Predicting the success of Laparoscopic Artificial Insemination in sheep

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Artificial insemination (AI) plays a critical role in facilitating rapid genetic and production gains for the sheep industry, yet variable success between sites, sires and breeding seasons places it at risk of waning adoption. While several factors are thought to influence sheep fertility, their relevance during laparoscopic AI and the interplay between factors is unknown. As such, this study examined how various female factors collected at the time of AI and male semen factors assessed *in vitro* influence the success of intrauterine laparoscopic AI in sheep.

Over 3 breeding seasons, data was collected during 30 different AI programs in Australia using Merino rams (N=387) and ewes (N= 30,254). Ewes were synchronised and laparoscopically inseminated as per industry standards. During AI, the ID tag, age, uterine tone score (1; pale/flaccid-5; turgid/pink), intra-abdominal fat score (1; little to no fat present-5; high fat), time of insemination post CIDR removal and sire used, was recorded for each ewe. A subset of the semen used for insemination underwent advanced *in vitro* semen assessment. Thawed samples were diluted (1:0.5) in PBS + 0.3% BSA and immediately assessed for volume (mL), subjective motility (phase contrast microscopy), sperm concentration (x10^6 spm/mL; NucleoCounter SP-100), and morphology (% abnormal; x400 objective). Samples were then incubated and aliquots were taken at 0, 3 and 6h for motility and kinematics assessment (Computer-assisted semen analysis; HT CASA IVOS II) as well as sperm viability and acrosome integrity (FITC-PNA/PI), membrane fluidity (M540/Yo-Pro), mitochondrial superoxide production (Mitosox Red/Sytox Green), lipid peroxidation (Bodipy C11) levels of intracellular Reactive Oxygen Species (H_2DCFDA) and DNA fragmentation (Acridine Orange) via flow cytometry (CytoFLEX and Aroura 3L).

Transcutaneous ultrasound was performed approx. 55 days post AI. Statistical analyses (RStudio) included correlations, CASA Principal Component Analysis (PCA) and a binomial multivariable regression analysis.

The concentration at which sperm were frozen ($p<0.001$), CASA PCA at 0h (PCA; $p=0.03$), the percentage of viable, acrosome intact sperm at 6h ($p=0.02$), percentage of abnormal sperm ($p<0.001$), as well as uterine tone ($P<0.001$) and intra-abdominal fat ($p=0.03$) of ewes significantly influenced the likelihood of pregnancy following AI. A 1% increase in the percentage of acrosome intact, viable sperm, a 1% increase in morphology abnormalities and an additional 100 x10^6 spm/mL frozen corresponded to a 1.01% increase (OR = 1.10, 95% CL: 0.34, 2.56), 1.07% decrease (OR = 0.99, 95% CL: 0.98, 1.00) and 5.09% increase (OR = 1.05, 95CL: 1.05, 1.05) in the probability of a ewe falling pregnant, respectively. Furthermore, each standard deviation away from the CASA PCA average led to a 0.37% decrease in the probability of pregnancy (OR = 1.00, 95% CL: 1.00, 0.99). Furthermore, the odds of pregnancy increased by 7.21% (OR = 1.07, 95% CL: 0.44, 2.60) and 6.05% (OR = 1.07, 95% CL: 0.78, 1.47) when semen was inseminated into an ewe with a uterine tone and intra-abdominal fat score of 4+5 compared to ewes which scored 1+2 ($p<0.001$ and $p=0.047$, respectively).

The results demonstrate the benefit of analysing multiple male *and* female factors recorded during AI on individual ewes. The identified factors could now be used to standardise and further enhance AI protocols. Screening semen before AI could help minimise variability in success rates, improve the adoption of reproductive technologies, and ultimately advance the rate of genetic gain for the sheep industry.