Animal Reproduction

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Physiology of reproduction in male and semen technology

Bovine oviductal fluid, the physiological additive for bovine sperm selection.

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Today, during a cycle for IVP of bovine embryos, the current blastocyst yield per oocyte is similar to the figures reported a decade ago, with only 30-40% of the collected oocytes reaching the blastocyst stage (Lonergan et al., Theriogenology, 81(1), 49-55, 2014). Since bovine oviductal fluid (BOF) is the physiological environment in which the last stages of capacitation and fertilization take place, we hypothesized that the inclusion of BOF into the composition of the sperm preparation medium might select a sperm population of better quality in terms of motility, viability and capacitation status, which will contribute to increasing the efficiency of the IVP process. Sperm quality was evaluated in terms of motility, viability, apoptosis, plasma membrane fluidity, and state of the acrosome. Asturian Valley bull sperm straws were thawed at 38 °C for 30 s. Each replicate (N=5) consisted of 4 straws from the same bull and a different bull was used in each replicate. BOF from the late follicular phase was acquired from EmbryoCloud (Murcia, Spain). Spermatozoa were selected by the swim-up method (Ruiz et al., Reproduction in Domestic Animals, 48(6) e81-e84, 2013). Two different media were used: Swim-up BSA, containing 6 mg/ml BSA (Parrish et al., Biology of Reproduction, 40(5), 1020-1025, 1988); and Swim-up BOF, where BSA was replaced by 1% BOF (v/v). To analyse sperm motility, sperm samples were evaluated at 38 ºC and 200X magnifications under a negative phase-contrast microscope coupled to a CASA system (ISASv1, ProiserR+D, Valencia, Spain). To evaluate viability, acrosomal integrity, and membrane fluidity of the samples by flow cytometry, the sperm concentration was set at $2x10^5$ spermatozoa per mL and the sperm suspension was incubated for 15 min at 37 °C with the following combinations of fluorochromes: i) 2.5 µg/mL propidium iodide and 1 µg/mL *Pisum sativum* lectin I conjugated with fluorescein isothiocyanate to assess sperm viability and acrosome integrity; and ii) 2.7 mM merocyanine-540 and 25 nM Yo-Pro1 to assess sperm viability and plasma membrane fluidity. Subsequently, samples were subjected to analysis by a Guava Easycyte 6-2L flow cytometer (Merck Millipore, Hayward, USA). Data, presented as mean ± SEM, were analyzed by the Student's t-test using the IBM SPSS Statistics package (IBM, Armonk, USA). Differences were considered significant when $P < 0.05$. BOF enabled a higher ($P < 0.01$) total and progressive motility, and higher kinematic parameters after swim-up, except for ALH and BCF. The percentages of spermatozoa viable, non-apoptotic and with non-reacted acrosome were similar in both groups. The proportion of spermatozoa with high membrane fluidity was higher after swim-up in BOF (37.5 ± 4.2%) than in BSA (21.9 ± 1.6%; $P < 0.05$). To sum up, the inclusion of BOF into the composition of the sperm preparation medium selects a population of functional spermatozoa with improved motility, which might contribute to increasing the fertilizing ability of impaired semen samples and so, enhance the efficiency of the IVP of bovine embryos. Supported by Fundación Séneca, Región de Murcia, Spain (21651/PDC/21).

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