## Reproduction biology

Time of ovulation and artificial insemination following superovulation in the southern hairy-nosed wombat (Lasiorhinus latifrons)

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The southern hairy-nosed (SHN) wombat (Lasiorhinus latifrons) is an ideal model for developing assisted reproductive techniques (ART) which may be used to increase the population size of its critically endangered sister species, the northern hairy-nosed (NHN) wombat (Lasiorhinus krefftii). Previously in SHN wombats, we demonstrated that multiple follicles are recruited and maturation to metaphase II occurs in 37 % of follicular oocytes recovered after treatment with 14. 3 mg porcine FSH (pFSH) every 12 h for 7 days, followed by a single 25 mg pLH injection 12 h later (DRUERY et al. 2003). The aims of this study were (1) to determine the timing of ovulation after superovulation and (2) to attempt artificial insemination (AI) after superovulation to produce live offspring. Six captive female wombats had their ovaries stimulated using the 7-day pFSH/pLH treatment protocol described above, and the timing of ovulation in relation to the pLH injection was determined using repeated laparoscopic examination (3 hourly). Ovulations were first observed 36 h after pLH injection (1 out of 33 mature follicles) and most follicles greater than 5 mm (39/51) had ovulated by 48 h post-pLH. Progesterone levels increased slowly after ovulation, indicating the presence of a luteal phase. This ovulation window was then used to time the delivery of semen by intra-uterine AI (IUAI) in SHN wombat females. Nine females were treated with the same pFSH/pLH protocol and inseminated 34h after the pLH injection with electro-ejaculated sperm (range  $1.5 - 3.0 \times 10^5$ /uterus). Females were examined using ultrasound and laparoscopy on ~day 18 post-IUAI for evidence of pregnancy and monitored for births at hourly intervals for 48 h from the expected time of parturition. Two pouch checks were then made at weekly intervals after the expected time of birth had passed. At the time of AI these females responded to pFSH by producing multiple ovarian follicles. By day 17 - 18 post-IUAI, laparoscopic observations of enlarged uteri and ultrasonographic images of structures resembling gestational sacs strongly suggested up to five females were pregnant. However, no live births were observed. The exact point at which embryo viability was compromised was not determined. This study has confirmed successful ovulation using the pFSH/pLH protocol and determined the window for ovulation in female SHN wombats to be 36 - 48 h postpLH injection. Further refinement of the insemination procedure is now required to increase fertilisation rates and improve post-conception survival, development and successful parturition of wombat neonates.

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