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6th Workshop on Mammalian folliculogenesis and oogenesis: from basic science to clinic

From primordial germ cells to oogonia

Kjeld Møllgård, Åse Jespersen, Melissa Lutterodt,
Claus Yding Andersen, Anne Grete Byskov
Laboratory of Reproductive Biology
Rigshospital and Copenhagen University, Denmark

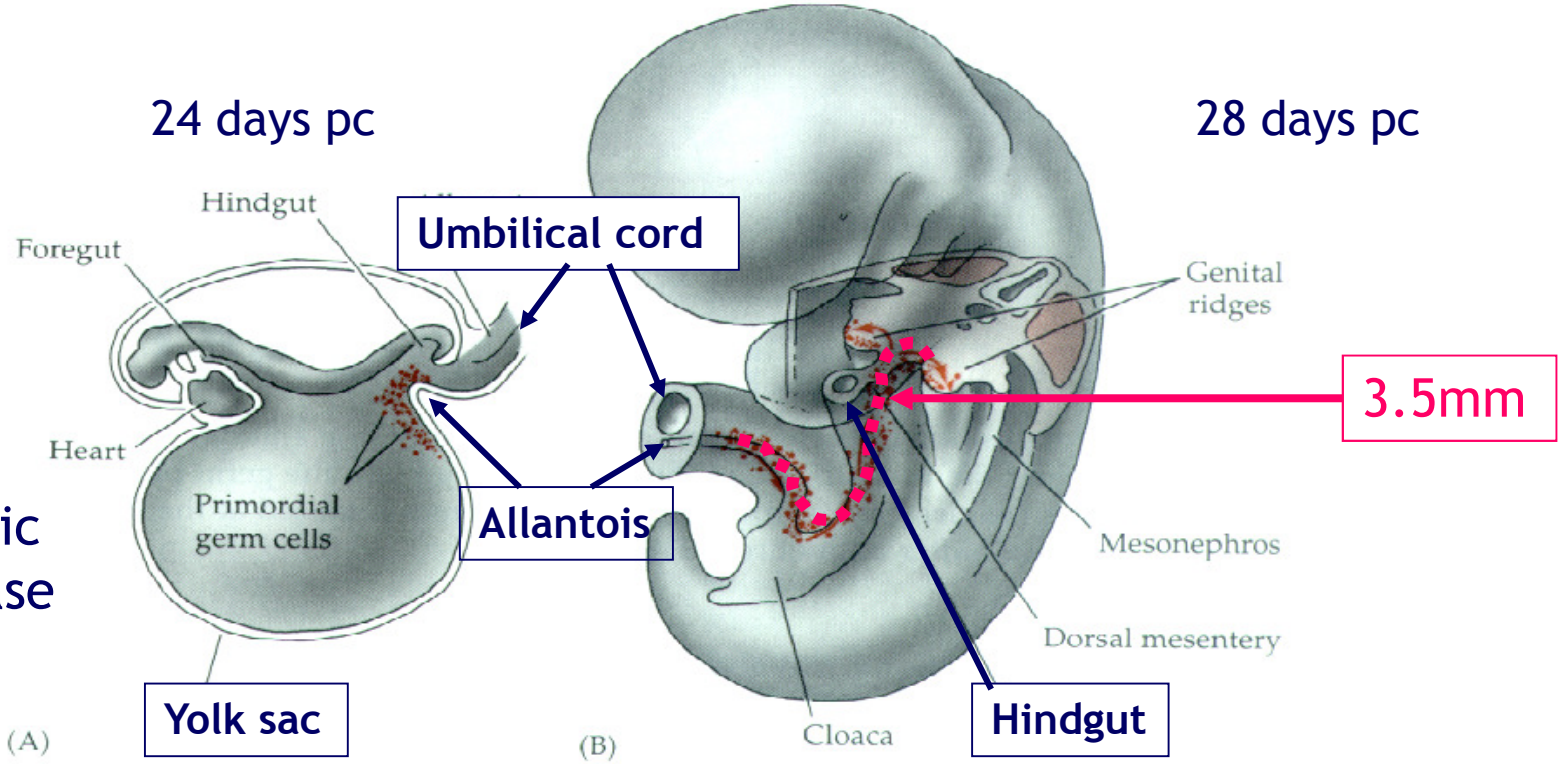
Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds (Witschi, 1948)

The Carnegy Collection of human embryos and fetuses

Whitschi concluded that the PGCs actively migrated from the yolk sac diverticle “allantois” to the gonads

i.e. PGCs should migrate 3.5 mm in 4 days - passing through the hindgut.

PGCs stained With alkalic phosphatase



Migration speed of the human PGCs

If the PGCs should move 3.5 mm in 4 days the speed would be 40mm per hour - if they go straight ahead towards the gonadal ridges

Mouse PGCs in vitro move 4 - 13mm per hour - but in random direction (Molyneaux et al., Development, 2003)

So, it seems unlikely that the human PGCs can migrate that fast without help.

Challenging Witschis concept of PGC migration

1. How do the primordial germ cells (PGCs) of the yolk sac reach the hindgut ?

2. How do the PGCs find their way from the hindgut to the gonadal-mesonephric area?

3. How do the PGCs actually enter the gonadal ridges?

1: How do the PGCs reach the hind gut?

Since the germ cells are not “born” in the area where the gonads will develop they must – in some manner - find their way from the site where they arise.

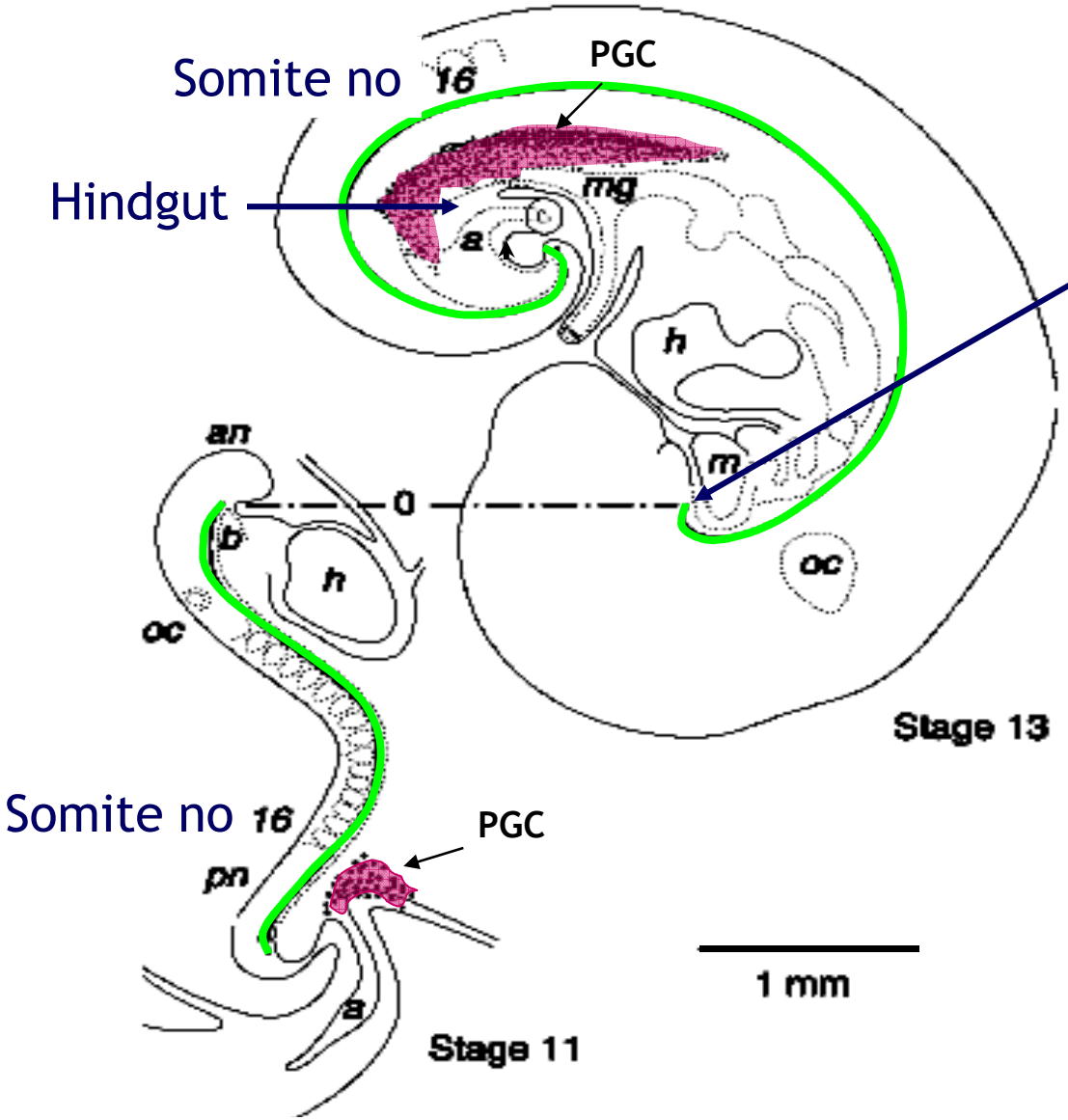
The PGCs must be able to:

Migrate or be translocated

Know where to go

”The active migration of germ cells in the embryos in mice and man is a myth”

(Freeman, Reproduction, 2003)



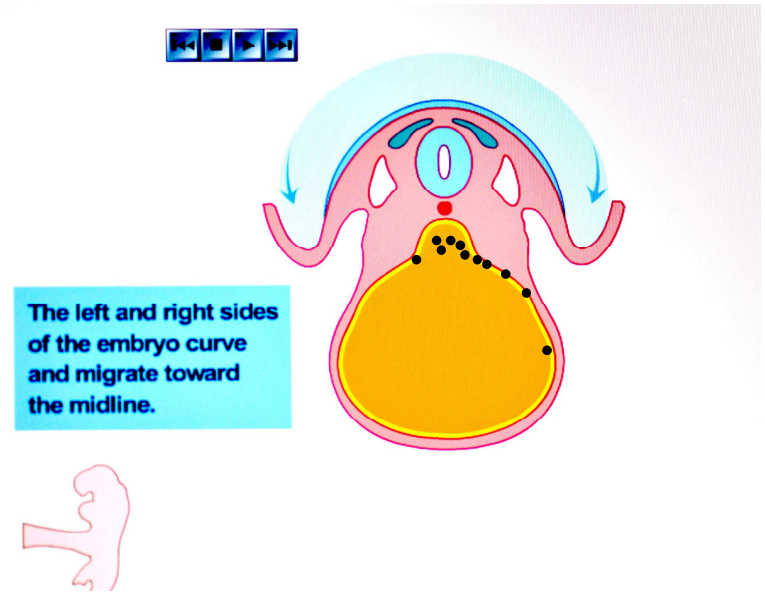
Cranial tip of Chorda used as fix point

Conclusion:

The PGC are mainly ”passively” translocated to the hindgut as the body grows and curves (i.e.**lateral folding**)

Lateral foldings of the human embryo from day 24 pc to day 28 pc

http://www.indiana.edu/%7eanat550/genanim/latfold/latfold.swf

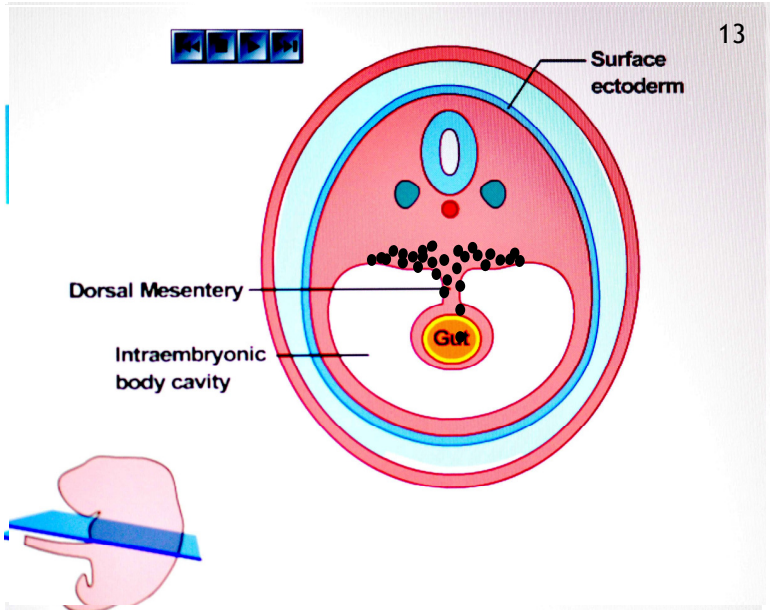


Indiana University Educational System

Lateral foldings of the human embryo from day 24 pc to day 28 pc

http://www.indiana.edu/%7eanat550/genanim/latfold/latfold.swf

From: Cartoon of Indiana University Educational System



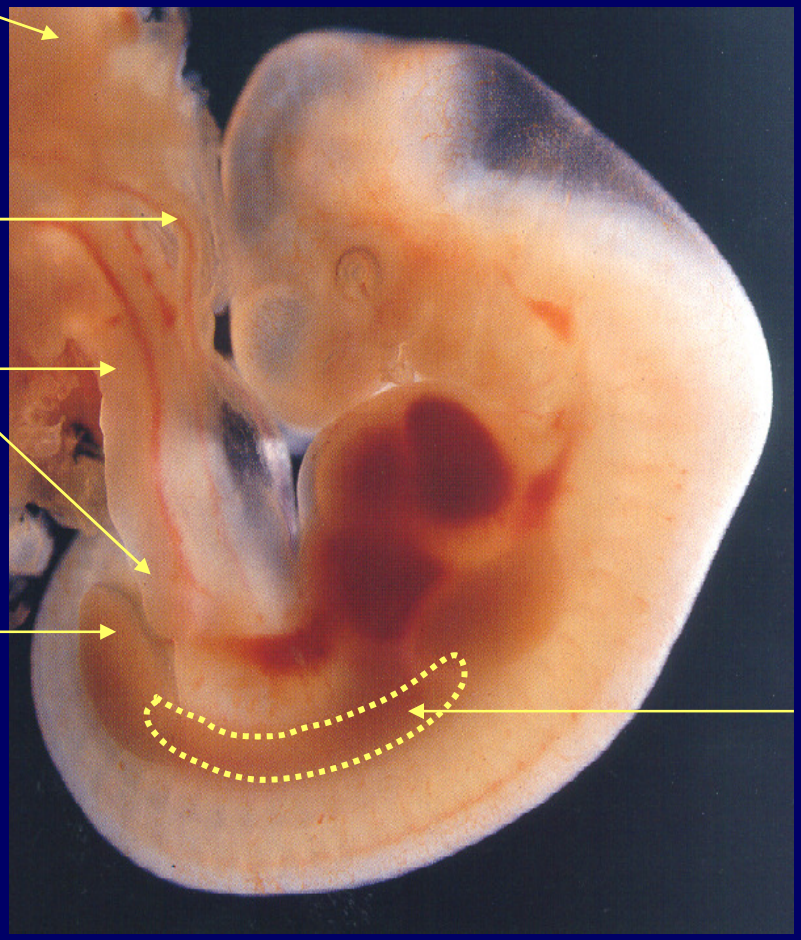
The yolk sac containing the PGCs lines the developing gut during early development

Thus, PGCs are part of the developing gut at all times before and during the lateral folding.

Therefore, PGCs just have to be translocated from the gut through the dorsal mesentery to the gonadal ridges – not from the yolk-sac

Human embryo

Stage 18, 5.2 weeks pc, CR: 14-15 mm



2. How do the PGCs find their way from the hindgut to the gonadal-mesonephric area?

Chemotaxis dependent receptor- ligand interaction ?
i.e. “directed migration”

SDF1/CXCL12 (Stromal cell Derived Factor 1) and
CXCR4 (its receptor): Mouse (Molyneaux et al., 2003)

Steel Factor (SCF): Mouse (deFelici et al.1994, Dolci et al.1991, Runyan et al. 2006; Gu et al. 2009)

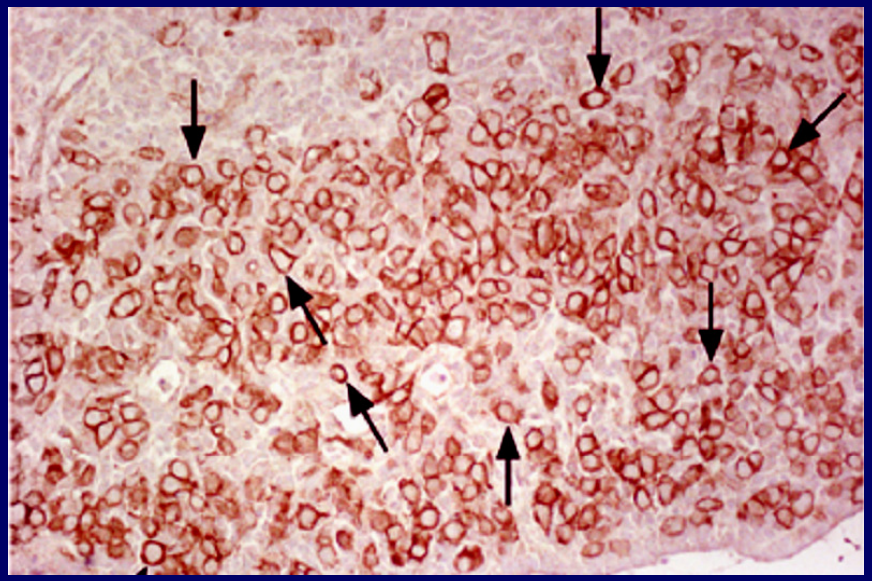
CKIT and SCF: Human (Høyer et al., Mol Cell Endocrinol, 2005)

Phospholipids: Drosophila (Renault, Curr Op Gen Dev, 2006)

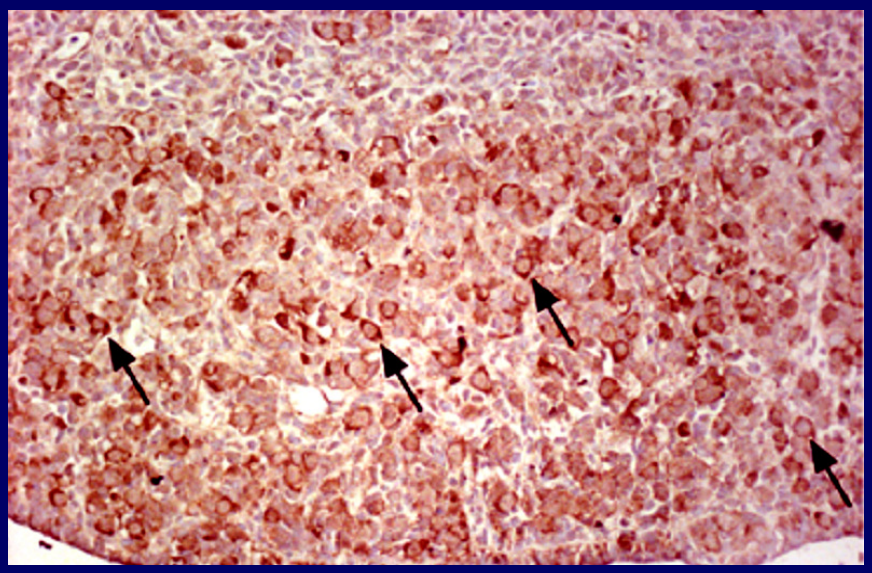
CKIT and SCF is expressed by oogonia

Human ovary 7.2 wpc (Hoyer et al., 2005)

CKIT



SCF



Gu et al. proposed that Steel factor (SCF) is essential for survival and proliferation of PGCs during migration (2009)

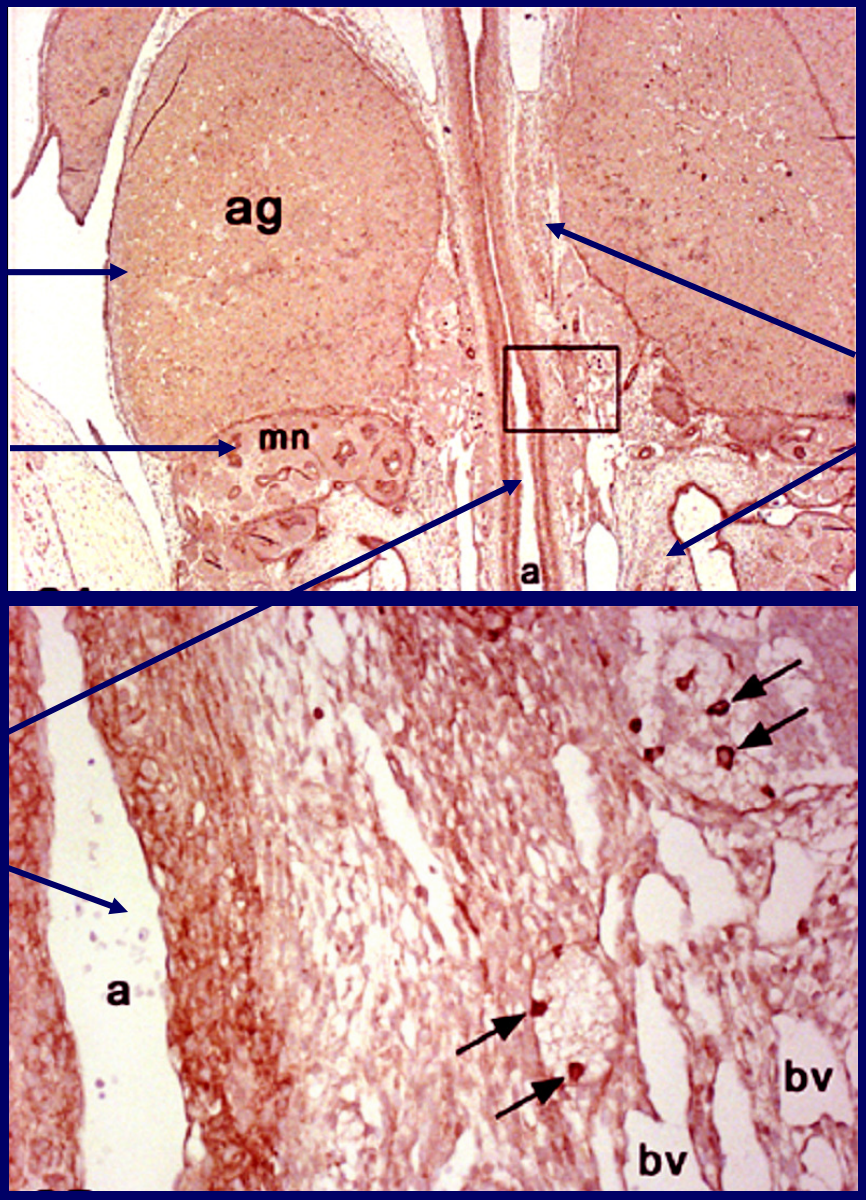
Expression of SCF in PGCs of the mesentery

Human female embryo 7,2 wpc stained for SCF (Hoyer et al., 2005)

Adrenal gland

Mesonephros

Aorta



Dorsal mesentery

PGC (?) stained for SCF within neurons (?) in the dorsal mesentery

Migration of PGCs in the dorsal mesentery

Are the nerve-like structures of the dorsal mesentery in fact nerves?

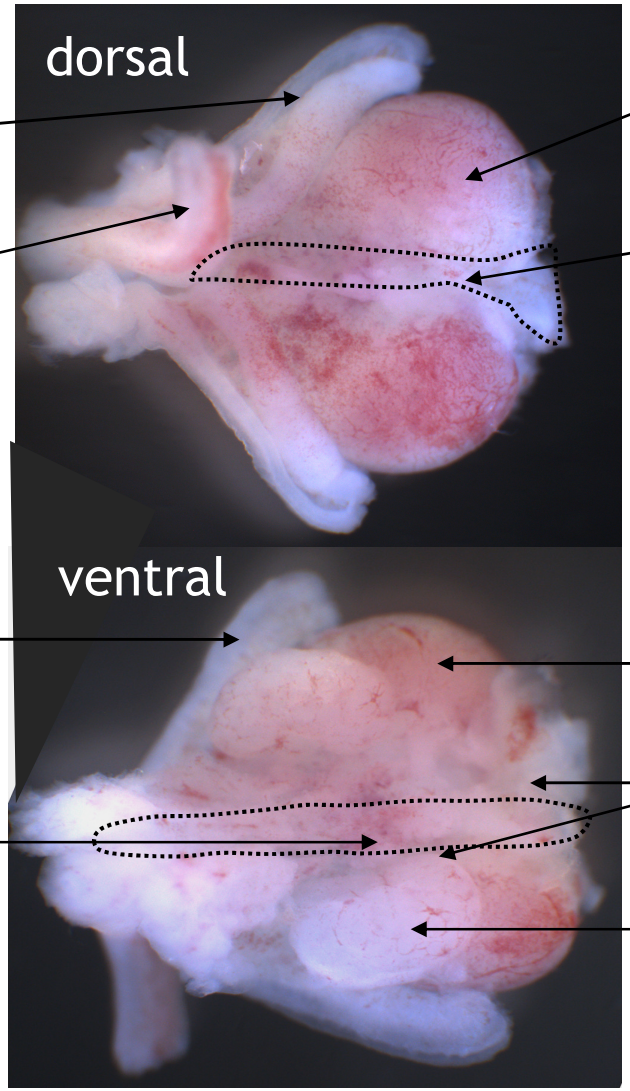
Are the large CKIT-and SCF-positive cells of the nerve-like structures in fact PGCs?

Staining of nerves and PGC

Antigen	
β III Tubulin	"neurotubuli": Immature nerve cells
NSE	"neuron specific enolase": perikaryon
PGP 9.5	"protein gene product": axons
GFAP	"glia fibrillary acetic protein": glia and axons
S100	"Schwann-100": Schwann-cells, glia
OCT 4	Embryonic stem cells
C-Kit/CD117	Embryonic stem cells
SCF	Stem Cell Factor (KIT ligand)
MAGE-A4	Cancer-testis antigen
GAGE	Cancer-testis antigen

Human aorta-gonadal-mesonephric region and the dorsal mesentery

Age 7,3 wpc



Mesonephros-gonadal complex

Hind gut

Adrenal gland

Dorsal mesentery
(Cut off from the hindgut)

Mesonephros-gonadal complex

Aorta

Adrenal gland

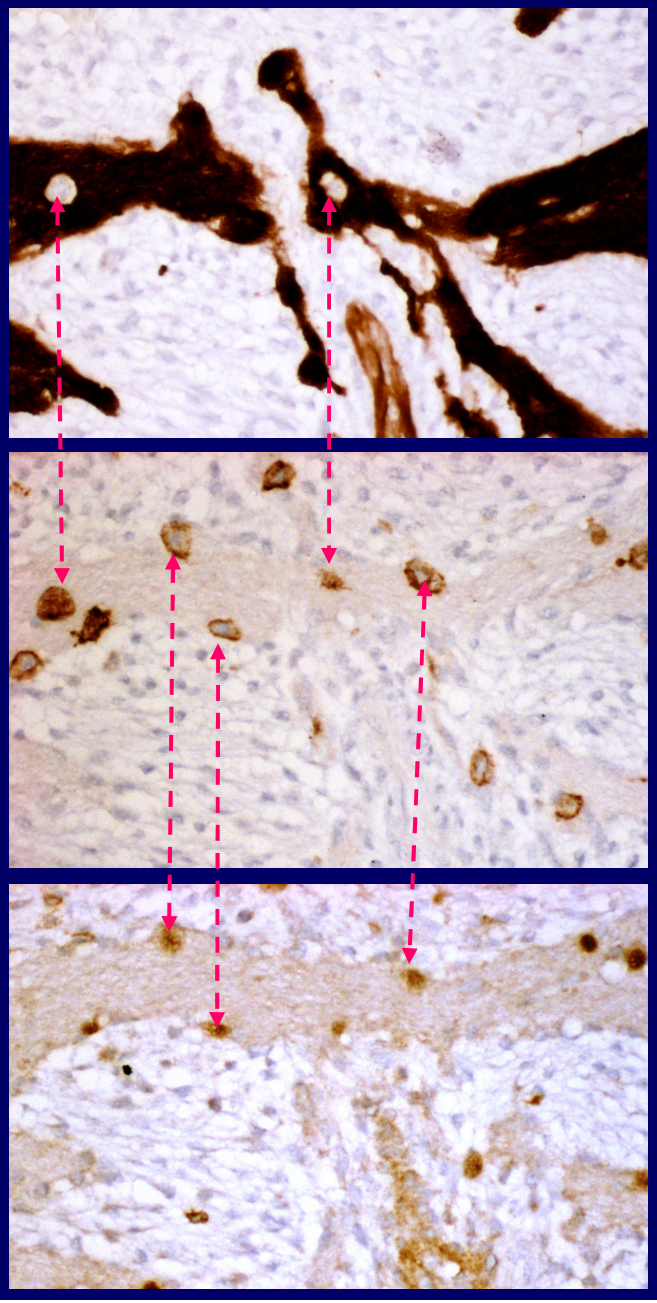
Dorsal mesentery

Kidney

Staining for PGCs in neurons of the mesentery

The neuron-like structures stain for βIII tubulin

CKIT-positive cells of the neurons also stain for OCT4



βIII tubulin (1st section)

CKIT (3 μm apart)

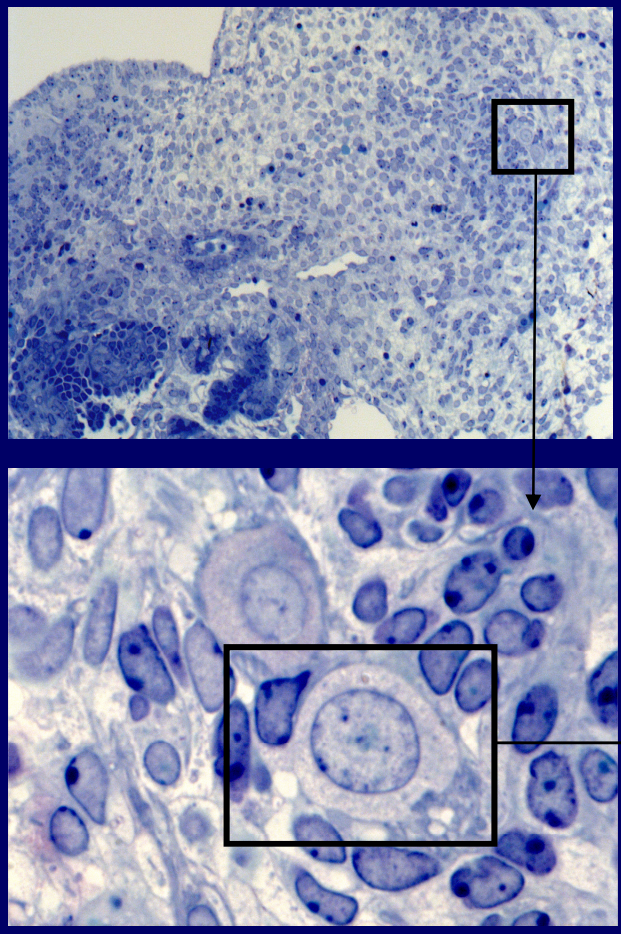
OCT4 (3 μm apart)

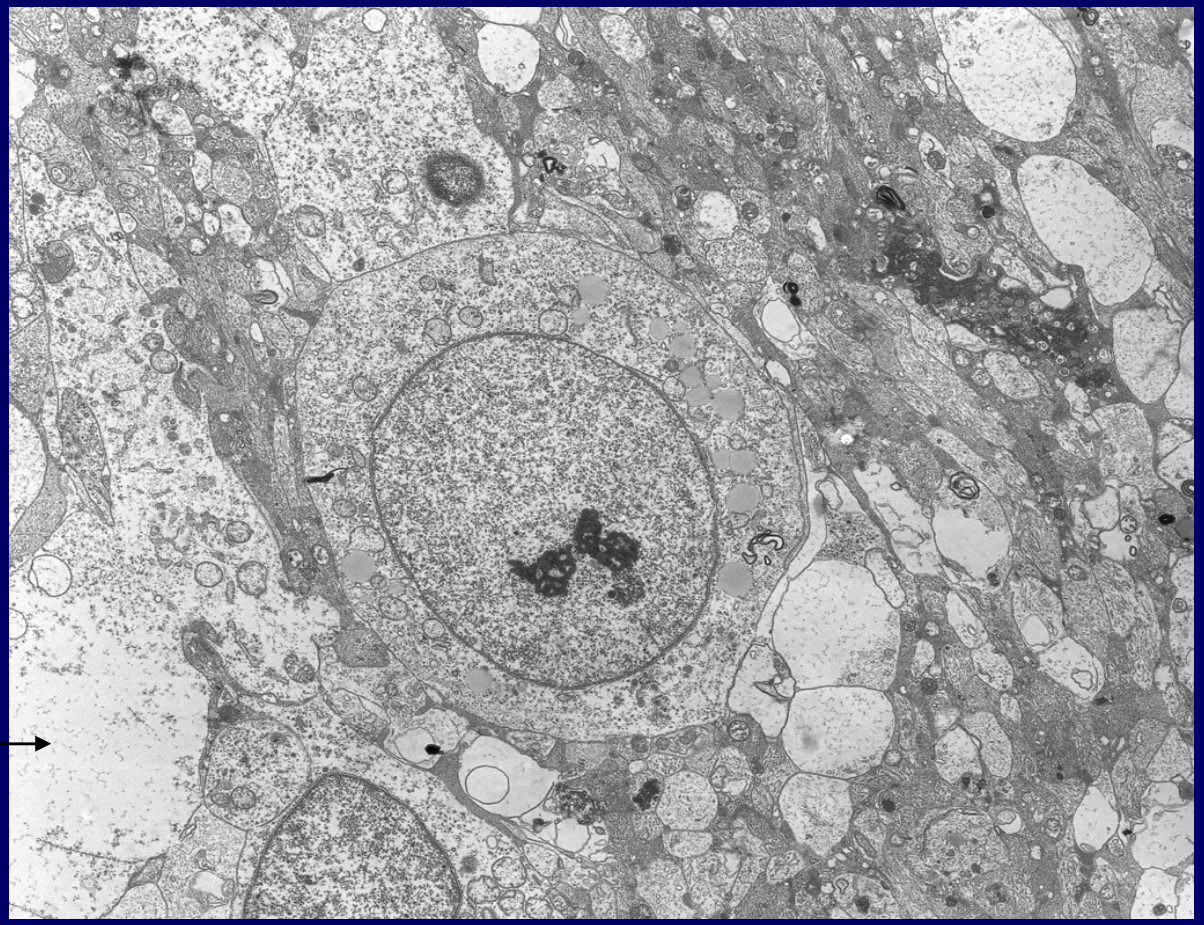
PGCs in neurons of the mesentery

Human embryoes prepared for TEM

Ovary

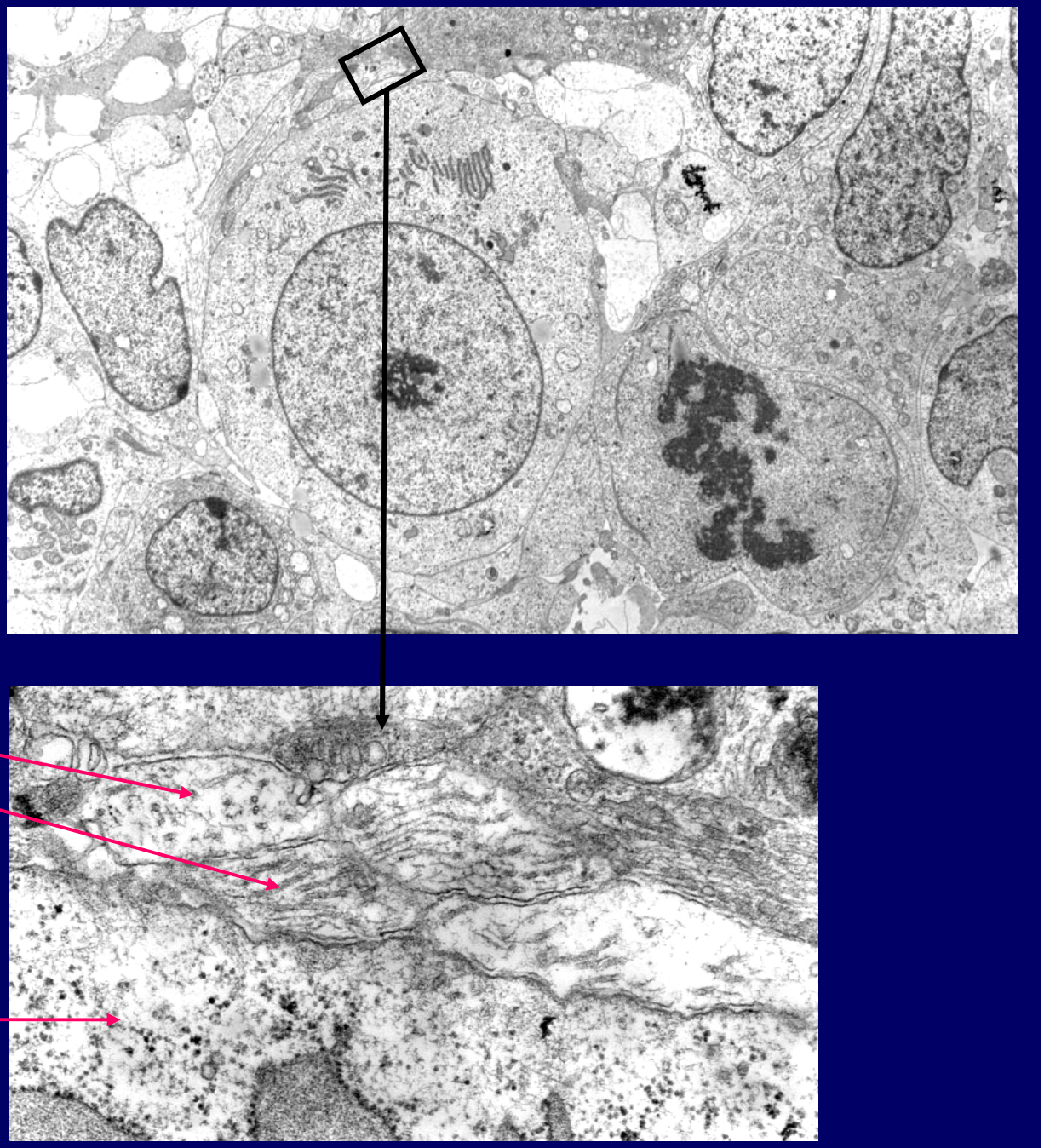
Mesentery





TEM of PGC in neurons of the mesentery

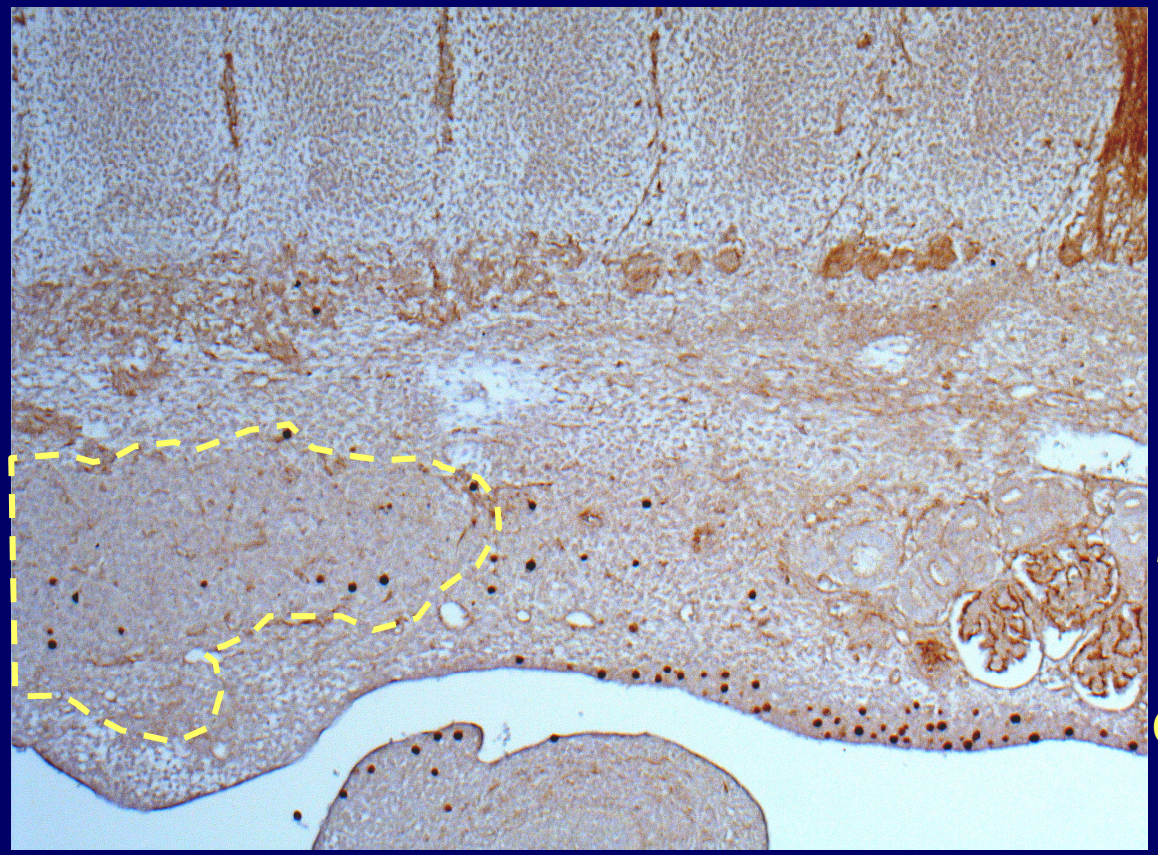
Human embryo 5.2 wpc



Cross and longitudinal section of neurons with neurotubuli

Cytoplasm of PGC

OCT4 expression
Human female embryo 4.2 wpc stained for OCT4

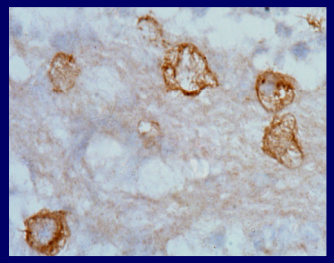


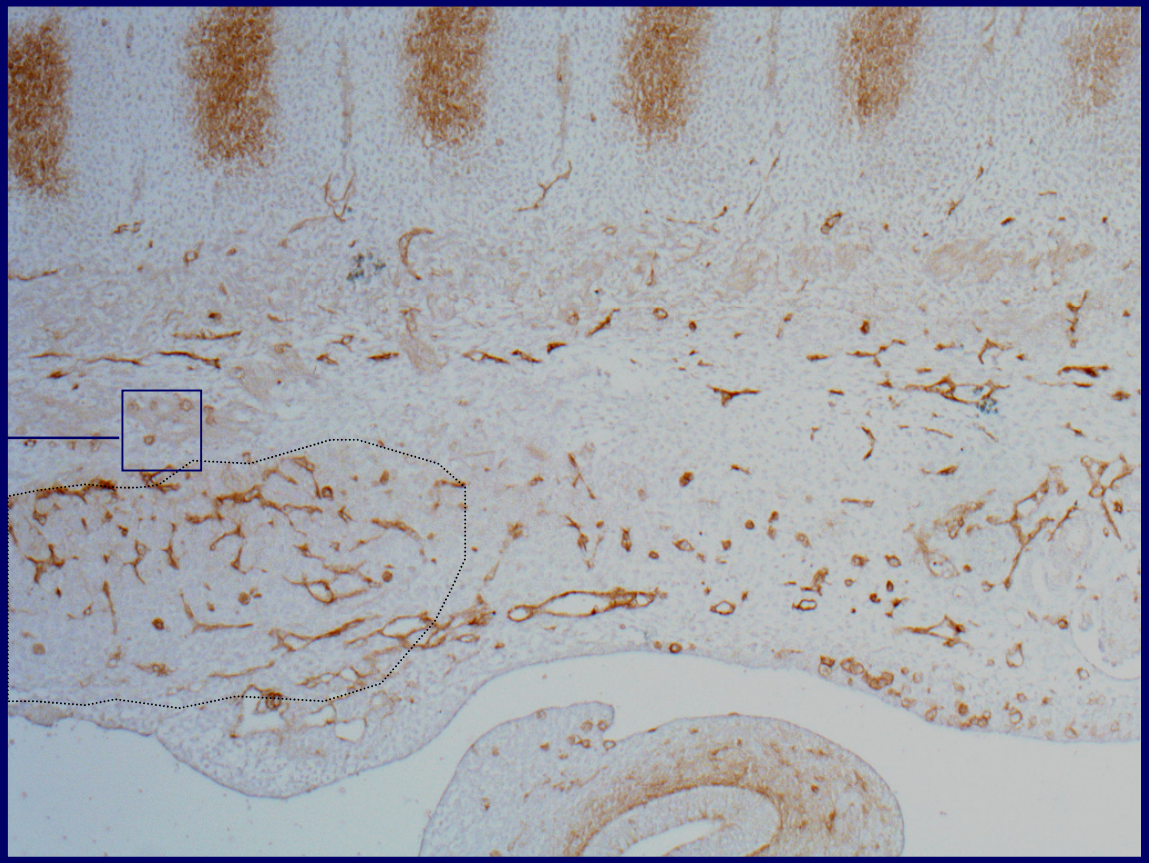
CKIT expression

Human female embryo 4.2 wpc stained for CKIT

Somite no 16

PGC in nerves





Adrenal gland

Mesonephros

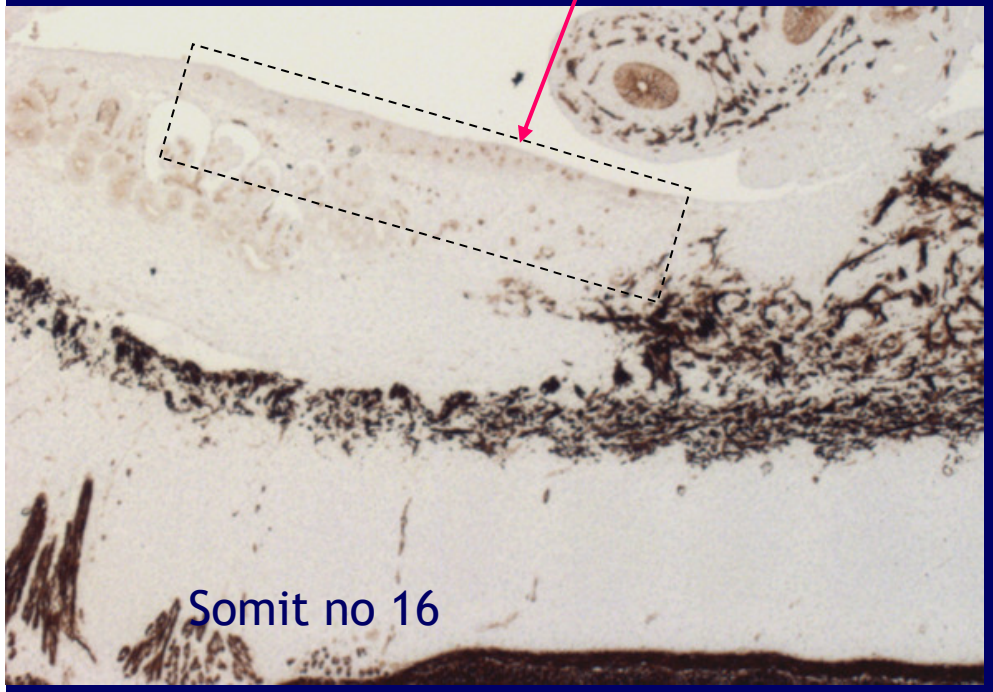
Gonadal ridge

Hindgut

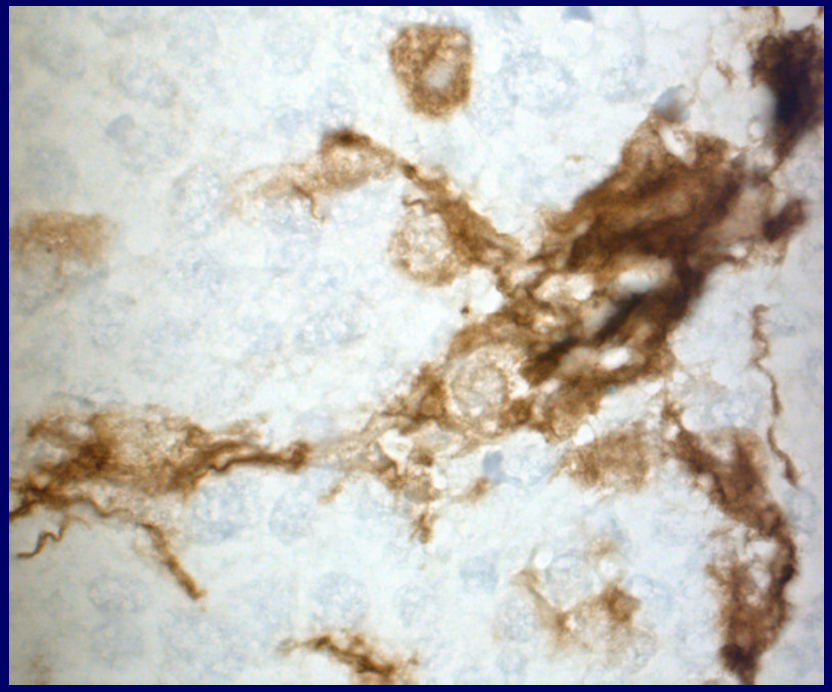
Autonomic nerve fibres (stained for βIII tubulin) reach from the mesentery into the ovarian anlage

Human embryo 5.0 wpc

Ovarian anlage

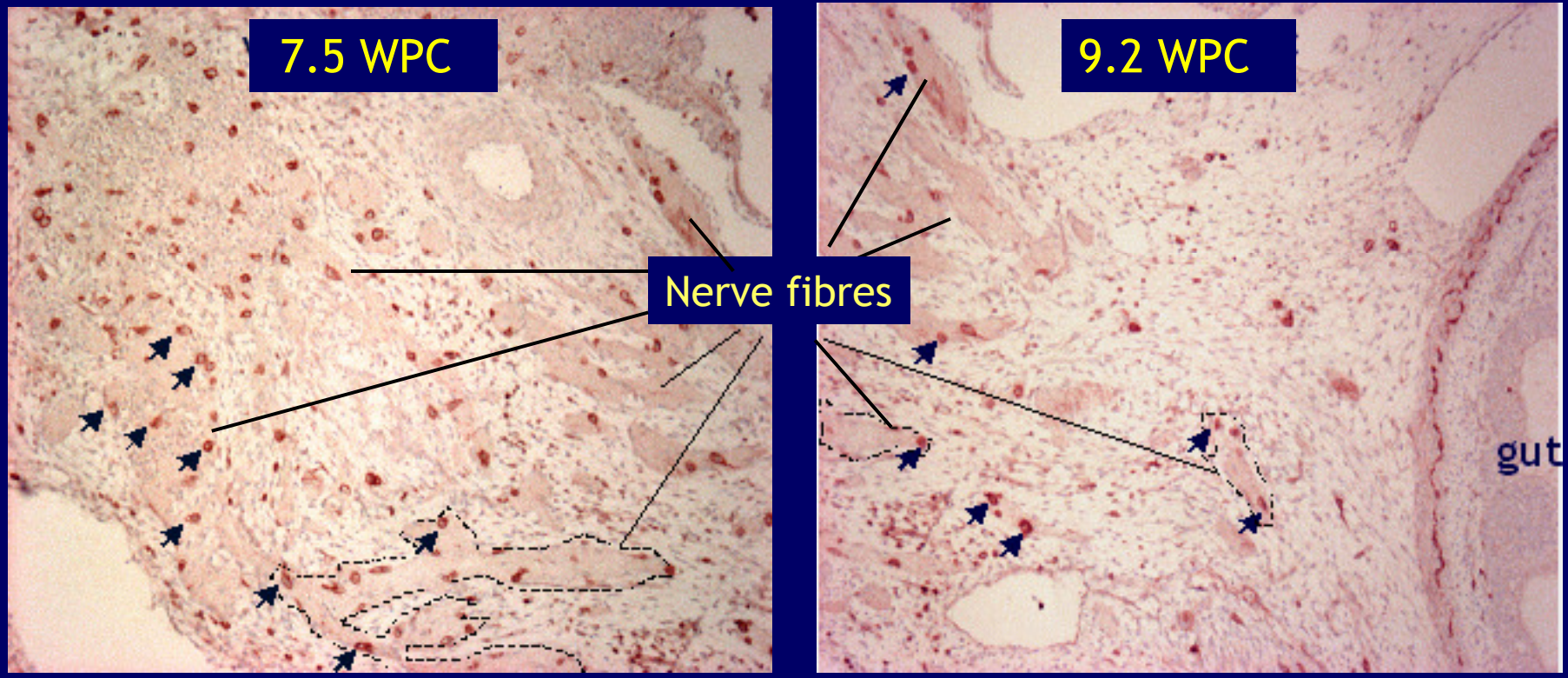


Nerve fibres embracing PGCs reaching into the ovarian anlage



PGCs in autonomic nerve fibres of the mesentery of human embryos

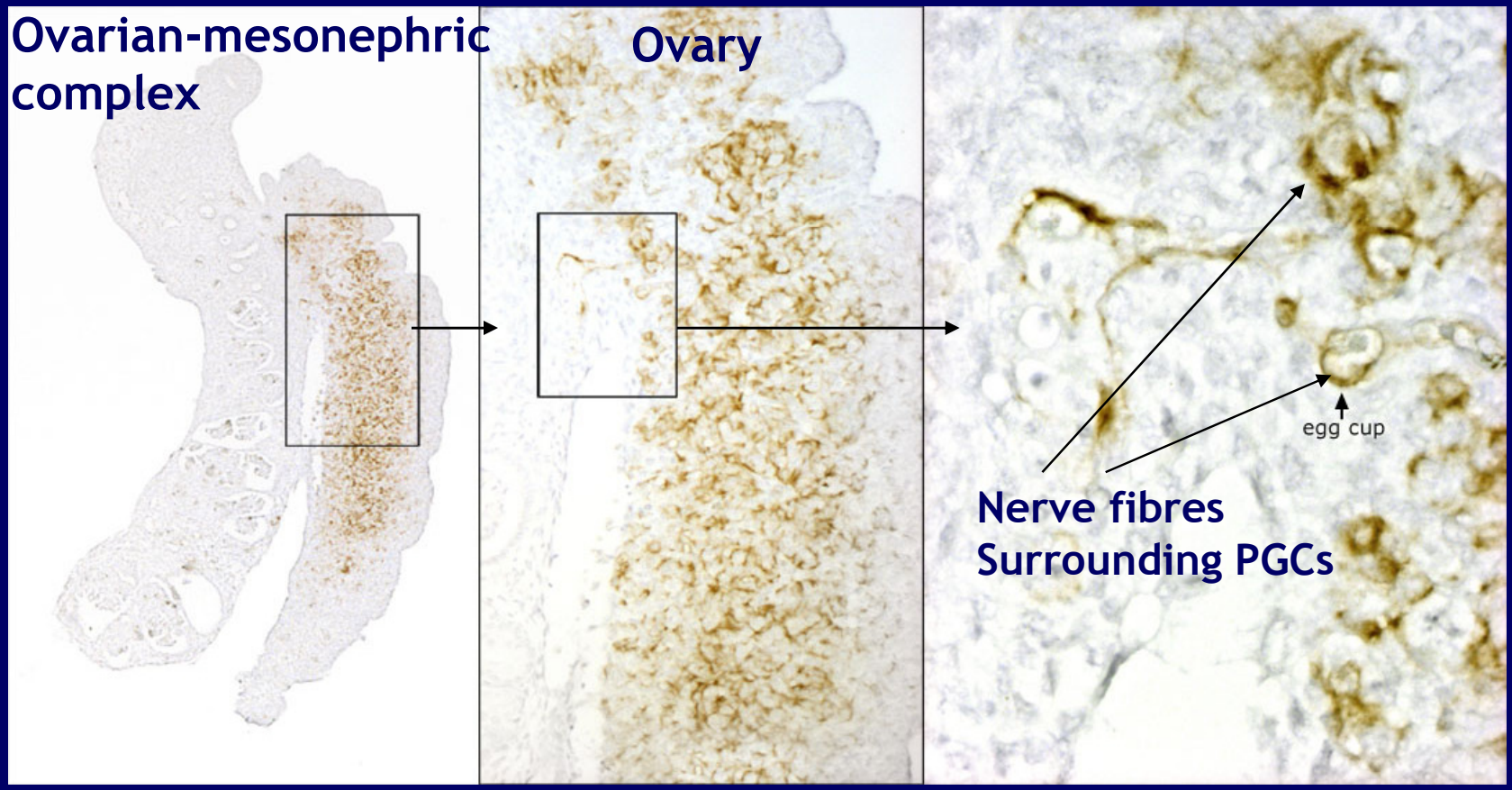
The arrows point at PGCs stained for SCF. Almost all are within nerve fibres



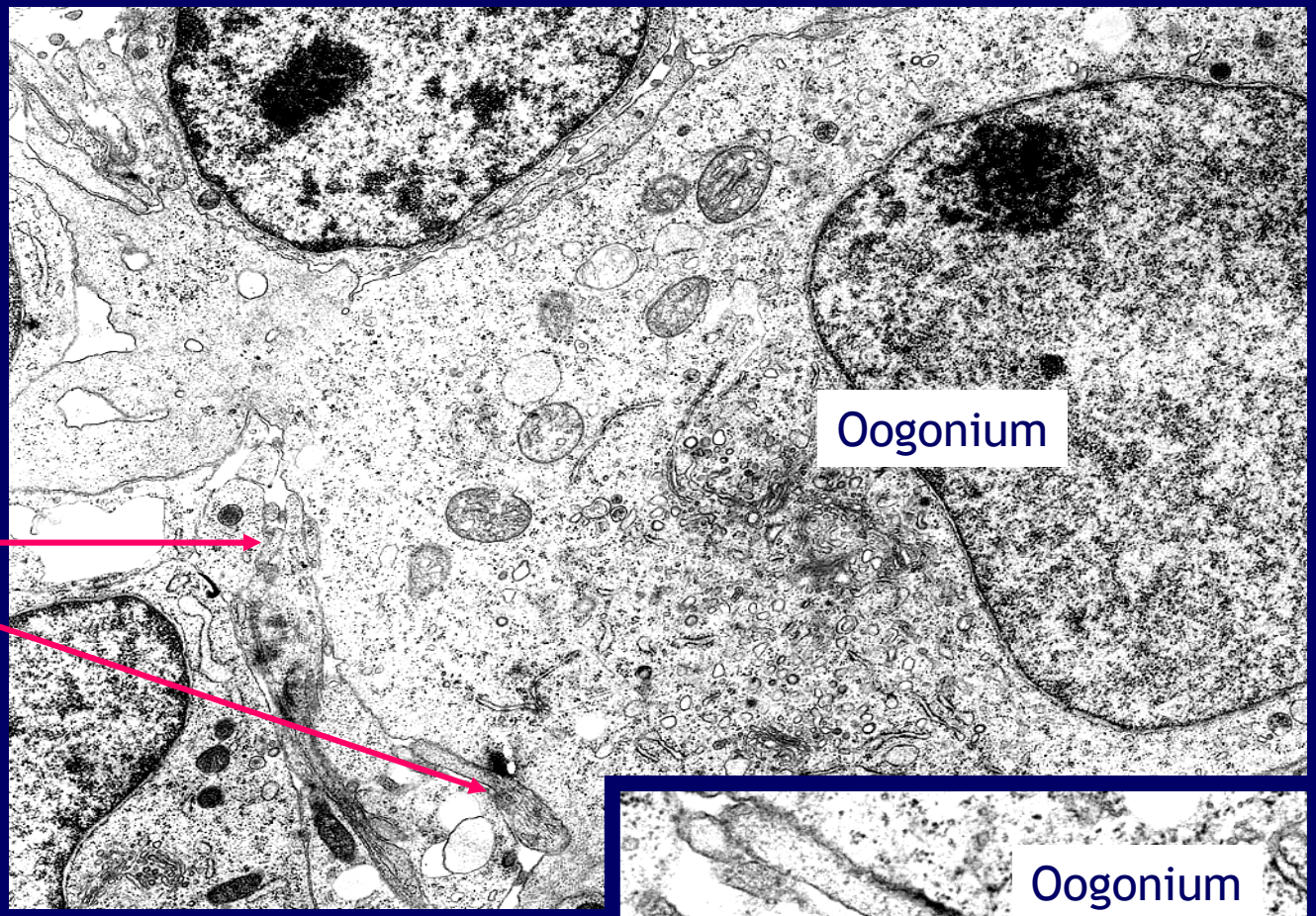
The number of PGCs in the mesenterium decreases with age

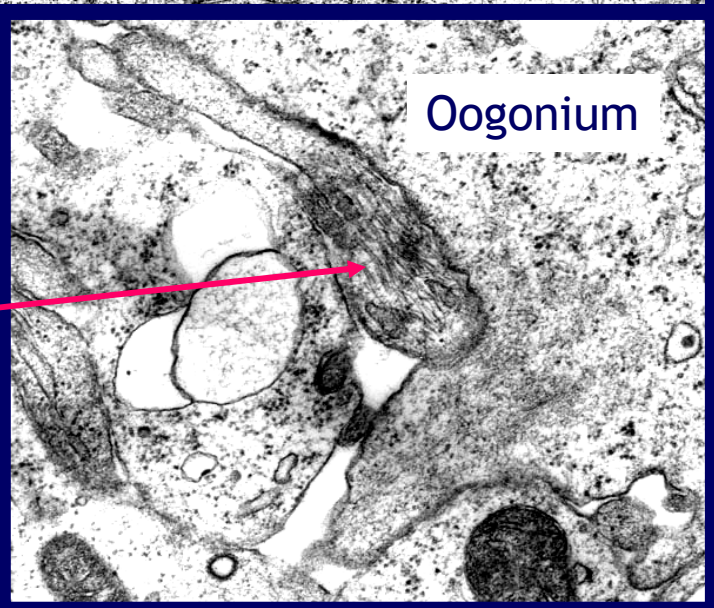
Autonomic nerve fibres (stained for βIII tubulin) reach from the mesonephric area into the ovary

Human embryo 9.0 wpc



Ovary of a human embryo 7.2 wpc - TEM





Axon with neurofilaments intimately connected to an oogonium

Summary / Conclusion

Human ovarian mesonephric complex, 10 wpc
(Lennart Nilsson, A.G.Byskov)

- PGCs in human are, at least partly, passively translocated from the yolk sac to the hind gut during the latteral folding
- PGCs are almost exclusively present in nerve fibres while translocated from the hindgut to the gonads
- Within the gonads the PGCs remain in close connection to neurons – until ??

Perhaps Leonardo da Vinci had a message when he proposed that the backbone is important for semen. In fact, the message goes right up into the brain

The drawing belongs to the British Queen and is located in Windsor Castle
(- the red line is added)

Migration of PGCs in human embryos

Melissa Lutterodt
LRB RH

Kjeld Møllgård
Susie Forckhammer
University of CPH

Aase Jespersen
University of CPH

LRB/Rigshospital

Inga Husum
LRB RH

Poul Erik Højer
University of CPH

Claus Yding Andersen
LRB RH