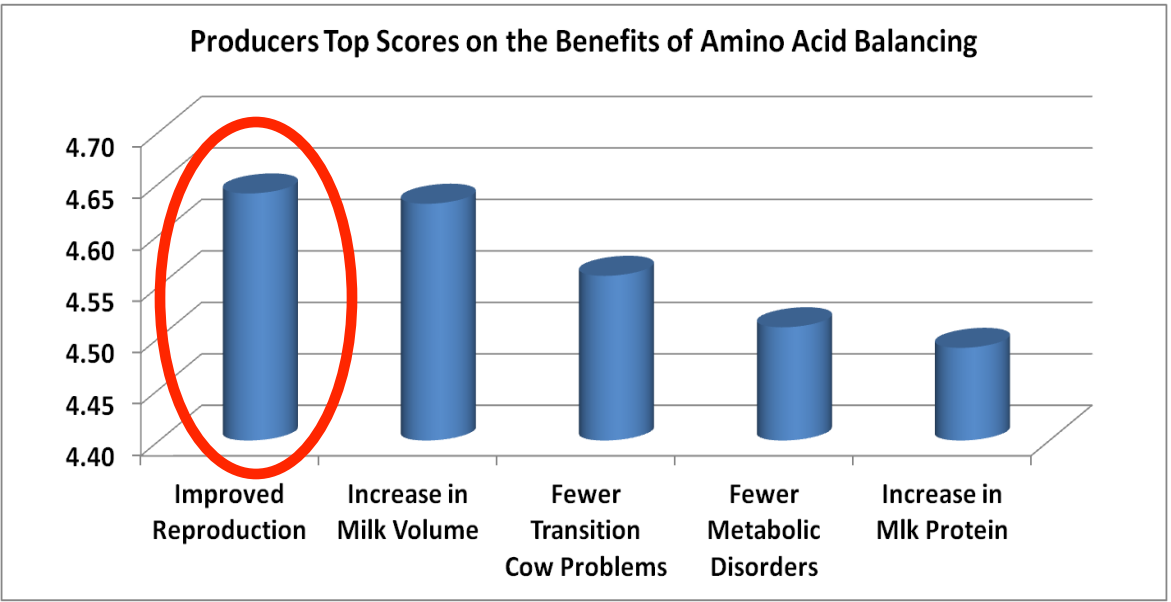
- Farm survey (n=50) of dietary nutrient composition relationships with measures of reproductive performance (Alex Souza)
- Farm survey (n=648) of relationship between milk fat/pro ratio and P/AI at 1st AI (Alex Souza)
- Relationship between postpartum changes in body weight or body condition score and reproductive performance (Paulo Carvalho)
- Changing insulin thru feed restriction or propylene glycol drench on reproductive performance (Robb Bender; Katie Hackbart)
- Feeding rumen-protected choline to transition cows & reproductive performance (Paul Fricke & Mason Amundson; Ric Grummer, Balchem)
- Feeding rumen-protected methionine & reproductive performance (Souza, Carvalho & Mateus Toledo; Daniel Luchini, Adisseo)

Producers and nutritionists were asked the same question:

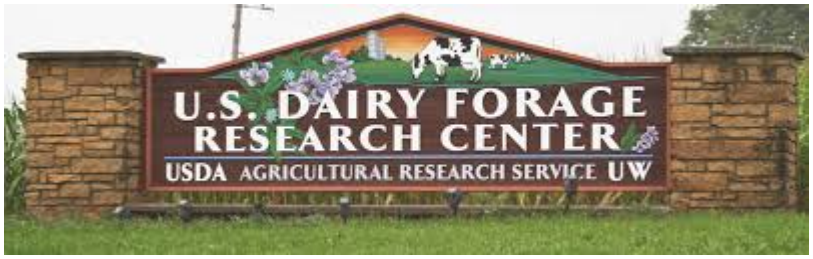
Rank the benefits of Amino Acid balancing (2012 DHM survey)

Nutritionists highest rankings were for more milk protein,then milk volume and lowering rations crude protein





Producers highest rankings were for improved reproduction, increased milk volume and fewer transition cow problems


U.S. DAIRY FORAGE
RESEARCH CENTER
USDA AGRICULTURAL RESEARCH SERVICE UW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Effect of dietary methionine supplementation in early lactation dairy cows:
I - Lactation performance & II - Embryo quality

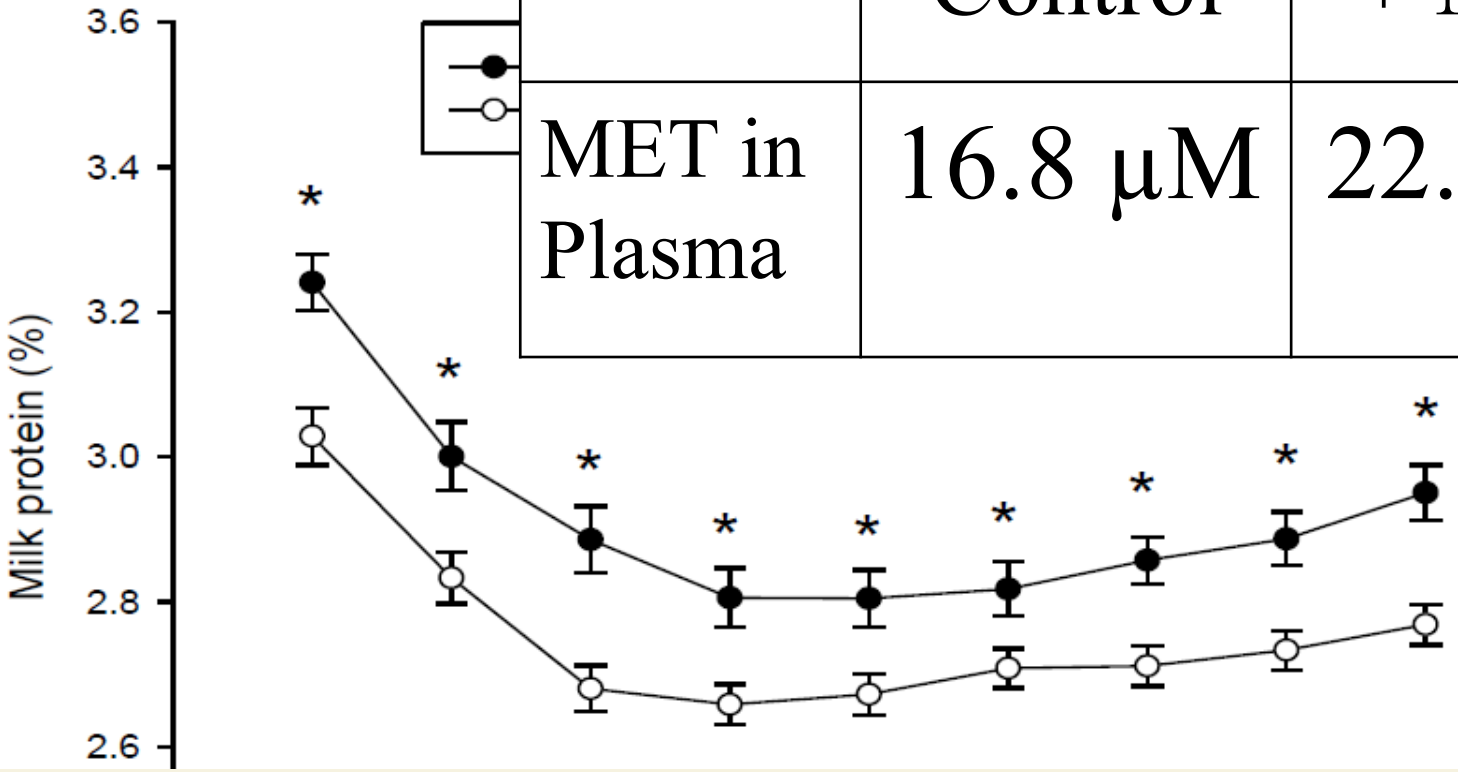
Souza, Carvalho, Dresch, Vieira, Hackbart, Luichini, Bertics, Betzold, Wiltbank & Shaver

2012 ADSA abstracts

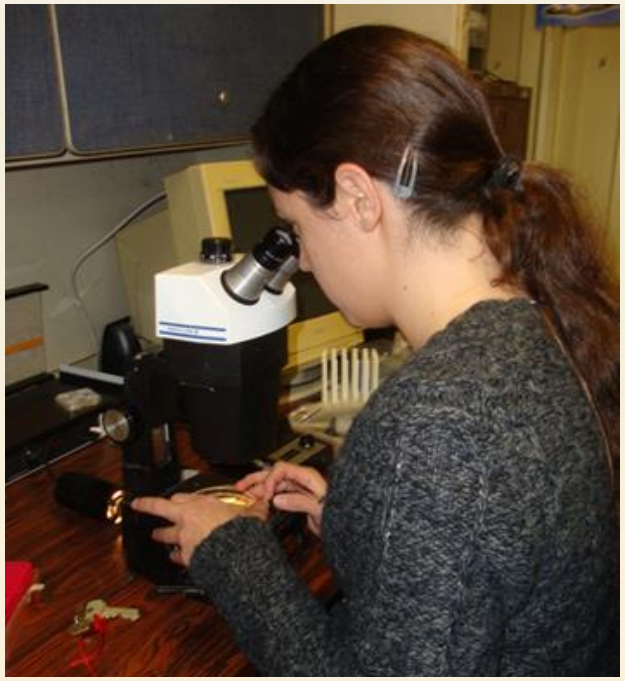
- Holstein cows (n=72)
- Dry period:
  - Housed in a single pen & fed same basal diet
- From calving to 70 DIM:
  - Individual tie-stalls and milked twice daily
- At calving, cows blocked by parity & calving date randomly assigned to two treatments differing in content methionine:
  - **MET**, formulated to deliver 2875g MP with **6.8 Lys %MP** & **2.43 Met %MP** (fed 26 g/d Smartamine® M)
  - **CON**, formulated to deliver 2875g MP with **6.8 Lys %MP** & **1.89 Met %MP**

PROTEIN%

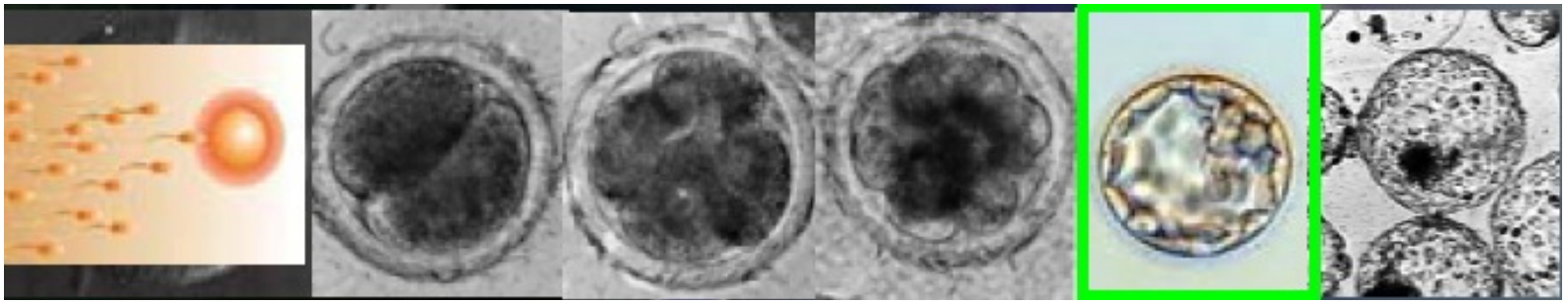
	Control	+ MET
MET in Plasma	16.8 µM	22.9µM



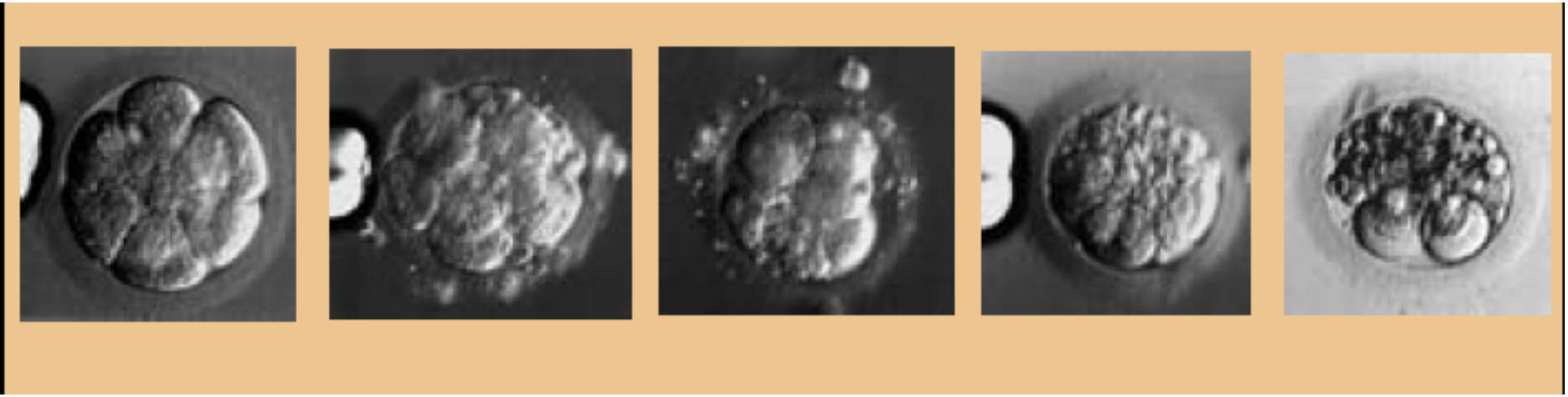
- Supplemental dietary methionine (rumen-protected) increased plasma methionine concentrations and milk protein concentration & milk protein yield.



Embryo grading



1 - Excellent 2 - Good 3 - Fair 4 - Poor 5 - Degenerate

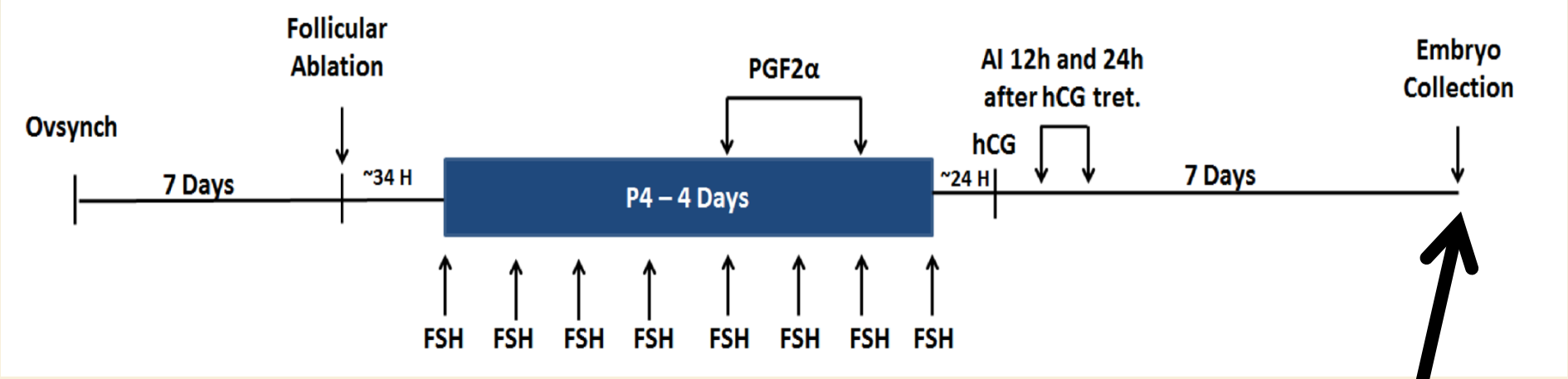


Embryos of superovulated cows fed MET or CON

	MET	CON	
Total 571 embryos/oocytes; n=	35	37	*P*-value
CL number	17.0 ± 1.3	17.7 ± 1.5	*0.90*
Total ova/embryos recovered	9.1 ± 1.4	6.8 ± 1.0	*0.18*
Number of fertilized ova	6.5± 1.1	5.5 ± 0.9	*0.56*
% Fertilized ova	74.7 ± 5.6	82.2 ± 3.8	*0.27*
Number of transferable embryos	5.0 ± 0.9	4.3 ± 0.1	*0.57*
% Transferable embryos	56.3 ± 6.5	62.5 ± 6.0	*0.49*
Number of degenerate embryos	1.5 ± 0.4	1.3 ± 0.4	*0.75*
% Degenerate embryos	18.5 ± 4.6	19.7 ± 4.7	*0.83*
% Degenerate of fertilized ova	25.1 ± 5.8	27.5 ± 6.0	*0.74*

± Methionine Supplementation (2.43 vs. 1.89 Met %MP)

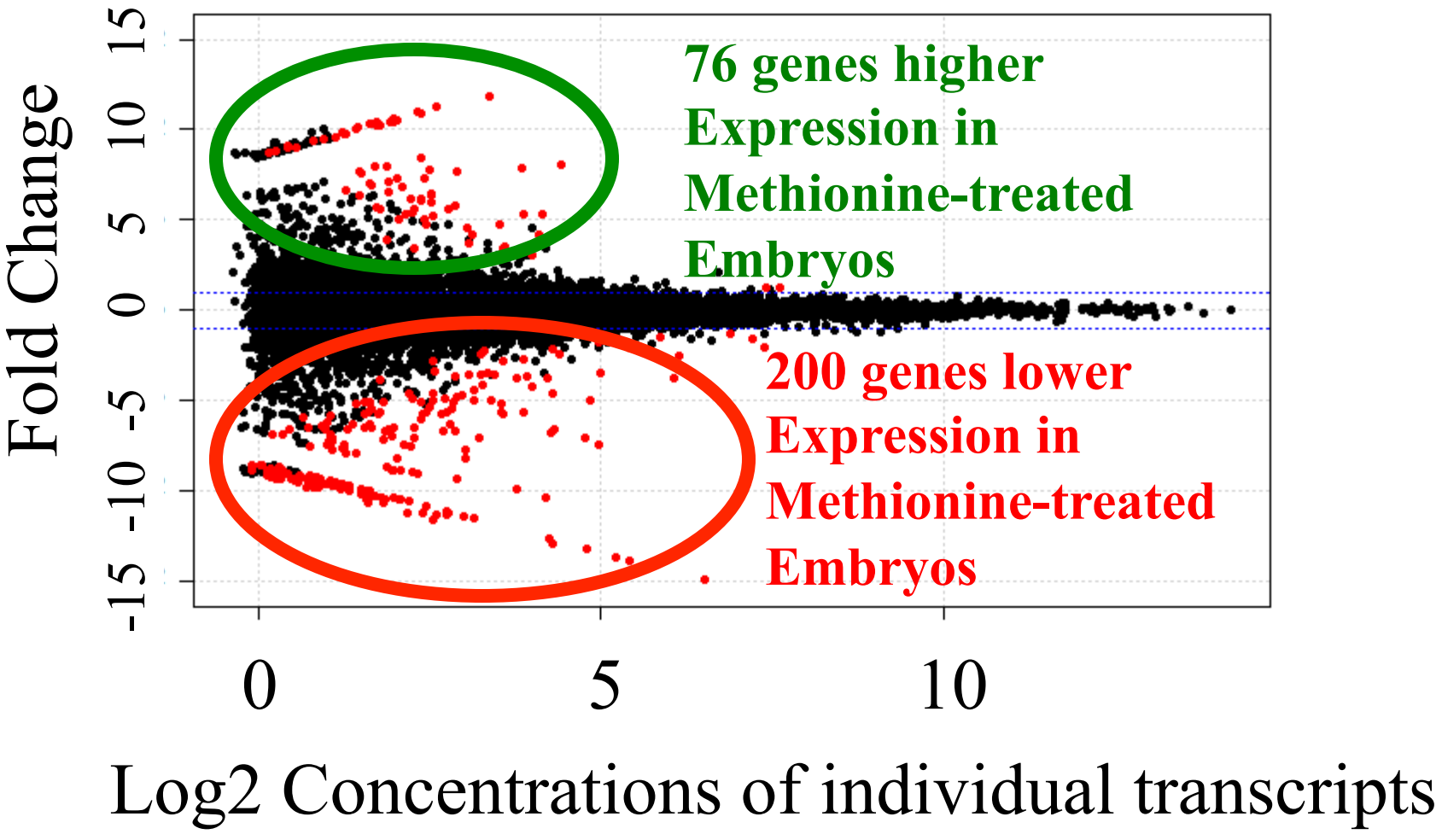
Synchronization and superovulation protocol



Evaluated Grade I Embryos by RNA-Sequencing

Plot of the log2 fold change against log2 average.

Differential expressed genes (FDR < 0.10) are highlighted in red.



Gene	Name	log2 FC	FDR
LAPTM5	Lysosomal protein transmembrane 5	-14.9	4.7×10^{-9}
NKG7	Natural killer cell group 7 sequence	-13.6	4.4×10^{-8}
VIM	Vimentin	-13.8	1.8×10^{-7}
TYROBP	TYRO protein tyrosine kinase binding protein	-13.2	3.2×10^{-6}
IFI6	Interferon, alpha-inducible protein 6	-12.6	1.5×10^{-5}
(			-5
L			-5
S			-5
L			-5
LCP1	plastin)	-9.9	1.1×10^{-4}

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PLOS ONE

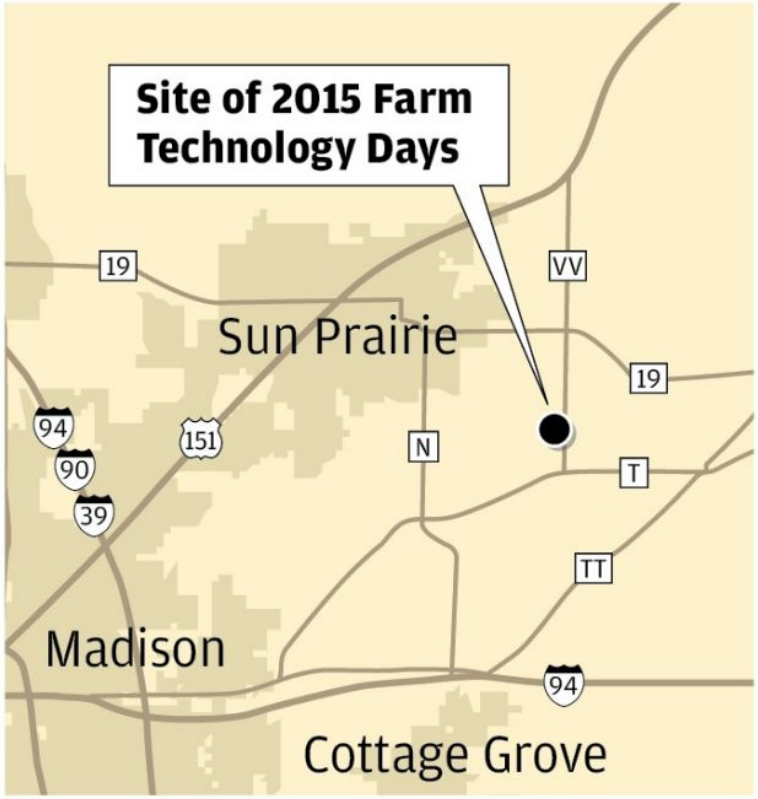
Effect of Maternal Methionine Supplementation on the Transcriptome of Bovine Preimplantation Embryos

Francisco Peñagaricano[1], Alex H. Souza[2], Paulo D. Carvalho[2], Ashley M. Driver[1], Rocio Gambra[1], Jenna Kropp[1], Katherine S. Hackbart[2], Daniel Luchini[3], Randy D. Shaver[2], Milo C. Wiltbank[2]*, Hasan Khatib[1]*

1 Department of Animal Sciences, University of Wisconsin, Madison, Wisconsin, United States of America, 2 Department of Dairy Science, University of Wisconsin, Madison, Wisconsin, United States of America, 3 Adisseo USA Inc., Alpharetta, Georgia, United States of America

Conclusions from RNA-Seq trial.

- Methionine supplementation of the dam during early embryo development changes gene expression in the embryo.
- Most genes are down-regulated by methionine supplementation.
- It is still unknown how these changes in gene expression will affect later pregnancy and calf physiology.
- Epigenetic effects may occur in embryo when dam is supplemented with methyl donors.



State Journal

Materials and Methods

- Holstein cows (n=309)
- From 28-35 to 121-128 days in milk
  milked twice

- Cows were blocked by parity randomly assigned to two treatments differing in content of methionine:

- **RPM** - 2477 g of MP with 6.9% of Lys & **2.34 %** of Met % MP (fed 21.1 g of Smartamine® M)

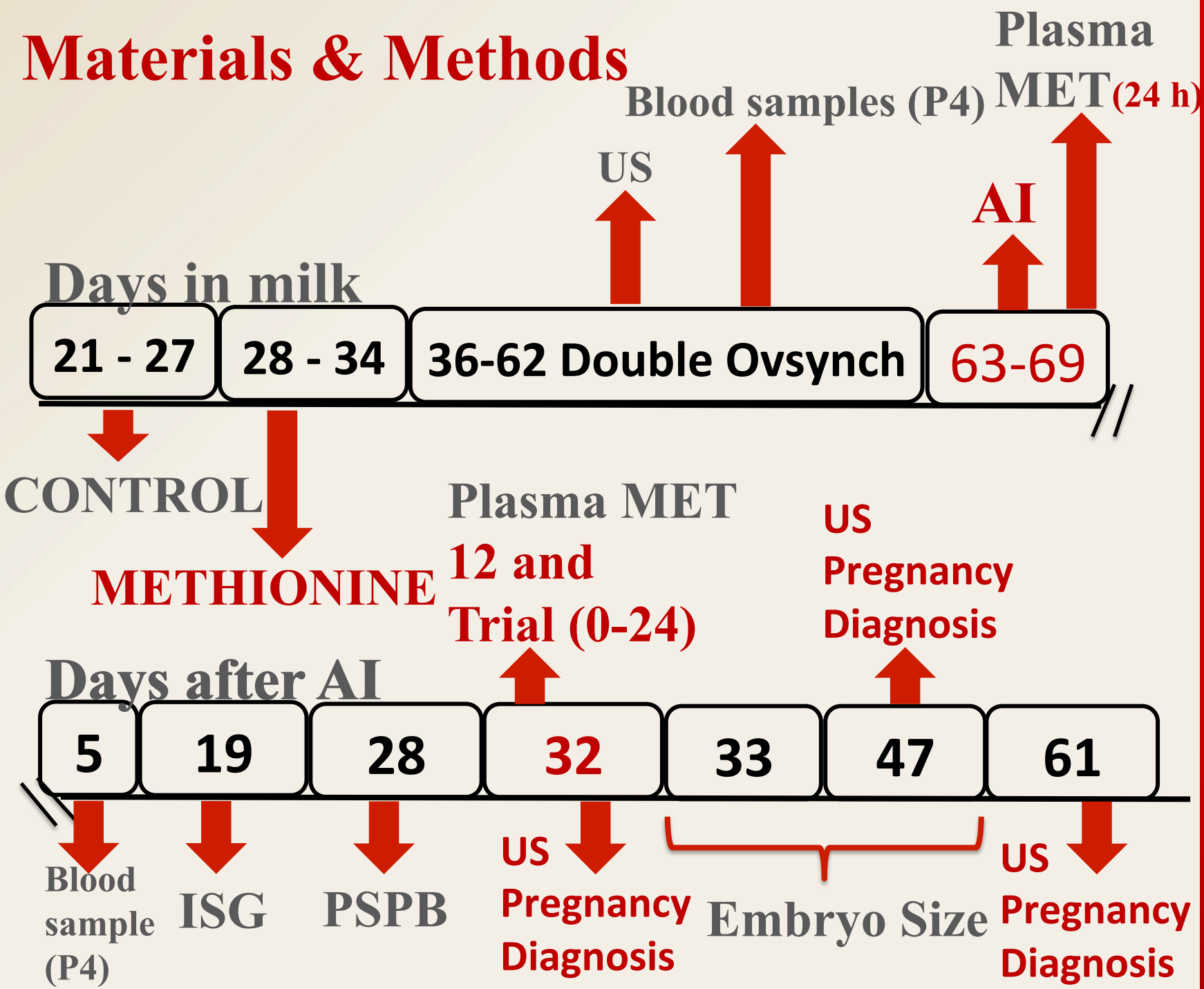
- **CON** - 2477 g of MP with 6.9% of Lys & **1.87 %** of Met % MP

- 21.1 g of SM by top-dressing
- 28-34 to 122-128 DIM
- 2.34 % MET of MP

Animals

	CONTROL	RPM	TOTAL
Primiparous	68	70	138
Multiparous	85	86	171
TOTAL	153	156	309

Materials & Methods

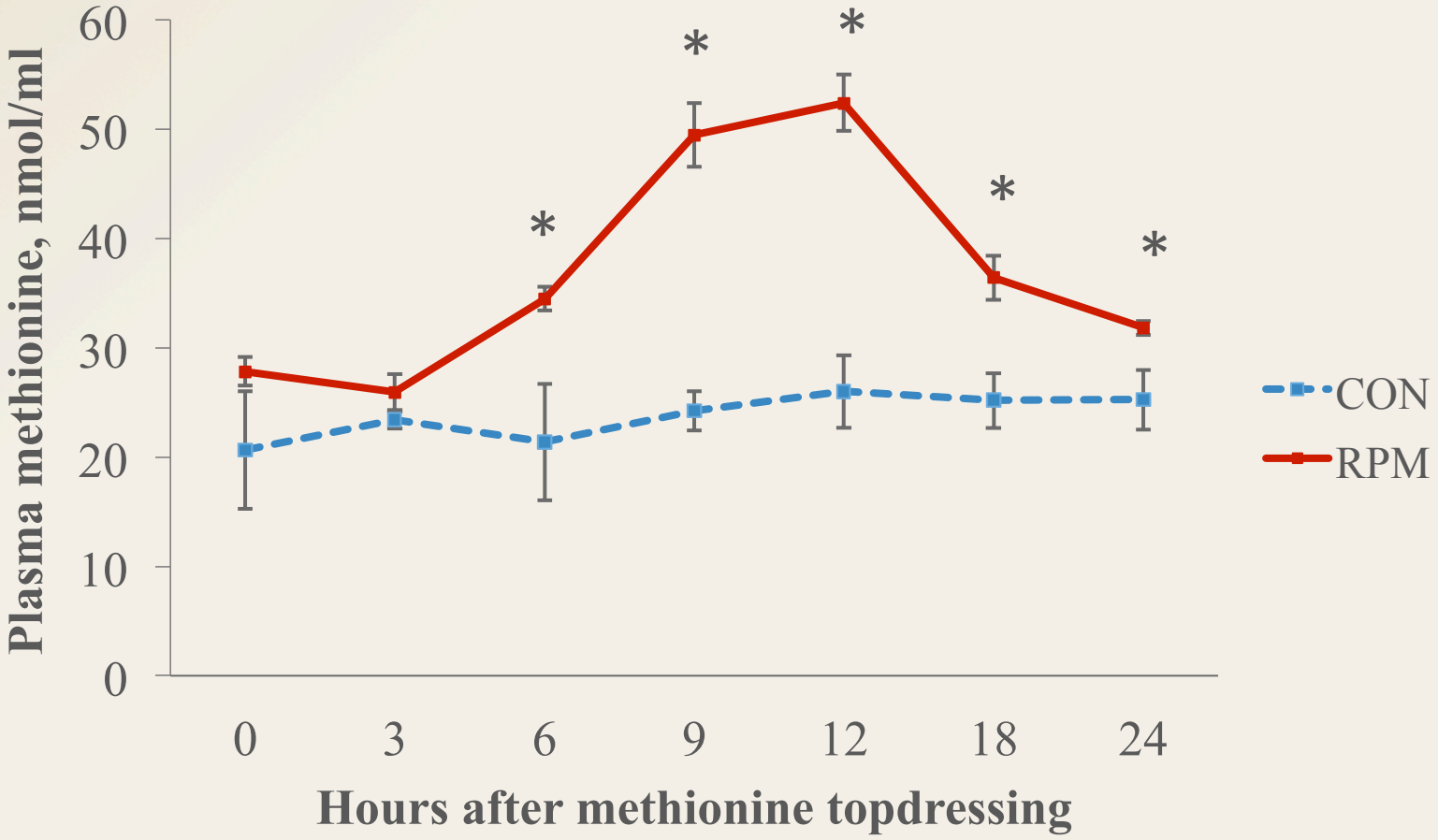


By Parity

Milk Test 3 (100-128)

	Primiparous		P-value	Multiparous		P-value
	CON	RPM		CON	RPM	
n	30	34		38	38	
DIM	113.2	113		113.1	112.8	
Milk Production (kg)	34.9	35.4	0.37	44.7	44.4	0.44
% fat	3.4	3.45	0.38	3.27	3.42	0.16
Kg fat	1.19	1.2	0.39	1.44	1.5	0.18
% protein	**3.12**	**3.16**	**0.18**	**3.00**	**3.08**	**0.05**
kg protein	1.08	1.11	0.22	1.33	1.38	0.27
3.5% FCM (kg)	34.3	34.8	0.37	42.69	43.7	0.29
ECM (kg)	34.2	34.8	0.33	43.4	43.4	0.28
SCC x 10^3	109.1	63.8	0.10	131.3	62.6	0.06

Plasma methionine peaked with 12 h after top-dressing



Control=2 pools, RPM=3 pools (n=4 cows each pool)

* *P-value* < 0.05. Comparisons are between CONTROL and RPM.

Conception rate and pregnancy losses only to cows that were synchronized

	CONTROL	RPM	*P-value*
P/AI at day 28	65.5% (91/139)	66.7% (96/144)	0.465
P/AI at day 32	58.6% (82/140)	61.37 (89/145)	0.358
P/AI at day 47	56.11 (78/139)	59.72 (86/144)	0.335
P/AI at day 61	54.34 (75/138)	58.27 (81/139)	0.295
Preg. loss 28-61	16.66 (15/90)	10.00 (9/90)	0.136
Preg. loss 28-32	8.88 (8/90)	6.25 6/96)	0.343
Preg. Loss 32-61	7.40 (6/81)	2.40 (2/83)	0.130

Item	Primiparous			Multiparous		
	CON	RPM	*P-value*	CON	RPM	*P-value*
P/AI at day 28	63.49 (40/63)	64.51 (42/63)	0.425	67.10 (51/76)	66.66 (54/81)	0.544
P/AI at day 32	58.73 (37/63)	60.31 (38/63)	0.500	58.44 (45/77)	62.19 (51/82)	0.373
P/AI at day 47	56.52 (35/62)	57.14 (36/63)	0.540	55.84 (43/77)	61.72 (50/81)	0.277
P/AI at day 61	54.83 (34/62)	56.45 (35/62)	0.500	53.94 (41/76)	59.74 (46/77)	0.287
Preg. Loss 28-61	12.82 (5/39)	14.63 (6/41)	0.536	19.60 (10/51)	6.12 (3/49)	0.042
Preg. Loss 32-61	5.55 (2/36)	5.40 (2/37)	0.682	8.88 (4/45)	0.00 (0/46)	0.055

Conclusion

Supplementation with RPM top-dressed:

- **Increased milk protein %.**
- **Elevated plasma methionine 1.8 fold over control, however it seems to be a different pattern of metabolism pattern than when supplementation is in the TMR. The effect of this pattern on reproduction and milk protein is unclear.**

Conclusion

- P/AI was not affected by RPM supplementation. However, a numerically difference of 4% overall and 5.8% in multiparous cows was showed on day 61.

- In general, only tended to decrease pregnancy losses. But, in multiparous cows, RPM treatment had lower pregnancy losses.

Embryo size



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- **Measurements – Software, Image J (National Institutes of Health, Bethesda, MD)**
- **Recorded for 15 seconds and the ideal position and orientation of the conceptus was selected**
- **2 independent people analyzed the videos**



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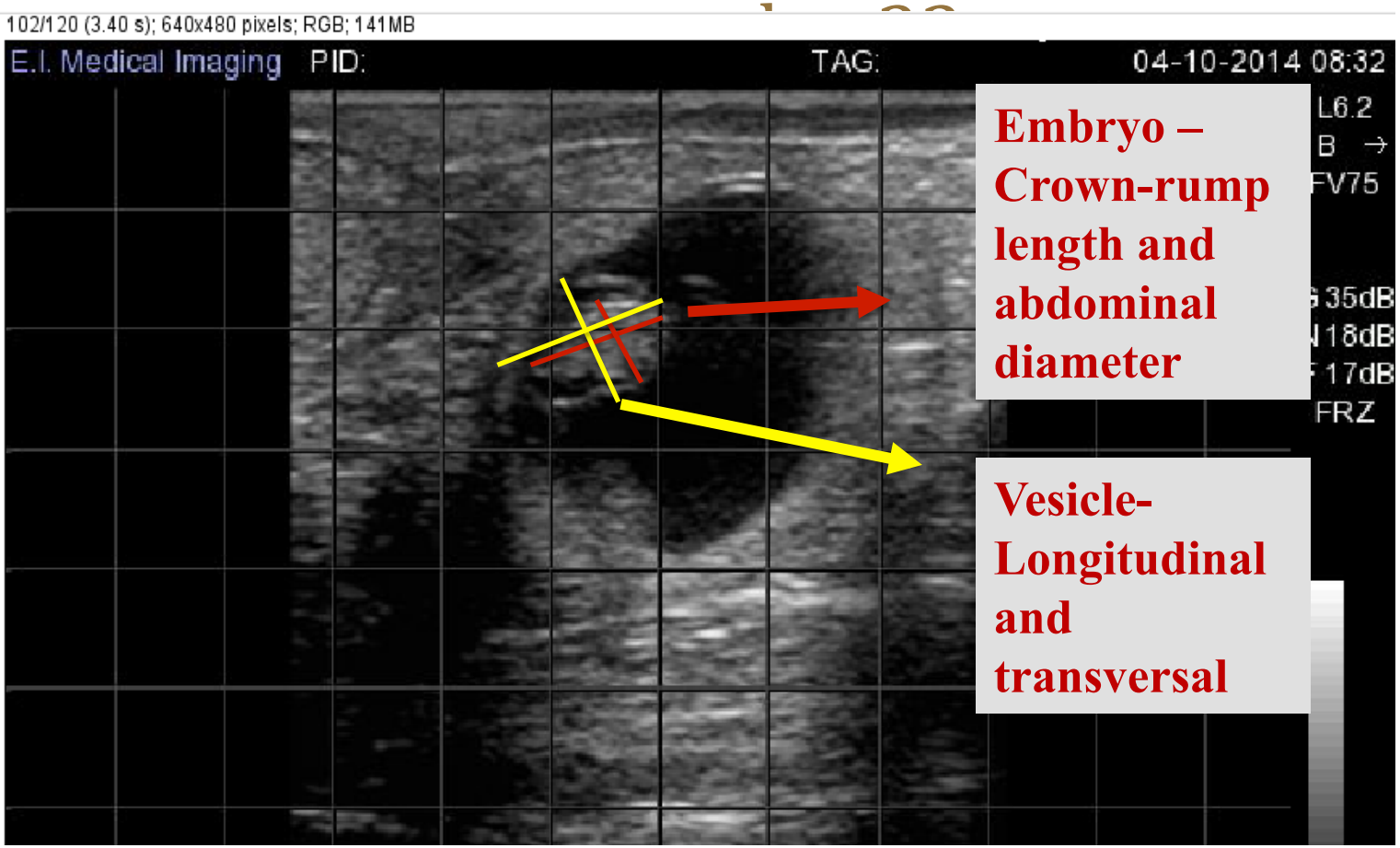
Embryo size- measuring embryo and vesicle



Effect of Rumen-protected Methionine on Amniotic Vesicle and Embryo size

	Amniotic Vesicle diameter (mm)			Embryo (mm)		
Day 33	**Conceptuses**	**Longitudinal**	**Transversal**	**Conceptuses**	**Crown-rump length**	**Abdominal diameter**
Overall						
Control	67	**13.1** ± 0.22	**8.7** ± 0.16	73	**10.5** ± 0.17	**5.4** ± 0.08
RPM	81	**13.7** ± 0.23	**8.9** ± 0.17	82	**11.0** ± 0.16	**5.8** ± 0.12
P-*value*	**-**	**0.02**	**0.20**	**-**	**0.03**	**0.008**
Primiparous						
Control	32	**13.6** ± 0.25	**9.1** ± 0.23	36	**10.5** ± 0.24	**5.5** ± 0.11
RPM	36	**14.0** ± 0.34	**8.9** ± 0.22	38	**10.9** ± 0.24	**5.7** ± 0.16
P-*value*	**-**	**0.17**	**0.26**	**-**	**0.08**	**0.19**
Multiparous						
Control	35	**12.7** ± 0.33	**8.3** ± 0.19	37	**10.6** ± 0.24	**5.3** ± 0.12
RPM	45	**13.5** ± 0.30	**8.9** ± 0.25	44	**11.0** ± 0.22	**5.9** ± 0.18
P-*value*	**-**	**0.03**	**0.04**	**-**	**0.10**	**0.01**

***Values are the average of two independent people. Data are presented with ± SEM.**

Mooving Forward.

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Questions?