Lipid mobilization, immune system and vitamin E in transition cows: an old story revisited

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Transition Period

It starts from week 3 prepartum until week 3 postpartum

Health Problems

Three main categories of metabolic problems

1. Energy metabolism (fatty liver, ketosis, acidosis)
2. Mineral metabolism (milk fever, subclinical hypocalcemia)
3. Immune system-related (mastitis, retained placenta, metritis)

Transition Period: The physiological basis

- Negative energy balance (imbalance between energy consumed and energy needed)
- Mobilization of body fatty reserves
- Increase of circulating NEFA and BHBA

NEFA – BHBA during transition period (1)

Time of sampling	NEFA (mmol/L)	BHBA (mmol/L)	α –T (µmol/L)	Ratio α-T/TC (x 10^3)
Dry off	$0.155^{a} \pm 0.017$	$0.394^{a} \pm 0.024$	$8.900^{a} \pm 0.206$	$2.422^{a} \pm 0.061$
Calving	$0.511^{b} \pm 0.017$	$0.512^{b} \pm 0.024$	$4.372^{b} \pm 0.206$	$1.863^{b} \pm 0.062$
30 d postpartum	$0.255^{c} \pm 0.017$	$0.620^{c} \pm 0.024$	$9.062^{a} \pm 0.212$	$2.361^{a} \pm 0.063$

a, b, c : Means within the same column followed by different letters differ at $P<0.05$

NEFA – BHBA during transition period

NEFA – BHBA during transition period (2)

Correlations between NEFA, BHBA, α-T and the ratio of α-T to total cholesterol (TC) during the periparturient period

			NEFA	BHBA	α-T	α-T/TC
Dry off	**NEFA**	Rho	1	0.114	-0.169	-0.002
		P	-	ns	ns (P=0.057)	ns
	BHBA	Rho		1	-0.370	-0.352
		P		-	***	***
	α-T	Rho			1	0.348
		P			-	***
	α-T/TC	Rho				1
		P				-
30 d postpartum	**NEFA**	Rho	1	-0.030	-0.300	0.028
		P	-	ns	***	ns
	BHBA	Rho		1	-0.104	-0.188
		P		-	ns	*
	α-T	Rho			1	0.388
		P			-	***
	α-T/TC	Rho				1
		P				-

* Correlation is significant at P <0.05 (2-tailed).

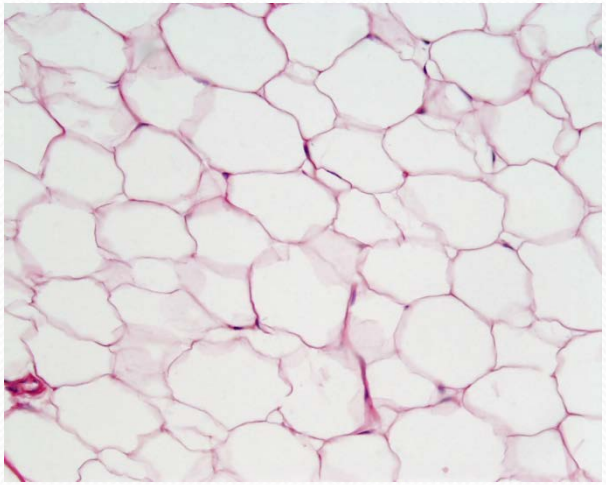
*** Correlation is significant at P< 0.001 (2-tailed).

ns: not significant

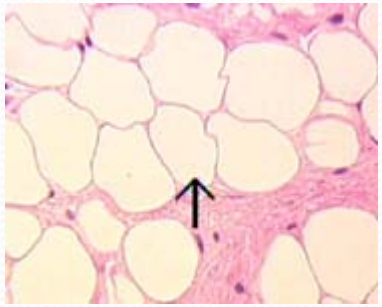

ADIPOSE TISSUE
is it an endocrine organ?

Adipose Tissue: Fundamental Facts

- Central role in controlling energy balance
- “Storage area” when energy balance is positive
- “Mobilization” of fatty acids when energy balance is negative



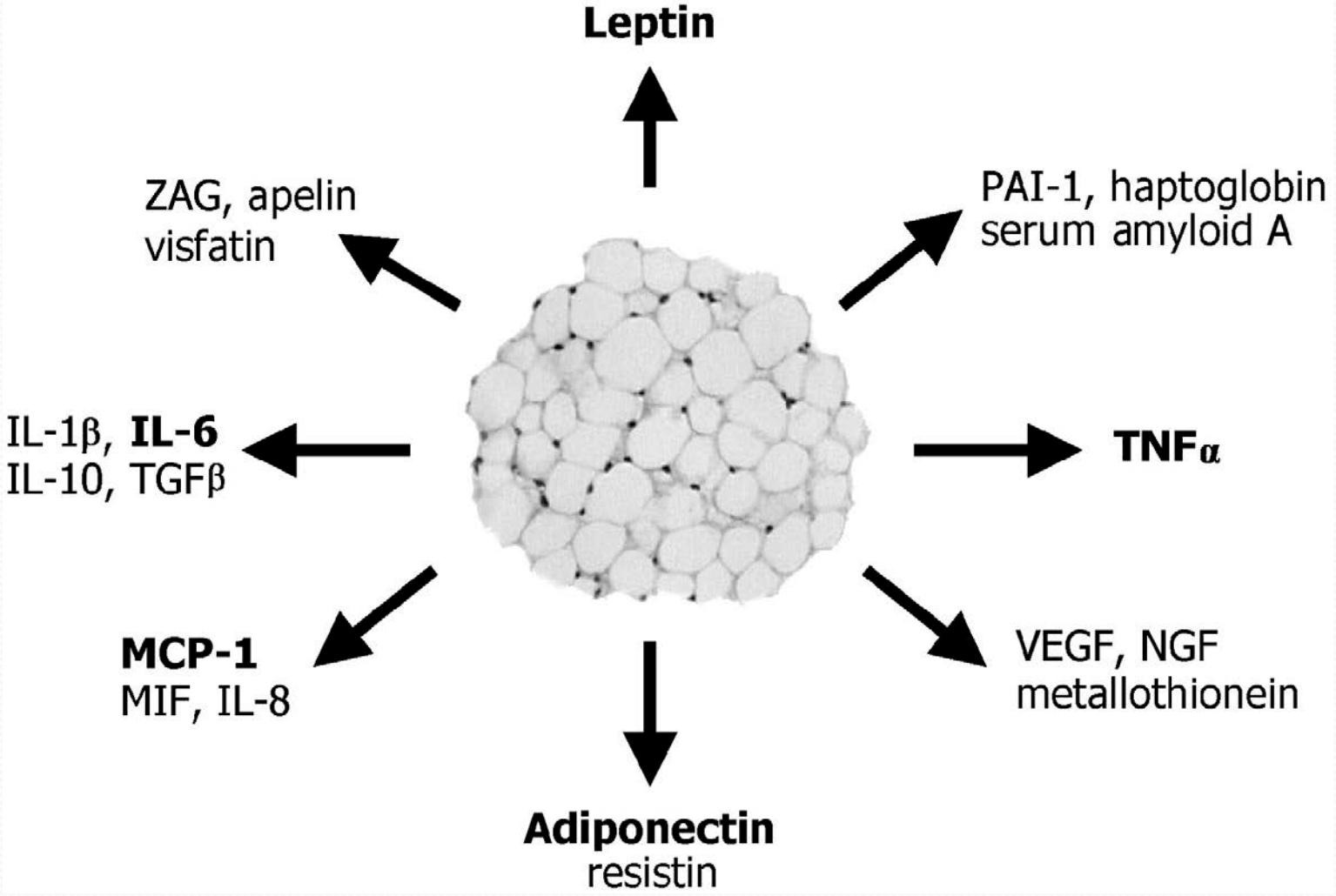
Adipose Tissue – Obesity



- Ectopic lipid deposition
- Reduced fatty acid oxidation
- “Low grade inflammation”

} Insulin Resistance

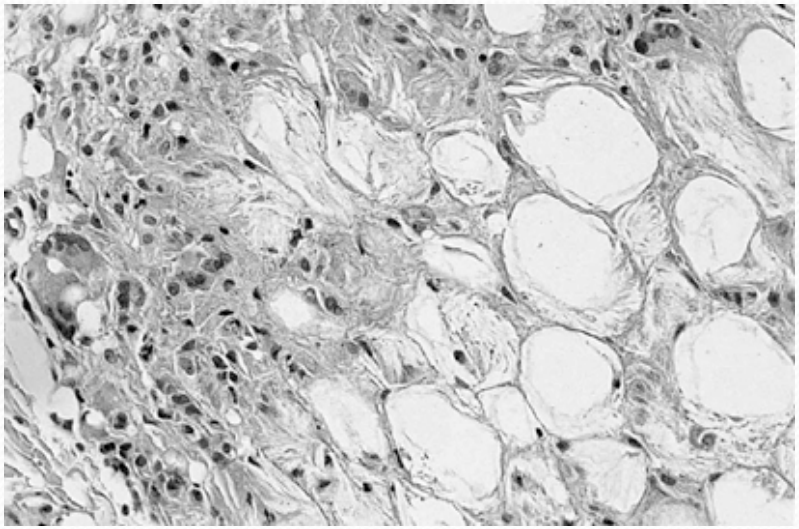
Adipose Tissue: a metabolic active organ?



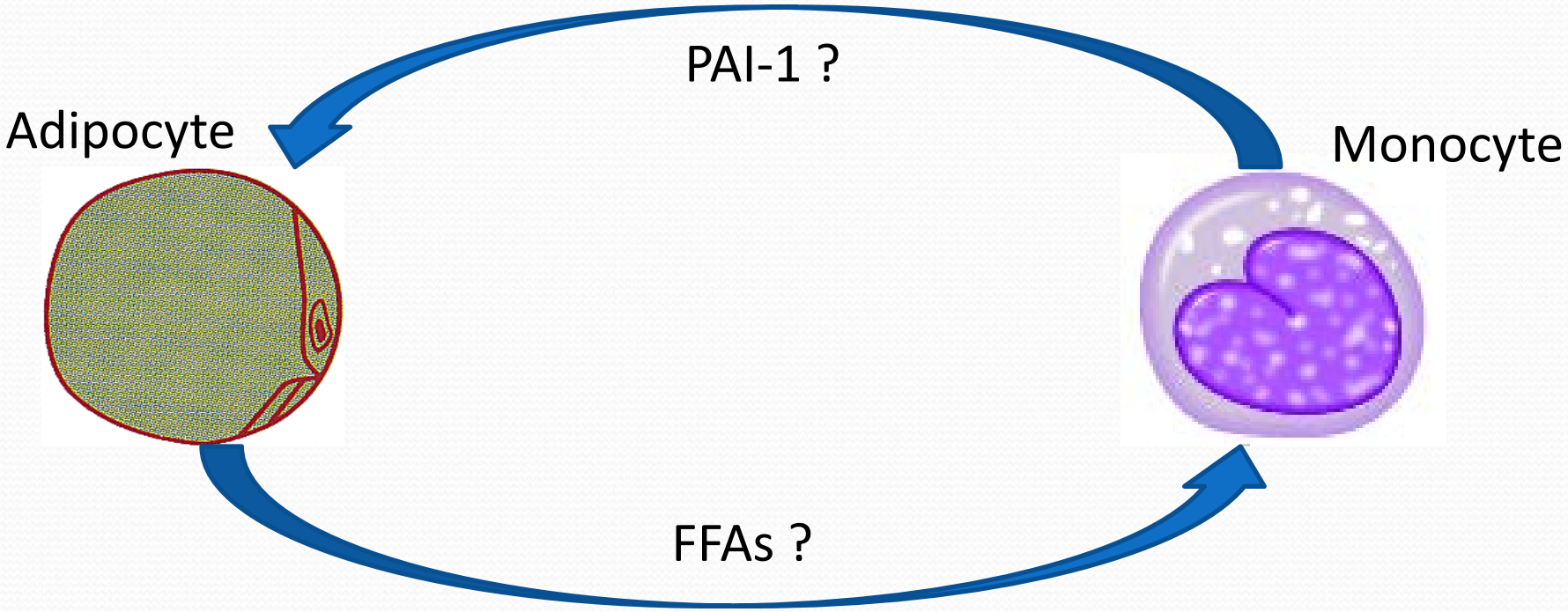
PAI-1 autocrine/paracrine feedback loop to limit adipose tissue expansion

Structure – Cell Type

- Adipocytes
- Fibroblasts
- Immunocompetent cells (monocytes, lymphocytes)
- Well-developed vasculature



Interaction between adipose tissue and immune system

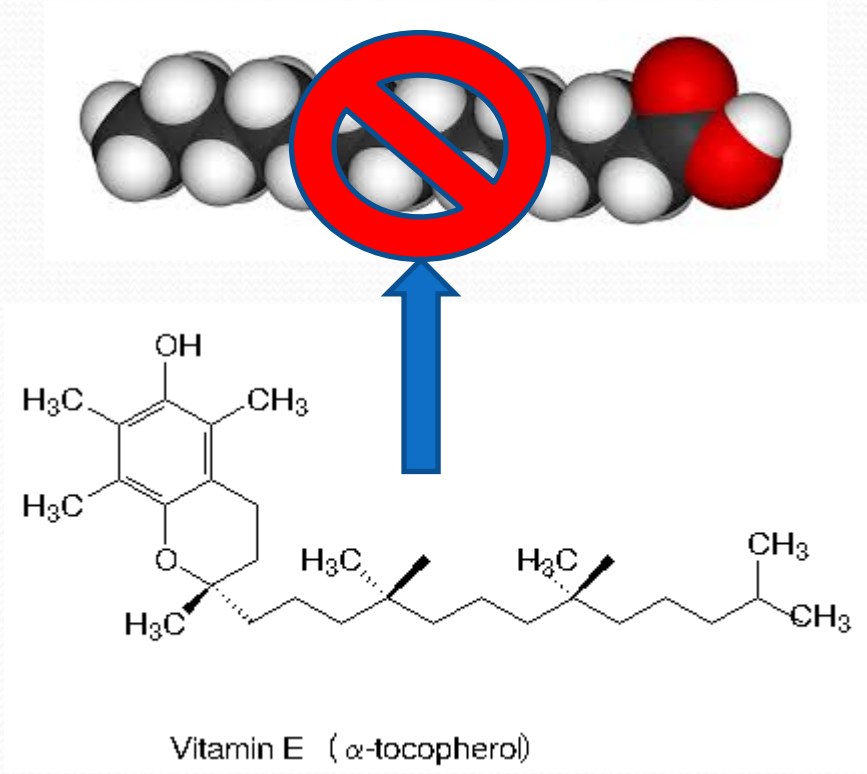


PAI-1 autocrine/paracrine feedback loop to limit adipose tissue expansion

EXPERIMENT

OBJECTIVES

- Are FFAs the metabolic "mediators" of inflammation towards ovine phagocytes?
- Does α-tocopherol block the activation initiated by FFAs?

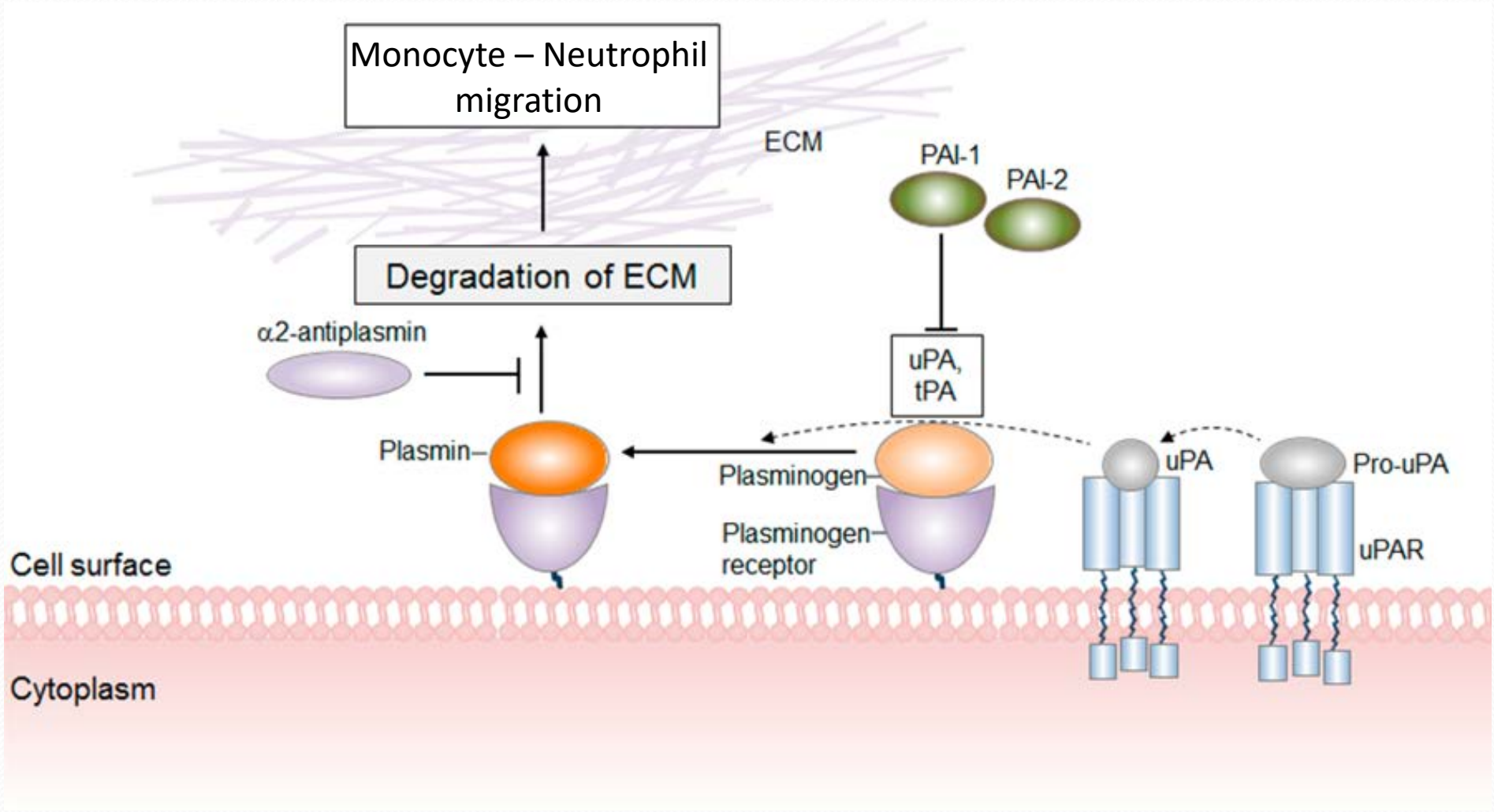


Vitamin E (α-tocopherol)

Methodology (I)

- 10 Dairy sheep (Chios Breed)
    - 4 samples during the dry period per animal
- Isolation of blood monocytes and neutrophils
- Isolated cells were cultured with various FAs (C14, C16, C18) with or without α-tocopherol
- Various forms of PA activity in monocytes and neutrophils

Methodology (II)



Effect of various fatty acids on u-PA related parameters of activated sheep monocytes.

Fatty acid (µM)	Membrane bound u-PA ΔA/h/10^6 cells	Free u-PA binding sites ΔA/h/10^6 cells
Control	0.26^a œ0.03	0.62^a œ0.08
Myristic (125)	0.28^a œ0.03	0.60^a œ0.08
Myristic (250)	0.34^b œ0.03	0.75^b œ0.08
Palmitic (125)	0.29^a œ0.03	0.60^a œ0.08
Palmitic (250)	0.42^c œ0.03	0.88^c œ0.08
Palmitoleic (125)	0.29^a œ0.03	0.64^a œ0.08
Palmitoleic (250)	0.42^c œ0.03	0.87^c œ0.08
Stearic (125)	0.40^c œ0.03	0.85^c œ0.08
Stearic (250)	**0.60^d ± 0.03**	**1.05^d ± 0.08**
Oleic (125)	0.42^c œ0.03	0.87^c œ0.08
Oleic (250)	**0.61^d ± 0.03**	**1.05^d ± 0.08**

Means within the same column with different superscripts are significantly different (P<0.05)

Effect of various fatty acids on u-PA related parameters of activated sheep neutrophils.

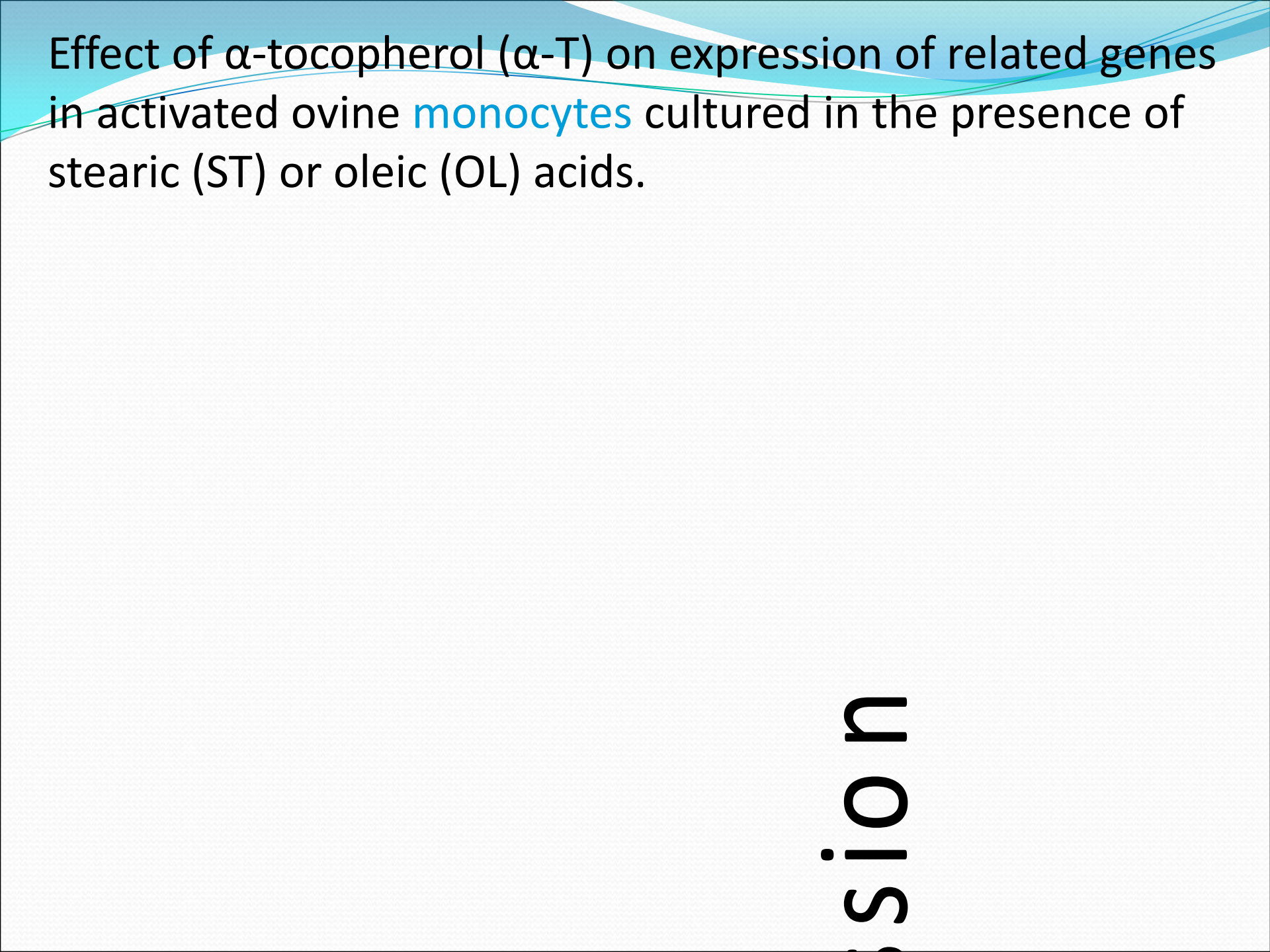
Fatty acid (µM)	Membrane bound u-PA ΔA/h/10^6 cells	Free u-PA binding sites ΔA/h/10^6 cells
Control	$0.52^a \pm 0.07$	$0.84^a \pm 0.10$
Myristic (125)	$0.55^a \pm 0.07$	$0.77^a \pm 0.10$
Myristic (250)	$0.53^a \pm 0.07$	$0.86^a \pm 0.10$
Palmitic (125)	$0.58^a \pm 0.07$	$0.75^a \pm 0.10$
Palmitic (250)	$0.49^a \pm 0.07$	$0.88^a \pm 0.10$
Palmitoleic (125)	$0.61^a \pm 0.07$	$0.90^a \pm 0.10$
Palmitoleic (250)	0.49^a œ0.07	0.79^a œ0.10
Stearic (125)	0.60^a œ0.07	0.87^a œ0.10
Stearic (250)	**$0.75^b \pm 0.07$**	**$1.22^b \pm 0.10$**
Oleic (125)	0.63^a œ0.07	0.76^a œ0.10
Oleic (250)	**$0.77^b \pm 0.07$**	**$1.25^b \pm 0.10$**

Means within the same column with different superscripts are significantly different ($P<0.05$)

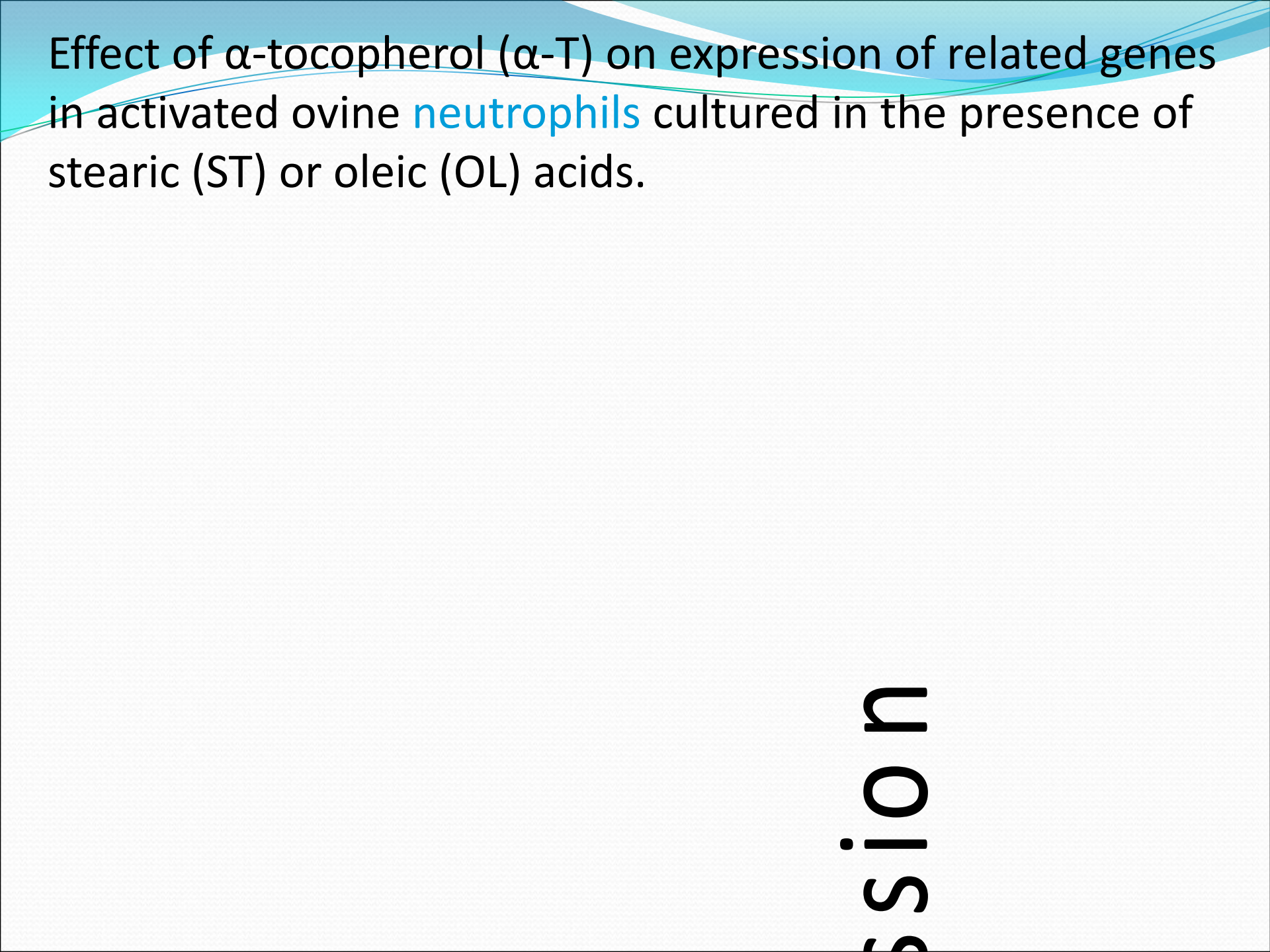
Effect of α-tocopherol (α-T) on expression of PA-related genes in activated ovine monocytes cultured in the presence of stearic (ST) or oleic (OL) acids.

Effect of α-tocopherol (α-T) on expression of PA-related genes in activated ovine neutrophils cultured in the presence of stearic (ST) or oleic (OL) acids.

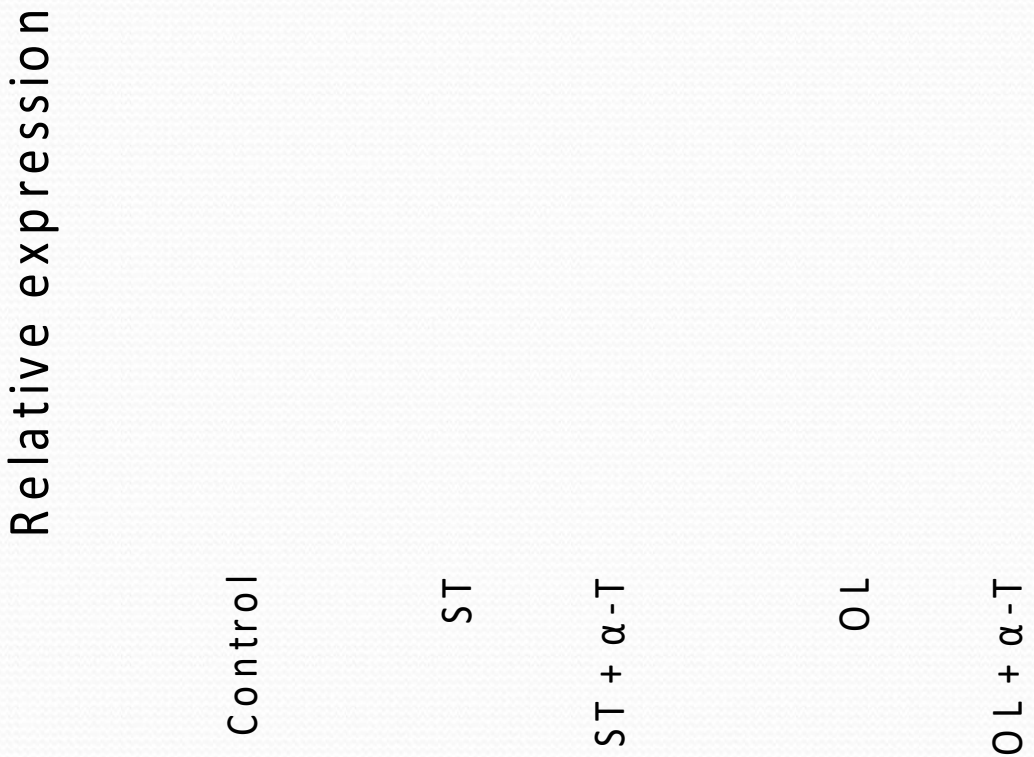
Effect of α-tocopherol (α-T) on expression of related genes in activated ovine monocytes cultured in the presence of stearic (ST) or oleic (OL) acids.



Effect of α-tocopherol (α-T) on expression of related genes in activated ovine neutrophils cultured in the presence of stearic (ST) or oleic (OL) acids.



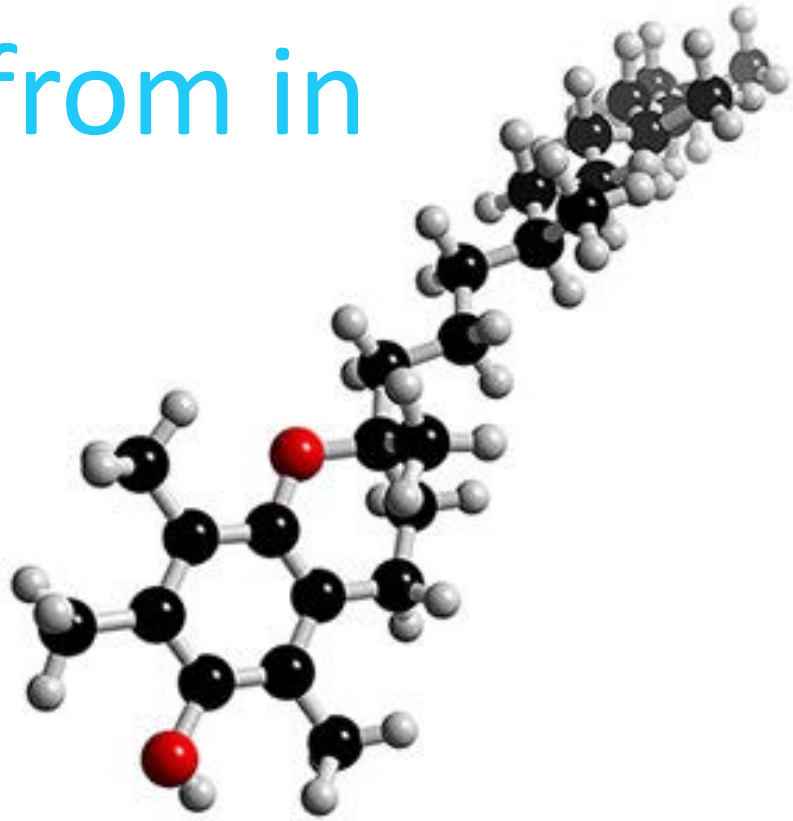
Effect of α-tocopherol (α-T) on expression of IL-10 in activated ovine monocytes cultured in the presence of stearic (ST) or oleic (OL) acids.



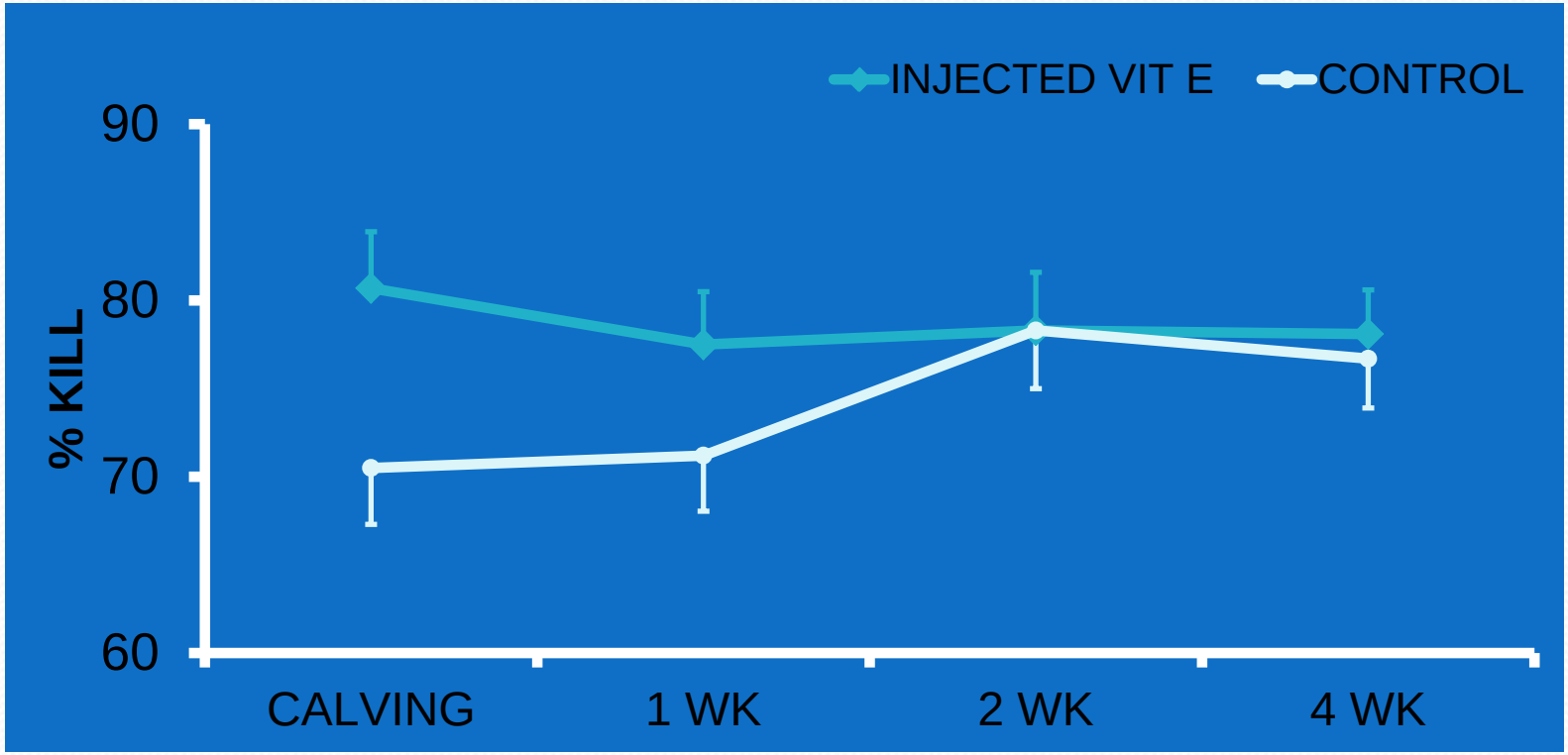
CONCLUSIONS

- FFAs activate ovine phagocytic cells
- Activation of monocytes > neutrophils
- $C_{18}>C_{16}>C_{14}$
- NS difference between saturated and unsaturated FFAs
- FFAs act in a pro-inflammatory manner
- Vit E does not block the inflammatory effect

Vitamin E and immune system: Lessons from in vivo studies

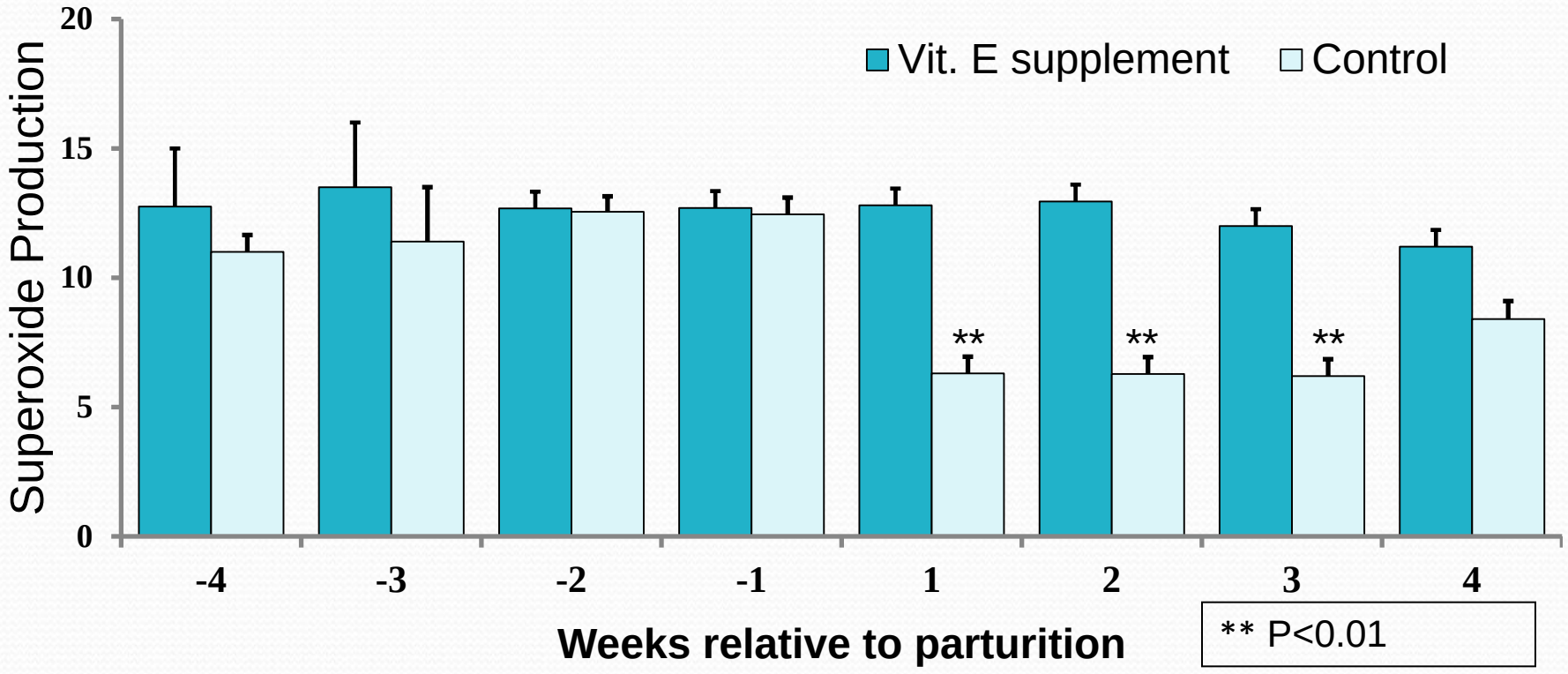


Vitamin E enhances the ability of neutrophils to kill ingested bacteria



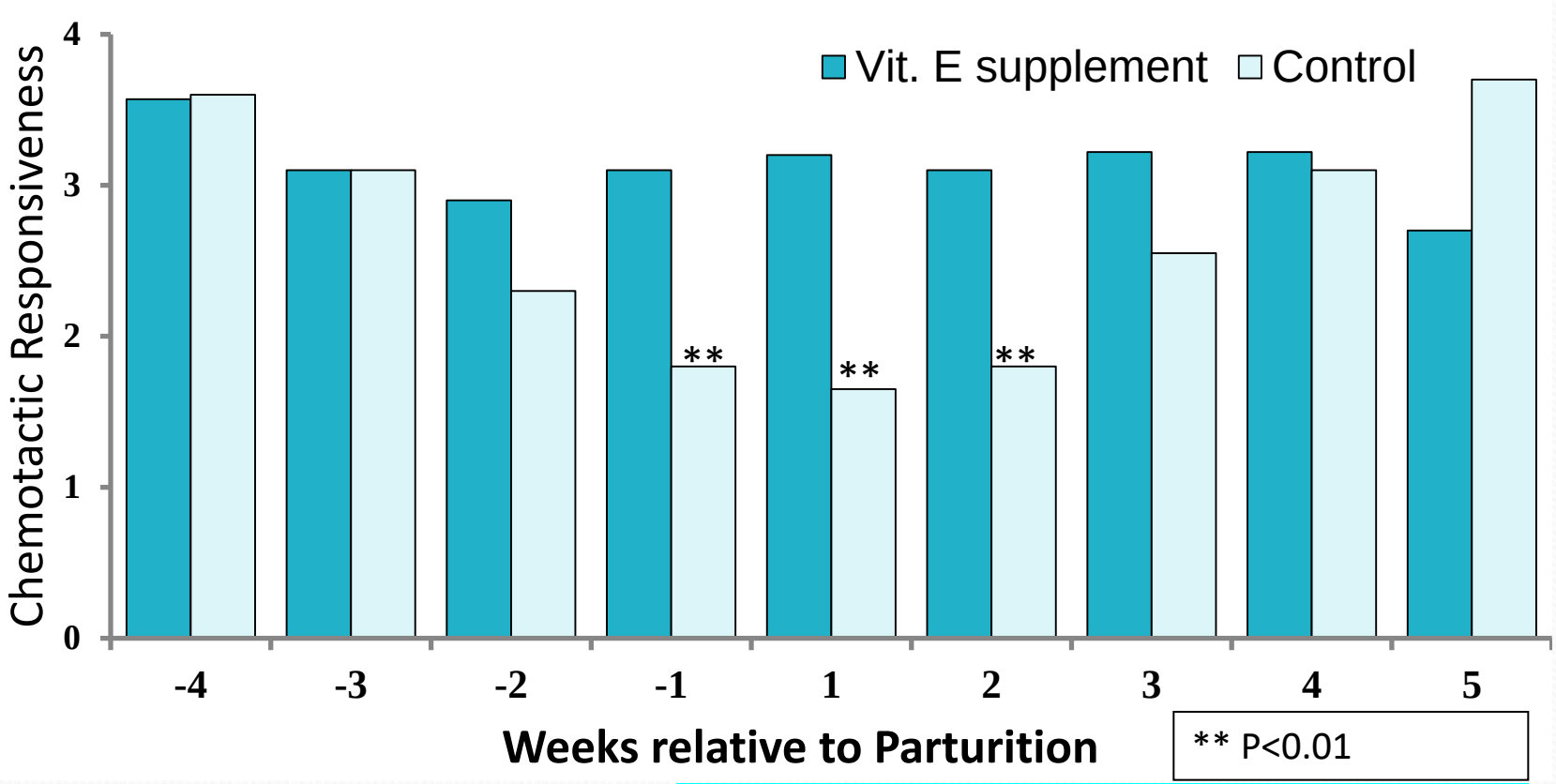
Hogan et al. (1992) J Dairy Sc. 75:399-405

Vitamin E enhances oxidative burst by neutrophils



Politis et al. (1995) Am J Vet Sci 56:179-184

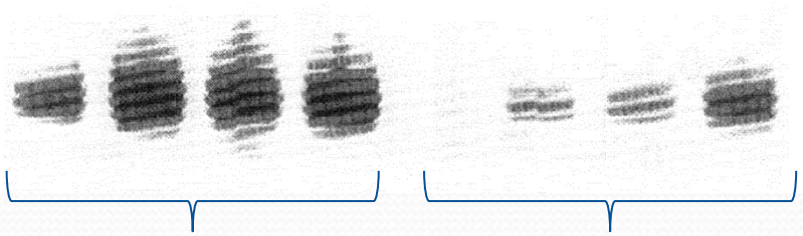
Vitamin E favors rapid recruitment of neutrophils in the mammary gland



Politis et al. (1997) Am J Vet Sci 57:468-471

Vitamin E upregulates u-PA system

* P<0.05



Vitamin E control

u-PAR Northern blot
week 1 postpartum

Politis et al. (2001) AJVR 62:1934-1938

Vitamin E – Immune system

Vitamin E enhances (restores)

proper immune function

during the transition period

Vitamin E levels – Type of diet

Type of diet	Vitamin E (IU/day)
Lactating	
Hay	400
Silage	1500
Pasture	2500
Dry	
Hay	200
Pasture	1800

Weiss et al. (1998)

Suggested levels of Vitamin E supplementation for lactating and dry cows

Stage in lactation cycle	Vitamin E (IU/day/cow)
Conditional or opposite effects must be repeated before changing Vitamin E recommendations	

EXTRA SLIDES

Veterinary-treated cases of clinical mastitis relative to blood α-tocopherol levels at dry-off and calving in dairy cows.

Period	α-tocopherol (µg/ml)	Mean	Median	P10	P90	Number of cows		
						Healthy	Mastitic	χ^2 p-value
Dry-off	> 6.25	7.2	7.0	6.3	8.5	30	4	1.47
	4.25-6.25	5.1	4.9	4.3	6.2	63	14	0.48
	< 4.25	3.6	3.7	2.8	4.1	27	8	
Calving	> 3	4.0	3.8	3.2	5.2	37	4	19.70
	2-3	2.5	2.6	2.0	3.0	57	5	P<0.001
	< 2	1.3	1.4	0.6	1.9	26	17	

P10: 10th percentile; P90: 90th percentile

Politis et al. (2012) J. Dairy Sci. 95:7331-7335

Reactive oxygen metabolites (ROM) relative to α-tocopherol levels in blood serum at dry-off and calving in dairy cows

Period	α-tocopherol groups (µg/ml)	ROM at dry-off (U/ml)	ROM at calving (U/ml)	P within rows
Dry-off	> 6.25	$40.8^{a} \pm 3.2$	$49.6^{a} \pm 3.2$	0.754
	4.25-6.25	$53.3^{ab} \pm 2.09$	$61.1^{b} \pm 2.1$	0.137
	< 4.25	$56.2^{b} \pm 3.1$	$64.4^{b} \pm 3.1$	0.960
Mean		50.0 ± 1.6	58.4 ± 1.6	<0.001
Calving	> 3	$41.5^{a} \pm 2.9$	53.0 ± 2.9	0.078
	2-3	$51.5^{ab} \pm 2.5$	61.2 ± 2.5	0.106
	< 2	$58.3^{b} \pm 2.6$	62.0 ± 2.6	1.000
Mean		50.4 ± 1.5	58.7 ± 1.5	<0.001

[a,b]Means within the same column and period followed by different letters differ at $P < 0.05$

Politis et al. (2012) J. Dairy Sci. 95:7331-7335

Thiol groups (SH) relative to α-tocopherol levels in blood serum at dry-off and calving in dairy cows

Period	α-tocopherol groups (µg/ml)	SH at dry-off (µmol/l)	SH at calving (µmol/l)	Probability within rows (P value)
Dry-off	> 6.25	319[a] ± 26	417[a] ± 26	0.137
	4.25-6.25	378[ab] ± 17	445[ab] ± 17	0.116
	< 4.25	402[b] ± 25	513[b] ± 26	0.039
Mean		366 ± 14	458 ± 14	<0.001
Calving	> 3	345 ± 24	428 ± 24	0.233
	2-3	364 ± 21	440 ± 21	0.160
	< 2	398 ± 21	492 ± 22	0.036
Mean		369 ± 13	453 ± 13	<0.001

[a,b]Means within the same column and period followed by different letters differ at $P < 0.05$

Politis et al. (2012) J. Dairy Sci. 95:7331-7335