EXPRESSION PROFILE OF GENES INVOLVED IN SEX DETERMINATION IN CATTLE

PLANELLS B[1,2], PERICUESTA E[1], LONERGAN P[2], GUTIERREZ-ADAN A[1]

[1]Department of Animal Reproduction, INIA, Madrid, Spain. [2]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland

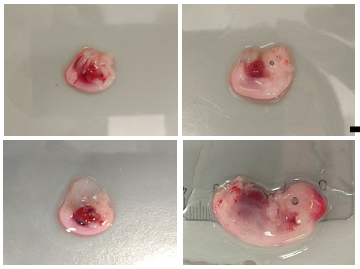
INTRODUCTION

The mechanisms controlling how sex is determined differ considerably among organism groups, even within closely related groups, the molecular control and the regulation of the factors involved in sex determination and gonad differentiation can be substantially different. Mammalian sex determination is the process by which a bipotential gonad develops into a testis or ovary depending on the genetic background of the individual. It is assumed that during mammalian sex determination of XY fetuses, SRY induces SOX9 in Sertoli cells, resulting in formation of testes. However, bovine SOX9 has lost the two transactivation motifs that are essential for sex determination in mammals. Therefore, alternative sex determination pathway should be responsible for sex determination in cattle.

OBJECTIVE

Determine when sexual differentiation of the gonad occurs in cattle by means of immunocytochemistry and analyse the expression during sex determination of several candidates genes involved in sex determination in other mammals

MATERIAL AND METHODS



Collection of bovine foetuses at the abattoir between day 32 and 56 of development (according to the crown rump length (CRL). Sex determined by PCR (amelogenin)

Genital ridge isolation from mesonephros

Fixation in Bouin's solution → Immunohistochemistry of Oct4 → Location of primordial germ cells (PGC) within the developing ridges

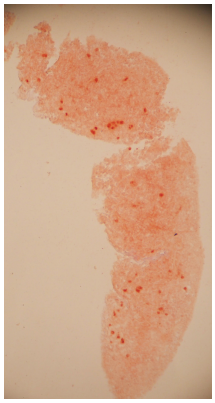
RNA extraction → cDNA → qPCR

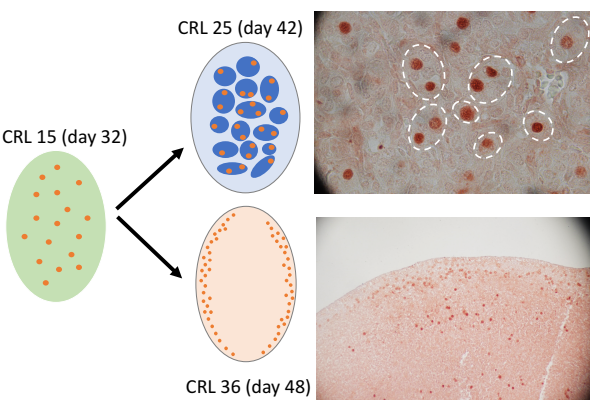
- *SF1, WT1* — Gonad formation
- *FOG2, GATA4, SRY, SOX9, DMRT1* — Testis determination and differentiation
- *WNT4, FOXL2* — Ovary development
- *ZRSRY2, SOX8, SOX10* — May be involved in male sex determination

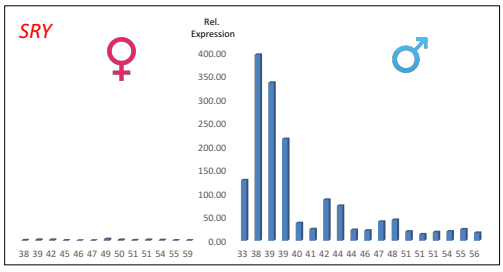
RESULTS

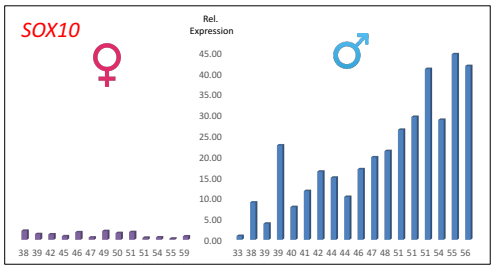
33 bovine embryos were collected from slaughterhouse, CRL measurements were taken at time of collection, and foetuses were sexed by PCR. CRL measurements were converted into age in days from conception (Pace et al., 2002).
CRL: measurement of the length of bovine fetuses from the top of the head to the bottom of the buttocks.

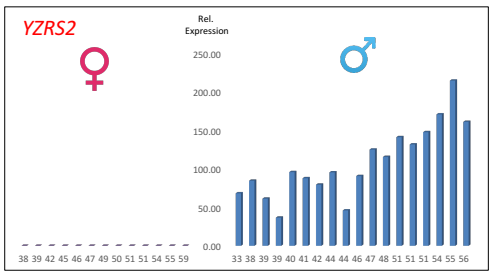
We identified that PGC follow two distinct patterns in males and females. Before SRY peak, PGC localize along the genital ridges of both sexes. After SRY peak, testis cords begin to be distinguishable at a CRL of 25 mm (Day 42) in males, with one to three PGC within each of the developing tubules. In the case of females, PGC tend first to distribute along the periphery of the developing ovary at a CRL of 36 mm.

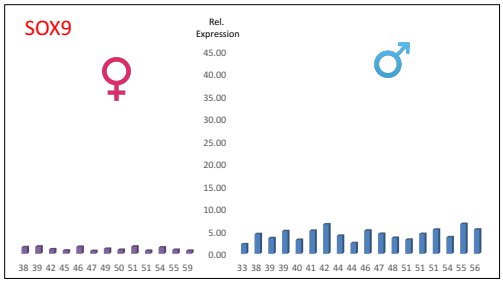


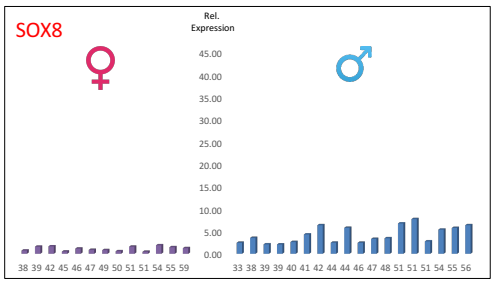


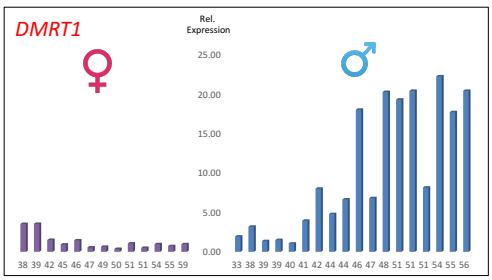












We found that *SRY* expression peaked at a CRL of 18 mm (Day 38). We detected expression of *SOX10* in male foetuses after the SRY peak (earlier than observed in mice and humans), and ZRSR2Y (a splicing factor related to RNA processing and RNA splicing) expression along all the stages analysed showing an increasing pattern from Day 33 to Day 56. Sox9 expression in the developing genital ridges do not follow the pattern observed in mice. We also observed an increase in DMRT1 expression once the testis cords have developed (Day 42). In human, DMRT1 is critically required for the development of the testis during fetal period, while in mice, this gene is involved in the maintenance and/or growth of the testis in the postnatal and adult period.

CONCLUSIONS

Sex determination in bovine genital ridges show characteristic features with SOX10 as possible co-transactivator to continue the gonadal differentiation after SRY peak, and ZRSR2Y as a splicing factor that could be involved in sex determination.

Funded by the European Union under grant agreement 675526-REP-BIOTECH–H2020-MSCA-Innovative Training Networks-2015.