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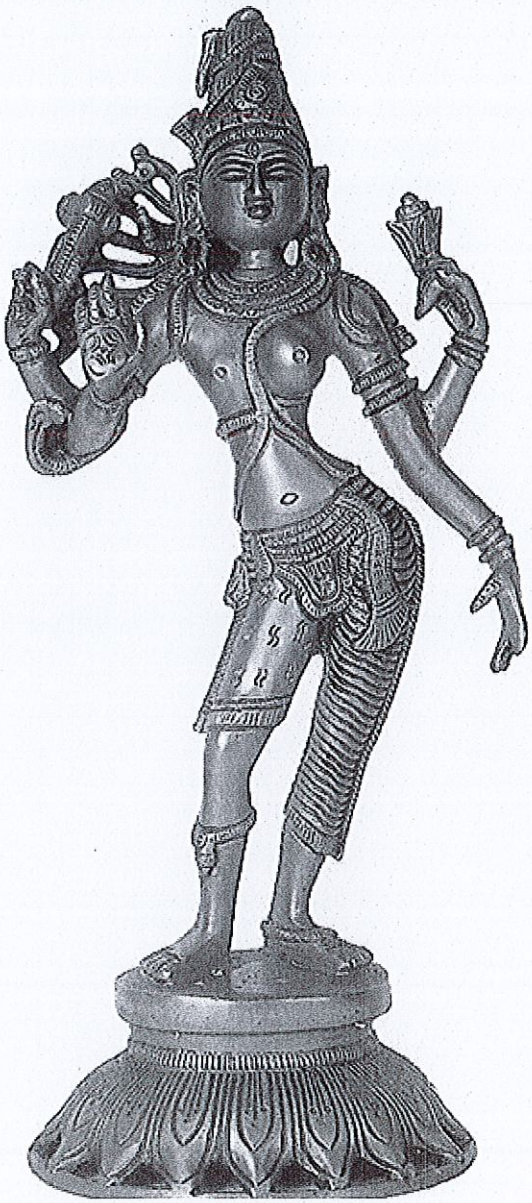

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ESTROGENS ARE KEY OVARIAN DIFFERENTIATING HORMONES IN RABBITS

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By the past, 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 **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). Blocking estrogens secretion in ovaries has been previously shown to induce testes differentiation in non-mammalian vertebrates, including birds [Wartenberg *et al.*, 1992; Nakamura, 2010]. Moreover, *Foxl2* with estrogen-receptors have been shown to inhibit *Sox9* in mouse adult granulosa cells [Uhlenhaut *et al.*, 2009] and consequently, to be key actors of granulosa cell identity maintenance.

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 from which of them three could be considered as estrogens-null mutants. Ovarian differentiation of these *CYP19*$^{-/-}$ mutants was profoundly affected since the earliest developmental stage studied, 22 days *post-coïtum*, i.e.: 4 days after the primary detection of *CYP19* ovarian mRNA. 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 birth, the few remaining oocytes engaged meiosis, then were included in few ovarian follicles that grow up until the pre-ovulatory stage at around 5 months as in control animals. After this 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 detected in their ovaries, except a very faint *SOX9* up-regulation at 7-months.

In conclusion these results demonstrate a critical role of estrogens in ovarian differentiation of mammalian species producing these hormones since the first stages of ovarian development, but in contrast with non-mammalian species, estrogens do not seem to be decisive for the gonadal cell fate in those mammals.

References

1. Boulanger L. *et al.* (2014). FOXL2 is a female sex-determining gene in the goat. *Current Biology,* 24: 404-408.
2. Elzaiat 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. Nakamura M., *et al.* (2010). The mechanism of sex determination in vertebrates: are sex steroids the key factor? *Journal of Experimental Zoology A*, 313: 381-398.
5. Pailhoux E., *et al.* (2001). A 11.7-kb deletion triggers intersexuality and polledness in goats. *Nature Genetics,* 29: 453-458.
6. 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.
7. 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.
8. Uhlenhaut N.H., *et al.* (2009). Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. *Cell*, 139: 1130-1142.
9. Wartenberg H., *et al.* (1992). Sexual differentiation and the germ cell in sex reversed gonads after aromatase inhibition in the chicken embryos. *Andrologia*, 24: 1-6.