125 FOLLICULAR FLUID AND GRANULOSA CELL RECOVERY FROM BOVINE FOLLICLES OF DIFFERENT DIAMETERS USING AN ADAPTED TRANSVAGINAL-GUIDED FOLLICULAR ASPIRATION SYSTEM

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Most studies of granulosa cell (GC) function have been performed *in vitro*, using follicular fluid (FF) and GC recovered from slaughterhouse ovaries. This approach does not consider the reproductive status and follicular developmental stage of the donor, limiting data interpretation and usefulness. The aim of this study was to evaluate the efficiency of an adapted ultrasound-guided transvaginal follicular aspiration (TVFA) procedure to recover FF and mural GC from live cows. A preliminary *in vitro* trial was performed to calculate fluid losses using a conventional TVFA circuit. A known volume of PBS, expected to be the volume of FF in follicles ranging from 4 to 12 mm in diameter, was aspirated using a 20 G needle connected to a Teflon circuit 80 cm long, with a 1.0-mm internal diameter, connected to a 1.5-mL tube. Losses of the expected volume of FF were 12.7 ± 1.1, 19.9 ± 2.5, 54.4 ± 4.0, 87.6 ± 4.3 and 100%, for follicles of 12, 10, 8, 6 and 4 mm in diameter (0.90, 0.52, 0.27, 0.11 and 0.03 mL, respectively). When an adapted system for small-volume recoveries was used, there was a decrease ($P < 0.05$) in fluid losses for follicles of 8, 6 and 4 mm. An experiment was performed *in vivo*, using the adapted system, to evaluate FF and GC recovery from follicles of 4 and 5 ($n = 16$), 6 ($n = 19$), 7 ($n = 7$), 8 ($n = 13$), 9 ($n = 15$), 10 ($n = 24$), 11 ($n = 11$), 12 ($n = 15$), 13 ($n = 13$), 14 ($n = 6$) and 16 ($n = 9$) mm in diameter. Follicular wave emergence was synchronized with 2 mg of oestradiol benzoate and an intravaginal progesterone device and follicles that reached the desired diameter were aspirated using an ultrasound machine equipped with a 7.5-MHz probe and disposable 20 G needle. The recovered FF volume was measured and centrifuged at $600 \times g$ for 10 min. The GC pellet was vortexed with 0.1% hyaluronidase (5 min) and washed twice in PBS and the number of cells was determined using a Neubauer chamber. Ribonucleic acid was extracted using an RNAeasy Microkit and quantified in NanoDrop. The efficiency of FF recovery was estimated by the difference between the recovered and expected volumes for each follicle diameter ($4/3\pi r^3$), which were compared by ANOVA. From all the follicles aspirated, the recovery of FF and GC was not successful in 2 (1.3%). Overall, FF recovery efficiency was 84.7%. The recovered volume ranged from 0.03 to 3.80 mL and increased with follicular diameter ($y = 0.011x^2 - 0.012x + 0.043$; $R^2 = 0.99$). Losses of FF were significant ($P < 0.05$) for follicles larger than 12 mm. The mean (±s.e.m.) number of mural GC recovered was $716{,}708 \pm 68{,}536$, providing 6.8 ± 0.7 samples of 100 000 cells with 14.8 ± 0.7 ng of RNA μL^{-1} for each punctured follicle. A high coefficient of variation (57.4%) was observed in cell recovery. There was no difference in the number of cells recovered from follicles of different diameters, but sample contamination with blood was more frequent (75%) in follicles larger than 10 mm. In conclusion, an adapted TVFA system can be used successfully on an individual basis and from follicles of different diameters for *in vivo* recovery of FF and GC for further endocrine and gene expression analyses.

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126 INFLUENCE OF PERIPHERAL PROGESTERONE CONCENTRATION ON MORPHOLOGICAL OOCYTE QUALITY IN REPEATED OVUM PICKUP SESSIONS

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Today, ovum pickup (OPU) followed by *in vitro* production (IVP) of bovine embryos is an integral part of many breeding programs. The quality of the obtained cumulus–oocyte complexes (COC) limits the success of embryo production. The developmental competence of the COC is dependent on several factors, including the stage of the oestrous cycle, the stage of follicular development and the follicular diameter. The aim of the present study was to determine the influence of circulating progesterone (P4) concentrations during repeated OPU sessions on the morphological quality of bovine COC. Cumulus–oocyte complexes were aspirated twice weekly for 5 to 6 weeks from 12 Holstein Friesian heifers. The first OPU session took place on Day 7 of the oestrous cycle (ovulation = Day 0). During each session, number and diameter of the punctuated follicles and diameter, consistency and cavities in the corpus luteum (CL) were recorded. Follicles were assigned to 3 groups according to their diameter (3 to 5 mm = small follicles; 6 to 8 mm = intermediate follicles; >8 mm = large follicles). Additionally, blood samples were taken at the time of each OPU session and blood P4 concentration was determined using a radioimmunoassay. The COC were categorised as IVP-suitable (round, ≥3 layers of cumulus cells, homo- or heterogeneous ooplasm) or unsuitable according to their morphological quality. All animals showed signs of oestrus accompanied by the presence of large follicles during the course of the OPU sessions. Statistical analysis was performed by an ANOVA followed by a Tukey test. A P-value of <0.05 was considered significant. The mean (±s.e.m.) cycle lengths for all heifers were 23.8 ± 4.6 days. Following the aspiration of a large follicle, a CL-like structure could be detected (referred to as "induced CL"). According to the P4 concentrations, the cycle was divided into 4 phases: natural CL phase (nCL; P4 ≥1 ng mL^{-1}), follicle phase 1 (Fp1; P4 <1 ng mL^{-1}), induced CL phase (iCL; P4 ≥1 ng mL^{-1}), or follicle phase 2 (Fp2; P4 <1 ng mL^{-1}). During the nCL phase, blood P4 concentrations were significantly higher than during the iCL phase (4.9 ± 2.3 ng mL^{-1}, $n = 12$ vs 3.0 ± 1.6 ng mL^{-1}, $n = 10$). There were no differences in follicle numbers, the diameter distribution of follicles, recovery rates, or number of retrieved IVP-suitable COC (nCL: 3.1 ± 3.4; Fp1: 3.3 ± 3.7; iCL: 2.7 ± 3.0; Fp2: 3.7 ± 3.7; Table 1). In summary, circulating P4 concentrations had no effect on follicle number, diameter, recovery rate, or IVP suitability of recovered COC.

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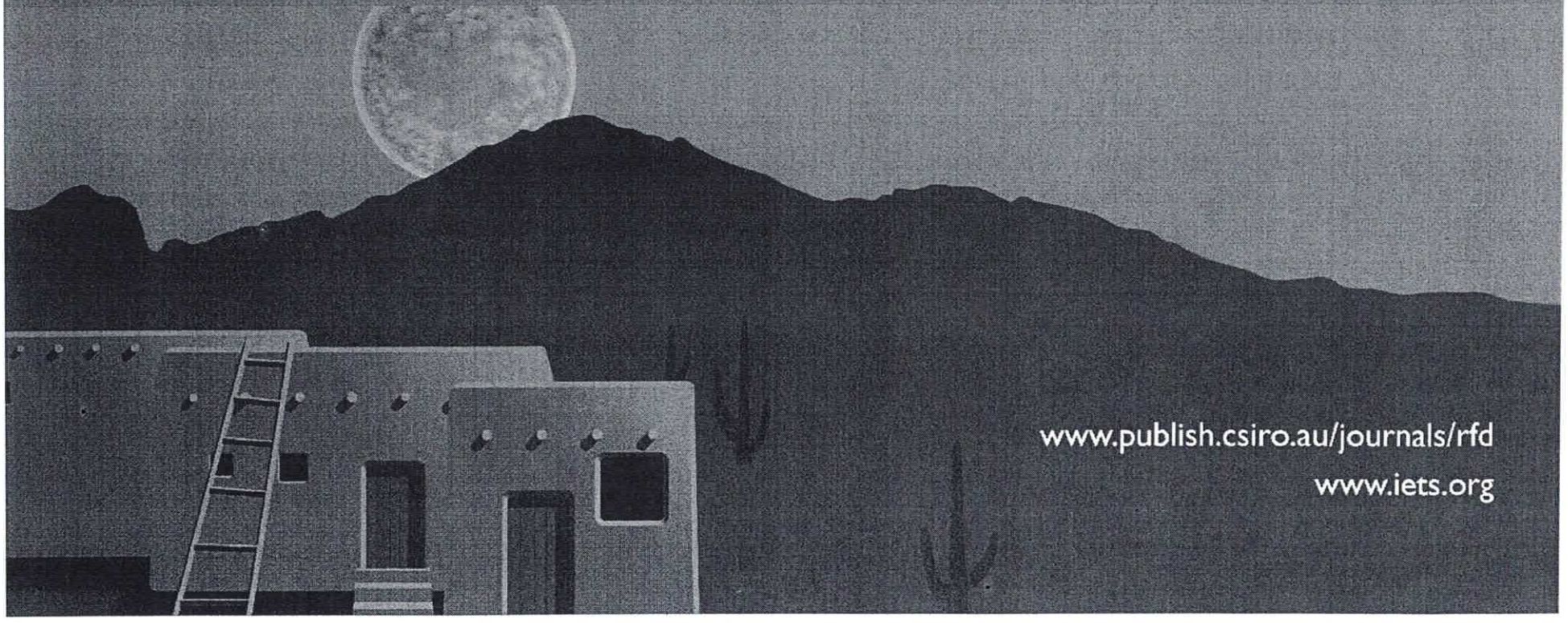

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