

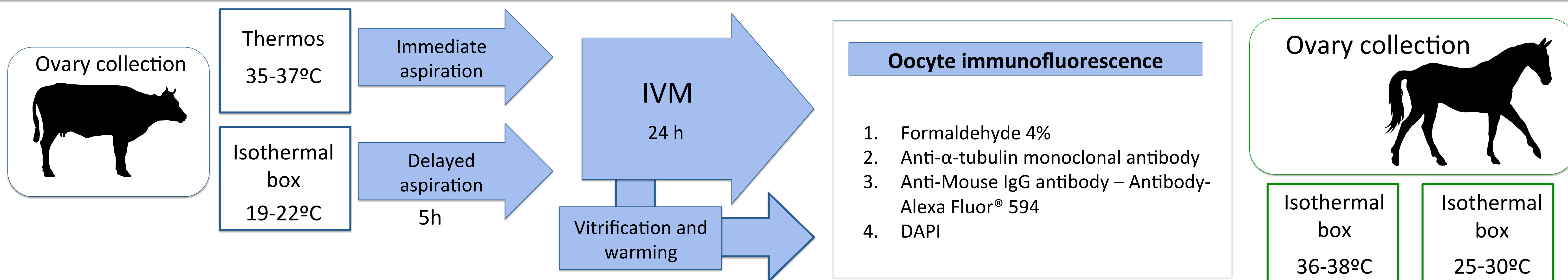
Introduction and Objectives

During ovaries transport from the abattoir to the laboratory, the oocytes in follicles undergo ischemic conditions. To alleviate adverse effects, time of ovary storage must be as short as possible. However, when it is not possible, decreasing the ovary storage temperature would be an strategy to avoid ischemic damage. Until today, ovaries processed at the Bovine IVF Laboratory at the Veterinary College of UAB have been transported to the laboratory at 35-37°C and processed upon arrival. A new protocol is proposed which consists in storing the ovaries at room temperature and delaying the processing of the ovaries for 5 hours.

The purposes of this study are:

- ✓ To evaluate the effect of different storage protocols of bovine ovaries on oocyte quality measured by the assessment of the maturation rate and the meiotic spindle configuration after IVM.
- ✓ To evaluate the effect of different storage protocols of bovine ovaries on the oocyte response to a vitrification/warming process, by the assessment of the maturation rate and the meiotic spindle configuration.
- ✓ To evaluate the effect of different storage protocols of equine ovaries on oocyte quality measured by the assessment of the nuclear maturation rate.
- ✓ To set up an optimum transport and storage protocol for slaughterhouse ovaries that maximizes the quality of the collected oocytes, both in bovine and equine species.

Materials and Methods



In vitro maturation stages and meiotic spindle morphologies

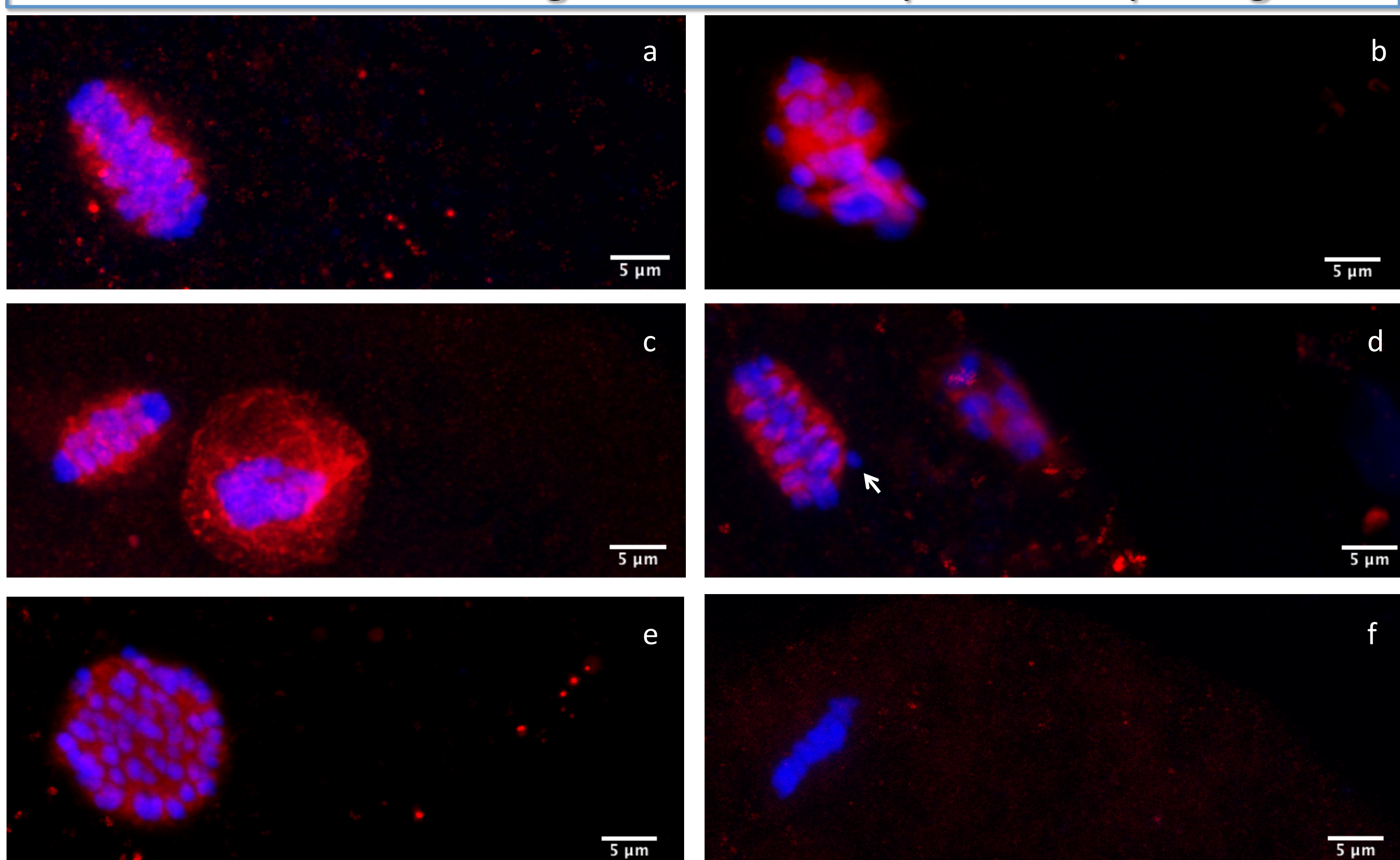


Figure 1. Confocal laser-scanning photomicrograph of bovine oocytes at different stages of *in vitro* maturation (a-c) and alterations observed in the spindle morphology and chromosome configuration of bovine MII oocytes (d-f).

Oocytes were immunocytochemically stained to visualize the microtubules (blue) and counterstained to visualize chromosomes (red). **a:** Metaphase I stage oocyte with microtubules having formed a clear meiotic spindle with chromosomes aligned at its equator. **b:** Anaphase I stage with chromosomes moving away from the the meiotic spindle equator. **c:** Normal barrel-shaped metaphase II spindle with compact chromosomes arranged at the equator of the structure. **d:** Abnormal meiotic spindle structures associated with disorganized chromosomes. **e:** Cross section of meiotic spindle with chromosomes partially dispersed and disorganized microtubules. **f:** Condensed and aligned chromosomes and absence of microtubules.

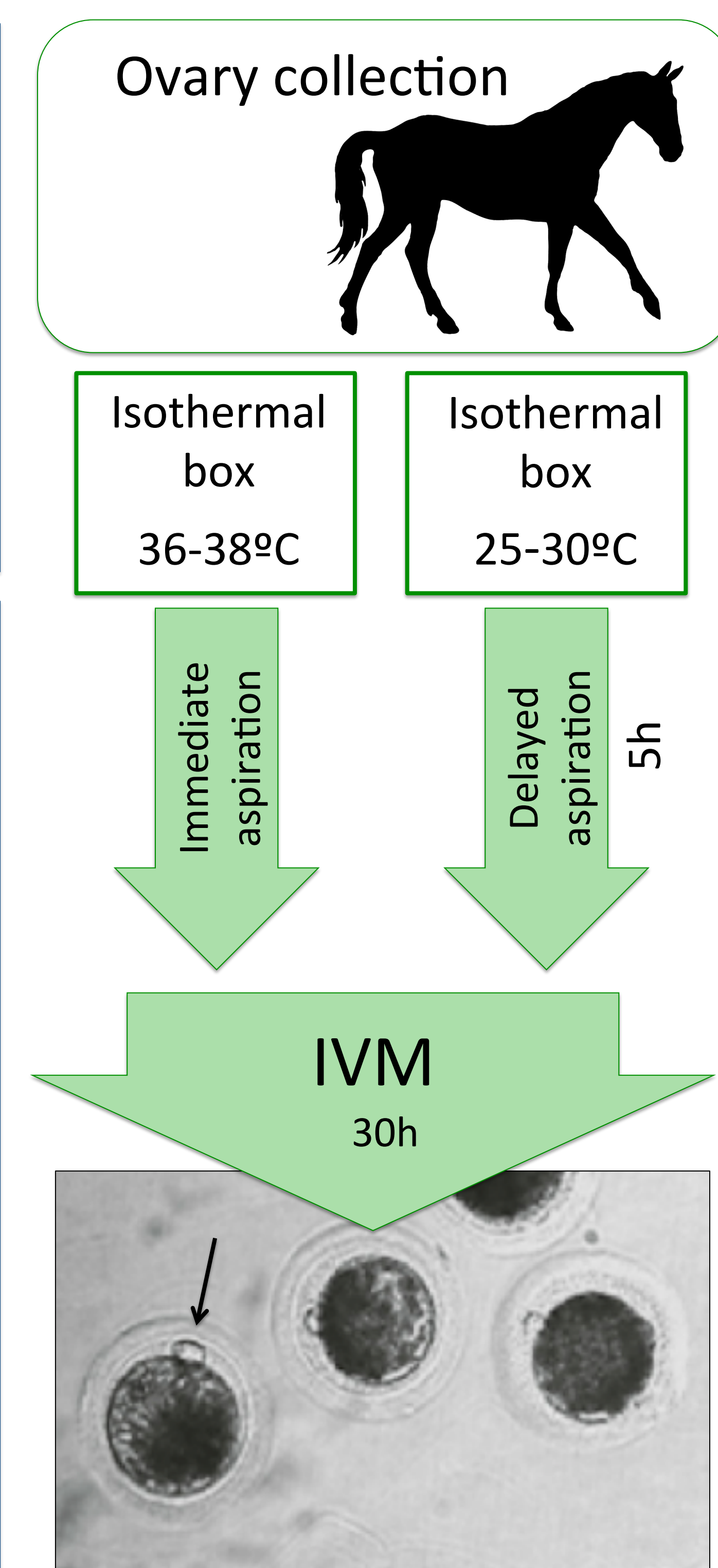
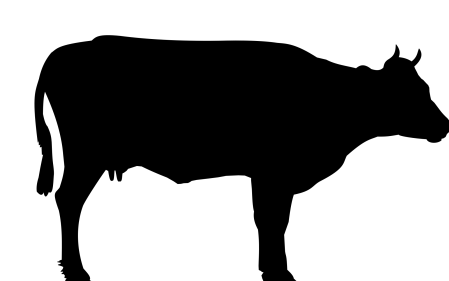


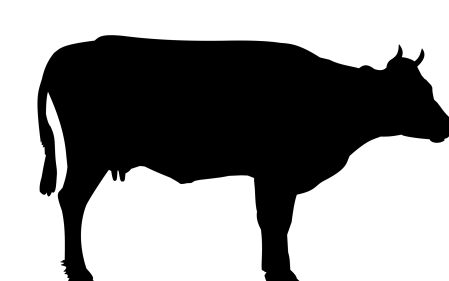
Figure 2. Equine matured oocytes with the first polar body extruded (from Curcio et al., 2014)

Results



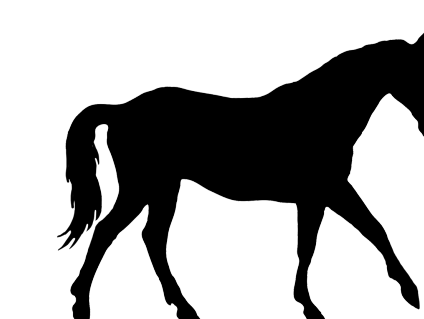
Effect of the ovary temperature during the transport on the maturation rate and meiotic spindle of fresh bovine oocytes

Treatment	n	MII, n (%)	Chromosome morphology			Microtubule morphology		
			Normal	Dispersed	Decondensed or absent	Normal	Dispersed	Absent
35-37°C	40	40 (100) ^a	30 (7,5) ^a	8 (20) ^a	2 (5) ^a	30 (75) ^a	8 (20) ^a	2 (5) ^a
19-22°C	188	87 (73,7) ^b	30 (34,5) ^b	56 (64,4) ^b	1 (1,1) ^b	30 (34,5) ^b	56 (64,4) ^b	1 (1,1) ^b



Effect of the ovary temperature during the transport on the maturation rate and meiotic spindle of vitrified/warmed bovine oocytes

Treatment	n	MII, n (%)	Chromosome morphology			Microtubule morphology		
			Normal	Dispersed	Decondensed or absent	Normal	Dispersed	Absent
35-37°C	74	53(71,6) ^a	15(28,3) ^a	21 (39,6) ^a	17 (32,1) ^a	16 (30,2) ^a	17 (32,1) ^a	20 (37,7) ^a
19-22°C	48	26(54,2) ^b	2(7,7) ^b	19 (73,1) ^b	5 (19,2) ^b	2 (7,7) ^b	23 (88,5) ^b	1 (3,8) ^b



Effect of the ovary temperature during the transport on the recovery rate and the nuclear maturation rate of equine oocytes

Treatment	n	Total oocyte	Recovery rate	% Nuclear maturation
36-38°C	48	107	2,23	78,5 ^a
25-30°C	96	200	2,08	43,5 ^b

Bovine oocytes from ovaries stored at room temperature showed a lower maturation rate, more abnormalities of the meiotic spindle and a worse response to vitrification and warming process

No significant differences were observed between treatments in the recovery rate of mare ovaries.

Significant differences were observed between treatments in the nuclear maturation rate of equine oocytes.

Conclusions

- ✓ Storing bovine ovaries at room temperature for 5 hours decreases the maturation rate and increases abnormalities in the meiotic spindle of oocytes.
- ✓ Response to vitrification and warming is worse when the bovine ovaries are transported and stored for 5 hours at room temperature.
- ✓ Storing equine ovaries at room temperature for 5 hours does not affect the recovery rate but decreases the nuclear maturation rate.
- ✓ The optimum protocol for bovine and equine ovaries consists in processing them upon arrival at the laboratory.