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Intrinsic direct role of dopamine in the regulation of rabbit (*Oryctolagus cuniculus*) corpora lutea: *in vitro* studyG Catone¹, M Maranesi², F Parillo¹, C Vullo¹, M Zerani¹, A Gobetti¹, C Boiti²¹Scuola di Bioscienze e Medicina Veterinaria, Matelica, Italy;²Dipartimento di Scienze Biopatologiche veterinarie ed Igiene delle produzioni animali e alimentari, Perugia, Italy

Dopamine (DA) is a catecholamine neurotransmitter that is distributed in the brain and also in different peripheral organs. In particular, DA receptors (DR) are expressed in luteal cells of various species, but the intrinsic role of the DA/DRs system on corpora lutea (CL) function is still unclear. The main objectives of the present study were to examine in rabbit CL the gene expression of DRs and DA and their immunolocalization, as well as the *in vitro* effects of DA on the production of progesterone, PGE₂, and PGF_{2α} during early, mid, and late luteal stages of pseudopregnancy. Immunoreactivity and gene expression for DR type 1 (D1R) decreased while that for D3R increased in luteal and blood vessel cells from early to late pseudopregnant stages. DA immunopositivity was evidenced only in luteal cells, DA and D1R agonist increased *in vitro* release of progesterone and prostaglandin E₂ (PGE₂) by early CL, whereas DA and D3R agonist decreased progesterone and increased PGF_{2α} *in vitro* release by mid and late CL. These results provide evidence that the DA/DR system exerts a dual modulatory function in controlling the lifespan of CL: the DA/D1R is luteotrophic, while the DA/D3R is luteolytic. The present data shed new light on the physiological mechanisms regulating luteal activity that might improve our ability to optimize reproductive efficiency in mammal species, including humans.

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Maturation and culture in the Corral[®] dish: effect on bovine embryo development

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Corral[®] dishes have been designed for group culture in human *in vitro* fertilization (IVF). Its quadrant design allows passage of medium and embryotrophic factors amongst the quadrants, but allows grouping of embryos per donor. We have tested its feasibility for culturing embryos in a model system for bovine ovum pick-up/IVF. Oocytes (n = 10) of individual cows (n = 64, 4 replicates) were matured in a quadrant of the Corral[®] dish in TCM-199 supplemented with 20 ng/ml EGF. After fertilization, 8 zygotes of each donor were cultured in a Corral[®] dish quadrant (corral) or in a single drop (drop) of SOF medium with 0.4% BSA and insulin-transferrin-selenium. Classical group culture in a 50 μl droplet, consisting of 25 embryos resulting from oocytes derived from different cows served as a control (embryo density 1 : 2). Cleavage rate was assessed at 45 h post insemination (pi) and was significantly lower in both test groups compared to the control (CTRL: 85.0 ± 2.52%; corral: 77.6 ± 2.66%; drop: 72.9 ± 2.78%) (p < 0.05). Blastocyst development was also significantly lower in the test groups compared to the control, both at day 7 pi (CTRL: 32.5 ± 3.31%; corral: 22.8 ± 2.67%; drop: 12.9 ± 2.10%) and day 8 pi (CTRL: 40.0 ± 3.46%; corral: 30.1 ± 2.92%; drop: 26.7 ± 2.77%). However, at day 7 pi, a significantly higher blastocyst rate was noted in the corral than the drop (after Bonferroni correction). In conclusion, allocating oocytes or embryos in the Corral[®] dish per cow did not match the benefits of group culture, probably due to the large distance between the quadrants and the lower embryo density (1 : 3.75).

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Effect of systematic preventive treatment using uterotonics on the occurrence of clinical endometritis in dairy cows – preliminary results

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The effect of repeated administration of oxytocin (day 1 and day 2 post partum) in combination with a single administration of ergometrin or dinoprost (day 7 p.p.) on the occurrence of clinical metritis in dairy cows was evaluated in this field trial. Cows without retained foetal membranes were divided into 3 groups. No treatment was performed in the control group (Group C, n = 156), repeated intramuscular administration of oxytocin (Oxytocin inj., Bioveta, 30 IU pro toto) in combination with dinoprost (Dinolytic inj., Pfizer, 10 mg pro toto; Group D, n = 76) or in combination with ergometrin (Ergometrin, Bioveta, 15 mg pro toto; Group E, n = 87) were performed in experimental groups. The vaginal discharge (score 1–5) and uterine content (score 1–3) were evaluated on D14–21 post partum. In addition, the numbers of interventions necessary for clinical endometritis treatments in groups C, D and E were evaluated. The average discharge score was 2.0; 2.31 and 2.03, the average contents score was 1.52; 1.64 and 1.60. The average number of interventions was 0.68; 0.86 and 0.78, the proportion of cows without treatment was 59.4%; 54.2% and 54.7% among groups C, D and E, respectively. There were no significant differences in all evaluated variables among the groups. The preliminary results did not show any favourable effect of post partum administration of oxytocin in combination with dinoprost or ergometrin on the occurrence of clinical metritis in dairy cows. (Supported by the grant IGA VFU Brno No. 68/2013/FVL).

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Reducing glycerol concentration with trehalose increases survival of bull spermatozoa

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Although glycerol has a known cytotoxic effects on bovine spermatozoa, it is used as a common cryoprotectant in TRIS based extenders. This study was conducted to analyse the effects of trehalose in semen extenders supplemented with low (3.5%) glycerol. Semen samples from 3 mature Sahiwal bulls were pooled, ejaculates were divided into 4 aliquots: I-Control (TRIS extender with 7% glycerol), II-Reduced glycerol (RG) 3.5%, trehalose 0 mM (T0), III-RGT100 mM, IV-RGT200 mM. Post thaw sperm motility, plasma membrane integrity (PMI) and functionality (HOST), normal acrosomal ridge (NAR) and DNA integrity (via acridine orange) were analysed. The post thaw motility (55 ± 1.58% vs. 42 ± 1.22%), viability (63 ± 0.89% vs. 51 ± 1.3%), HOS (54.2 ± 0.37% vs. 41.4 ± 1.67%) and DNA integrity (97.62 ± 0.48% vs. 96.51 ± 0.37%) were significantly (p < 0.05) higher in RGT200 compared to Control. NAR % was significantly (p < 0.05) higher in RGT100 compared to Control (57.2 ± 0.58 vs. 40 ± 0.71). In conclusion, extenders with low fraction of glycerol (3.5%) supplemented with gradual increase in trehalose concentration (up to 200 mM) resulted in better sperm survival.