ABSTRACTS

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MORPHOLOGY AND EXPRESSION OF ANDROGEN RECEPTOR, STAR AND 3ß-HSD DURING RECRUDESCENCE OF SPERMATOGENESIS FOLLOWING DOWNREGULATION WITH A GNRH-IMPLANT IN THE DOG

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Introduction - Aberrations in semen quality causing infertility must often be classified as idiopathic as no other deviations from normal, also in respect to peripheral hormone levels, can be found. Hence not an absolute deficit but rather aberrations in the local availability of hormonal control factors may be responsible for disruption of spermatogenesis.

Materials and methods - Consequently recrudescence of spermatogenesis was monitored after having achieved downregulation of testicular function with a GnRH-implant (Gonazon®, Intervet 18.5 mg Azagly-Nafarelin) in 30 Beagles. Implant removal was after 5 months (week 0) and 3-4 dogs were castrated at weeks 0, 3, 6, 9, 12, 15, 18, 21 and 24; the testes were conserved for further examination. Testosterone (T) and estradiol-17ß (E) were estimated in blood collected during downregulation and at castration. To assess morphological changes approximately 200 tubule-cross-sections were evaluated and grouped according to the most developed germ cell observed: Gr. A, spermatocytes (n=4); Gr. B, round spermatids (n=3); Gr. C, elongating spermatids (n=6) and Gr. D, elongated spermatids (n=17). On the mRNA-level expression of the androgen receptor (AR), Steroid Acute Regulatory Protein (StAR) and 3ß-hydroxysteroid dehydrogenase (3ß-HSD) was tested by real time RT-PCR using specific canine probes. On the protein level, expression was assessed by immunohistochemistry (IHC). Specificity of primary antibodies was verified by Western Blot.

Results - Timing of recrudescence showed distinct individual differences yielding different numbers of dogs per group as indicated above. T and E concentrations increased (p<0.05) from Gr. A to B (T: 0.14 ± 0.10 to 2.54 ±1.57ng/ml; E: 6.40 ± 2.19 to 9.73 ± 4.16pg/ml) and were constant thereafter. Relative gene expression for AR was not significantly different between Gr. A-D, but a tendency of a decreased expression in Gr. D was obvious. Expression of 3ß-HSD was significantly lower in groups A and D compared to group C (p<0.01). StAR expression was highest in Gr. B and different (p<0.05) from Gr. D. IHC located expression of AR primarily in the Sertoli cells and to a lesser extent in the Leydig cells. Expression of 3ß-HSD was primarily located in the Leydig cells, but also some Sertoli cells stained positive. Similarly StAR expression was primarily located in Leydig cells and to some extent also in the cytoplasm of Sertoli cells. Interestingly IHC for StAR also stained the forming acrosomal cap in round/elongating spermatids. These first results imply that AR-expression is not depressed by downregulation but rather by the increasing T-concentrations following removal of the implant. Onset of spermatogenesis following implant removal is indicated by an increased expression of StAR and 3ß-HSD as shown on the mRNA- and protein level. Onset of spermatogenesis occurs very early at still low T-concentrations, T-levels plateau with the occurrence of elongating spermatids. To our knowledge, this is the first study giving detailed information about recrudescence of spermatogenesis of the dog following downregulation.