

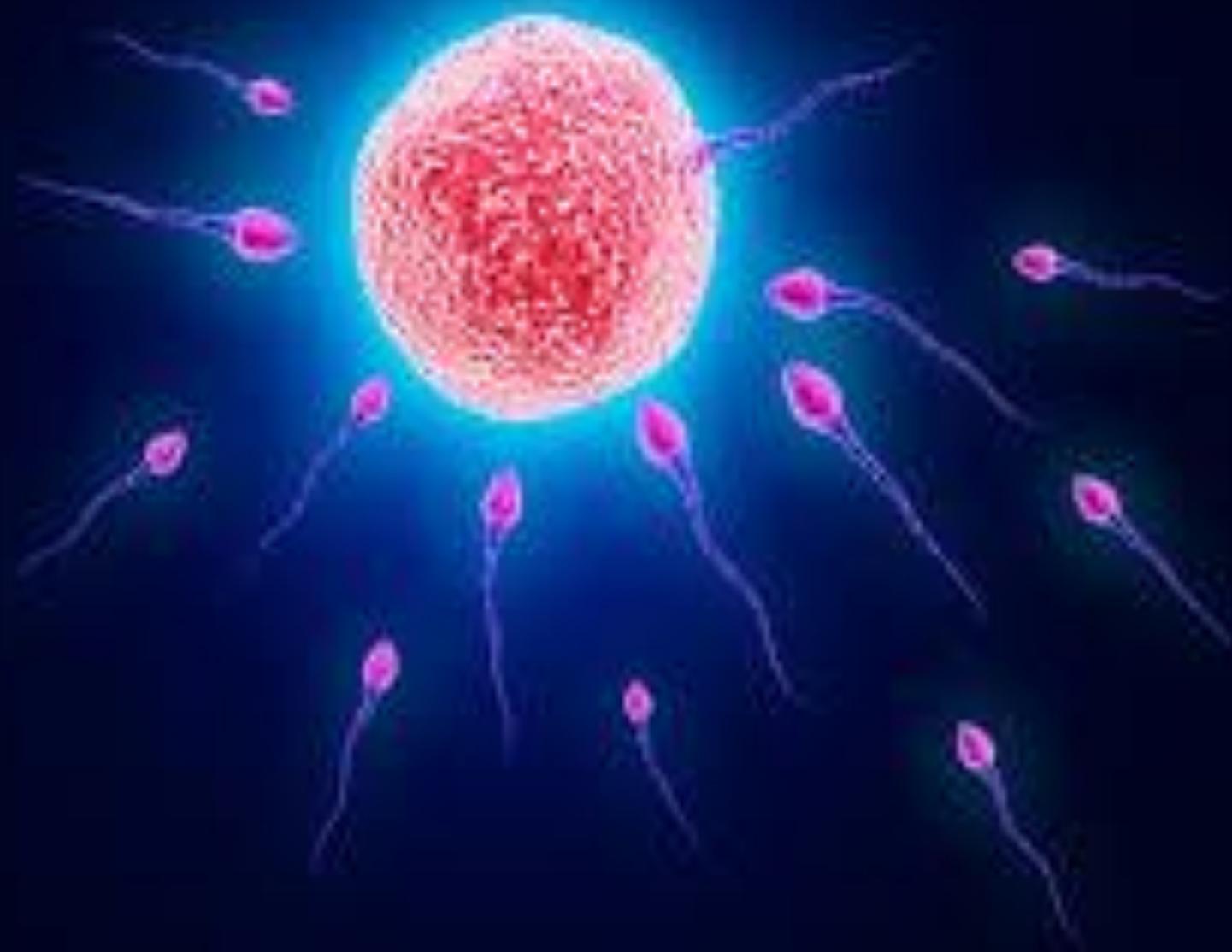
Immunohistochemical And Ultrastructural Analysis Of The Effect Of Omega-3 On Embryonic Implantation In An Experimental Mouse Model

Kemal Sarsmaz*, Ashlı Göker*, Serap Cilaker Mıçılı**

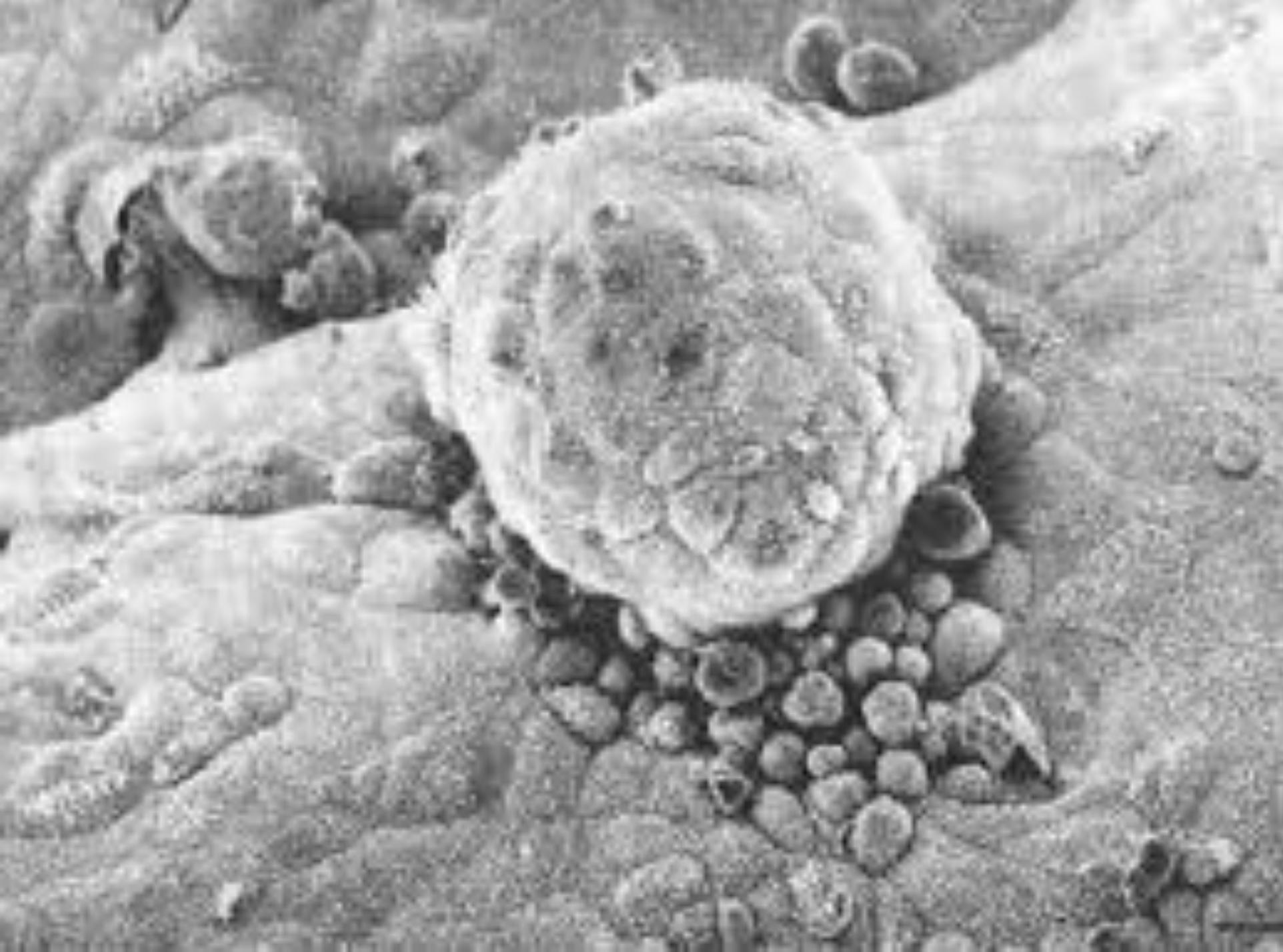
Bekir Uğur Ergür, Naci Kemal Kuşçu***

**Obstetrics and Gynecology Celal Bayar University Manisa,*

***Histology and Embryology Dokuz Eylül University Izmir*







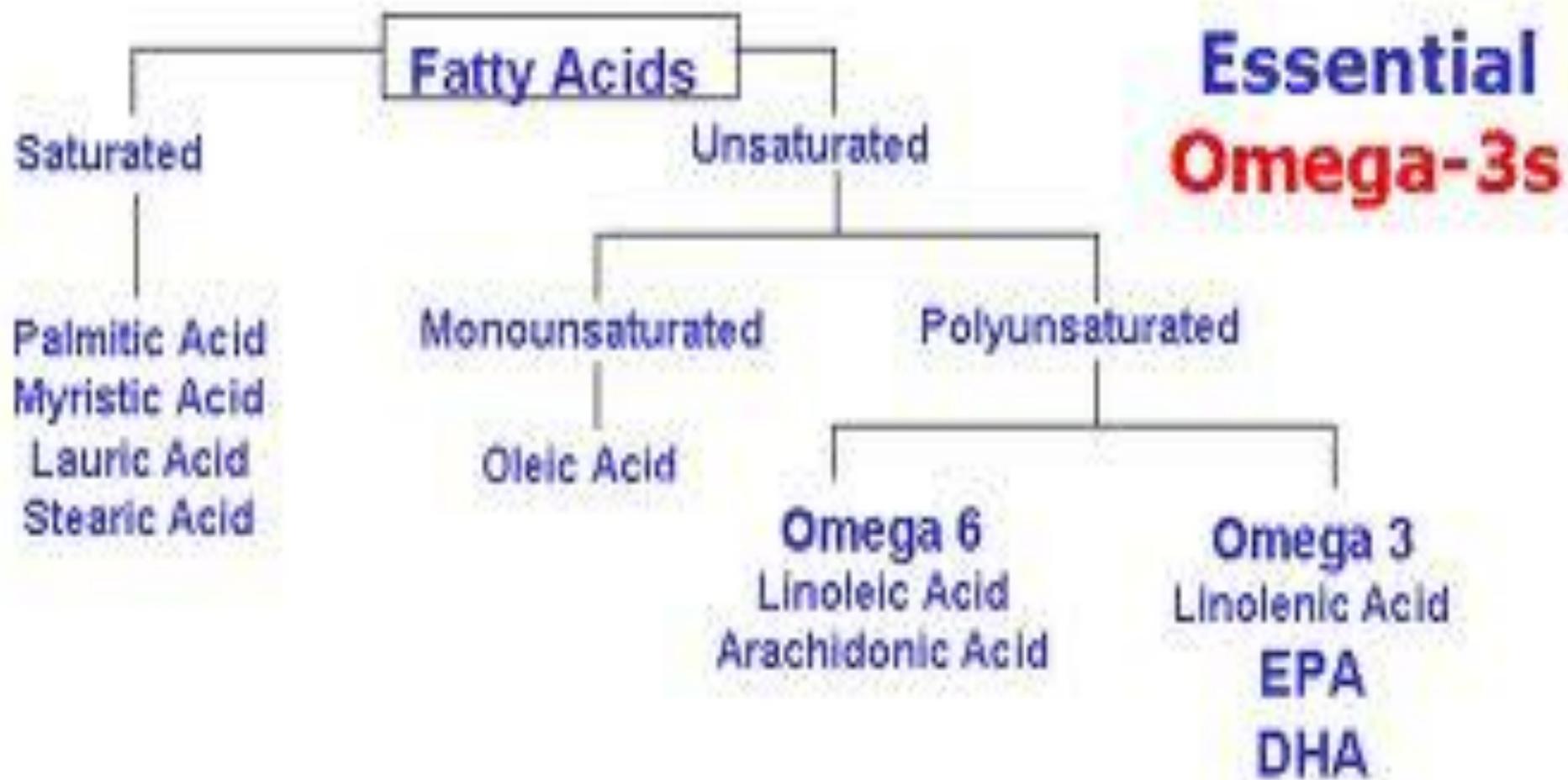
Implantation

- Steroid hormones, cytokines, integrins, growth factors, adhesion molecules, pinopodes
- The implantation window: the blastocyst interacts with the endometrial epithelium and is in the receptive stage

Implantation

- During implantation the interaction between trophoectoderm and luminal epithelium triggers a remodelling in epithelial cell organisation.
- Cells flatten and lose their microvilli and the polarity between apical-basal luminal epithelium decreases
- The success of implantation depends on correct timing of the blastocyst-endometrium encounter.





Saturated

Fatty Acids

Unsaturated

- Palmitic Acid
- Myristic Acid
- Lauric Acid
- Stearic Acid

Monounsaturated

Polyunsaturated

Oleic Acid

Omega 6

- Linoleic Acid
- Arachidonic Acid

Omega 3

- Linolenic Acid
- EPA**
- DHA**

Essential Fatty Acids

- Are used in the synthesis of prostaglandins, thromboxanes and leucotrienes
- Are structural components of cell membranes and are needed for cell functioning

Omega-3

- Insufficient Omega-3 fatty acid may lead to increase in triglycerid and cholesterole, **growth retardation**, **hypertension**, impairment in wound healing, hair loss, depression of the immune system and **postpartum depression**
- Omega-3 integrates into the phospholipids of the cell membrane and is important for mitochondri specific functions

LIF

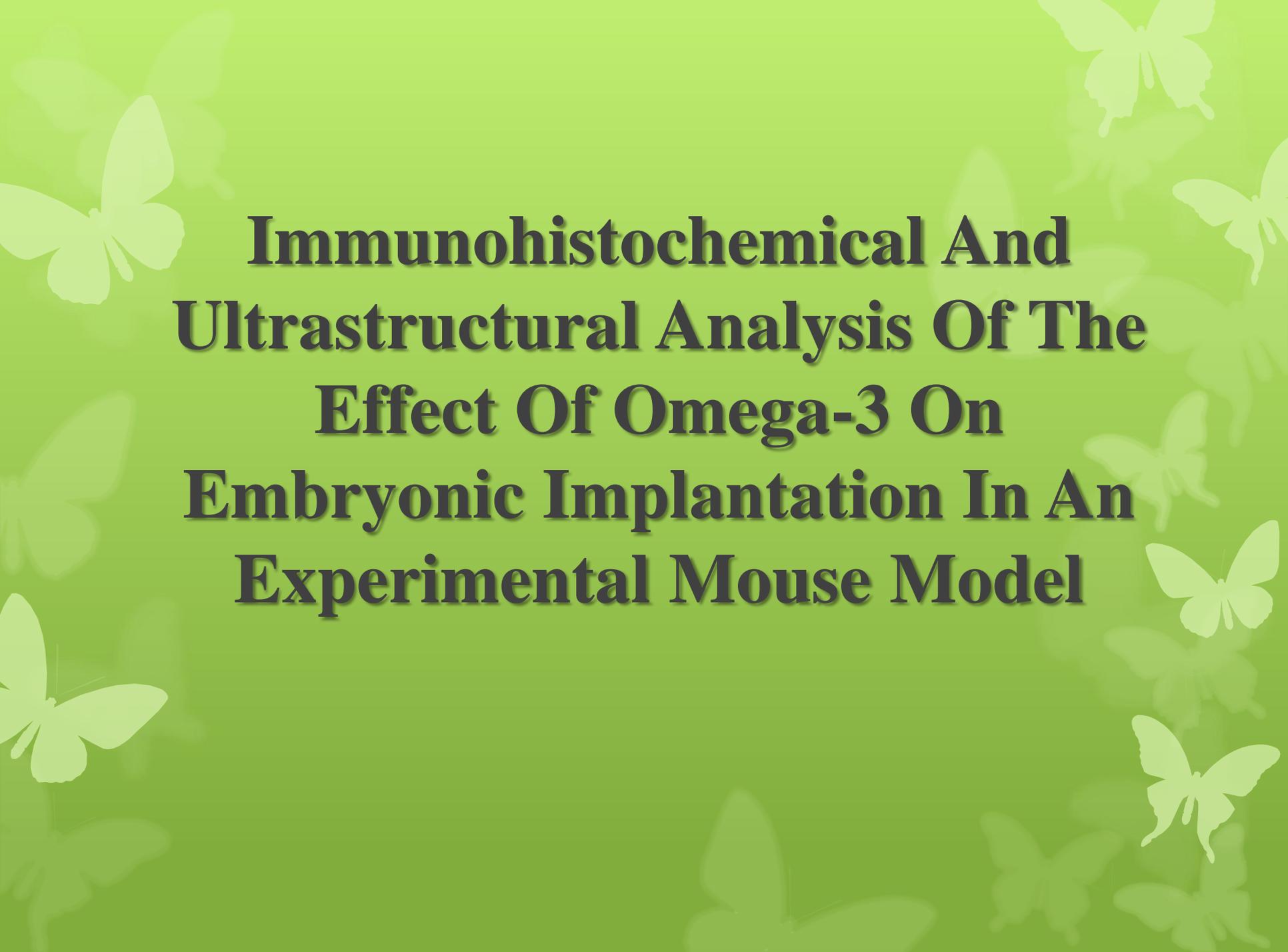
- Endometrium of mice at implantation contain LIF
- Human endometrium contains LIF during blastocyst implantation
- LIF also contributes to trophoblast adhesion and differentiation
- Women with high LIF immunoreactivity during the implantation period are shown to have high pregnancy rates
- Infertile women with endometriosis do not express LIF in their endometrium

Laminin

- Laminin is an extracellular matrix protein that increases in basal membrane after implantation
- Contributes to embryogenesis, cell migration, differentiation and cell growth
- Laminin favors trophoblastic invasion into the extracellular matrix

Morphology during Implantation

- Decrease in microvilli in apical membranes of secretory cells and the formation of pinopodes



**Immunohistochemical And
Ultrastructural Analysis Of The
Effect Of Omega-3 On
Embryonic Implantation In An
Experimental Mouse Model**

Aim of the study

To investigate the effect of
Omega-3 fatty acid
supplementation on implantation

Materials and methods

- 21 albino mice (*mus musculus* (C/C)) weighing 18-22 g
- Regular menstrual periods of the mice were determined by vaginal smears



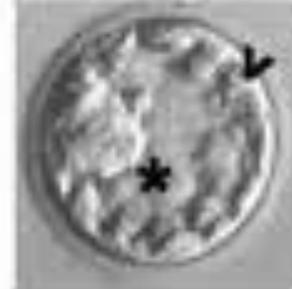
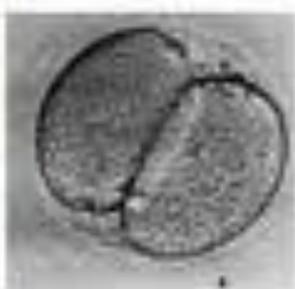
● Group I :standard animal food pellets

● Group II + low dose Omega-3

(400 mg/kg Omega -3)

● Group III + high dose Omega-3

(1000 mg/kg Omega-3)



zygote
mouse D0
human D1

2-cell
D1
D1-2

4-cell
D1-2
D2

multi-cell
D2
D3

morula
D2-3
D3-4

blastocyst
D3-4
D5

* inner cells of the morula form the inner cell mass; ^ outer cells of the morula form the trophoblast

Images are courtesy of Dr. B. Behr and the Stanford University IVF clinic.

- the mice were left for mating.
- Vaginal plaque was checked for pregnancy the following day and time at 12.00 was considered as embryonic day E:0.5.
- The mice were sacrificed on expected day of implantation, namely day 3.5.
- Chicago Blue was applied intravenously, after ten minutes laparotomy was performed and foci on uterine horns which were blue in color were determined as implantation regions.



Results

● LIF Immunohistochemical scoring

	Lumen Epithelium	Gland Epithelium	Stroma
Control	1,00±0,57	0,57±0,53	1,14±0,37
Low dose	1,14±0,37*	0,71±0,48*	1,14±0,37*
High dose	2,28±0,48*	1,71±0,48*	2,00±0,57*

Results

● Laminin Immunohistochemical scoring

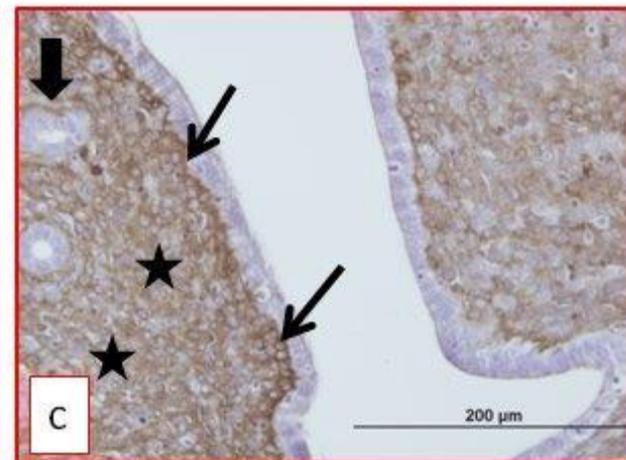
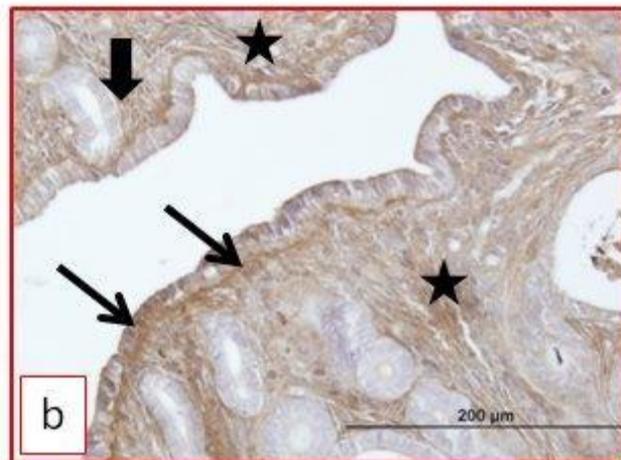
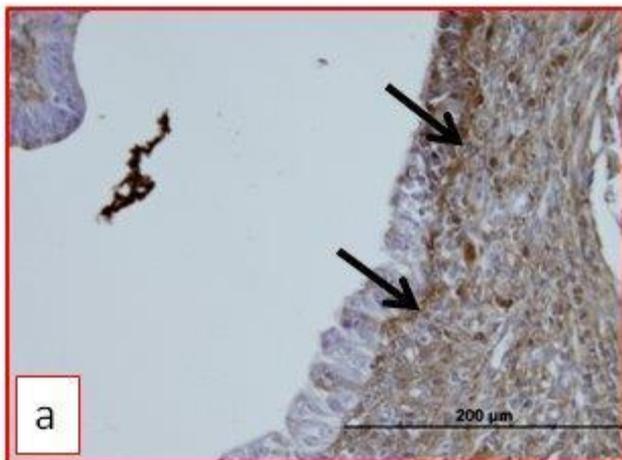
	Lumen Epithelium	Gland epithelium	Stroma
Control	1,71±0,48	1,57±0,53	1,57±0,53
Low dose	1,57±0,53	1,42±0,53	1,85±0,37
High dose	2,42±0,53*	2,42±0,53*	2,42±0,53*

Group I

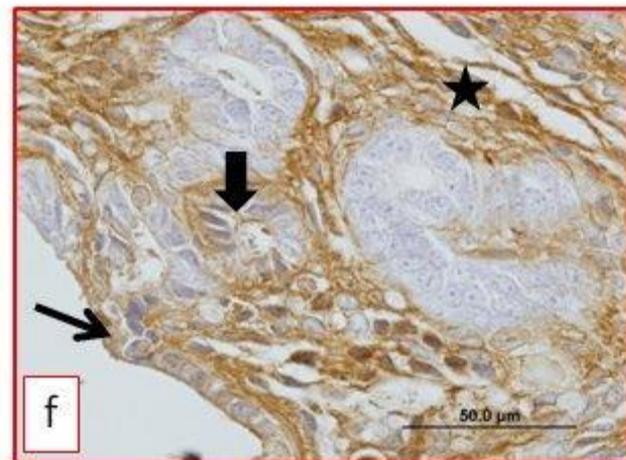
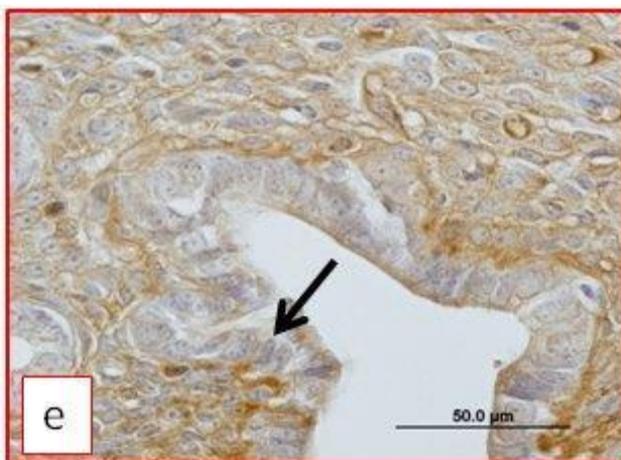
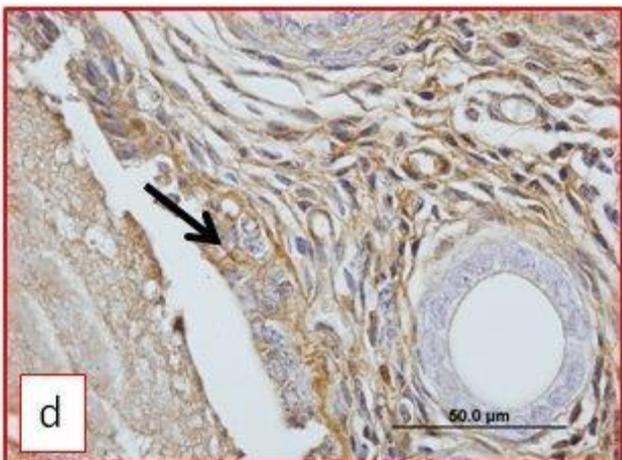
Group II

Group III

Laminin

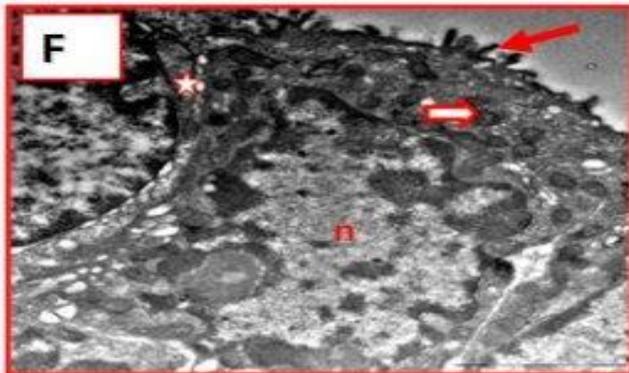
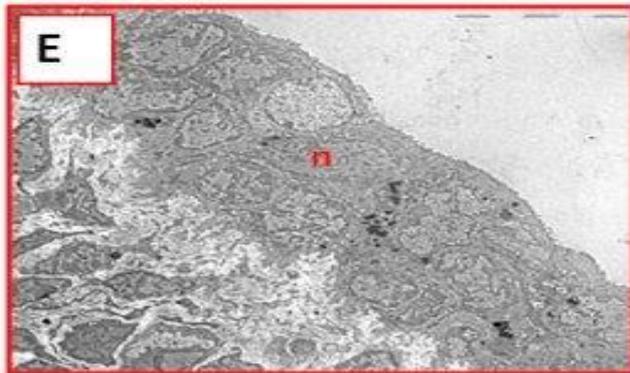
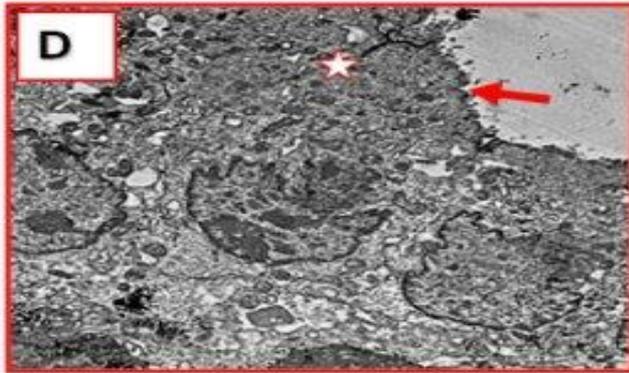
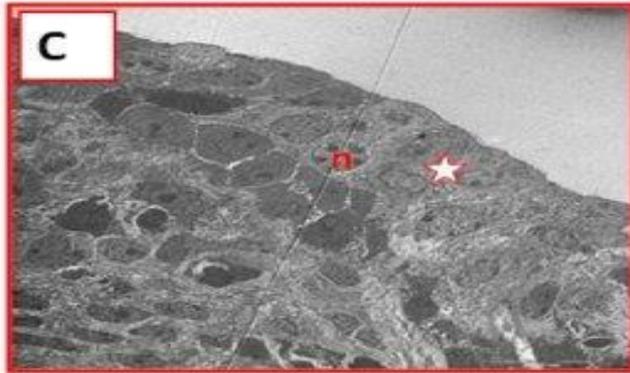
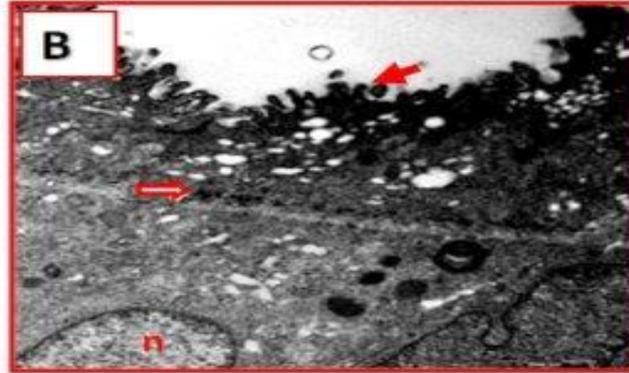
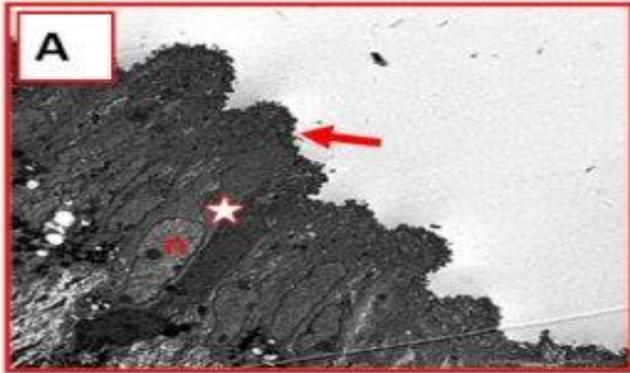


LIF



● Number of microvilli per unit area and Lumen epithelium height (ultrastructural)

Groups	Microvillus number/1000nm	Mann Whitney U Test (p value)	Epithelium average height (nm) ± Standard deviation	p value
Control	3,80	0,599	18175,94 ± 2979,3	0,008
Low dose Omega-3	2,67	0,000*	17844,71± 719,9*	0,004
High dose Omega-3	2,16	0,000*	7051,08± 682,3*	0,004



Conclusion

Mice that received Omega-3 supplementation

- Had an increased secretion of LIF during the implantation window
- Had an increase in Laminin immunoreactivity
- Had a decrease in microvillus number
- Had a decrease in uterus surface epithelial height

Omega-3 supplementation seems to have good effects on implantation and reproduction