UMR Biologie du Developpement et Reproduction

Identification of transcripts involved in meiosis and follicle formation during ovine ovary development

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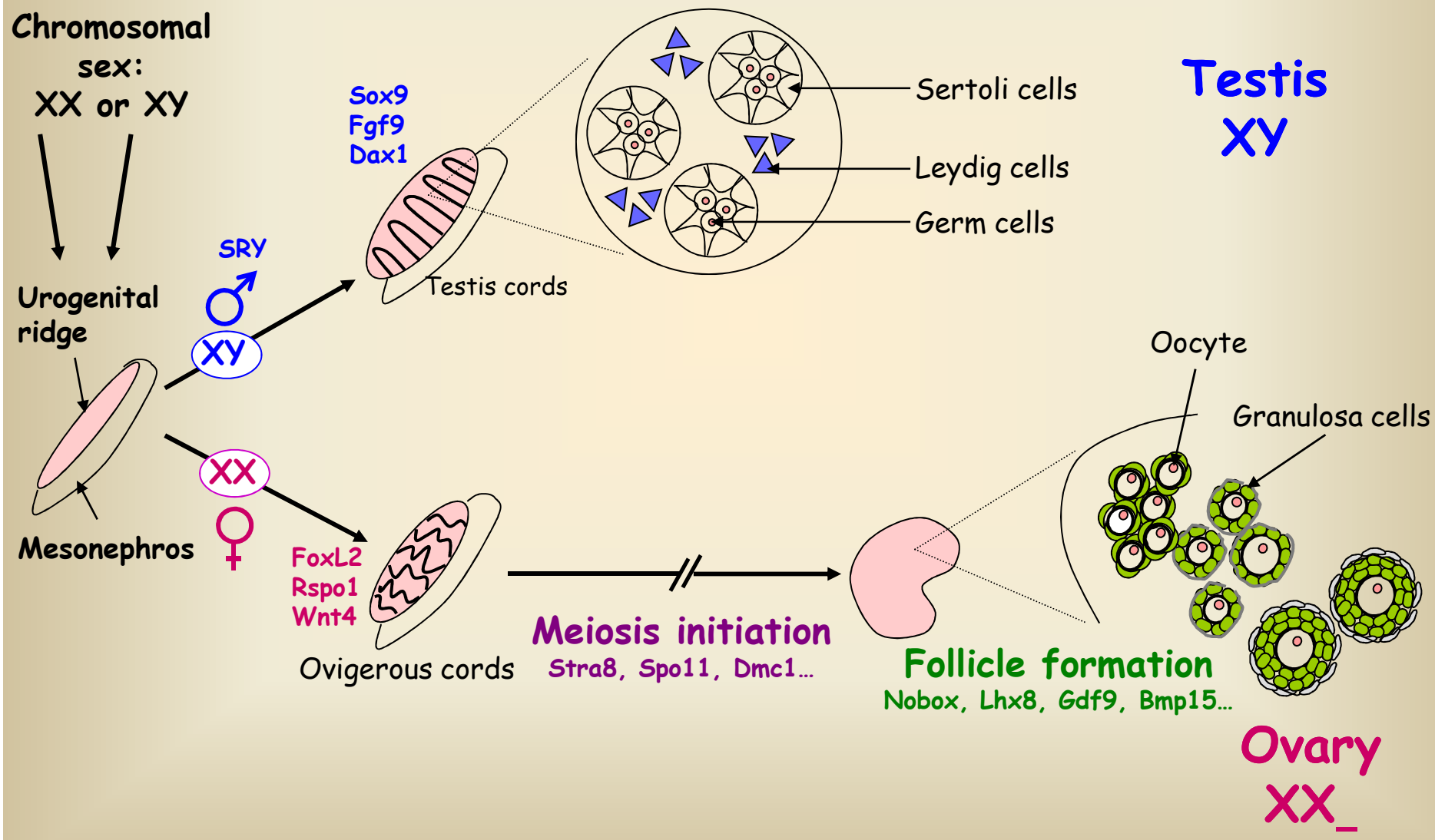
INRA

Jouy en Josas, France

6th Workshop on Mammalian folliculogenesis and oogenesis: from basic science to the clinic, Postdam, 8-10 October 2009

Gonad differentiation in mammals



Chronological events and histological aspects of ovine ovarian development

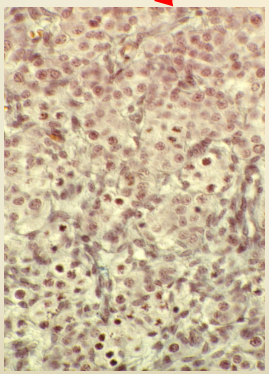
Genetic Sex (XX) → **Gonadal sex** → **Phenotypic sex**

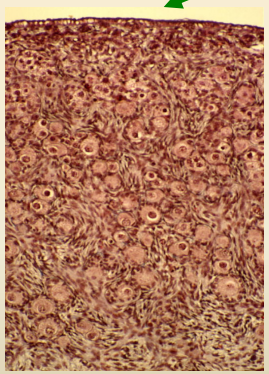
- SRY

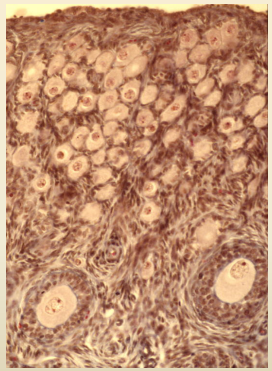
CONCEPTION — **Ovarian Differentiation** — **Female Meiosis** — **Folliculogenesis** — **BIRTH**

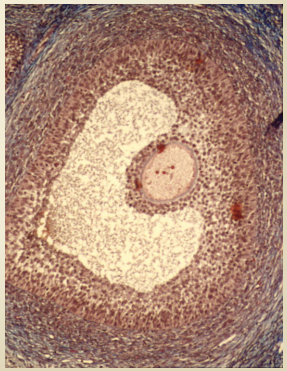
- Urogenital ridge formation
- Gonadal sex differentiation
- 1st primordial follicle
- 1st primary follicle
- 1st secondary follicle
- 1st tertiary follicle

0 — 23 — 30-32 — 55 — 75 — 100 — 120 — 135 — 145 dpc


56 dpc

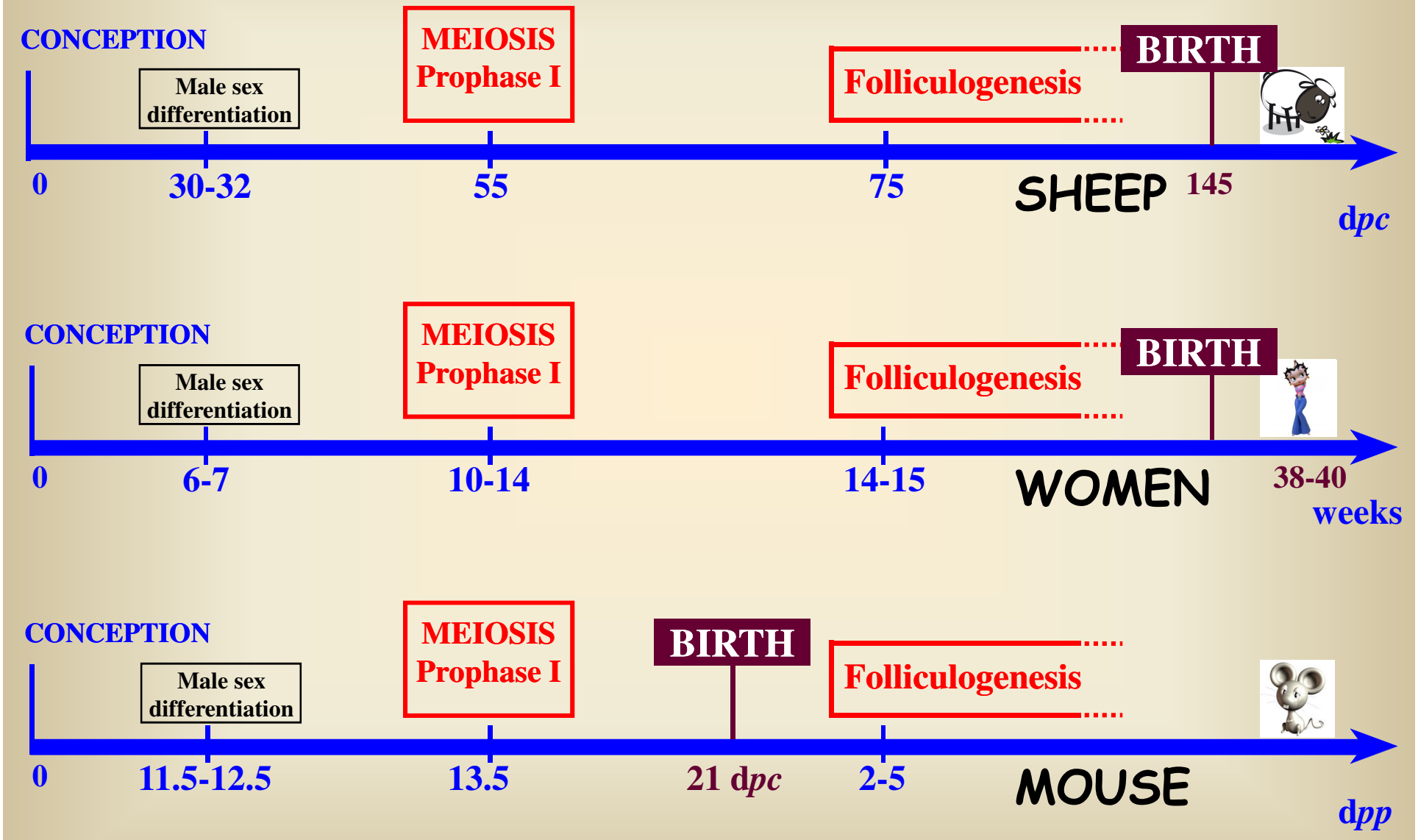

82 dpc


0 dpp

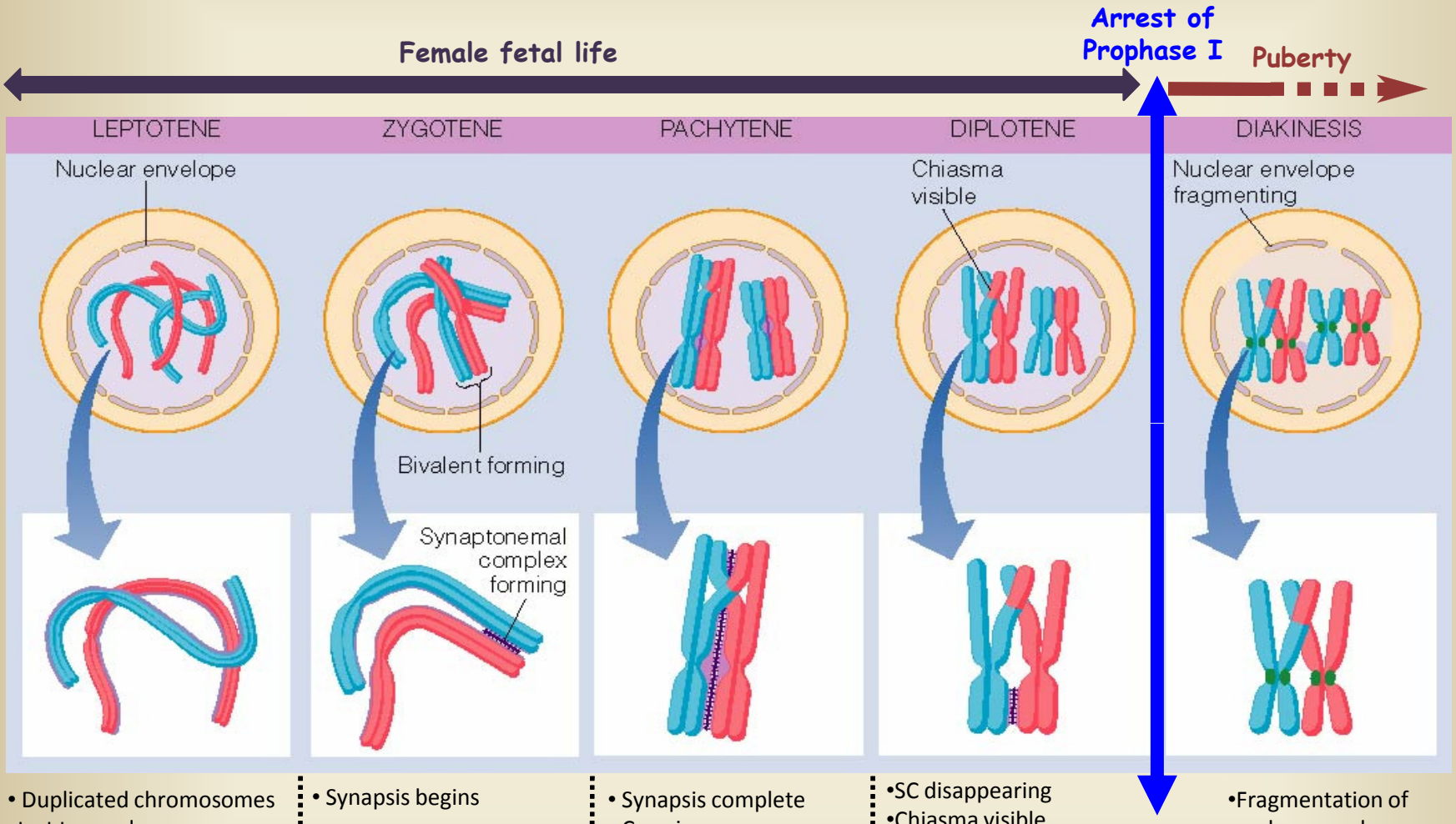

Adulthood

Mandon-Pépin et al., 2003 Biol Reprod

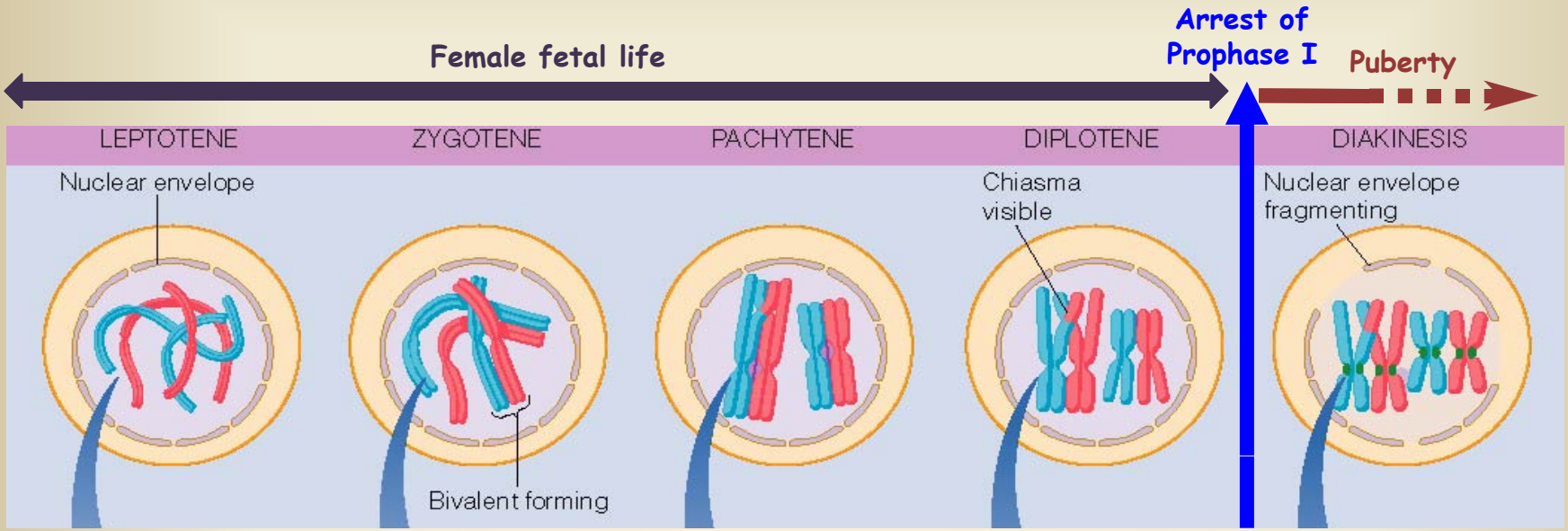
Ovarian differentiation in mammals



Prophase I of meiosis



Prophase I of meiosis



Stra8 → Initiation of meiosis

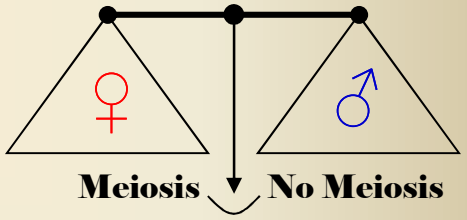
Spo11 → Meiotic DSB formation

Sycp2/ Sycp3 → Components of the SC

Dmc1/ Rad51 → Meiotic homologous recombination

Msh4/ Msh5/ Tex11/ Tex15

Msh4/ Msh5/ Mlh1/ Mlh3

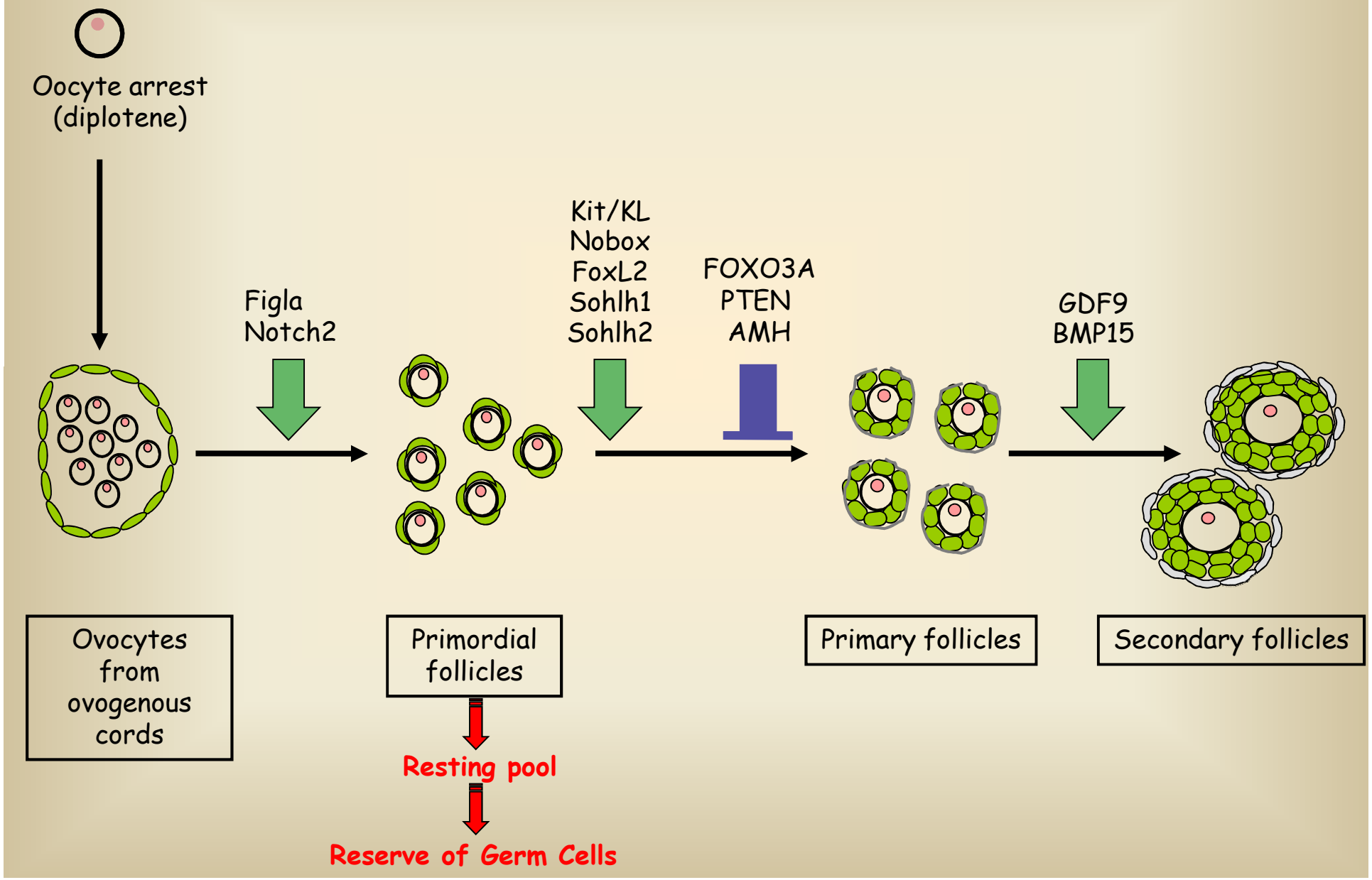


♂ ⊖ Retinoïc acid

♀ ⊕ Retinoïc acid

KO of most of these genes ⇨ arrest of gametogenesis

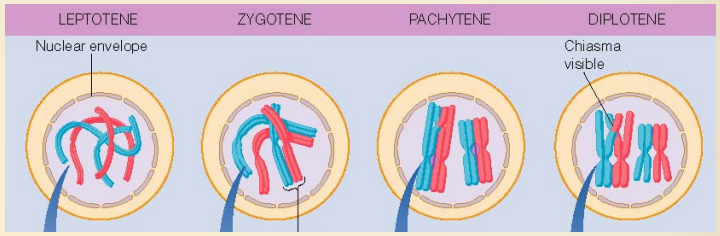
Follicles formation: early folliculogenesis



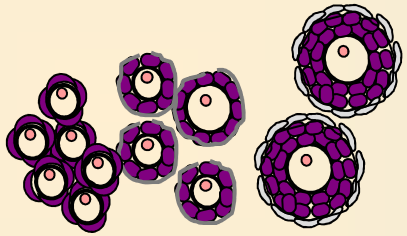
Aim of our study

Identification of **transcripts** involved in the main steps of ovary differentiation :

- **Prophase I of meiosis**



- **follicle formation**



Animal model : sheep

Constitute a transcript catalogue

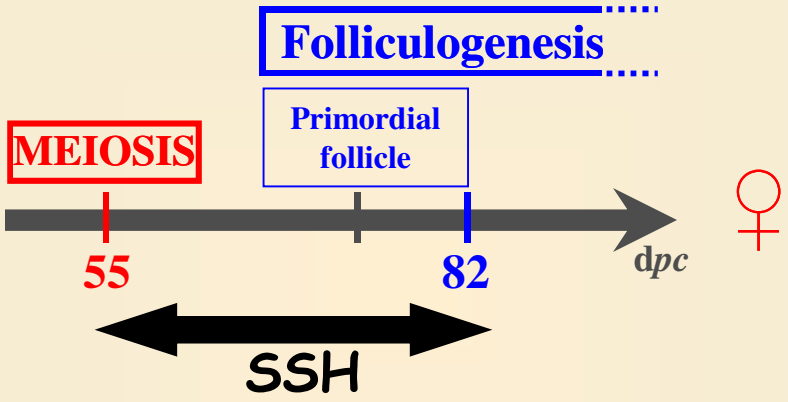
dedicated to early ovarian function in ruminants

Interests of using the "Sheep model"

- Common physiological characteristics with human:
  - mono-ovulatory cycle
  - ovarian steroidogenesis during fetal life
  - follicle formation before birth (≠ rodents)
- Opposite effects between natural mutation in sheep and knockout in mouse (BMP15)
- Relatively good knowledge of the chronology of ovarian differentiation
- Agronomical interest (INRA)

How did we isolate ovine ovarian transcrits?

Suppressive Subtractive Hybridization (SSH)



Getting transcripts expressed in:

- **M library**: **meiosis initiation and arrest in diplotene of prophase I** (55/82)
- **F library**: **follicle formation** (82/55)

Distribution of clones generated from SSH libraries

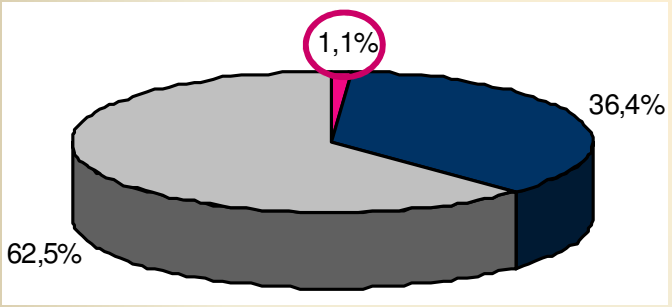
6 080 sequenced clones

2 535 contigs

2 101 unique transcripts (Blast)

Meiosis library

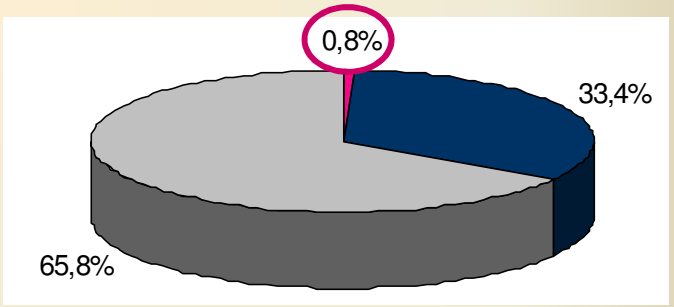
1 361 transcripts
15 unknown (1,1%)



Nucleic annotation in M library

Folliculogenesis library

740 transcripts
6 unknown (0,8%)



Nucleic annotation in F library

Unknown contigs | Bovine and human homologous contigs | Ovine homologous contigs

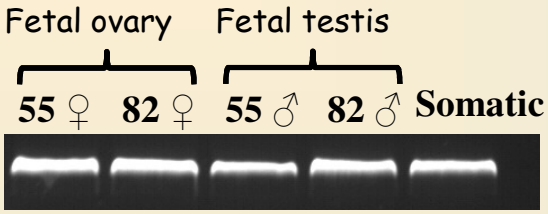
2 101 transcripts dedicated to the ovarian development of sheep

Identification of differentially expressed ovary-specific transcripts

Bioinformatics comparison: Identification of previously described genes in mice

-Meiosis: Stk31, Smc3, Dmrt7, Zp148, Mutl1, Dazl, Stathmin, Msh2, Msh5…
-Folliculogenesis: Foxo3A, Lhx8, BMP4…

RT-PCR screening used:



-Mixture of cDNAs from 4 different fetal somatic tissues (kidney, liver, heart, brain)

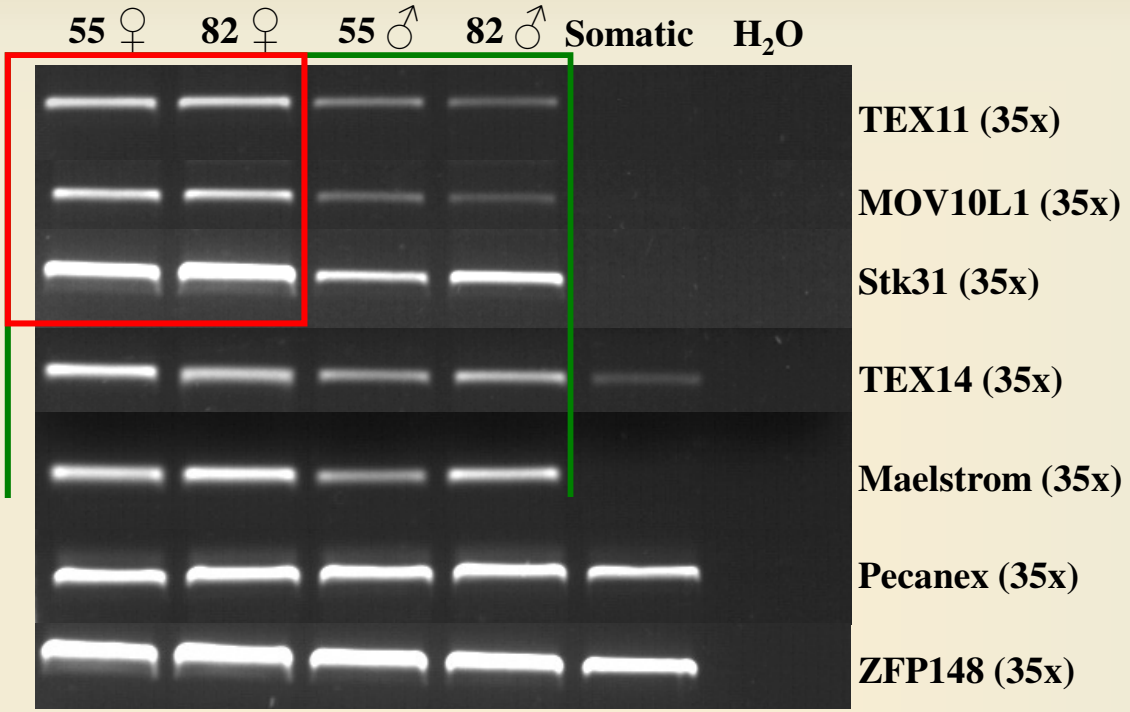
-cDNA from female and male gonads at 2 different developmental stages (D55 and D82)

Baillet et al., 2008 BMC Genomics

Identification of differentially expressed ovary-specific transcripts (RT-PCR)

Predominantly ovary-expressed transcripts

Predominantly gonad-expressed transcripts



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