

TITLE: EVALUATION OF FOLLICULAR ASPIRATE TO DETECT *LEPTOSPIRA* SPP. DNA IN THE REPRODUCTIVE TRACT OF NATURALLY INFECTED COWS

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ABSTRACT:

Bovine genital leptospirosis (BGL) is a syndrome characterized by reproductive disorders that leads to severe economic losses. Although the pathogenesis of BGL is unclear, an experimental study demonstrated that oocyte can be infected by leptospires, and then, be injured. Considering this, the present study aimed to investigate the presence of leptospiral DNA in the ovarian follicle of slaughtered cows. This research was approved by Ethical Committee for Animal Use of Federal Fluminense University (protocol 863/2016). Follicular aspirate from ovaries of 65 non-pregnant cows were collected. During the slaughter procedures, the reproductive system from which cows was set apart and then follicular samples were collected through aspiration in 1mL syringes with needle 6mm x 0.25mm (31G). All follicle content from the two ovaries of the same cow was collected and transferred to identified cryotubes, frozen (-20° C) and properly transported to the Federal Fluminense University. The DNA extracted from those samples were submitted to PCR targeting *LipL32* gene (only present in pathogenic *Leptospira* spp.). Samples positive in *LipL32*-PCR were submitted to a nested PCR targeting *secY*, and with its amplicon, nucleotide sequencing was proceeded. In total, 7/65 (10.8%) of the samples were positive, confirming the presence of leptospiral DNA in the ovarian follicle of naturally infected cows. It was possible to amplify and sequence *secY* fragments from two amplicons, identifying them as *L. interrogans* and *L. santarosai*. This study demonstrated for the first time the presence of *Leptospira* spp. in the ovaries of naturally infected cows, indicating early oocyte infection. This result demonstrates the relevance of ovarian infection which could induces embryonic damage, leading to embryonic death, reproductive failure and, consequently, estrus repetition in animals and economic hazards. This finding is important for future studies that concern the pathogenesis of the infection and its consequences on animal reproduction.

Keywords: Leptospirosis, PCR, *SecY*, sequencing

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