Global endometrial gene expression during simulated diestrus, estrus and anestrus in the bitch

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It has been shown in an in vivo canine pyometra disease model that the disease can only be
induced reliably if E. coli is inoculated into the uterus during simulated diestrus; inoculation during
simulated estrus or anestrus does not cause pyometra.1 These observations raise the possibility that the
uterine immune response may differ during different stages of the estrous cycle.
The objectives of this study were to characterize differences in host immune defences at different
stages of the estrous cycle in order to elucidate the differences in disease susceptibility in relation to
ovarian hormone concentrations.

Twelve post-pubertal, ovariectomized greyhound bitches were treated with estradiol benzoate
(Intervet, Bendigo East, Victoria, Australia) and/or megestrol acetate (Jurox Pty Ltd., Rutherford NSW,
Australia) to simulate estrus or diestrus. Uteri were obtained either on day 4 of simulated estrus or on day
10 of simulated diestrus (n=4 per group). Untreated animals served as anestrous controls (n=4). Punch
biopsies of uterine tissue were preserved in RNAlater® (Applied Biosystems, Scoresby, Victoria,
Australia) and total RNA was extracted, mRNA purified and randomly transcribed into cDNA. Pooled
cDNA libraries from simulated diestrus, estrus or anestrus groups were paired-end sequenced using the
Illumina platform (Illumina® Genome Analyzer II). Paired-end reads were mapped to the annotated
canine genome (Broad Institute, Massachusetts Institute of Technology and Harvard University,
Cambridge, MA). The total number of mapped reads was normalized for gene length and pair-wise
statistical differences in gene expression were determined using a false-discovery rate corrected P-value
of ≤ 0.01, a minimum 4-fold difference in expression and filtration for genes with more than 100 reads
mapped in at least one treatment group.

Comparison of biopsies from uteri in simulated diestrus to those from uteri in simulated estrus
identified 496 genes that were differentially expressed (264 genes were upregulated and 232 were
downregulated in simulated diestrus). Comparison of biopsies from uteri in simulated diestrus to those
from uteri in simulated anestrus revealed 708 differentially expressed genes (325 were upregulated and
383 downregulated in simulated diestrus). Several of the most highly up- and downregulated genes play a
role in innate immunity, including beta-defensins, aquaporins, cadherins, lactoferrin, serum amyloid A
and alpha-antitrypsin.

In conclusion, cycle stage-specific uterine tissues revealed pronounced differential gene
expression, suggesting that ovarian hormone profiles play an important role in the pathogenesis of canine
pyometra.

Keywords: Dog; pyometra; RNA-Seq; estrous cycle

Reference