212 EFFECT OF A LOW DOSE OF ECG ON SUPEROVULATION AND EMBRYO COLLECTION IN WOOD BISON DURING THE BREEDING SEASON

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In an effort to conserve a threatened Canadian species, *Bison bison athabascae*, we developed an embryo collection protocol subsequent to superovulatory treatment involving two doses of FSH diluted in hyaluronan given 48 h apart. The follicular response to superstimulatory treatment was satisfactory, but many follicles did not ovulate, thus limiting the number of embryos collected. Based on recent results in cattle, where replacement of the final doses of FSH with a low dose of eCG resulted in the recovery of a greater number of ova/embryos, the objective of the present study was to evaluate the effect of adding eCG to the superovulatory protocol to increase ovulation rate, embryo collection, and embryo quality in wood bison during the breeding season (September). Ovarian synchronization was induced in wood bison ($n = 24$) by treatment with a luteolytic dose of prostaglandin (500 mcg, Cloprostenol) followed 8 days later by transvaginal ultrasound-guided follicular ablation. Follicular wave emergence (Day 0) was defined as the day after follicle ablation. Bison were assigned randomly to two groups: FSH ($n = 12$) and FSH$^+$eCG ($n = 12$). FSH was diluted in hyaluronan (5 mg mL^{-1}, MAP-5, Bioniche Animal Health, Belleville, ON, Canada) and given intramuscularly on Day 0 (300 mg) and Day 2 (100 mg) in both groups. Bison in the FSH$^+$eCG group received 450 IU eCG (Pregnecol, Bioniche Animal Health) intramuscularly on Day 3, and bison in both groups were administered a luteolytic dose of prostaglandin on Day 3. On Day 5, bison were given 2500 IU hCG (Chorulon, Merck Animal Health, Summit, NJ, USA) intramuscularly to induce ovulation. The bison were artificially inseminated with chilled semen 12 and 24 h after hCG treatment. Nonsurgical embryo collection was performed on Day 13. The ovaries were examined by transrectal ultrasonography on Days 5, 7, and 13 to record the follicular response, ovulation rate, and number of corpora lutea (CL), respectively. Results were compared between groups by *t*-test or chi-square test (Table 1). The number of ovulatory-sized follicles (≥9 mm), ovulation rate (number ovulations/ovulatory-sized follicles), number of CL, number of ova/embryos, and number of transferable embryos were not different between groups ($P = 0.23$, $P = 0.19$, $P = 0.25$, $P = 0.18$, $P = 0.09$, respectively). In conclusion, the superovulatory response and embryo collection rate in wood bison approached that observed in cattle, but were not improved by the addition of a low dose of eCG.

Table 1. Response of wood bison (mean ± s.e.m.) to superovulatory treatment with or without eCG

	n	No. of follicles ≥9 mm	Ovulation rate	No. of CL	No. of ova/embryo	No. of transferable embryos
FSH	12	8.2 ± 2.4	79%	6.9 ± 0.98	4.2 ± 0.46	2.9 ± 0.71
FSH$^+$eCG	12	7.2 ± 2.4	69%	5.4 ± 0.86	3.7 ± 0.96	1.7 ± 0.25

213 USE OF ACTIVE CASPASE 3 AND TUNEL ASSAYS TO ESTIMATE EMBRYONIC QUALITY IN *IN VIVO* SANTA INES EWE EMBRYOS

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This study was designed to quantify the percentage of apoptotic cells using active caspase 3 and TUNEL assays, in order to estimate the quality of ovine embryos produced *in vivo*. For that, 60 Santa Ines ewes ($n = 10$ per group) were submitted to superovulation with FSH treatment started near the different follicular wave emergence of the protocols (G-1 or G-2), during breeding season, transition, and nonbreeding season. Follicular wave emergence days were defined in a previous study that evaluated the follicular dynamic in oestrus synchronization treatments (Oliveira *et al.* 2011 Acta Sci. Vet. **38**, 361). On Day 0, all ewes received a P4 device (CIDR®) and 37.5 μg of D-cloprostenol. The P4 device was replaced by a new one on Day 7 just for G-2 in the transition period. The superovulation treatment started on Day 4, 4, and 6 of protocol for G-1 and on Day 10, 10, and 11 for G-2 in nonbreeding, transition, and breeding season, respectively. The FSH treatment consisted of eight injections of pFSH administrated twice a day in descending order (40, 30, 20, and 10 mg of pFSH). The P4 device was removed two days after beginning of FSH treatment. At these times, all ewes received another injection of 37.5 μg of D-cloprostenol and a dose of 200 IU of eCG. Ewes were mated with a fertile ram for 4 days after P4 device removal. Embryo recoveries were carried out by laparotomy, 7 days after CIDR withdrawal. Embryos were morphologically classified. Grade I to III morulas and blastocysts were considered viable. A representative sample of each treatment was fixed and stained by active caspase 3 and TUNEL assays to assess the apoptotic cells percentage. Data were analysed by GLIMMIX using SAS comparing mean values (±s.e.m.) between groups at each season ($P = 0.05$). Pearson correlation was estimated between active caspase 3 and TUNEL assays. No effect was detected between treatments in each season on the number of viable embryos (3.2 ± 0.8 *v.* 1.8 ± 0.8, 3.9 ± 1.9 *v.* 5.7 ± 1.4, and 3.8 ± 1.5 *v.* 3.4 ± 0.8 for G-1 *v.* G-2 in nonbreeding, transition and breeding season, respectively). The treatment G-2 increased ($P < 0.05$) apoptotic cells percentage in nonbreeding season group, assessed by active caspase 3 (G-1: 3.1 ± 1.6% and G-2: 12.8 ± 4.3%) and TUNEL (G-1: 1.6 ± 0.5% and G-2: 11.1 ± 3.5%) assays. The apoptotic cells percentage remained unaltered for Transition and Breeding season groups, assessed by either active caspase 3 (G-1: 6.0 ± 0.9% and 5.6 ± 1.5%; G-2: 5.6 ± 1.1% and 5.1 ± 0.5%) and by TUNEL (G-1: 7.5 ± 1.3% and 5.2 ± 1.0%; G-2: 5.0 ± 0.9% and 6.4 ± 1.1%). The Pearson correlation between

active caspase 3 and TUNEL tests was $r^2 = 0.436$ ($P < 0.0001$). In conclusion, the active caspase 3 and TUNEL assays presented similar results for apoptosis level assessment in Santa Ines ewes *in vivo* produced embryos, and both assays were considered appropriate for this purpose. The increased apoptosis levels detected in the G-2 nonbreeding season group suggest that this treatment is harmful for Santa Ines ewe embryos.

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214 SUPEROVULATION IN A/J MICE USING A COMBINATION OF GONADOTROPINS AND THE PTEN INHIBITOR BPV(PIC)

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Strain and individual differences in superovulation constitute a serious problem in mice. Gonadotropins stimulate the maturation of developing ovarian follicles, but not primordial follicle activation (PFA), which is negatively controlled by the Phosphatase and Tensin Homologue Deleted from Chromosome 10 (PTEN). PTEN inhibitors may increase the number of developing follicles by promoting PFA. If so, subsequent gonadotropin injections may make more follicles ovulate. This study tested whether PTEN inhibitors promote superovulation by gonadotropins and examined the fertilizability of ovulated oocytes *in vitro*. Method: Immature females of the low responder A/J mouse strain were used. Based on preliminary results regarding peaks in the daily changes in the ovary weight/body weight ratio and ovarian anti-Müllerian hormone protein content after injections of the PTEN inhibitor, dipotassium bisperoxo (picolinato) oxovanadate (V) [bpV(pic)], the number of ovulated oocytes was examined using four combinations of the bpV(pic) dose and interval between the bpV(pic) and pregnant mare serum gonadotropin (PMSG) injections: 1) 3 μg and PMSG on Day 3; 2) 3 μg and PMSG on Day 4; 3) 30 μg and PMSG on Day 1; and 4) 30 μg and PMSG on Day 2. Ovulation was induced by hCG 48 h after PMSG injection. *In vitro* fertilization of the obtained oocytes was performed using TYH medium and epididymal sperm from adult ICR male mice (Day 1). Subsequently, the oocytes were cultured in KSOMaa+0.1% bovine serum albumin for 4 days. The numbers of two-cell embryos and blastocysts were recorded on Days 2 and 5, respectively. Results: The average number of oocytes collected in treatments 1 to 4 was 1) 7.2 ± 0.7 *v.* 9.4 ± 1.6 [control *v.* bpV(pic), mean ± s.e.m., $n = 5$]; 2) 7.8 ± 0.7 *v.* 9.0 ± 1.4; 3) 9.8 ± 1.6 *v.* 11.2 ± 1.0; and 4) 9.4 ± 1.0 *v.* 7.4 ± 1.9. More oocytes tended to be collected in the bpV(pic) groups in treatments 1 to 3, but the differences were not significant on ANOVA. The corresponding percentages of two-cell embryos on Day 2 after insemination were 1) $78.7 \pm 7.7\%$ *v.* $83.7 \pm 5.6\%$; 2) $76.0 \pm 4.2\%$ *v.* $65.2 \pm 4.2\%$; 3) $53.5 \pm 13.0\%$ *v.* $68.6 \pm 9.1\%$; and 4) $78.2 \pm 9.7\%$ *v.* $55.2 \pm 5.7\%$. The respective percentages of blastocysts on Day 5 after insemination were 1) $62.7 \pm 6.5\%$ *v.* $69.5 \pm 8.1\%$; 2) $67.9 \pm 7.7\%$ *v.* $41.6 \pm 5.6\%$; 3) $74.9 \pm 11.3\%$ *v.* $79.2 \pm 7.8\%$; and 4) $78.0 \pm 3.8\%$ *v.* $60.5 \pm 4.3\%$. On weighted ANOVA with angular transformations, the two-cell embryo and blastocyst rates were significantly lower in bpV(pic) groups than in control groups for treatments 2 and 4. Discussion: More oocytes tended to be collected in the bpV(pic) groups in 3 of the 4 experimental treatments. Different injection intervals between bpV(pic) and PMSG influenced both the superovulation efficiency and fertilizability of the oocytes. This new method using both PTEN inhibitors and gonadotropins is a promising method for improving superovulation efficiency, although the optimal injection scheme should be determined.

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