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## Using genome editing approaches to decipher the gonadal differentiation pathway in non-rodent mammals

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Sex determination in mammals relies on the presence of the *SRY* gene (Sex-determining Region of Y chromosome) that induced the undifferentiated gonad into testicular differentiation. Since the discovery of *SRY* in 1990, many efforts have been done to decipher the genetic cascade downstream *SRY*. In mice *SRY* has been proved to induce *Sox9* expression; *Sox9* being a critical testis differentiating gene.

In goats and most of mammals except rodents we have accumulated evidences showing that *SRY* should inhibit the *FOXL2* gene in addition to upregulate *SOX9*. Indeed, we have demonstrated that *FOXL2* is an ovarian determining gene in goats [Boulanger *et al.*, 2014]. *FOXL2* loss-of-function in goat ovaries has been achieved either by genome editing [Boulanger *et al.*, 2014], or in the context of the Polled Intersex Syndrome (**PIS** natural mutation) where *FOXL2* regulatory elements are disturbed [Pailhoux *et al.*, 2001; Pannetier *et al.*, 2012]. Its silencing in XX undifferentiated gonads led to their trans-differentiation into testes from the primary stages of gonadal development, then to XX female-to-male sex reversal from the first third of gestation in goats, a stage that is before initiation of meiosis in XX normal ovaries.

Among *FOXL2* ovarian gene targets, *DMRT1* and estrogens emerge as key elements that could explain gonadal sex reversal. Indeed on one hand, *FOXL2* inhibits *DMRT1* in the goat XX somatic lineage [Elzaïat *et al.*, 2014], and *DMRT1* has been shown to be required for *SOX9* up-regulation and testis differentiation in humans [Murphy *et al.*, 2015]. On another hand, *FOXL2* is a critical factor of estrogens synthesis by up-regulating *CYP19* aromatase gene and inhibiting most of the genes encoding steroidogenic enzymes required for androgen synthesis [Pannetier *et al.*, 2006; Elzaïat *et al.*, 2014]. Consequently, *FOXL2* loss-of-function in XX goat gonads leads primarily to an inversion of steroidogenesis from female to male (i.e.: XX *FOXL2* KO gonads do not produce estrogens but secrete androgens to comparable male levels able to induce a fully masculinised internal and external genitalia).

In order to evaluate the role of estrogens in early developing ovaries (before germ cell meiosis) in a mammalian species producing ovarian estrogens, we engineered different *CYP19* KO rabbit lines. Mutant ovaries showed a drastic decrease of the number of germ cells, histological differences of the ovarian surface epithelium and a global disorganization of both cortical and medullar areas. After the first wave of folliculogenesis, *CYP19*<sup>-/-</sup> ovaries remained completely devoid of germ cells at 7-month of age, consecutively to an absence of ovarian reserve evidenced at 2 months of age. *CYP19*<sup>-/-</sup> females were completely sterile but no clear signs of XX sex-reversal could be detected in their ovaries.

In conclusion, our results demonstrate a critical role of estrogens in ovarian differentiation of non-rodent mammalian species producing these hormones since the first stages of ovarian development. This finding should be taken into account for the study of endocrine disruptors on female fertility, because many of EDC compounds are xenoestrogens and should have an impact on the ovarian reserve, as it has been shown in sheep by our team [Lea *et al.*, 2016].

## References

1. Boulanger L. *et al.* (2014). FOXL2 is a female sex-determining gene in the goat. *Current Biology*, 24: 404-408.
2. Elzaïat M., *et al.* (2014). High-throughput sequencing analyses of XX genital ridges lacking *FOXL2* reveal *DMRT1* up-regulation before *SOX9* expression during the sex-reversal process in goats. *Biology of Reproduction*, 91:153.
3. Murphy M.W., *et al.* (2015). An ancient protein-DNA interaction underlying metazoan sex determination. *Nature Structural & Molecular Biology*, 22: 442-451.
4. Pailhoux E., *et al.* (2001). A 11.7-kb deletion triggers intersexuality and polledness in goats. *Nature Genetics*, 29: 453-458.
5. Pannetier M., *et al.* (2012). Telling the story of XX sex reversal in the goat: highlighting the sex-crossroad in domestic mammals. *Sexual Development*, 6: 33-45.
6. Pannetier M., *et al.* (2006). FOXL2 activates *P450 aromatase* gene transcription: towards a better characterization of the early steps of mammalian ovarian development. *Journal of Molecular Endocrinology*, 36: 399-413.
7. Lea R.G., *et al.* (2016). The fetal ovary exhibits temporal sensitivity to a 'real-life' mixture of environmental chemicals. *Sci Rep*, 6: 22279.