

***In Vitro* Embryo Production can be Achieved After Delayed Harvest of Bovine Oocytes from Abattoir-derived Ovaries**

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Oocytes from abattoir-derived ovaries are widely utilized for *in vitro* production (IVP) of bovine embryos. Most current protocols require that cumulus-oocyte-complexes (COC) be harvested from the ovary within a few hours of collection. Opportunities exist for additional research and flexibility in IVP embryo protocols if abattoir ovaries can be maintained for longer periods of time before COC collection. We hypothesized that blastocysts can be produced from ovaries maintained at 15°C for 17 or 24 hours (h) beyond normal COC harvesting time. Ovaries were obtained from an abattoir and transported 3.5 h to the laboratory at 20 – 25°C in saline containing antibiotics and antimycotics. Ovaries were then either used immediately (control) or maintained at 15°C for an additional 17 h or 24 h. All COCs were matured (25 COCs/drop; 4 drops/treatment/replicate; 8 replicates) and fertilized using pooled Holstein semen. Presumptive zygotes were cultured in SOF-BE1 drops with cleavage visually evaluated on d 2 post-fertilization. Blastocyst development was assessed on d 7.5. A portion of blastocysts (25 embryos/treatment) were assessed by immunofluorescent detection of CDX2, a trophoblast marker (TE) marker, and DAPI counterstaining for total nuclei. Cleavage rate was reduced ($P < 0.003$) for the 17h group ($61.1\% \pm 3.5\%$) and the 24h group ($59.4\% \pm 3.7\%$) compared to the control group ($78.0\% \pm 2.1\%$). The percentage of blastocysts generated from total zygotes was greatest in the control group ($29.2\% \pm 2.5\%$), tended to be reduced ($P = 0.06$) in the 17 h group ($24.0\% \pm 2.3\%$), and was reduced ($P = 0.002$) in the 24 h group ($10.6\% \pm 1.1\%$). Blastocyst formation as a percentage of cleaved embryos demonstrated no differences between the control and 17 h group ($48.2\% \pm 5.9\%$ vs $41.7\% \pm 3.2\%$, respectively), but the percentage of blastocysts formed was reduced ($P = 0.02$) in the 24 h group ($20.1\% \pm 4.1\%$). Total cell number, TE cell number and ICM:TE ratio did not differ for blastocysts in each treatment, but ICM cell number was reduced ($P = 0.04$) in the 24h group when compared to the control group. No differences in ICM cell number were detected between the control and 17 h treatment groups. In summary, abattoir ovaries maintained in saline at 15°C for 17 h prior to COC collection contained a slight reduction in cleavage rates but otherwise produced blastocysts at the same rate with the same TE and ICM composition as COCs harvested at the conventional time. Delaying harvest by 24 h was harmful to blastocyst yields and quality. This project was supported by the Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-34485 from the USDA National Institute of Food and Agriculture.