191 TIMING OF OVULATION IN THE GONADOTROPHIN-STIMULATED SOUTHERN HAIRY-NOSED WOMBAT, *LASIORHINUS LATIFRONS*

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The southern hairy-nosed wombat (SHW), *Lasiorhinus latifrons*, is a model species in which to develop assisted breeding techniques for the endangered northern hairy-nosed wombat, *Lasiorhinus krefftii*. We recently showed that anoestrus SHW respond to eutherian gonadotrophins by production of multiple ovarian follicles, but ovulation had not occurred at the time of examination 24 h post-LH (Druery GV *et al*. 2003 Theriogenology 59, 391 abst). This study investigated the timing of ovulation in six anoestrus captive adult female SHW ($n = 3$ per group) after ovarian superstimulation using porcine FSH (200 mg total, Folltropin-V, Bioniche, Belleville, Ontario, Canada) administered s.c. at 12-h intervals over 7 days. Ovulation was triggered by a single s.c. dose of porcine LH (25 mg Lutropin-V, Bioniche) 12 h after the final FSH injection. Superstimulatory response was determined by laparoscopy immediately after the final FSH injection on Day 7 prior to LH. Group 1 was re-examined at 33, 36, and 39 h post-LH, and Group 2 at 42, 45, and 48 h post-LH, for evidence of ovulations using laparoscopy and transabdominal ultrasonography. Laparoscopy on Day 7 revealed an ovarian follicular response in all six females, which coincided with the highest levels of estradiol. The reproductive tract also responded to the treatment (swollen fimbriae and enlarged, highly vascular uteri). Multiple follicles (range 16–31) up to 11 mm in diameter were observed in five females. One female had ovulated, as determined by the presence of corpora lutea. Transabdominal ultrasonographic imaging was unable to confirm the number of follicles in stimulated ovaries. Ovulation had commenced by 36 h post-LH, with the majority occurring 39–45 h post-LH. Ovulation was recorded as having occurred if a dark red, highly vascular crater on the surface of the newly formed corpus hemorrhagicum was observed. Increased circulating levels of progesterone were confirmed 9 days after the last laparoscopies. These results have important implications for the development of assisted reproductive technologies in the SHW: (1) transabdominal ultrasound imaging is ineffective for determining ovarian activity; (2) laparoscopy is a well-tolerated, repeatable minor surgical procedure that can be used for intrauterine AI in this species in which nonsurgical AI is unlikely to succeed (Paris DBBP *et al*. 2003 Theriogenology 59, 401 abst); and (3) knowledge of the timing of ovulation will enable insemination of spermatozoa into the uterus prior to ovulation.

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192 PROBLEMS USING JC-1 TO ASSESS MITOCHONDRIAL STATUS IN BROWN BEAR (*URSUS ARCTOS*) SEMEN

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Brown bear is a highly endangered species in Spain and could benefit from biological resource banking. Currently, we are studying several reproductive aspects in order to aquire the knowledge for establishment of a germplasm bank for this species. One of our objectives is to develop adequate protocols for the evaluation of bear sperm before and after cryopreservation. We have used the fluorescent probe JC-1 protocol, which differentially stains mitochondria, according to its activity (Garner DL *et al*. 1997 Biol. Reprod. 57, 1401–1406). Here we describe one problem that arose using this staining for evaluation of extended bear semen. We electroejaculated 13 adult brown bears (*Ursus arctos*) (206–311 kg) housed in a half-freedom regime in the Cabarceno Park (Cantabria, Spain). Anesthesia was performed with tiletamine + zolazepan (Zoletil 100®, 7 mg/kg; Virbac, Spain), and ketamine (Imalgene 1000®, 2 mg/kg; Mericl, Sain). We used an electroejaculator (PT Electronics®; Boring, OR, USA) with a 3-electrode transrectal probe (26 mm in diameter, 320 mm long). Ejaculation occurred at 10 V/250 mA. Samples were extended (prepared in our laboratory, Anel L *et al*. 2003 Theriogenology 60, 1293–1308; M3 modified) and cooled to 5°C for 70 min (pre-freezing protocol). We analyzed individual (MI) and progressive (MP) motility by means of an automated motility analyzer (Hamilton Thorne Biosciences, Inc., Beverly, MA, USA), using a phase contrast microscope (Nikon, ×10). Mitochondrial status was analyzed after diluting the sample 1:100 with buffered medium (20 mM HEPES, 153 mM NaCl, 2.5 mM KOH, 10 mM glucose; Sigma, Madrid, Spain) and adding JC-1 (6.8 μM final; Molecular Probes, The Netherlands). After 30 min at 37°C, 100 cells were counted with an epifluorescence microscope (Nikon, ×400), determining the percentage of sperm with orange-stained (active) mitochondria. We analyzed a total of 55 samples in three different models: fresh, pre-freezing, and thawed. We divided the samples into successful JC-1 staining (valids: V) or failed JC-1 staining (not valid: NV) (depending on the aspect of the stained cells). In not-valid samples we observed a greenish background, with almost no fluorescent spermatozoa. These observations were consistent in a given sample, giving the same V or NV result when we repeated the staining. In fresh and thawed groups there were no NV samples, but in the pre-freezing group there were 40 NV samples (73%). We calculated Pearson correlations (SAS; SAS Institute, Inc., Cary, NC, USA) between percent JC-1 orange population and MI and MP in fresh ($r = 0.40$ and 0.33; $P < 0.001$), thawed ($r = 0.61$ and 0.43; $P < 0.001$) and pre-freezing samples ($r = -0.11$ and -0.24; $P > 0.05$), all respectively. When pre-freezing samples were split between V and NV, the former had good correlations ($r = 0.74$ and 0.49; $P < 0.05$), and NV still did not ($r = -0.17$, -0.27; $P > 0.05$). We conclude that JC-1 staining is not reliable for the pre-freezing analysis of bear sperm, at least under the conditions described here. This could be due to the interaction of the extender or the refrigeration treatment with the sperm. However, this problem did not occur in the analysis of fresh and thawed samples. Nevertheless, it may be advisable to test other mitochondrial probes for analyzing this kind of samples.