PUFA as tool to improve fertility: hype or significant benefit?

Sébastien ELIS
UMR Physiology of Reproduction and Behaviors,
Centre INRAE Val de Loire, France

Fatty acids

Number and position of double bonds

Saturated fatty acids
SFA  
Stearic Acid 18:0

Monounsaturated fatty acids
MUFA  
Oleic Acid 18:1, n-9

Polyunsaturated fatty acids
n-6 PUFA  
Linoleic Acid 18:2, n-6

n-3 PUFA  
α-Linolenic Acid 18:3, n-3

INRAE
PUFA as tool to improve fertility: hype or significant benefit?
05.02.2020 / Wilrijk, Belgium / Sébastien El is
**n-3 PUFA**

- **α-linolenic acid** (ALA)  \[18:3, \text{n-3}\]
- **Eicosapentaenoic acid** (EPA)  \[20:5, \text{n-3}\]
- **Docosahexaenoic acid** (DHA)  \[22:6, \text{n-3}\]

Grass, lin seeds...

Marine origin: *Fish oil, algae...*

---

**How does it work?**

**Prostaglandins:**

- Arachidonic acid  \[20:4 \text{n-6}\]
- Eicosapentaenoic acid (EPA)  \[20:5 \text{n-3}\]
- Docosahexaenoic acid (DHA)  \[22:6 \text{n-3}\]

**Membrane structure/composition:** elasticity

**Signal transduction:** lipid rafts

**Activation of specific receptors:** fatty acid receptors


Calder 2015, *Biochim and Biophys Acta*, 1851:469-484
n-3 PUFA supplementation and reproduction

In vitro (oocyte maturation medium)
Increased embryo developmental rates

In vivo experiments:
Greater pregnancy rate
Lower early embryo mortality
Decrease in PGF2α (uterus level) → facilitating embryo implantation
Increase in pre-ovulatory follicle size in small follicle number improve embryo production

Discrepancies / confounders
- negative effects on reproduction
- FA, dose, form (ruminal protection?), duration, physiological stage ...
- control used in the study
  SFA (palmitic acid)
  n-6 PUFA (linoleic acid: soy, maize...)
  also positive effects

2 reviews:
Dietary fat
N-3 PUFA

INRAE
PUFA as tool to improve fertility: hype or significant benefit?
05-02-2020 / Wilrijk, Belgium / Sébastien ELIS
Objectives:

• Investigate EPA / DHA effects on oocyte quality

• AI, embryo production, \textit{in vitro} studies

• Comparison with n-6 PUFA

• Moderate dose, several diet duration (folliculogenesis stage)
Experimental designs:

**Exp 1**

- Effects on fertility through the oocyte?

- 3 month for an oocyte to grow and mature (basal folliculogenesis targeted)
- The diet could affect oocyte growth BUT not embryo implantation (high n-3 PUFA clearance during lactation)

→ Follicular population and conception rates after the 1st AI

**Exp 2**

- Effects on embryo production? oocyte quality?
- Both basal and terminal folliculogenesis targeted
- Several durations

→ Developmental rates

---

**INRAE**
PUFA as tool to improve fertility: hype or significant benefit? 05-02-2020 / Wilrijk, Belgium / Sébastien ELIS


**INRAE**
PUFA as tool to improve fertility: hype or significant benefit? 05-02-2020 / Wilrijk, Belgium / Sébastien ELIS

**Freret et al. 2019, Reproduction, doi: 10.1530/REP-18-0644**
Experimental designs:

- Terminal folliculogenesis only
- Several concentrations
- Insight on mechanisms of action

In vitro exp. DHA supplementation in IVM medium during 24h

Developmental rates

Effects on oocyte quality?

Oseikria et al. 2016, Therio, 85:1625-1634

Ellis et al. 2017, J Ovarian Res, 10:74

Results:

A. Total number of follicles

C. Number of medium follicles (5 ≤ diameter < 10 mm)

B. Number of medium follicles

D. Number of large follicles (10 ≤ diameter < 25 mm)

Increase in number of follicles

**Results:**

**Conception rates**

<table>
<thead>
<tr>
<th></th>
<th>n-3</th>
<th>n-6</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-EEM</td>
<td>13.5</td>
<td>38.8</td>
<td>0.086</td>
</tr>
<tr>
<td>CRAI1 35D after AI1</td>
<td>72.9</td>
<td>48.5</td>
<td>0.129</td>
</tr>
<tr>
<td>CRAI1 90D after AI1</td>
<td>56.1</td>
<td>48.1</td>
<td>0.629</td>
</tr>
</tbody>
</table>

Increase in number of follicles

Reduction in the non fertilization – early embryo mortality %

**Ovarian effects** (not only through uterus and prostaglandins)

**Embryo production**

**Oocyte lipid composition**

<table>
<thead>
<tr>
<th></th>
<th>n-3</th>
<th>n-6</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% recovered COC</td>
<td>38.0±1.6 *</td>
<td>32.8±1.6</td>
<td>0.0035</td>
</tr>
<tr>
<td>% cleaved embryos</td>
<td>77.3±3.8 #</td>
<td>82.3±3.3</td>
<td>0.1033</td>
</tr>
<tr>
<td>% blastocysts</td>
<td>49.6±5.5 #</td>
<td>42.3±5.5</td>
<td>0.0865</td>
</tr>
<tr>
<td>% blastocyst Q1-Q2</td>
<td>42.2±7.7 *</td>
<td>32.7±7.1</td>
<td>0.0217</td>
</tr>
</tbody>
</table>

**Diet : changes in oocyte lipid composition**

Increase in blastocyst rate (freezable quality)

A short duration of supplementation might be enough (less than a month)

**Results:**

*In vitro experiment: DHA in IVM – 24h*

<table>
<thead>
<tr>
<th></th>
<th>% blastocysts</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.6 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>DHA 1 µM</td>
<td>30.6 ± 4.1</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

DHA 1 µM improves blastocyst rate

No effect of DHA 10 µM

Toxic effect of DHA 100 µM

---

Oseikria et al. 2016, Therio, 85:1625-1634

---

**Similar effect by activating a FA receptor: FFAR4**

---

Elis et al. 2017, J Ovarian Res, 10:74
Are n-3 PUFA supplementations effective?

- N-3 PUFA supplementation is effective
  - enhancement in reproductive parameters (most of the studies)
  - action through ovary and not only uterus
  - even when compared to n-6 PUFA

BUT

- Moderate improvement only

- High cost / competition with human feed – limited resources
  
  - grass, lin seed, algae (similar efficiency?)

Take-home messages:

- N-3 PUFA → positive effects on the oocyte quality
- Interesting when dealing with moderate quality oocyte
  
  (→ high genetic merit cows)

BUT

- Cost - benefit evaluation depends on your activity

- Embryo producing companies: low cost solutions, short supplementation

- Farms: too expensive for reproduction purpose only
  
  (→ increasing dairy product value : n-3 enriched products?)
Thank you for your attention

Bingo team
Virginia Maillard
Svetlana Uzbekova
Sandrine Fréret
Alice Desmarchais
Jean-Luc Touzé
Juliette Cognié
Valérie Labas
Mouhamad Oseikria
Maëva Durcin
Céline Hivelin

UEPAO:
Eric Briant
Mickaël Delanoue
Mickaël Dupont
Ludovic Métivier
Christophe Mouaze

Allice
Pascal Salvetti
Daniel Le Bourhis
Olivier Desnoes
Laurene LeBerre

Funding

INRAE
PUFA as tool to improve fertility: hype or significant benefit?
05-02-2020 / Wilrijk, Belgium / Sébastien Elis