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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 prove 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 demonstrate 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 **P**olled **I**ntersex **S**yndrome (**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 [Elzaiat *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; Elzaiat *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 induced 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*$^{-/-}$ 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*$^{-/-}$ females were completely sterile but no clear signs of XX sex-reversal could be detect 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 take 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 show in sheep by our team [Lea *et al.*, 2016].

References

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