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### 43 | Impact of a maternal diabetes on embryonic DNA methylation in rabbit blastocyst Einfluss eines mütterlichen Diabetes mellitus auf die DNA-Methylierung des Embryos beim Kaninchen

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In early pregnancy maternal diabetes leads to delay in embryo development and changes in nutritional and hormonal signals of the uterine environment. The current study focuses on consequences of maternal diabetes on embryonic tissue formation and its DNA methylation. Therefore we investigated the expression and promoter methylation of epiblast lineage specifier Oct4 in 6 day old rabbit blastocysts at early gastrulation stage. The expression of Oct4 was higher in epiblast of diabetic blastocysts, accompanied by upregulation of Nanog and Sox2. Specific methylation of the POU5F1 (Oct4) promoter region was investigated by bisulphite sequencing. The Oct4 promoter was hypomethylated in hypoblasts and trophoblasts of diabetic rabbits, implying also a mark of delay in differentiation. The global DNA methylation of male and female blastocysts from diabetic and healthy rabbits was examined, employing embryoblast and trophoblast tissue separately, using LUMinometric Methylation Assay (LUMA). No significant changes in global DNA methylation were observed. Furthermore we verified that concentrations of methyl group donor S-adenosyl methionine (SAM) and the product of the methylation reactions S-adenosylhomocysteine (SAH) were changed in diabetic pregnancy. SAM and SAH were measured by use of a modified liquid chromatography–tandem mass spectrometry in rabbit blood plasma collected at the day 6 post-coitum. Our data showed that maternal diabetes mellitus affects the Oct4 promoter methylation in a specific way with consequences for Oct4 gene transcription and embryo development. Alteration of Oct4 methylation was not caused by global methylation changes.

### 44 | Urospermia in an Entlebucher Mountain Dog with ectopic ureters—a case report Urospermie bei einem Entlebucher Sennenhund mit ektopischen Ureteren—Ein Fallbericht

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Urospermia/US may result from retarded closure of the urinary bladder neck during semen emission due to local inflammation or insufficient  $\alpha$ -adrenergic stimulation of the smooth muscle layers. By this urine passes the bladder neck when pre-secretion and the sperm rich fraction are reaching the pelvic urethra. In the present case US is suspected to be the result of a bilateral ectopic ureter (EU), a congenital malformation, with the ureteral orifice terminating intravesically near the bladder neck or extravesically at the prostate, deferent duct, or urethra. Case report: A 4-year-old Entlebucher Mountain Dog was presented for breeding soundness evaluation due to an unsuccessful mating 5 months ago. At the age of 2 years an abnormal course of both ureters in the area of the bladder neck had been diagnosed sonographically. As the finding was not classified as serious, the dog had been approved for breeding. The current andrological examination revealed both testes and epididymides of adequate size and shape. The collected ejaculate showed normal volume and total sperm count, but severe deficiencies in sperm motility, plasma and acrosome membrane integrity and sperm morphology. The most striking macroscopic finding was a yellowish appearance showing continuously increasing intensity in the watery prostatic secretion. Odour and pH-value revealed admixture of urine. A repeat ultrasound examination confirmed a bilateral intramural EU entering the urinary bladder about 1 cm cranial of the vesico-urethral transition with the opening pointing caudally. This finding may not per se indicate a causal connection between EU and US. Nevertheless the latter may be a functional indicator of a urine leakage possibly existing as additional congenital abnormality in the caudal vesical/cranial urethral area.

### 45 | Factors affecting semen evaluation of native and frozen-thawed goat semen Einflussfaktoren auf spermatologische Untersuchungsergebnisse und den Erfolg der Kryokonservierung von Ziegensperma

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Parameters collected during semen evaluation are the basis to establish the suitability of an ejaculate. Therefore, the conditions must be kept as constant as possible. The aim of this study was to evaluate possible factors affecting semen analysis of native and frozen-thawed goat semen. A total of 20 ejaculates from 5 healthy peacock goat bucks were obtained using an artificial vagina. After a macroscopic evaluation 2 aliquots were assessed at different handling temperatures (20 and 37 °C) and time points (TP) (3, 10, 15 and 30 min. after collection). The pH of each sample was evaluated with indicator-paper every 10 min. for 1 h. Density and progressive motility (PM) were assessed using computer assisted sperm analysis (AndroVision<sup>®</sup>, Minitüb, Germany). Evaluation of semen viability was performed using bromophenol-nigrosine staining. For cryopreservation, a commercial