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Folliculogenesis, oogenesis, and superovulation

Optimization of a superovulation protocol for the collection of in vivo matured oocytes from Holstein Friesian heifers

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In vivo matured oocytes have superior developmental competence to those matured *in vitro*. As such, the study of *in vivo* oocyte maturation supports the refinement of the *in vitro* maturation system. Therefore, this work aimed to optimize a protocol to collect *in vivo* matured oocytes from Holstein Friesian heifers. To do so, animals (n=5) were synchronized using an intravaginal device (CIDR, Zoetis Belgium). Eight days later, the device was removed and heifers received 0.5 mg of cloprostenol (2 ml of Cyclix (prostaglandin; PG) Virbac, France). Estrus (=day 0) was confirmed two days later by ultrasound. To induce ovulation, the heifers were treated with 10 µg of buserelin (2.5 ml of Receptal (GnRH), Intervet, Germany) on day 8 of the estrous cycle. From day 10, the heifers were superovulated with 180 mg of FSH (Folltropin, Vetoquinol, Canada) administered twice daily for four days in decreasing doses (1.5, 1.5, 1.25, 1.25, 1, 1, 0.75 and 0.75 ml respectively). On day 12, animals received 0.75 mg (3 ml) of PG and LH surge was induced with 10 µg (2.5 ml) GnRH administered 40 h after the PG injection. To determine the optimum timing of follicle aspiration, we tested four timing protocols (P1, P2, P3, P4) based on the time-post-GnRH treatment (P1=20h, P2=21.5h, P3=22.5h and P4=24h) at which OPU was performed. Only one animal was used in each protocol, except in P4 which included two heifers. Follicles ≥ 8mm in diameter were aspirated using a 18 gauge needle. A 130 mmHg vacuum pressure was used due to the presence of expanded and sticky cumulus cells characteristic of oocyte maturation. Before follicle aspiration, the OPU tubing system was rinsed with PVP medium (0.3% PVP (PVP-360; Sigma) in Ca- and Mg-free PBS + 10 IU/ml heparin (Sigma)). The follicular content was collected in a 50 ml conical tube and cumulus-oocyte complexes were recovered under a stereomicroscope and stored in EmXcell medium without BSA (imv-technologies, France) until the end of the collection procedure. To assess maturation status, all oocytes (n=45) were denuded in 0.1% hyaluronidase (Sigma) in PVP medium for approximately 3 min and by pipetting until all cumulus cells were removed from the oocyte. Subsequently, the presence of the first polar body (PBI) was evaluated and the denuded oocytes were stored for further molecular analysis. In P1, a total of 8 oocytes were collected and the PBI-extrusion rate was 0%. Similarly, in P2, we collected 3 oocytes and the PBI-extrusion rate was 0%. Interestingly, at 22.5h after GnRH injection (P3), the PBI extrusion rate was 20% (2/10). A greater PBI extrusion rate was observed in P4 where the rate was 37.5% for both heifers (3/8 and 6/16) used in this protocol. The present results suggest that the optimum timing of aspiration of *in vivo* matured oocytes is beyond 24h after GnRH injection. Moreover, the hormonal stimulation treatment we used could be considered an efficient method to collect in vivo matured bovine oocytes. This work also provides insights into using defined and serum-free media when performing non-standard experiments on livestock for further molecular studies on *in vivo* oocyte maturation.

Keywords: OPU, oocyte, cattle