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Towards serum-free culture conditions for bovine endometrial explants: some preliminary results

Davoud Eshghi Chaharborj^{1,2}, Bartłomiej M. Jaśkowski^{1,3}, Ugur Comlekcioglu¹, Mojtaba Kafi², Osvaldo Bogado Pascottini^{1,4}, Geert Opsomer¹

¹Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium;

²Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; ³Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Poland; ⁴Gamete Research Center, Laboratory for Veterinary Physiology and Biochemistry, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium; davoud.eshghi@ugent.be

Bovine endometrial explant culture has emerged as a model for the study of *in vitro* uterine function. Culture medium for endometrial explants is routinely supplemented with fetal bovine serum (FBS) as it contains hormones, vitamins, transport proteins, and growth factors that optimize *in vitro* endometrial function. However, there is an increasing interest in using serum-free conditions as a more defined medium and due to sanitary and animal-welfare concerns (FBS is an animal-derived product). Yet, the optimal serum-free culture medium composition and incubation time window for endometrial explants remain to be elucidated. This study aimed to evaluate the effect of serum and serum-free culture medium and incubation time on endometrial explant viability markers. Five uteri from healthy Belgian Blue cows at the diestrus stage (stage I corpus luteum) with no evident signs of gross inflammation were collected from the slaughterhouse and transported to the laboratory within 1 h. Intact endometrium tissue samples (8 mm in diameter; 1–2 mm in thickness) were obtained using a sterile 8-mm punch biopsy. The explants were cultured for 48 h in Dulbecco's modified Eagle's medium (DMEM) containing 50 µg/mL gentamicin and supplemented with, (1) control (no supplementation), (2) 10% FBS, (3) 10% serum replacement (SR) (Knockout™ SR, Gibco), and (4) 1% bovine serum albumin (BSA). Spent culture medium samples were collected at 6, 12, 18, 24, 30, 36, 42, and 48 h of incubation and assayed for interleukins (IL-1β and IL-6) using bovine-specific ELISA kits. Indirect assessment of tissue viability was measured through lactate dehydrogenase (LDH) activity (colorimetric test) in the spent culture media. The effect of culture medium composition, incubation time, and their interaction on IL-1β, IL-6, and LDH concentrations were fitted in linear regression models in RStudio. No differences ($P > 0.05$) within the first 24 h of culture were found among experimental groups for IL-1β. Reduced IL-1β concentrations ($P < 0.05$) were found in FBS compared with SR at 30 and 42 h of incubation. Control and FBS culture medium had lower IL-6 concentrations at 12, 18 and, 24 h compared with the other groups ($P < 0.05$). The LDH activity was higher ($P < 0.05$) for FBS than SR (6 and 18 h) and BSA (12, 18, 24, and 30 h). The present study shows that a serum-free medium is a valid alternative for short-term bovine endometrial explant culture. However, high LDH activity may suggest that endometrial explants viability significantly declines after 24 h of incubation irrespective of culture conditions.

Keywords: Endometrium, explant culture, serum-free medium