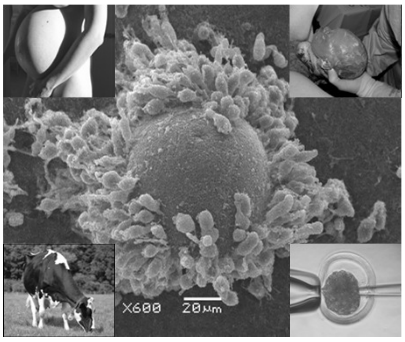
Maternal metabolic health in relation to oocyte and embryo quality;

the vet's perspective of a dairy cow tale



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Vereniging voor Klinische Embryologie – 14 jan 2015

In this talk

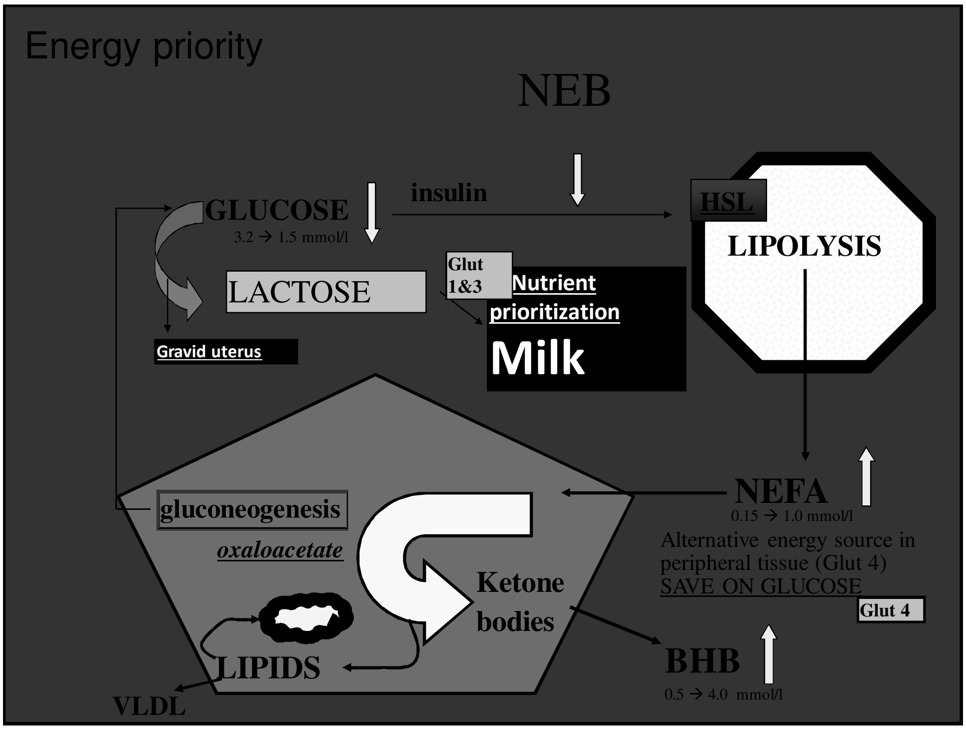
- Our central hypothesis in dairy cows and … in women
- The oocyte's "preprogramming" micro-environment
- Britt's hypothesis: "difficult to investigate"
- Conclusions

Reproduction: the underground of pathways…

Estrus cyclicity	Pregnancy at first service	Staying pregnant
Onset of ovarian activity and ovulation before day 25 post partum	*Being pregnant with < 2 AI's at 100 days post partum*	*Preg loss < 5% until day 60 of pregnancy*

Confounders: *management factors and genetic merit for milk production*

Estrus cyclicity	Pregnancy at first service	Staying pregnant
Endocrine communication Healthy follicle Timed ovulation Energy balance Nutrition Stress (heat, social, pain, …) Infectious disease	Oocyte quality Embryo quality Corpus luteum quality Early uterine environment Carry over effects of energy balance Nutrition Stress (heat, social, pain, …) Infectious disease	Uterine environment Corpus luteum quality (epi)Genetic errors Infectious disease



- Reduced oocyte quality in **diary cows** has been associated with:
  - NEB, timing post partum
  - Lactation
  - BCS changes
  - Diet (composition, energy content)
  - Heat stress
  - Inflammation and infection

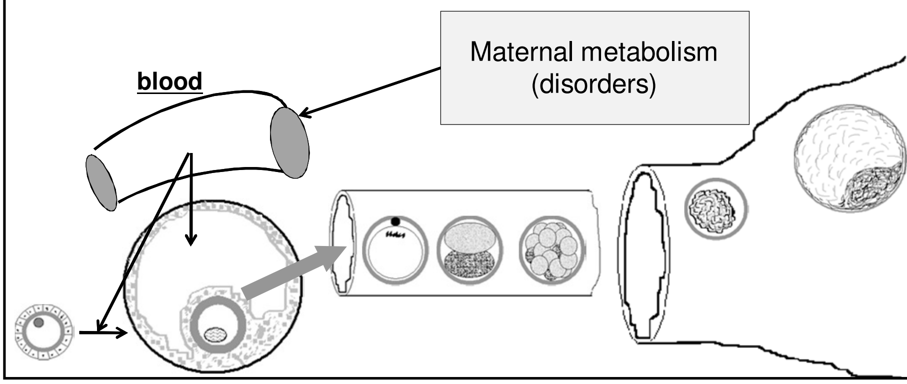
- And in **women**? Reduced oocyte quality in patients with obesity, insulin resistance

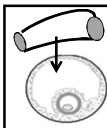
- Obesity (abdominal)
- Upregulated lipolysis
- Elevated NEFA and ketone concentrations
- High glucose concentrations
- High insulin concentrations
- High growth hormone and high IGF-I concentrations
- Inflammatory signals
- *Reduced fertility*:
  - *Hyperandrogenism*
  - *Anovulation or delayed ovulation of an aged oocyte*
  - *PCOS*
  - *Increased rates of embryo mortality*
  - *Miscarriages and obstetrical problems*

- Weight loss
- Upregulated lipolysis
- Elevated NEFA and ketone concentrations
- Low glucose concentrations
- Low insulin concentrations
- High growth hormone and low IGF-I concentrations
- Inflammatory signals
- *Reduced fertility*:
  - *Low sex steroids*
  - *Anoestrus*
  - *Anovulation or delayed ovulation of an aged oocyte*
  - *Cystic ovarian disease*
  - *Increased rates of embryo mortality*

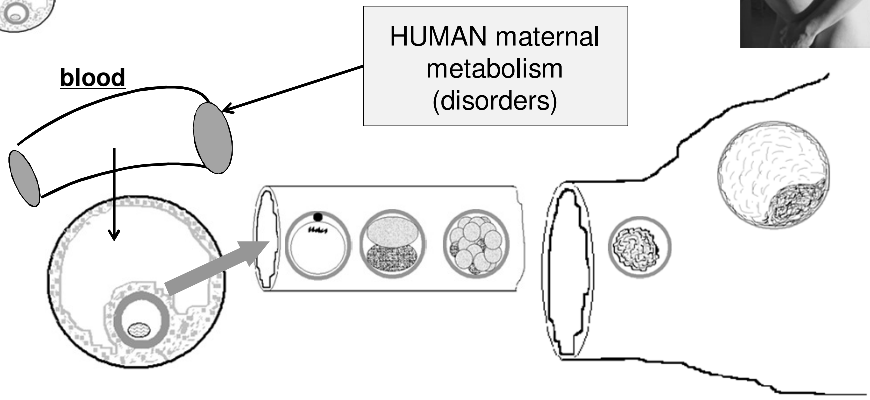
CENTRAL HYPOTHESIS:

Maternal metabolic health affects oocyte quality (what is that?) and thus fertility in general through changes in the micro-environment of the pre-ovulatory oocyte.





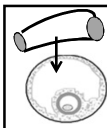
Metabolism and
micro-environment (2)



Hypothesis:

Obese women subjected to assisted reproduction (OPU) have an altered metabolic blood composition which is **reflected** in the follicular fluid.

This altered oocyte micro-environment hampers the **oocyte's developmental competence** and the **quality of the pre-implantation embryo**.



Metabolism and
micro-environment (3)

Subgroups of parameters	Parameter*	Serum	FF	Spearman
Standard biochemical	Ureum (mg/dl)	23.3 ± 6.0	25.4 ± 9.1	0.763 (P < 0.01)
	Total protein (g/dl)	6.7 ± 0.4	5.6 ± 0.5	0.605 (P < 0.01)
	Albumin (g/dl)	4.4 ± 0.3	4.0 ± 0.3	0.578 (P < 0.01)
	CRP (mg/l)	2.9 ± 3.4	1.6 ± 2.1	0.946 (P < 0.01)
Fat metabolism related	Cholesterol (mg/dl)	163.7 ± 24.5	30.9 ± 7.3	0.249 (P = 0.013)
	HDL cholesterol (mg/dl)	61.1 ± 12.5	28.7 ± 7.1	0.628 (P < 0.01)
	Triglycerides (mg/dl)	95.5 ± 49.3	9.0 ± 4.6	0.168 (P = 0.098)
	NEFA (mmol/l)	0.6 ± 0.2	0.3 ± 0.1	0.262 (P = 0.009)
	Apo A1 (mg/dl)	157.7 ± 25.3	104.2 ± 17.5	0.683 (P < 0.01)
	Apo B (mg/dl)	61.15 ± 15.59	-	-
	Free carnitine (μmol/l)	24.4 ± 6.1	28.2 ± 6.9	0.778 (P < 0.01)
	Total carnitine (μmol/l)	34.7 ± 6.2	35.9 ± 7.2	0.913 (P < 0.01)
Carbohydrate metabolism related	Glucose (mg/dl)	75.2 ± 7.7	56.0 ± 15.2	-0.045 (P = 0.655)
	Lactate (mg/dl)	11.4 ± 3.7	36.2 ± 11.3	0.146 (P = 0.148)
Hormonal	Insulin (mU/l)	11.5 ± 7.8	9.0 ± 6.0	0.396 (P < 0.01)
	IGF-1 (ng/ml)	179.4 ± 58.0	124.5 ± 42.6	0.807 (P < 0.01)
	IGFBP3 (ng/ml)	-	4106.67 ± 838.15	-

Data are presented as means ± standard deviations. * Every parameter's concentration differed significantly (*P* < 0.05) between serum and FF.

Valckx et al., 2012

Metabolism and
micro-environment (4)

Fat fraction	Normal weight	Overweight	Obese
Phospholipids	1165.57 ± 180.55	1147.93 ± 247.63	1199.95 ± 296.46
Cholesteryl esters	920.04 ± 196.64	891.22 ± 117.35	1067.29 ± 269.40
Triglycerides	215.91 ± 57.89*	355.39 ± 226.44*	244.78 ± 74.65
NEFA	221.67 ± 23.00[a]	245.55 ± 35.98[a]	315.53 ± 82.68[b]
Total concentration	2598.56 ± 422.68	2769.88 ± 477.07	2931.80 ± 684.58

Data are presented as means ± standard deviation. [abc]Data with a different superscript differ significantly ($P < 0.05$). * Differences tend to be significant ($P < 0.1$).

Metabolism and
micro-environment (6)

TABLE 2

IVF cycle date for patients completing IVF cycles.

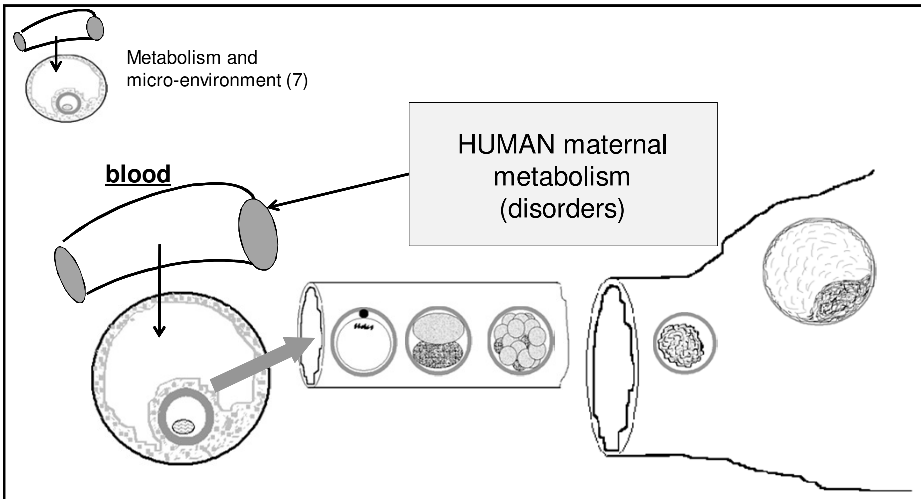
Characteristic	BMI <40 kg/m^2 (n = 53)	BMI ≥ 40 kg/m^2 (n = 19)	*P* Value
Cycle cancellation[a]	6	1	.7
Days of stimulation	10.8 ± 2.6	11.9 ± 3.4	.18
Gonadotropin use (IU)	1924.5 ± 720.5	2606.8 ± 1180.1	.03
Follicles ≥ 12 mm	19.1 ± 6.8	17.7 ± 8.0	.5
Follicles ≥ 16 mm	8.3 ± 3.7	8.2 ± 3.5	.87
Peak E_2 (pg/mL)	2144.5 ± 793.9	1478.7 ± 701.08	.002
Ooctyes retrieved	13.6 ± 5.1	8.9 ± 4.5	.0006
ICSI cases	22	12	.19
Fertilization rate (%)	69	59	.03
Complete failure to fertilize	0	3	.02
Average embryo cell stage	6.5 ± 1.2	6.2 ± 0.5	.5
Average embryo fragmentation	4.3 ± 6.5	10.1 ± 9.6	.01
Day-3 ET	24	12[b]	.28
Day-5 ET	29	2	.002
No. of embryos transferred	2.06 ± 0.4	1.7 ± 0.87	.14
Implantation rate (%)	47	30	.14

Note: Values are mean ± SD or total number of cases unless otherwise noted.
[a] These patients were not included in the remainder of the analyses in this table because they were canceled at various time points in their cycles and did not make it to oocyte retrieval.
[b] Two patients had a day-2 transfer, and three patients had no embryos to transfer.

Jungheim. Morbid obesity in PCOS harms IVF success. Fertil Steril 2009.

triglycerides (A) ($r = 0.45$; $P = 0.0003$), decreased SHBG (B) ($r = -0.41$; $P = 0.001$), increased free androgen index (C) ($r = 0.44$; $P = 0.0003$), and increased C-reactive protein (D) ($r = 0.63$; $P < 0.0001$) in follicular fluid.

Valckx et al., 2011
Jungheim et al., 2009
Robker et al., 2009



Findings:

Follicular fluid **reflects** the changes in the blood associated with obesity (cfr bovine model)
Obesity → **reduced developmental competence** of the oocyte (why, how?)
This is associated with a **lower quality** of the surviving **embryo** (why, how?)

70 women: OPU

Exclusion criteria

- Age older than 38
- BMI < 18kg/m²
- PCOS
- Blood contamination of the sample
- < 6 oocytes aspirated
- < 50% fertilization
- < azoospermia / cryptospermia

FF collected from three patient groups

1. NEG FF:
   - ✓ ≤30% top embryos on Day 3 p.i.
   - ✓ Oocyte originating from FF sample → no development or low quality embryo
   - ✓ BMI < 30kg/m²
2. POS FF:
   - ✓ ≥50% top embryos on Day 3 p.i.
   - ✓ Oocyte originating from FF sample → good embryo quality
   - ✓ BMI < 30kg/m²
3. OBESE FF: BMI > 30 kg/m²

FF added in 25% to bovine oocyte in vitro maturation model

Hypothesis: bad embryo development is (partially) caused by differences in the pre-ovulatory follicular environment

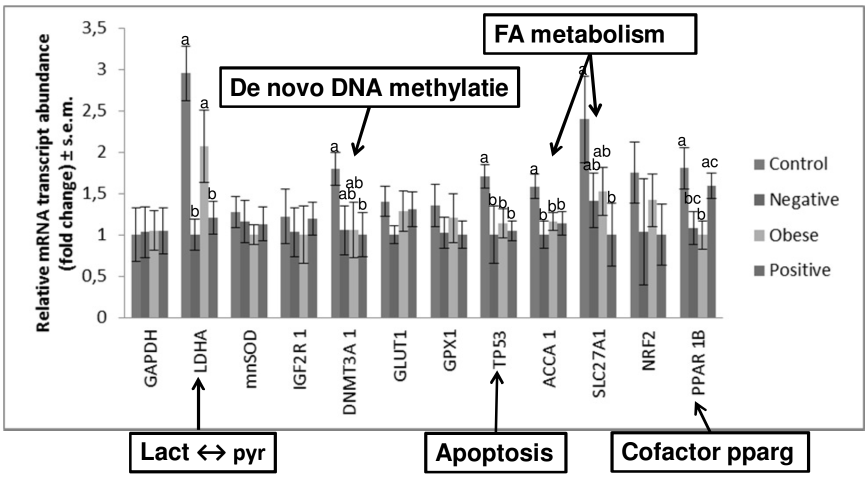
Results

Bovine oocytes matured in human follicular fluid

	Control	NEG FF	POS FF	OBESE
Maturation degree (%)	74 (n = 19)	92 (n = 24)	78 (n = 23)	62 (n = 21)
Fertilisation degree (%)	79 (n = 24)	63 (n = 24)	79 (n = 24)	76 (n = 21)
Cleavage (%)	80^{a} (n = 327)	74^{b} (n = 317)	77^{ab} (n = 331)	76^{ab} (n = 301)
Blastocyst formation (%)	31^{A} (n = 327)	25^{B} (n = 317)	27^{AB} (n = 331)	24^{B} (n = 301)
Blastocyst formation from cleaved zygotes (%)	38^{A} (n = 263)	34^{AB} (n = 233)	35^{AB} (n = 256)	31^{B} (n = 230)
Hatching rate (%)	39 (n = 101)	28 (n = 79)	36 (n = 89)	32 (n = 72)
Cell number (n)	108 (n = 25)	110 (n = 26)	101 (n = 27)	110 (n = 26)
Apoptotic cell index (%)	5,9 (n = 25)	5,8 (n = 26)	6,4 (n = 27)	4,1 (n = 26)

^{ab}Data with a different superscript differ significantly (P < 0.05). ^{AB}Differences tend to be significant (P < 0.1).

Characterization of human follicular fluid



16

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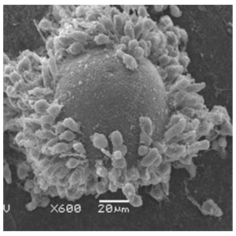
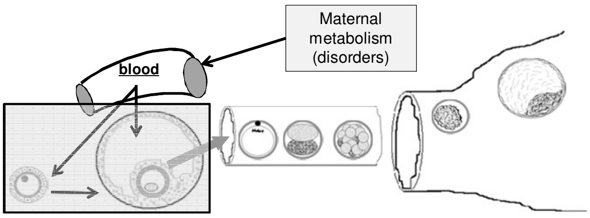
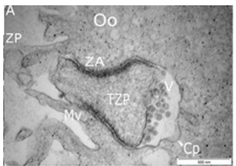
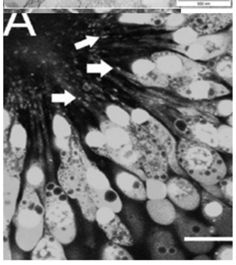




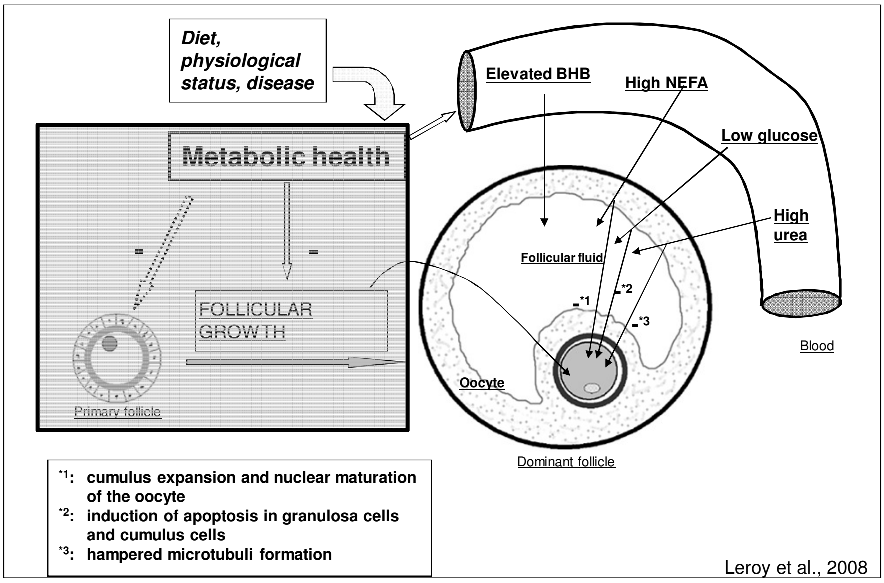




During follicular <u>growth from preantral to antral</u> the oocyte changes:

1. Drives the follicle activation
2. Deposition of the zona pelucida and synthesis of cortical granules
3. Intens contact with somatic cells
4. DNA transcription and mRNA accumulation
5. Oocyte growth untill follicular diameter of 3 mm
6. Epigenetic modifications
7. Reach meiotic competence
8. Reach developmental (cytoplasmic) competence
9. Somatic cell transcriptional support to the oocyte
(Macaulay et al., BOR 2014 in press)

A deviant follicular growth path affects oocyte quality: "Checkpoints of compentence"



Metabolic disturbances in HP dairy cows directly affect the oocyte's micro-environment and may affect follicular physiology and the oocyte's developmental competence.

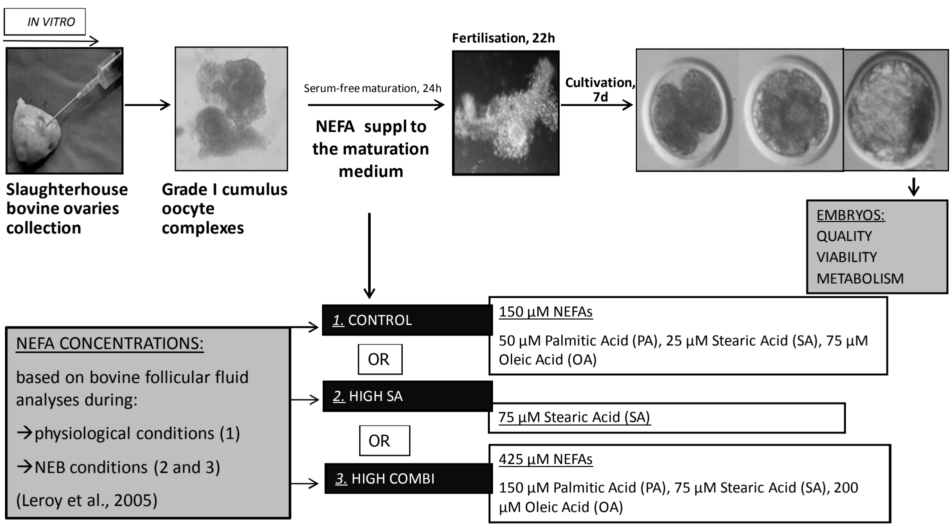
→ **Patho-physiological relevance of NEFA:**

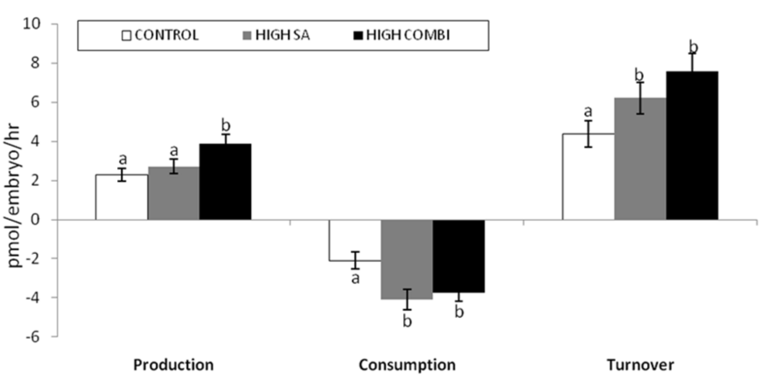
- → In dairy cow
- → In human

→ **Cytotoxic relevance of NEFA**

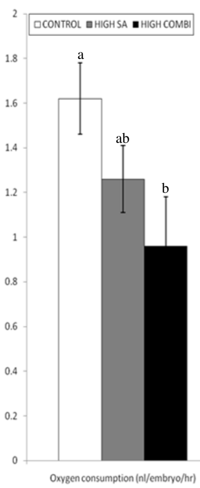
- → Beta cell toxicity
- → Insulin resistance in peripheral tissue
- → Nerve cell toxicity
- → Granulosa cell toxicity
- → Reduces oocyte's developmental competence

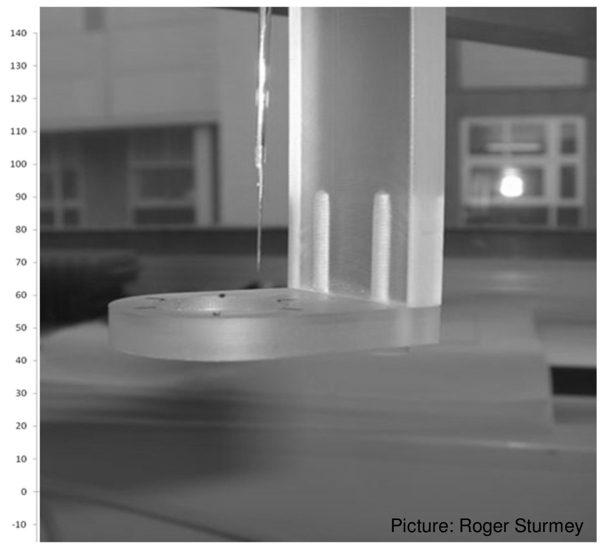
We hypothesize that maturation under elevated NEFA conditions may alter the quality and the physiology of the resulting pre-implantation embryo.





Van Hoeck et al., PLoS ONE, 2011

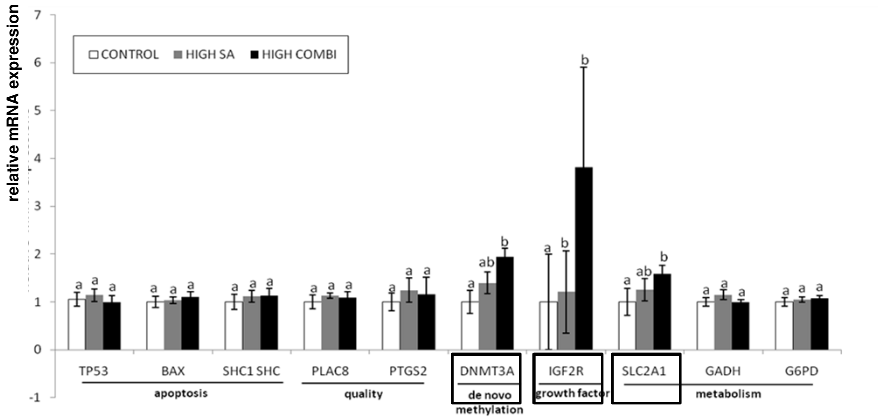




Picture: Roger Sturmey

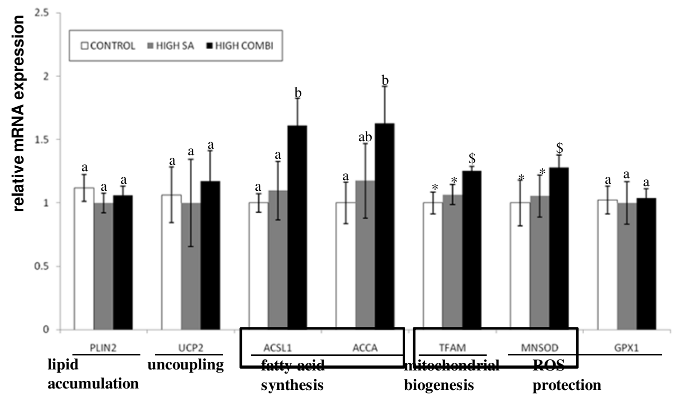
[a,b] Different superscripts per barr indicate a significant difference between treatment groups ($P < 0.05$).

NEFA concentrations and oocyte quality (6)



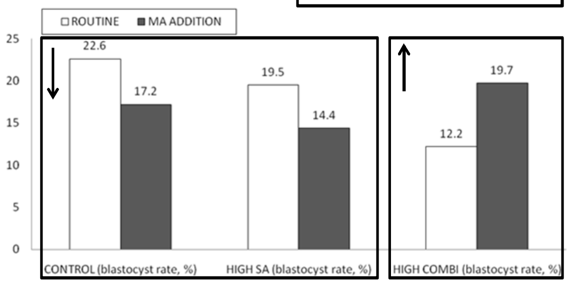
a,b Different superscripts indicate a significant difference between treatment groups ($P < 0.05$).

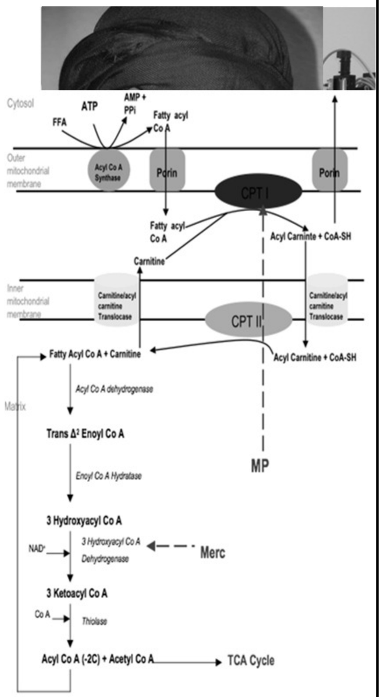
NEFA concentrations and oocyte quality (7)



superscripts a, b: $P < 0.05$. superscripts *,$: $P < 0.1$

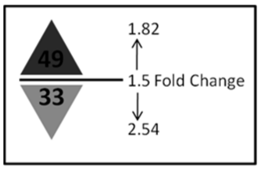
METABOLITE (ACETYL-COA) IS RESPONSIBLE FOR THE REDUCED DC



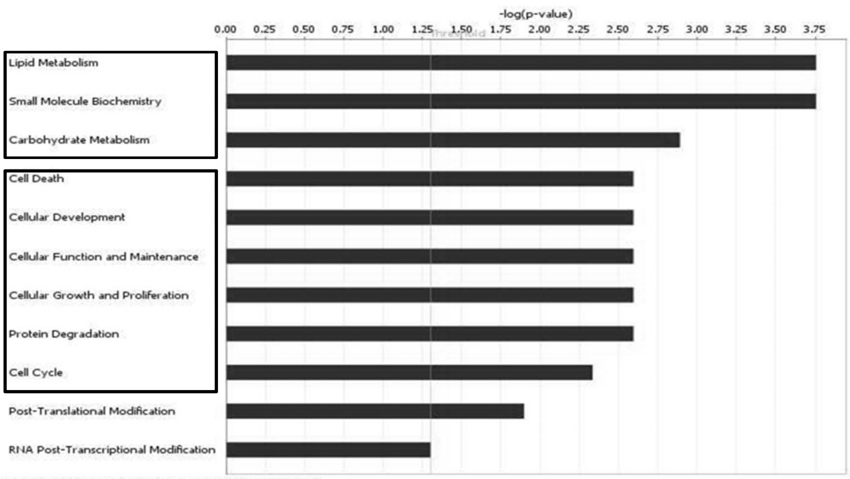


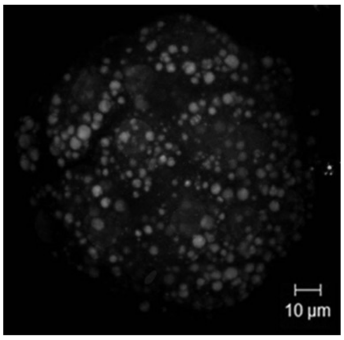
In the HIGH COMBI treatment blastocyst production increased after supplementation of 0.1mM β-mercapto-acetate to the maturation medium.

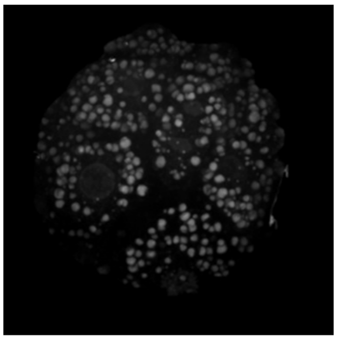
Van Hoeck et al., Reproduction, 2012



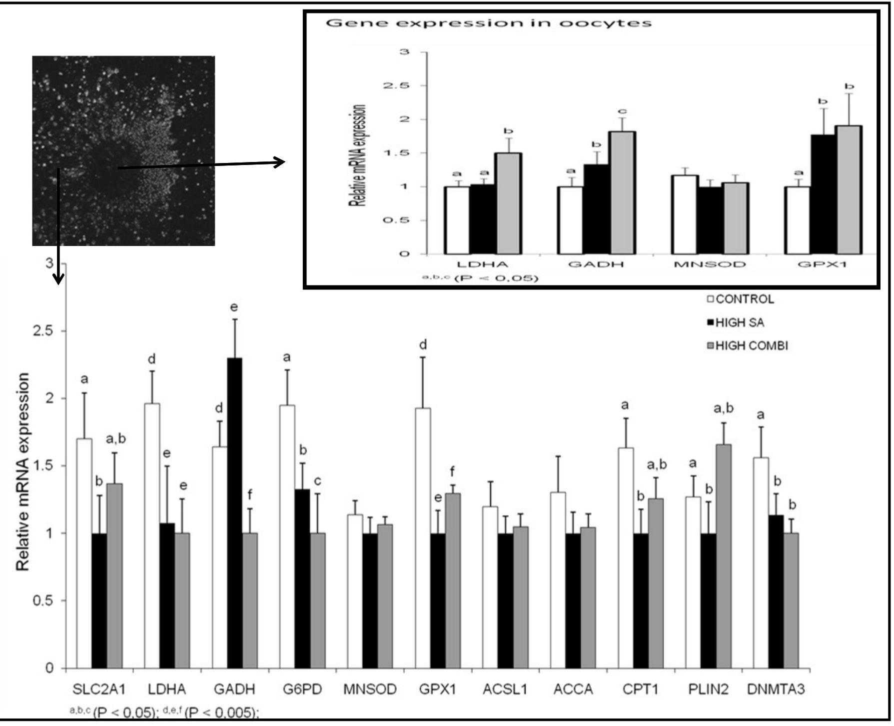
HIGH COMBI vs. CONTROL



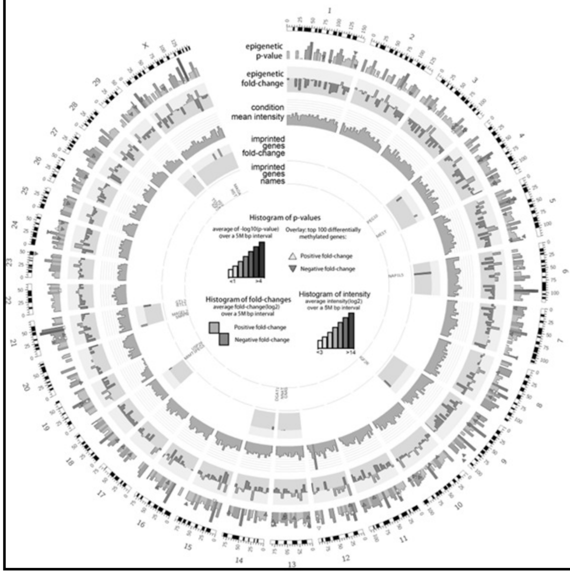




	control	HIGH SA	HIGH COMBI
Volume lipid droplets per cell (µm³)	2440.25 ± 236.52 [ab]	1927.59 ± 234.57 [a]	3482.89 ± 491.13 [b]
Size lipid droplets (µm³)	14.33 ± 1.23 [a]	16.68 ± 1.66 [a]	17.56 ± 1.92 [a]
Number of lipid droplets per cell	178.48 ± 14.84 [a]	113.10 ± 5.76 [b]	185.07 ± 19.39 [a]



DNA methylation profile



up-regulated expression of genes associated with epigenetic regulation (*DNMT3A*, *HIST1H2BN*)

Hypermethylation	Hypomethylation
191 genes	91 genes

Most important epigenetically modified pathways

- Apoptosis
- Lipid metabolism
- Gene transcription
- Inflammatory response
- Metabolic disorders

Embryogene 2013 - 2014

Obesity warning issued to women wanting babies

Failed pregnancies can result from fatty ovaries, researchers claim

Press Association
The Guardian, Wednesday 17 August 2011 22.01 BST

Scientists say their findings add further weight to the recommendations which emphasise being a healthy weight before starting a pregnancy. Photograph: Corbis Cusp/Alamy

Fatty ovaries can upset embryonic development and lead to failed pregnancies, research suggests.

The findings, from a study carried out on cow eggs, supports advice to women to avoid being overweight if they want to conceive.

They may help explain why obese women and those with diabetes often struggle to conceive, say researchers. These individuals tend to metabolise more stored fat, leading to higher fatty acid levels in the ovary.

The study involved exposing eggs from cows to high concentrations of saturated fatty acids. Researchers discovered embryos created from the fat-exposed eggs had fewer cells, and underwent changes in gene and metabolic activity.

Lead scientist Professor Jo Leroy, from the University of Antwerp in Belgium, said: "We know from our previous research that high levels of fatty acids can affect the development of eggs in the ovary, but this is the first time we've been able to follow through to show a negative impact on the surviving embryo."

British colleague Dr Roger Sturmey, from the University of Hull, said: "Our findings add further weight to the public health recommendations which emphasise the importance

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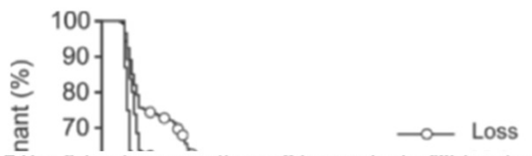


Table 7. Embryo characteristics of lactating Holstein cows based on BW change from first to third week postpartum (values presented as mean ± SEM)

Item	Quartile group[1]: Lost BW+: fourth quartile	Lost BW: third quartile	Maintained BW: second quartile	Gained BW: first quartile	P-value
Corpora lutea (no.)	18.4 ± 2.6	18.4 ± 1.7	19.0 ± 1.7	16.0 ± 2.0	0.67
Total ova/embryos (no.)	9.6 ± 2.5	10.6 ± 1.7	6.4 ± 1.2	7.4 ± 1.4	0.31
Fertilized structures (no.)	7.6 ± 2.1	7.3 ± 1.1	4.8 ± 1.1	5.8 ± 1.4	0.43
Degenerated embryos (no.)	2.7 ± 0.7^{a}	1.7 ± 0.7^{ab}	0.7 ± 0.2^{b}	0.6 ± 0.2^{b}	0.02
Quality 1 and 2 (no.)	4.2 ± 1.4	5.3 ± 0.9	3.9 ± 1.1	4.9 ± 1.4	0.47
Quality 1, 2, and 3 (no.)	4.9 ± 1.6	5.6 ± 0.8	4.1 ± 1.1	5.3 ± 1.4	0.49
Fertilized (%)	76.9 ± 7.1	77.0 ± 6.6	77.6 ± 7.6	78.4 ± 7.1	0.99
Degenerated (%)	35.2 ± 8.5^{a}	12.6 ± 4.6^{b}	14.5 ± 6.3^{b}	9.6 ± 3.7^{b}	0.02
Quality 1 and 2 (%)	$38.0 \pm 8.7^{b,B}$	$61.3 \pm 8.2^{ab,A}$	$60.6 \pm 9.4^{ab,A}$	$63.4 \pm 8.6^{a,A}$	0.14
Quality 1, 2, and 3 (%)	$41.7 \pm 8.8^{b,B}$	$64.4 \pm 8.2^{ab,A}$	$63.1 \pm 9.3^{ab,A}$	$68.9 \pm 8.7^{a,A}$	0.13
Degenerated of fertilized (%)	$46.9 \pm 9.6^{a,A}$	$17.4 \pm 6.4^{b,B}$	$24.8 \pm 9.3^{ab,A}$	$16.2 \pm 7.0^{b,B}$	0.04
Quality 1 and 2 of fertilized (%)	48.4 ± 9.5^{b}	78.3 ± 6.6^{a}	72.6 ± 9.5^{a}	77.7 ± 7.4^{a}	0.05
Quality 1, 2, and 3 of fertilized (%)	$53.2 \pm 9.6^{b,B}$	$82.6 \pm 6.4^{a,A}$	$75.2 \pm 9.3^{a,AB}$	$83.8 \pm 7.0^{a,A}$	0.04
Recovery rate (%)	45.6 ± 7.4	55.1 ± 6.9	35.4 ± 6.7	45.3 ± 5.8	0.25

[a,b]Values within a row with different superscript lowercase letters differ at $P < 0.05$.

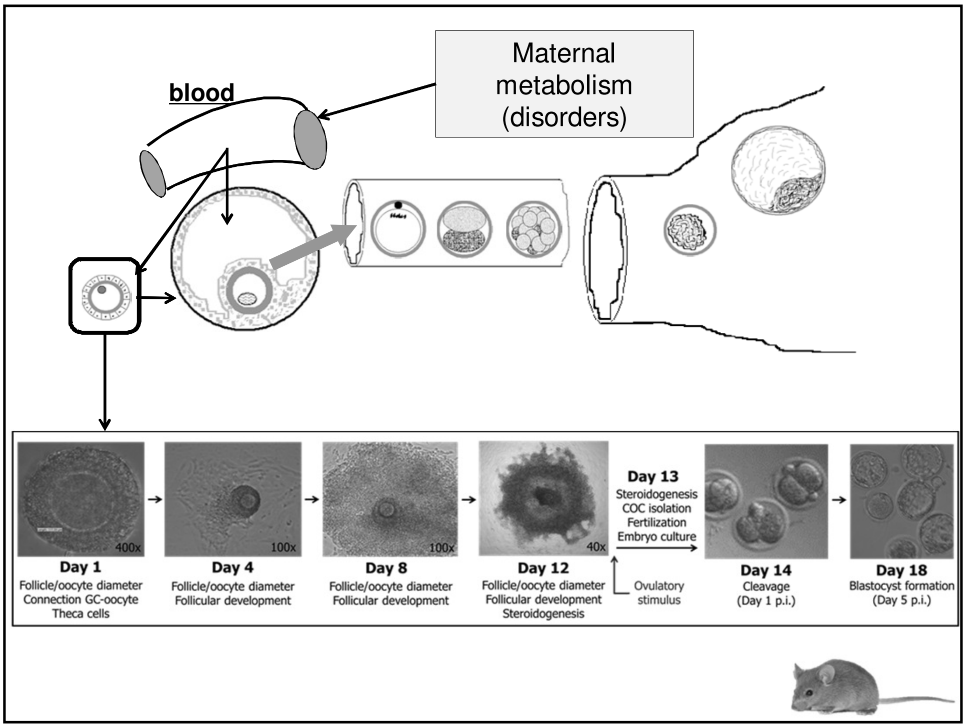
[A,B]Values within a row with different superscript capital letters differ at $P < 0.15$.

[1]Fourth quartile = most BW (BW+) loss.

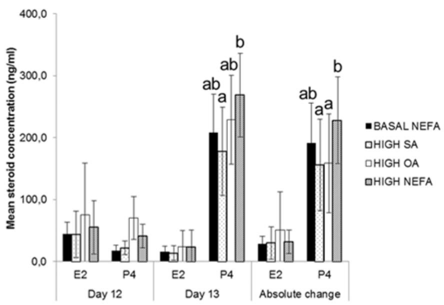
Cows gaining BCS between calving and 21 d postpartum had a median [illegible] compared with 113 d for cow [illegible] BCS [illegible] for cows losing BCS between calving and 21 d postpartum. The symbols on each line represent a censoring event (cow removed from analysis due to death, culling, or designation as "do not breed").

Britt's Hypothesis

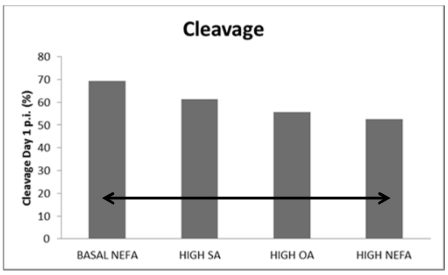
Carvalho et al., JDS 2014

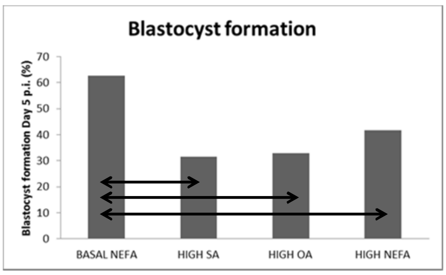


- Day 12 antrum formation
- Cleavage rate (Day 1 p.i.) and blastocyst formation (Day 5 p.i.)

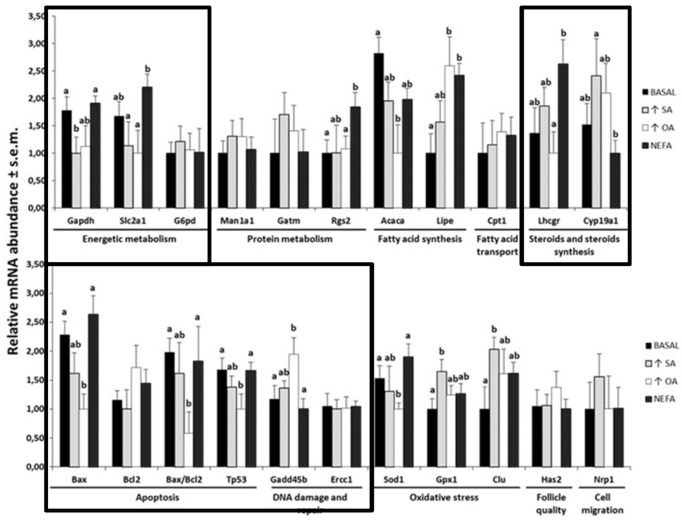


Valckx et al., Fert Ster in press

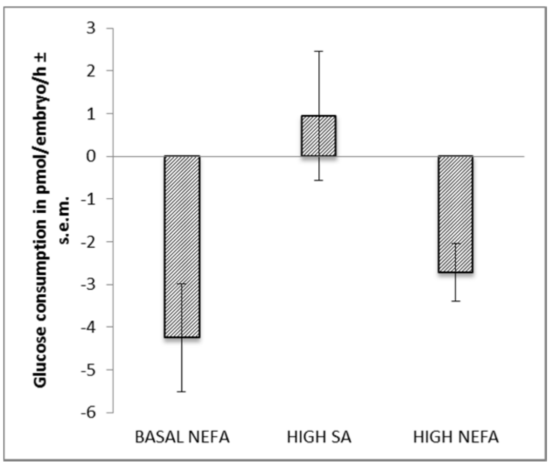




Day 13 granulosa cell gene expression patterns

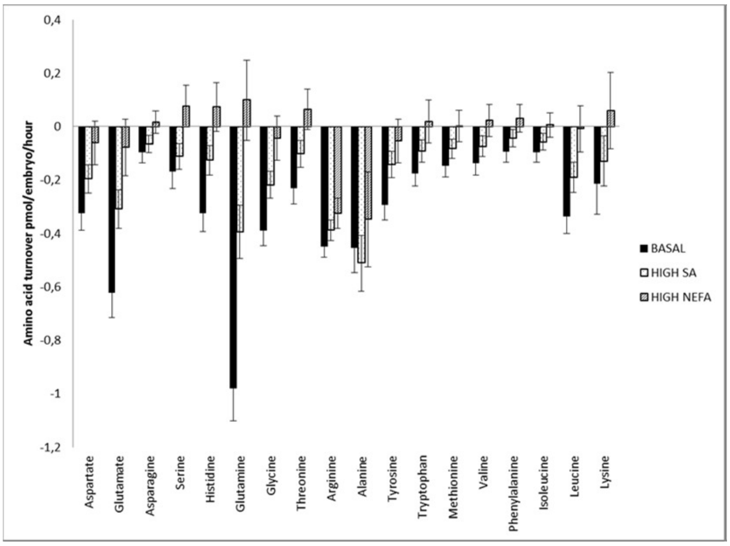


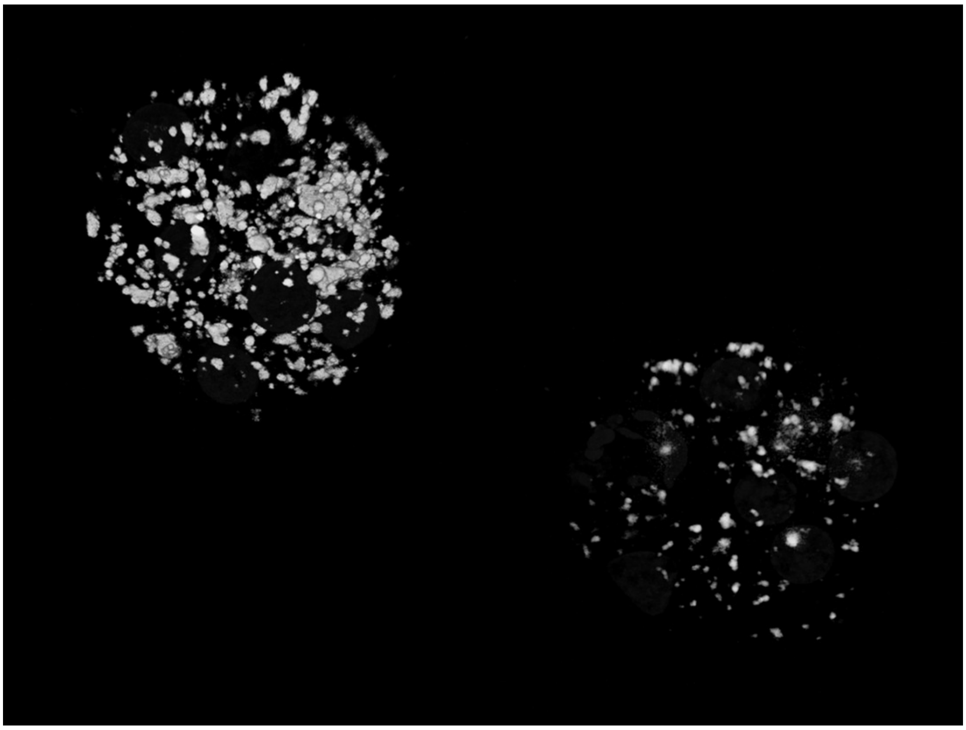
Glucose metabolism



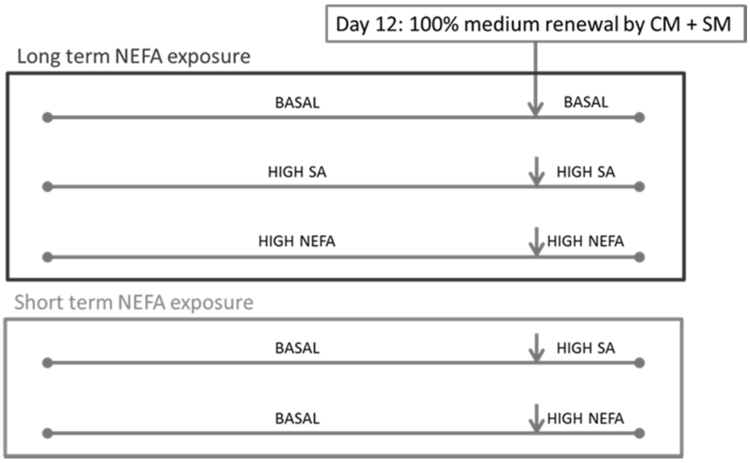
No glucose consumption for HIGH SA embryos:
significant difference between BASAL NEFA and HIGH NEFA vs. HIGH SA

Amino Acid turnover

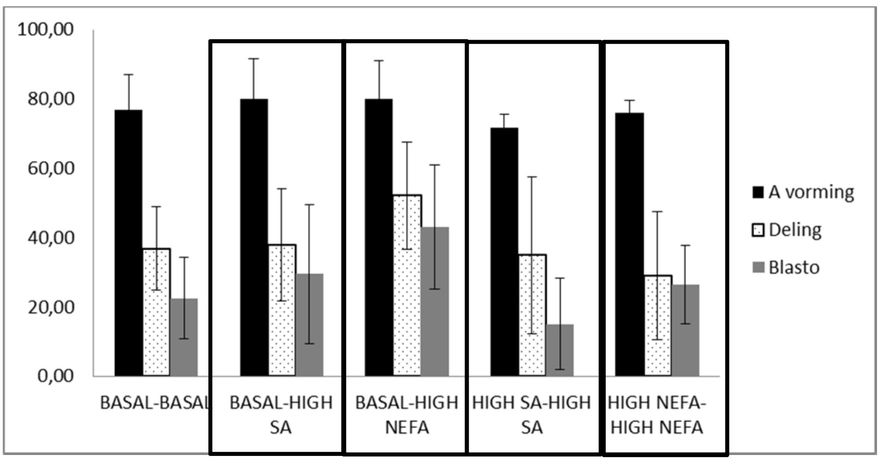




Short vs. long term NEFA exposure



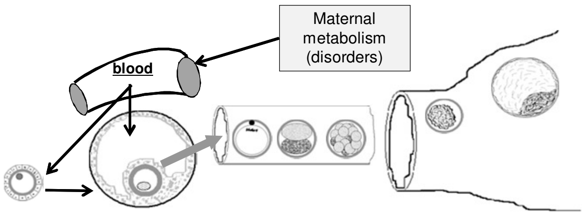
Short vs. long term NEFA exposure



In this talk

- Our central hypothesis in dairy cows and … in women
- The oocyte's "preprogramming" micro-environment
- Britt's hypothesis: "difficult to investigate"
- Conclusions

Conclusions



1. The micro-environment of the female gamete is affected by maternal metabolic changes.
2. Some of these changes do have a direct effect on oocyte and pre-implantation embryo physiology.
3. NEFA are an intriguing metabolic key in the relation between metabolic disorders associated with upregulated lipolysis and subfertility.
4. The fatty acid type affects the biological response and affected pathways
5. How valid is our model?
    1. Acute or chronic exposure, the "Britt's hypothesis" …
    2. Translation to in vivo situation (Roth et al., 2008; Matoba et al., 2012)

However, do we need all thesis “omics” to discover why this animal won’t reproduce? What about human subfertility?

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