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Involvement of retinoic acid (ATRA) signaling and DMRT1 in rabbit male and female germ cell fates

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Gonadal differentiation and its disturbances (DGP)
UMR BREED ; INRAE Jouy-en-Josas

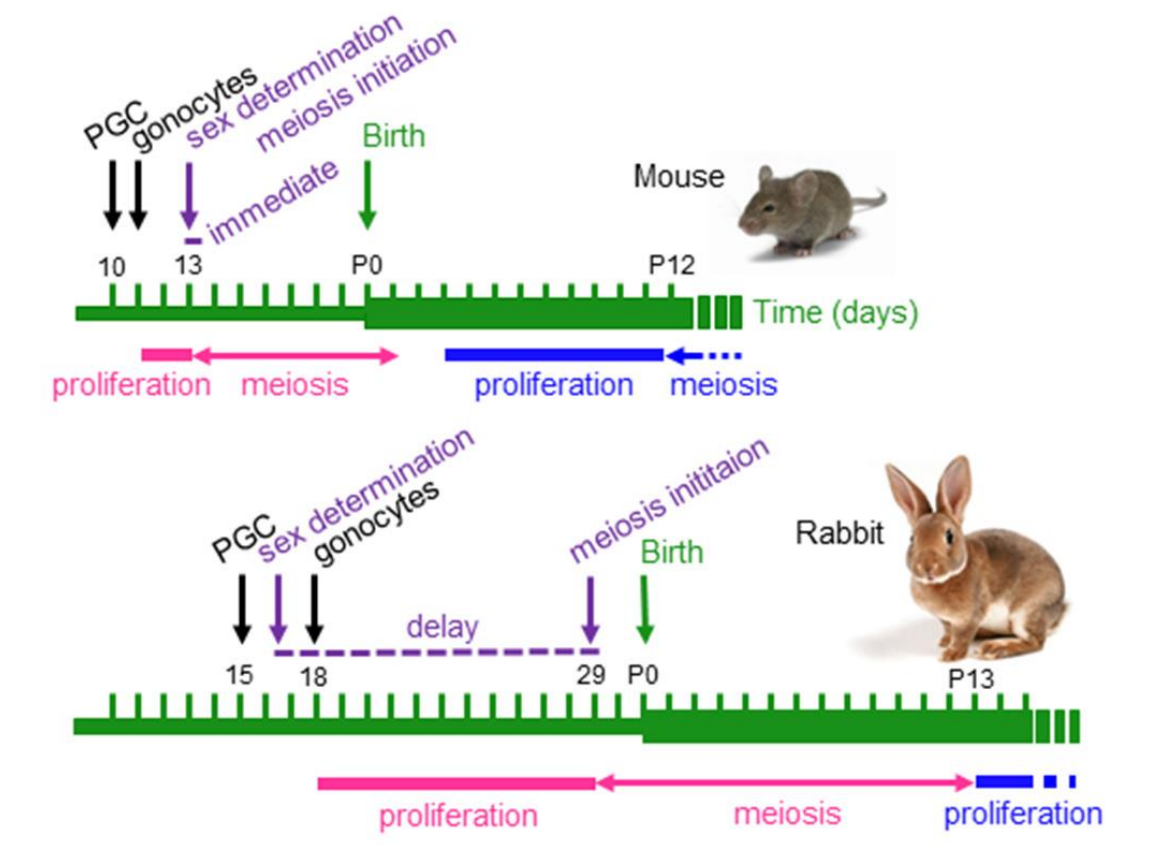


Involvement of retinoic acid (ATRA) signaling and *DMRT1* in rabbit male and female germ cell fates

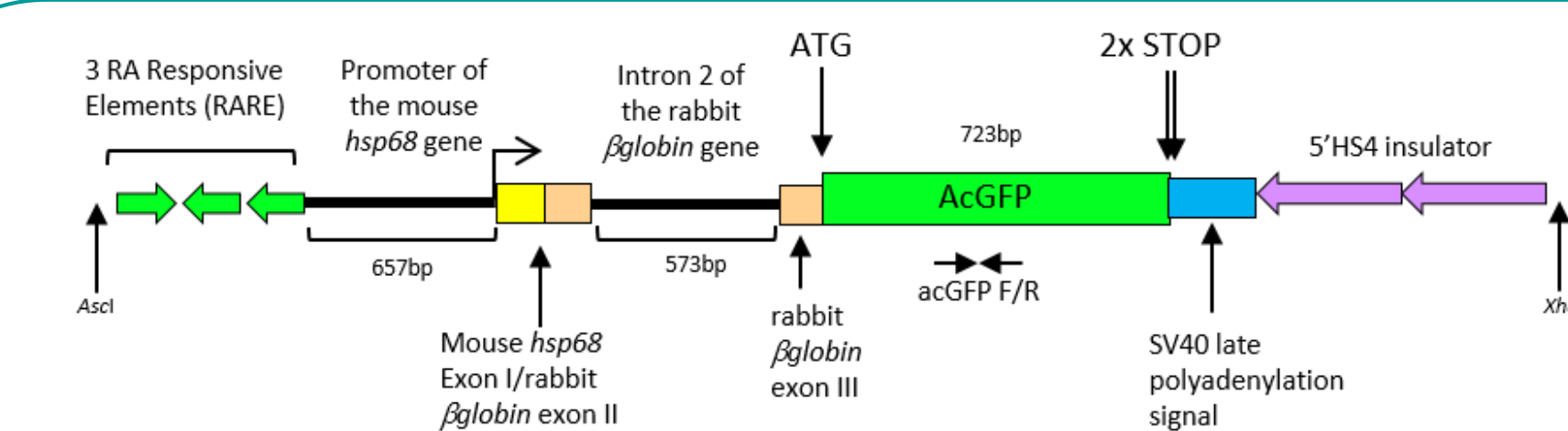
Iris Barka^{1,2*}, Emilie Dujardin^{1,2*}, Aurélie Dewaele^{1,2}, Marjolaine André^{1,2}, Béatrice Mandon-Pépin^{1,2}, Maëlle Pannetier^{1,2}, Geneviève Jolivet^{1,2} & Eric Pailhoux^{1,2}

Introduction

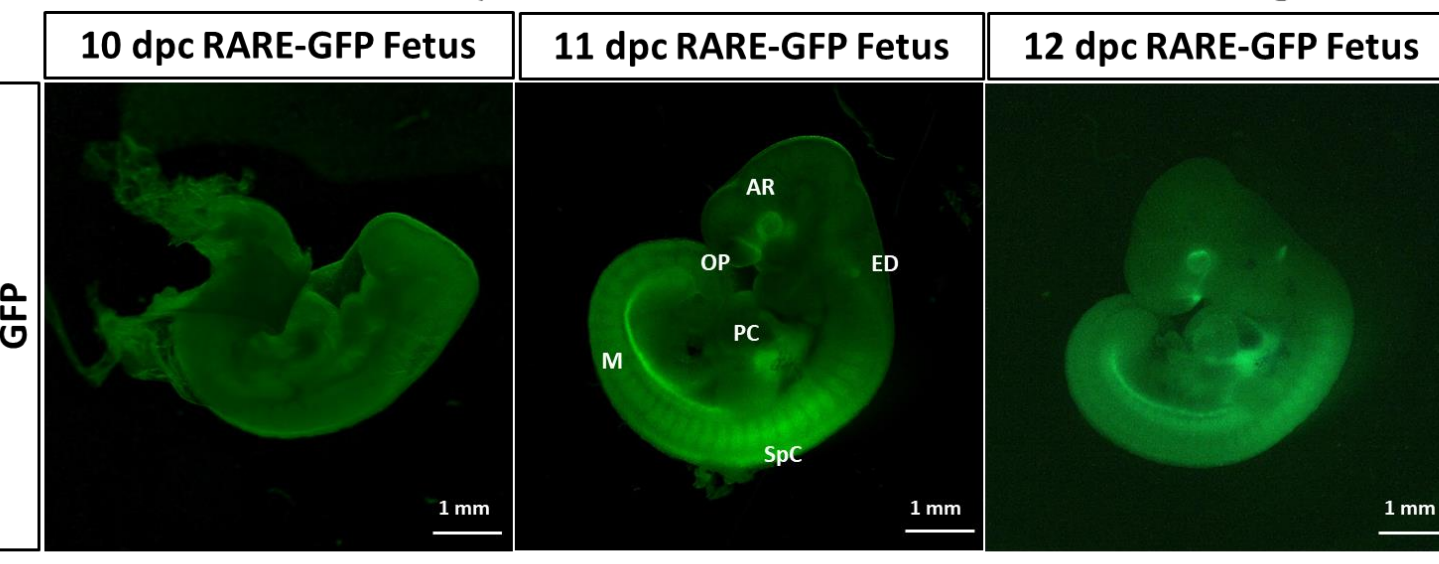
Retinoic acid (ATRA), a metabolite of vitamin A, is a significant player in cell differentiation from the primary stages of vertebrate embryo development to the adult. In germ cells, there is a controversy about the action of retinoic acid. It has been proposed that retinoic acid plays a key role in meiosis, acting as the meiosis inducing substance (MIS) in both female and male gonads. This concept was challenged after studies showed that female germ cells will initiate and progress into meiosis, even when the ATRA signaling pathway was disrupted. In males, retinoic acid plays an essential role in the early differentiation of spermatogonia. Indeed, it has been described that the absence of ATRA synthesis enzymes will prevent the differentiation of type B spermatogonia. In the mouse, the delay between the time of sex determination and the time of meiosis initiation in female is very short (less than 1 day), making the mouse a species with “immediate” meiosis. In the rabbit, the delay between sex determination and germ cell meiosis is about 10 day-long, making the rabbit a species displaying “delayed” meiosis, as humans. Since the majority of the previous studies were carried on mice, our lab generated a transgenic rabbit model expressing a recombinant RA-sensitive transcriptional gene, including the green fluorescent protein (GFP) reporter gene: **RARE-GFP rabbit model**. This model allows us to follow ATRA signaling at different developmental stages and better understand its role in germ cells of both sexes.



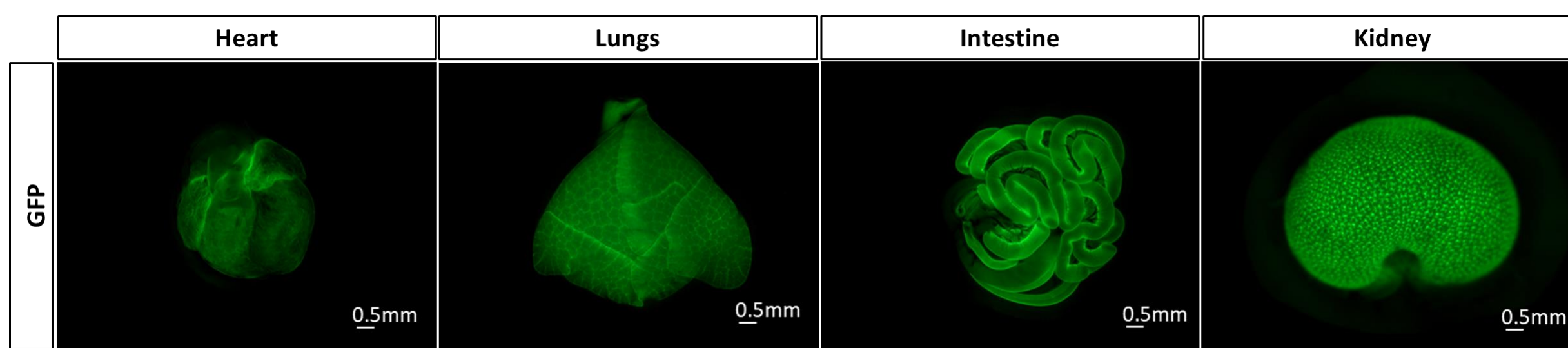
Description of the RARE-GFP rabbit model



Transgenic rabbit model RARE-GFP: A genetic construct was designed to follow retinoic acid signaling in rabbit germ cells. The ATRA response elements were placed upstream of the GFP reporter gene, whose expression is driven by the promoter of the *Hsp68* gene. So, when cells respond to the ATRA signaling pathway, they express the green fluorescent protein.

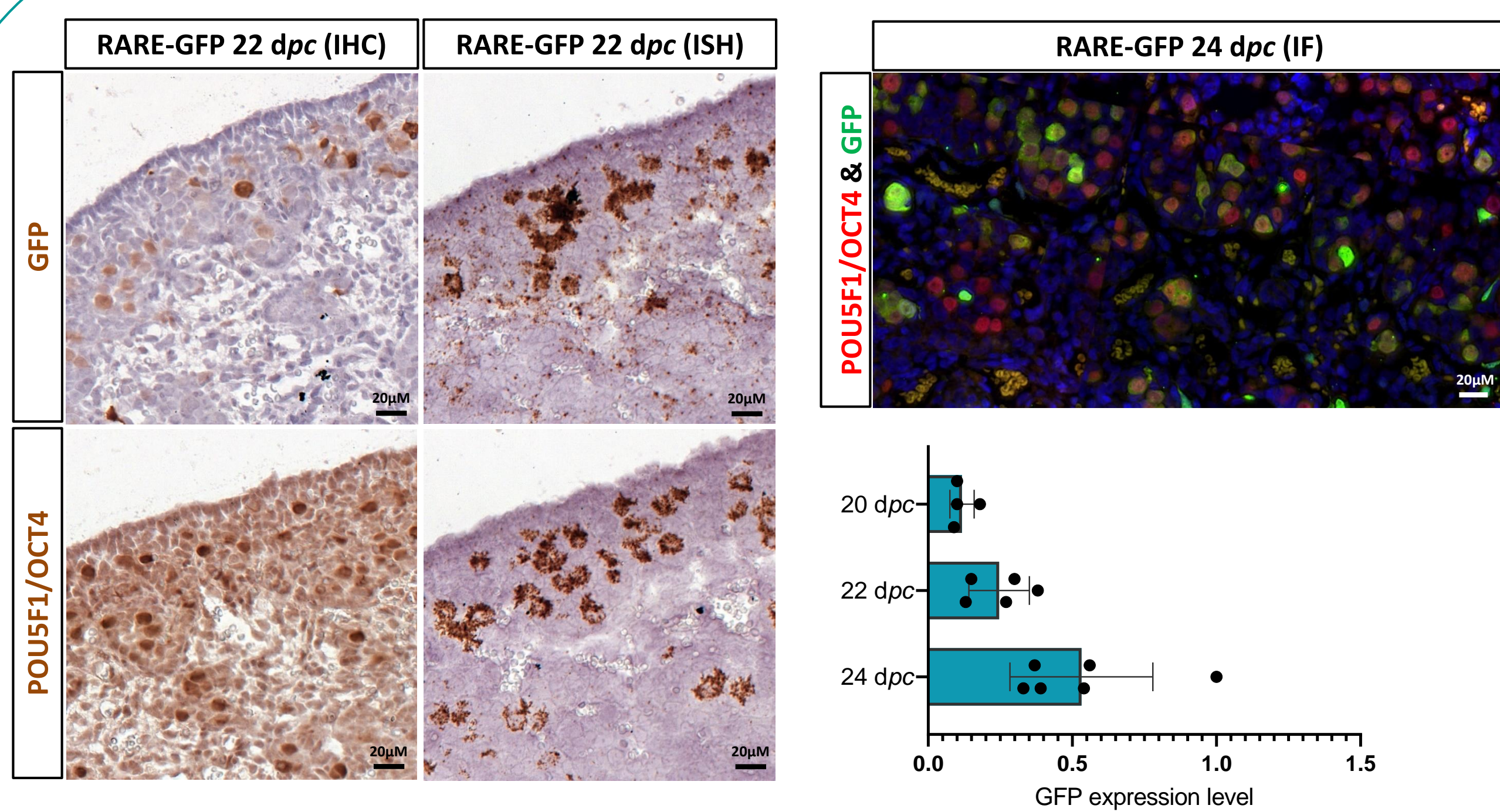


Detection of GFP at 11 dpc in rabbit fetuses



GFP expression in various organs at 22 dpc

ATRA signaling in female germ cells

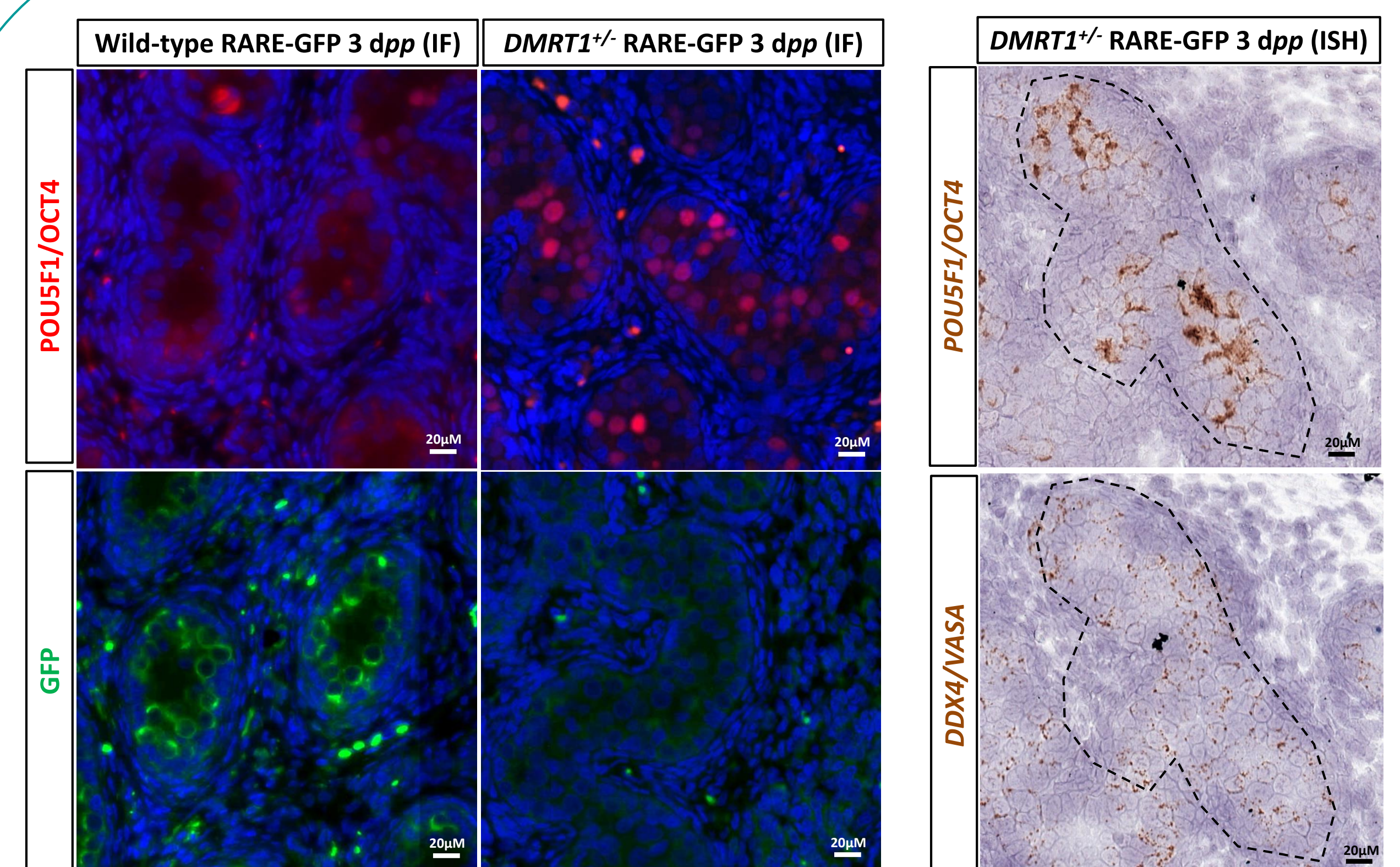


Retinoic acid signaling begins between 20 and 22 dpc in female germ cells:

- Detection of GFP in germ cells at 22 dpc ;
- Co-expression of OCT4 and GFP at 24 dpc in the majority of germ cells;
- The expression level of GFP increases between 20 and 24 dpc.

➤ **Retinoic acid signaling begins more than 10 days before meiosis in rabbit female germ cells**

ATRA signaling in male germ cells



- **Delayed of germ cells differentiation in *DMRT1*^{-/-} rabbits:** Maintenance of pluripotency with the presence of OCT4 at 3 dpp;
- **Delayed of ATRA signaling in *DMRT1*^{-/-} rabbits:** Loss of GFP expression at 3 dpp.

➤ **Evidence for a link between loss of germ cell pluripotency and ATRA signaling in male rabbit germ cells**

Perspectives

- Culture of fetal ovaries in the presence or the absence of ATRA receptor antagonists → RA targets at these stages?
- Cell sorting from fetal ovaries at 22 dpc using our OCT4-GFP VASA-TOMATO rabbit model and comparing with RARE-GFP germ cells → RA targets at these stages?
- Study of the testicular phenotype of *DMRT1*^{-/-} rabbits during puberty → Understanding secondary infertility in heterozygous males.

