Analysis of the histosection showed a changed structure of some liver lobes, as well as a significant amount of bilirubin in hepatocytes. Single focal necrosis of hepatocytes with discomplexation of liver tissue and disruption of beams, pronounced hemosiderosis were observed.

Discussion. Research results have shown that a preliminary clinical diagnosis of babesiosis in carnivores can be made on the basis of blood smear examination in combination with medical history, hemato-biochemical, post-mortem and histopathological changes. The obtained results of pathohistological studies are important for scientists both theoretically and practically, so further scientific research will be aimed at studying more detailed histomorphological aspects of the early stages of the pathological process.

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THE IMPACT OF GnRH AGONIST ON SPERMATOGONIAL STEM CELLS IN MALE DOGS

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Introduction and aim. Gonadotropin-releasing hormone agonist slow-release implants (GnRH SRI) are widely used in veterinary practice as an alternative to surgical castration. These implants, such as buserelin and deslorelin, effectively suppress male testicular, endocrine, and germinative functions. Beyond medical applications like treating benign prostatic hyperplasia

(BPH), GnRH SRIs play a crucial role in dog population management and behaviour modification by reducing testosterone-influenced behaviours, contributing to a healthier life for animals (Driancourt and Briggs, 2020). GnRH SRIs continuously stimulate the release of GnRH, leading to a sustained downregulation of the pituitary gland's response, thereby reducing the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). This inhibition suppresses testosterone production and testicular function. After implant removal, testosterone levels normalize within two weeks, and spermatogenesis resumes within 7-12 weeks, with full semen quality recovery observed after 12-19 weeks (Stempel et al., 2022).

Spermatogenesis involves the renewal and proliferation of spermatogonial stem cells (SSCs), progression through meiosis, and spermatogenesis (Miura and Miura, 2002). Key biomarkers in this process include PGP 9.5, FOXO1, DMRT1, and PCNA. PGP 9.5 is used to identify germ cells and is essential for SSC self-renewal and differentiation. FOXO1 regulates SSC maintenance and meiosis initiation, with its inactivation leading to developmental defects (Goertz et al., 2011; Shen et al., 2022). DMRT1 promotes SSC self-renewal and differentiation, preventing premature meiosis initiation (Matson et al., 2010). PCNA is crucial for DNA replication and repair during cell proliferation, serving as a marker for proliferating spermatogenic cells (Tang et al., 2019, 2022).

Despite extensive studies on germ cells in rodent models, canine models offer valuable insights due to their physiological and genetic similarities to humans (Schaffer 2019). Research on SSC dynamics in canines during GnRH SRI treatment is limited but essential for understanding spermatogenesis and reproductive health, offering potential applications in reproductive medicine (de Souza et al., 2021).

In summary, GnRH SRIs are effective tools in veterinary medicine for managing reproduction and behavior in male dogs. Their impact on spermatogenesis, mediated through key biomarkers, underscores the importance of further research in canine models to enhance our understanding of reproductive biology.

Materials and methods. Nineteen healthy male dogs received an azagly-nafarelin GnRH slow-release implant (GnRH-SRI), which remained in place for 5 months. After the 5-month period, the implants were surgically removed. To assess the immediate and delayed effects of implant removal on spermatogenesis, 3-5 dogs were castrated at specific intervals: immediately at week 0 (W0), and at 3, 6, 9, 12, and 24 weeks post-removal (W3, W6, W9, W12, W24). In addition, three dogs each received buserelin (PG) and deslorelin (SG) implants and were castrated after 5 months, corresponding to W0. Control samples were collected from the testes of five healthy adult males (CG) and three juvenile 2-month-old males (JG) to provide baseline comparisons. Testicular tissues from all groups were processed for immunohistochemical analysis using antibodies against PGP9.5, FOXO1, DMRT1, and PCNA. These markers were chosen to identify and quantify spermatogonia at various stages of development. Immunopositive spermatogonia were counted in approximately 20 round seminiferous tubules per sample at 200x magnification. This allowed for a detailed assessment of the presence and quantity of different spermatogonial populations.

Statistical analysis was conducted using GraphPad Prism10. The analysis was divided into two main comparisons: The downregulation phase (W0, PG, SG) was compared with the adult and juvenile controls (CG, JG). The recovery phase (W0, W3, W6, W9, W12, W24) was compared with the adult controls (CG).

Results. Immunohistochemistry revealed positive staining for PGP9.5 in the nuclei and cytoplasm of spermatogonia and early spermatocytes located at the basal membrane of the seminiferous tubules. In the recovery dataset, significant differences were observed between recovery groups and the control group (CG) (ANOVA, $p<0.05$). Notably, PGP9.5 expression was significantly higher at W3, W6, and W12 compared to W0. The intensity was strongest at W12 ($p<0.01$). In the downregulation dataset, significant differences were noted between downregulated groups and CG (ANOVA, $p<0.001$), with JG showing significantly lower expression compared to CG, SG, and W3.

DMRT1 staining was prominent in the nuclei of spermatogonia and mildly in Sertoli cells. No expression was observed in JG. In the recovery dataset, significant differences were noted among

recovery groups and W0 (ANOVA, $p<0.01$). The number of immunopositive germ cells rapidly increased at W3 and W6. In the downregulation dataset, significant differences were found between downregulated groups and CG (ANOVA, $p<0.0001$), with JG showing significantly lower expression compared to CG and other downregulated groups.

FOXO1 staining was observed in the cytoplasm of spermatogonia across all groups. In the recovery dataset, significant differences were found between groups (ANOVA, $p<0.05$), with a significant increase at W6 compared to W0. In the downregulation dataset, significant differences were found between downregulated groups and CG (ANOVA, $p<0.001$), with lower FOXO1 expression at W0 compared to CG.

PCNA staining showed clear signals in the nuclei of spermatogonia and early spermatocytes, with variation among animals. In the recovery dataset, significant differences were observed (ANOVA, $p<0.01$), with significant increases at W6, W9, W12, and W24 compared to W0. In the downregulation dataset, significant differences were observed between downregulated and control groups (ANOVA, $p<0.0001$), with lower expression at W0 compared to CG and higher expression in SG compared to JG.

Overall, significant differences in the numbers of PGP9.5, FOXO1, DMRT1, and PCNA-positive spermatogonia were observed in both datasets. During downregulation, the lowest counts were in JG, with significantly fewer FOXO1, DMRT1, and PCNA-positive spermatogonia compared to CG. In recovery, there was a rapid restoration of these markers to levels comparable to CG.

Conclusions. Our results highlights the significant impact of GnRH-SRI on spermatogonial stem cell (SSC) activity and spermatogenesis in male dogs. The observed reduction in DMRT1 and PCNA-positive spermatogonia during downregulation indicates that GnRH-SRI treatment adversely affects mitotic proliferation, contributing to the temporary arrest of spermatogenesis at the spermatogonia and spermatocyte stages. The similar outcomes across different GnRH agonists suggest a consistent mode of action. The decreased number of FOXO1-positive spermatogonia suggests a potential depletion of undifferentiated spermatogonia; however, the reversibility of this effect, evidenced by the rapid restoration of SSC markers and recovery of spermatogenesis, contradicts this hypothesis. These findings underscore the reversible nature of GnRH-SRI treatment on testicular function and highlight the need for further research to fully understand the mechanisms underlying these effects.

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АКТИВНІСТЬ ЕНЗИМІВ В КРОВІ СВИНЕЙ ЗА ЗАСТОСУВАННЯ НАНОСПОЛУК МІКРОЕЛЕМЕНТІВ

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