Application: Bovine embryo *in vitro* production remains inefficient. One of the problems is the acquisition of competence by aspirated oocytes to develop to an embryo after *in vitro* maturation. Meiosis inhibitors, such as C-type natriuretic peptide used at the pre-maturation step to increase oocyte competence, may help to improve blastocyst outcome.
Introduction: In an ovary, meiotic arrest enables oocyte growth, along with gaining developmental competence. Oocytes collected by ovum pick up require maturation regardless of insufficient competence level. The proportion of oocytes that develop to the blastocysts stage following *in vitro* maturation (IVM) and *in vitro* embryo production remains low (Lonergan et al., 2006). The inclusion of a pre-maturation step, which inhibits resumption of meiotic maturation, but facilitates improvement of oocyte developmental competence may improve blastocyst yield (Xi et al., 2018).
Materials and Methods: Bovine ovaries were obtained from a local slaughterhouse. Cumulus Oocyte Complexes (n = 729) were collected by aspiration. During the pre-maturation step oocytes were cultured in media supplemented with 200 nM (group 1; n = 259) or 200 nM C-type natriuretic peptide and 100 nM 17β-oestradiol (group 2; n = 289) for 6 h, followed by IVM for the next 24 h. The control group (n = 181) was matured for 24 h without pre-maturation. After 10 h of fertilization presumptive zygotes were transferred to culture medium for 8 days. Cleavage (day 3), blastocyst (day 7) and hatched blastocyst (day 8) rates were recorded and compared. A chi-square test (Statistica 13.3.) was performed to analyse whether the pre-maturation step affects the rate of cleavage, blastocysts on day 7 and 8 or hatching. The effect was considered significant at $P \leq 0.05$.
Results: Inclusion of the pre-maturation step in group 2 did not improve cleavage, day 8 blastocyst or hatched blastocyst rate: 200 (69.2%), 82 (28.3%), 54 (18.6%) compared to control group: 130 (71.8%), 45 (24.9%), 31 (17.1%), respectively. However, the day 8 and hatched blastocyst ratio in group 1 was significantly lower ($P \leq 0.05$) than that of the control group: 44 (17.0%), 25 (9.7%), respectively.
Conclusions: Despite the use of the pre-maturation step with C-type natriuretic peptide and oestradiol, a higher outcome of blastocysts was not obtained. Further studies of other combinations of meiosis inhibitors are in progress.
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References

Lonergan, P., Fair, T., Corcoran, D., Evans, A.C.O., 2006. Theriogenology 65, 137–152.
Xi, G., An, L., Jia, Z., Tan, K., Zhang, J., Wang, Z., Zhang, C., Miao, K., Wu, Z., Tian, J., 2018. Theriogenology 106, 198–209.

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Measuring cumulus expansion of bovine cumulus-oocyte complexes: comparing the reliability of three methods

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Application: To define the most repeatable method for non-invasive cumulus expansion measurement.
Introduction: Evaluating cumulus expansion is a commonly used approach to determine oocyte quality. Although several methods have been described to assess cumulus expansion non-invasively, all methods are subjective and no gold standard is declared. Therefore, three methods were compared to determine the most reliable evaluation for cumulus expansion measurements.
Materials and Methods: Bovine cumulus-oocyte complexes (n = 232) were individually matured *in vitro* for 22 h in 20 µl droplets of tissue culture medium-199 in 5% CO_2 in humidified air at 38.5°C and their images were acquired at 0 h (immature) and 22 h (mature) of *in vitro* maturation. Three observers evaluated cumulus expansion from these images, whereby every observer applied three methods: (1) area (comparing the area of immature vs mature cumulus-oocyte complexes); (2) 3-distance (calculating the shortest, median and longest distance between zona pellucida and extreme of cumulus before and after maturation); and (3) scoring (scoring on a Likert scale ranging from 0 to 4, with 0 = no expansion and 4 = complete expansion). Observers performed all evaluations in duplicate. The reliability of each method was calculated in Python, using a two-way random effects model for inter-observer agreement and a one-way random effects model for intra-observer agreement. Consequently, intra-class correlation coefficients (ICC (95% confidence interval)) were calculated and interpreted as follows: <0.50, poor; 0.50–0.75, moderate; 0.75–0.90, good; >0.90, excellent agreement.
Results: Inter-observer agreement (ICC (95% CI) was good for the area method (0.90 (0.88–0.92)), moderate for the 3-distance method (0.56 (0.49–0.63)) and poor for the scoring method (0.23 (0.12–0.34)). Similarly, for intra-observer agreements, the area method resulted in a good to excellent agreement for observer 1, 2 and 3 (0.87 (0.84–0.9); 0.90 (0.87–0.92); 0.96 (0.95–0.97) respectively). Intra-observer agreement (ICC (95% CI) was moderate for the 3-distance method for observer 1, 2 and 3 (0.61 (0.53–0.69), 0.59 (0.5–0.67), and 0.64 (0.56–0.71), respectively). The scoring method resulted in a poor to moderate intra-observer agreement (0.69 (0.63–0.76), 0.11 (−0.01 to 0.24) and 0.51 (0.42–0.6)) for observer 1, 2 and 3 respectively.
Conclusions: Although scoring cumulus expansion on a Likert scale is the most cited method, this study showed that it was the most vulnerable to subjective interpretation. Application of the area method resulted in the most reliable evaluation of cumulus expansion, since this method achieved best inter- and intra-observer agreements.

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Transcriptome response of oocytes to seasonal heat stress in beef cows

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Application: Identify pathways and associated genes that may play an important role in the response of oocytes to elevated temperatures in cows.
Introduction: Reduced reproductive performance due to seasonal heat stress is a major problem in the beef and dairy industries. Examining the effects of heat stress on the molecular mechanism of bovine oocytes can help to better understand their alterations on a transcriptional level and to plan and mitigate those effects. We aimed to study the response of oocytes to seasonal thermal stress.
Materials and Methods: Multiparous angus dry beef cows ($n = 11$) were kept together during the study and subjected to synchronisation and stimulation of follicular growth using a 5-day CIDR and follicle-stimulating hormone (FSH) protocol. Ovum pick-up (OPU) was conducted on all animals in the winter (January) and summer (August). Cumulus-oocyte-complexes (COCs) were isolated from the follicular fluid aspirated during the OPU procedure. Denuded oocytes were further isolated from the COCs, snap frozen, and stored at −80 °C until further use for library sequencing, RNA sequencing, bioinformatics and gene enrichment analysis. Additionally, rectal temperatures were recorded on the day of each OPU. Environmental data were collected daily three weeks before the day of each OPU using the Florida Automated Weather Network. Statistical analysis for average rectal temperature included overall mean (average rectal temperature), treatment effects (season), and the residual. Treatment effect was considered significant at $P \leq 0.05$. Data are presented as mean ± standard error of the mean. RNA was extracted from five biological replicates/pools of oocytes (each containing $n = 2$) followed by library preparation and sequencing (NovaSeq; Illumina).
Results: As expected, environmental conditions were contrasting [average air temperature (11.5 °C vs 27.5 °C), average max air temperature (16.9 °C vs 33.7 °C), relative humidity (83.5% vs 82.3%), and temperature-humidity index (53.39 vs 79.16) for winter and summer, respectively]. Average rectal temperature was greater ($P = 0.03$) in summer (39.2 ± 0.2 °C) than in the winter (38.8 ± 0.2 °C). Data analysis revealed an up-regulation of 446 transcripts and a down-regulation of 940 transcripts in oocytes collected during summer compared to winter (Fold Change ≥ 2; FDR P-value ≤ 0.05). Upregulated transcripts are involved in protein digestion and absorption, ATP-binding cassette transporter, oocyte meiosis, and progesterone-mediated oocyte maturation pathways. Conversely, down-regulated transcripts are involved in pathways related to extracellular matrix-receptor interaction, focal adhesion, and PI3K-Akt signaling.
Conclusions: In conclusion, exposure of cows to thermal stress can significantly alter the transcriptome of oocytes, which may negatively impact subsequent developmental competence.

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Effect of oocyte recovery method on bovine embryo development using an individual *in vitro* culture system

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Application: Acquire data on molecular factors affecting oocyte competence to refine *in vitro* bovine embryo production.
Introduction: Developing suitable techniques for oocyte collection and maturation is necessary to efficiently study molecular markers of oocyte competence using slaughterhouse bovine ovaries. An individual *in vitro* culture system allows tracking the development of immature oocytes to the blastocyst stage. However, the conventional technique of follicle aspiration (FA) to retrieve oocytes precludes information on the follicle of origin, critical for molecular studies. We aimed to evaluate follicular dissection (FD) as an alternative technique to recover oocytes when investigating molecular dynamics of oocyte competence.
Materials and Methods: Slaughterhouse-derived genital tracts were collected and ovaries were selected if a corpus luteum was present in the contralateral ovary and no follicles ≥15 mm were present in the ovary used for oocyte collection. Selected ovaries were cut in half before processing them for FD or FA respectively. Cumulus-oocyte complexes (COCs; $n = 47$) were liberated after FD by rupturing the follicles. Recovery of COCs ($n = 64$) by FA was performed using a 10 ml syringe fitted with an 18-gauge needle. Only 2–8 mm follicles were considered and only COCs with ≥5 compact cumulus layers were selected for individual *in vitro* maturation-fertilization-culture as described previously (Azari-Dolatabad et al., 2019). Group culture (GC) of COCs ($n = 310$) selected from a random pool of ovaries was per-