Embryonic gonadal gene expression provides insights into canine gonadal sex determination.

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Lack of information regarding gene expression during canine gonadal development has been an obstacle to the study of inherited disorders that adversely affect reproduction, such as XX and XY Disorders of Sexual Development (DSD), and the utility of the dog as a model to study the human equivalents. The goal of this project is to provide a qualitative and quantitative database of canine gonadal gene expression from the period of gonadal sex determination to early differentiation. Gestation was timed from the day of the preovulatory serum luteinizing hormone surge (d0) and concomitant rise in serum progesterone concentration in the dam. By this method, unassisted parturition occurs at d65 (+/- 2d). This method has been successfully used to time gestation for the purpose of canine embryo transfer and canine in vitro fertilization. Embryos were collected from d34-44, in accordance with the National Institutes of Health Guidelines for Vertebrate Animal Research. Gonad pairs were microdissected from each embryo, then flash frozen and stored in liquid nitrogen until RNA extraction. The sex of each embryo was determined by canine SRY polymerase chain reaction (PCR) assay in genomic DNA templates. Embryos were developmentally staged from photographs of external and internal morphology taken during microdissection. An RNA-seq library was prepared from the gonad pair of each individual by extracting total RNA (RNeasy Plus Mini kit, Qiagen Inc., Alameda, CA), producing a barcoded cDNA library (TruSeq RNA Library Preparation Kit, Illumina), and sequencing the pooled barcoded libraries (HiSeq 2000, Illumina). The barcoding approach allows one to identify the transcriptome data derived from each embryonic gonad pair. Reads were aligned to the canine genome sequence (CanFam3, UCSC and Ensembl). Transcription levels were quantitated in units of FPKM (Fragments Per Kilobase of exon per Million fragments mapped) using the Tuxedo package, which provides expression measurements for all genes expressed, even if the gene is unknown. Transcriptome quantification was guided by, but not limited to, the reference CanFam3 transcriptome (Ensembl). The mean FPKM for genes of interest was tested for statistically significant differences between males and females using the Tuxedo package. The timing of testis and ovary pathway gene expression in canine embryos was found to be similar to that of humans and some domestic animals. A number of genes have expression patterns similar to SOX9 or WNT4 during the sex determination period, which may indicate they are downstream in testis or ovary determination. Other genes found to be expressed later in gestation, and primarily in one sex, are likely involved in testis or ovarian differentiation. These results provide a framework in which to investigate the genetic mechanisms of abnormal canine gonadal development, such as in XX DSD and XY DSD. Furthermore, results indicate that the canine gonad is an appropriate model for investigating inherited disorders of gonadal development in humans and other mammals.