Effect of potential oocyte transport protocols on blastocyst rates after intracytoplasmic sperm injection in the horse

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Summary

Reasons for performing study: Intracytoplasmic sperm injection (ICSI) is used to produce foals from otherwise infertile mares and from stallions with limited sperm stores, but requires expensive equipment and is technically demanding. Methods to transport oocytes to ICSI laboratories would allow collection of oocytes by the referring veterinarian and enable greater application of this technique.

Objectives: This study was conducted to evaluate protocols that could be used to transport immature and maturing oocytes for ICSI.

Study design: In vitro experiment.

Methods: Oocytes were recovered by transvaginal ultrasound-guided follicular aspiration either from dominant follicles 24 h after deslorelin administration (dominant stimulated follicle [DSF]), or from subordinate (immature) follicles at the same time. To mimic transport, DSF oocytes were incubated overnight under differing conditions before ICSI; immature oocytes were placed in varying conditions overnight before in vitro maturation, followed by ICSI. The rate of blastocyst production was compared among treatments.

Results: Blastocysts were produced in all groups. Dominant stimulated follicle oocytes held in sealed tubes in pre-equilibrated control maturation medium maintained at 37°C yielded blastocyst development equal to that obtained for control incubated oocytes (70%). Dominant stimulated follicle oocytes held similarly in a warm passive device yielded poor blastocyst development (10%). Immature oocytes held for one or 2 nights in modified M199 medium, or for one night in commercial embryo holding solution, in air at room temperature, yielded 35–37% blastocyst development per injected oocyte.

Conclusions: A commercially available medium can be used for shipping immature oocytes at room temperature with good resulting blastocyst rates. Better blastocyst rates per oocyte are obtained from DSF oocytes; however, these require maintenance at 37°C and as they are already maturing at the time of collection, are more sensitive to delays. This new, practical information supporting transport of both immature and DSF oocytes for ICSI may allow wider use of this procedure.

Keywords: horse; oocyte; intracytoplasmic sperm injection; embryo transfer; in vitro fertilisation

Introduction

In vitro embryo production via intracytoplasmic sperm injection (ICSI) is a useful tool to produce foals from mares unable to produce their own embryos, from stallions with limited sperm numbers or sperm quality and even from mares post mortem [1–4]. Unfortunately, ICSI requires expensive equipment and is technically demanding, limiting its availability to a few locations. Development of systems for transporting oocytes would expand access to the technique; oocytes could be recovered by the referring veterinarian and shipped to the ICSI facility for embryo production. This would allow use of ICSI by owners or veterinarians who do not currently have access to it. Some commercial ICSI is already occurring using transported oocytes [4], but limited information is available concerning optimal shipping protocols or results.

Equine oocytes of 2 types can be collected for clinical assisted reproduction: in vivo maturing oocytes recovered from the preovulatory follicle of a mare in oestrus, 24–35 h after gonadotropin stimulation (dominant stimulated follicle [DSF]) and immature oocytes collected from small or subordinate (immature) follicles at any time in the oestrous cycle. Oocytes from DSF have the advantage of natural selection and physiological maturation and therefore have higher developmental competence than do oocytes matured in vitro [3,5,6]. In addition, DSF oocytes are easier to recover than are immature oocytes, having a typical recovery rate of 75–85% [7,8]. However, DSF oocytes may be damaged during handling because they are actively in meiosis; even minor cooling can cause depolymerisation of the meiotic spindle, with limited ability for recovery [9–11]. Furthermore, because meiosis has resumed in these oocytes, a delay during shipping could result in oocyte ageing and suboptimal timing for fertilisation.

Collection of immature oocytes from smaller immature follicles via transvaginal ultrasound-guided aspiration can be performed whenever follicles are present, greatly facilitating scheduling, and can be set up on a fixed biweekly or similar timetable [5]. Although not all oocytes recovered will mature, embryo production may be greater for immature aspiration than for DSF aspiration alone, because more oocytes may be obtained per aspiration session [5,12]. Notably, immature oocytes can be held at room temperature overnight before in vitro maturation without detrimental effects on maturation rate or blastocyst development, thus facilitating shipping [4,13].

This study was performed to evaluate media and conditions potentially suitable for shipping of DSF and immature oocytes before ICSI. Rates of oocyte maturation to metaphase II and blastocyst production after ICSI were compared among treatments.

Materials and methods

Follicle aspiration

Mares: The study was conducted from September to December of one year. Oocyte donors were 28 Quarter-type mares 8–12 years of age, housed outside in paddocks and fed grain daily and ad libitum hay and water. All experimental procedures were performed according to the United States Government Principles for the Utilisation and Care of Vertebrate Animals Used in Testing, Research and Training.

Procedure: Mares were examined by ultrasonography per rectum daily during oestrus. When the diameter of a growing dominant follicle was ≥35 mm in the presence of uterine oedema, 1.8 mg deslorelin (SuproMate®) was administered i.m. Follicular aspiration was performed 24–27 h later.

For aspiration, a Sonosite Micromax ultrasound machine with a 5–10 MHz microconvex ultrasound probe and Minitube transvaginal needle guide was used. The probe was inserted transvaginally. The ovary was manipulated transrectally to the cranial vaginal wall to visualise the dominant follicle, which was punctured and aspirated using a 12-gauge
Transport of equine oocytes for ICSI

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TABLE 1: Embryo development after intracytoplasmic sperm injection (ICSI) of oocytes recovered from stimulated dominant follicles

<table>
<thead>
<tr>
<th>Group</th>
<th>Medium</th>
<th>Equilibration in mixed gas</th>
<th>Incubation conditions</th>
<th>Oocytes injected (n)</th>
<th>Cleaved (n)</th>
<th>Blastocysts (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MatM</td>
<td>Yes</td>
<td>37°C/mixed gas</td>
<td>10</td>
<td>8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>MatM</td>
<td>Yes</td>
<td>Sealed/37°C air</td>
<td>10</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>mEH</td>
<td>Yes</td>
<td>Sealed/37°C air</td>
<td>10</td>
<td>8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>1990HT</td>
<td>Yes</td>
<td>Sealed/37°C air</td>
<td>10</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>mEH</td>
<td>No</td>
<td>Sealed/37°C air</td>
<td>10</td>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>M199H</td>
<td>No</td>
<td>Sealed/37°C air</td>
<td>10</td>
<td>8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>mEH</td>
<td>Yes</td>
<td>Sealed/dark Equitainer/air</td>
<td>10</td>
<td>8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Within columns, values with different superscripts differ significantly (P<0.05).

**Oocytes from dominant stimulated follicles**

Treatments: Fluid recovered from DSF was distributed in 150-mm Petri dishes on a warming tray regulated to maintain fluid temperature near 37°C and oocytes located using a dissecting microscope at 10–25x. Recovered oocytes were assigned to one of 7 incubation groups, without regard to morphology, with 10 oocytes per group. In all treatment groups, additional treatments were added based on the results obtained. However, an effort was made to continue to assign oocytes to the different groups over the entire course of the experiment. Treatments were as follows (Table 1); all media listed as ‘pre-equilibrated’ were pre-equilibrated in mixed gas (5% CO₂, 5% O₂ and 90% N₂): Group 1 (Control) oocytes were placed in 5 ml of standard DSF maturation medium (MatM; 45% Medium 199 with Earle’s salts, 45% DMEM (Dulbecco’s modified Eagle’s medium)/F-12 with 15 mmol/L HEPES, 1% newborn calf serum, 0.15 mmol/L sodium pyruvate and 25 μg/ml gentamicin) in a 30 mm Petri dish and incubated at 37.9°C in a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂ (mixed gas); Group 2, oocytes were placed in a 5 ml polypropylene culture tube (pc tube) filled to near the top (with a minimal air gap) with MatM, pre-equilibrated in an incubator under Control conditions with the cap loosened for 26 h. The tube was then sealed with parafilm and placed in an ambient-air incubator at 37°C to mimic shipment in a portable incubator. Group 3 as for Group 1 except pre-equilibrated EH medium was used (13) consisting of 40% Medium 199 with Earle’s salts, 40% Medium 199 with Hanks’ salts, 20% newborn calf serum and 25 μg/ml gentamicin, modified by the addition of 0.3 mmol/L sodium pyruvate (modified EH, mEH). Group 4 as for Group 2 except a pre-equilibrated medium consisting of 40% DMEM/F12 with 15 mmol/L HEPES, 20% Medium 199 with Hanks’ salts, 20% Medium 199 with Earle’s salts, 20% newborn calf serum, 25 μg/ml gentamicin and 0.3 mmol/L sodium pyruvate (1990HT) was used. Group 5 as for Group 2 but nonequilibrated mEH was used. Group 6, as for Group 2 but nonequilibrated Medium 199 containing Hanks’ salts, 20% newborn calf serum, 25 μg/ml gentamicin and 0.3 mmol/L sodium pyruvate (M199H) was used. Group 7 used pre-equilibrated mEH, but the sealed tubes were placed in a 50 ml centrifuge tube filled with commercial embryo flushing solution at 37°C. The centrifuge tube was sealed and placed in a room temperature (22–23°C) passive cooling device (Equitainer) in which the coolant cans, isothermaler and ballast bags had been warmed to 37°C. The Equitainer was maintained overnight at room temperature, and 4 h before ICSI, the oocytes were removed and placed in Control conditions.

**Intracytoplasmic sperm injection and embryo culture:** Intracytoplasmic sperm injection (ICSI) was conducted 42 h after administration of deslorelin to the oocyte donors. Straws of cryopreserved semen from one fertile stallion were sectioned under liquid nitrogen; approximately one-tenth of a straw was used per ICSI session. The cut section of the straw was placed in a pc tube containing 3 ml commercial embryo flushing medium at 37°C and spermatozoa were allowed to swim up for 15 min in ambient room air and temperature. Oocytes were denuded by repeated pipetting in Medium 199 with Hanks’ salts with 10% newborn calf serum, 25 μg/ml gentamicin and 0.1% hyaluronidase and placed in a 30 μl droplet of commercial ICSI medium (GMOPS PLUS) on a plate. Two μl of sperm suspension, collected from immediately above the straw in the swim-up tube, were placed in a 5 μl drop of a commercial 10% PVP solution on the manipulation plate. Intracytoplasmic sperm injection was performed with a standard sharp ICSI pipette with a Piezo drill. After sperm injection, oocytes were placed in groups of 1–2 in 30 μl droplets of embryo culture medium (54% DMEM/F12 with 15 mmol/L HEPES and 25 μg/ml gentamicin, 36% Global medium [a low-glucose commercial human embryo culture medium, used to achieve lower glucose levels from that of straight DMEM/F-12, 17 mmol/L], 5% newborn calf serum, 5% serum substitute and 0.1 mmol/L sodium pyruvate) under mineral oil and maintained in an incubator at 37.9°C in a humidified atmosphere under mixed gas. Embryos were moved to fresh culture medium on Day 4 of culture. Cleavage was assessed on Days 1 and 2 and blastocyst formation, characterised by development of a layer of presumptive trophoblast (Fig 1) was evaluated on Days 6–10.

**Oocytes from subordinate follicles**

Oocyte holding and maturation: Oocytes recovered from immature follicles were subjected to one of 7 different holding treatments before in vitro maturation without morphological evaluation. Not all treatments were performed concurrently: Groups A, B and C were evaluated in September and October, Groups D and E in October and November and Groups F and G in November and December. Oocytes (4–8 per tube) were
placed in 5 ml media in a parafilm-sealed pc tube and held overnight (∼15 h) unless otherwise noted (Table 2). Group A (Control) used pre-equilibrated mEH and tubes were placed in a cabinet at room temperature. Group B oocytes were placed in nonequilibrated M199H and held in an ambient-air incubator at 37°C. Group C oocytes were placed in pre-equilibrated mEH and held in an ambient-air incubator at 37°C. Group D was prepared as for Group A, but was held for 2 nights (∼39 h). Group E was prepared as for Group A, but was held for 3 nights (∼63 h). Group F was performed as for Group A but used nonequilibrated M199H. Group G was performed as for Group A but used nonequilibrated embryo holding solution (Emcare Holding Solution; EM).

Maturation medium for all immature groups consisted of 49.5% DMEM/F12 with 15 mmol/l HEPES and 25 μg/ml gentamicin, 32.5% Global medium, 4.5% newborn calf serum, 4.5% serum substitute, 0.1 mmol/l sodium pyruvate, 5 μmol/l follicle stimulating hormone (FSH), 1 μmol/l somatotropin, 10 μmol/l commercial insulin-transferrin-selenium solution (ITS) and 9% frozen-thawed pooled equine follicular fluid from dominant stimulated preovulatory follicles. Units of FSH and somatotropin were as defined by the manufacturer on the individual lot of hormone used. Oocytes were cultured in 30 μl drops under mineral oil in mixed gas at 37.9°C for 28–30 h before ICSI.

**Table 2: Maturation and embryo development after intracytoplasmic sperm injection (ICSI) of oocytes recovered from subordinate follicles**

<table>
<thead>
<tr>
<th>Group</th>
<th>Medium</th>
<th>Equilibration in mixed gas</th>
<th>Holding time (h)</th>
<th>Holding temperature</th>
<th>MII (%)</th>
<th>Cleaved (%) of MII</th>
<th>Blastocysts (%) of MII</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>mEH</td>
<td>Yes</td>
<td>15</td>
<td>23°C</td>
<td>36</td>
<td>29 (81)</td>
<td>10 (35)</td>
</tr>
<tr>
<td>B</td>
<td>M199H</td>
<td>No</td>
<td>15</td>
<td>37°C</td>
<td>31</td>
<td>27 (87)</td>
<td>23 (85)</td>
</tr>
<tr>
<td>C</td>
<td>mEH</td>
<td>Yes</td>
<td>15</td>
<td>37°C</td>
<td>34</td>
<td>22 (65)</td>
<td>15 (68)</td>
</tr>
<tr>
<td>D</td>
<td>mEH</td>
<td>Yes</td>
<td>39</td>
<td>23°C</td>
<td>43</td>
<td>37 (86)</td>
<td>26 (70)</td>
</tr>
<tr>
<td>E</td>
<td>mEH</td>
<td>Yes</td>
<td>63</td>
<td>23°C</td>
<td>41</td>
<td>34 (83)</td>
<td>24 (71)</td>
</tr>
<tr>
<td>F</td>
<td>M199H</td>
<td>No</td>
<td>15</td>
<td>23°C</td>
<td>40</td>
<td>35 (88)</td>
<td>26 (74)</td>
</tr>
<tr>
<td>G</td>
<td>EM</td>
<td>No</td>
<td>15</td>
<td>23°C</td>
<td>41</td>
<td>35 (85)</td>
<td>27 (77)</td>
</tr>
</tbody>
</table>

**a,b** Within columns, values with different superscripts differ significantly (P<0.05). MII = metaphase II.

**Oocytes from subordinate follicles**

Immature oocytes were aspirated during 56 of the 84 DSF aspiration sessions. This yielded 266 oocytes (4.75 immature oocytes per mare undergoing immature aspiration); the number of immature follicles aspirated was not recorded. Individual mares underwent immature follicle aspiration from one to four times. Maturation, cleavage and blastocyst rates of immature oocytes held in the different treatments before in vivo maturation are presented in Table 2. After in vivo maturation culture, maturation, as determined by the presence of a polar body, was similar in all groups (80.5–87.5%) with the exception of Group C, mEH at 37°C (64.7%). The highest production was in oocytes held in mEH at room temperature for one or 2 nights, or in EM overnight (Groups A, D and G; 35, 35 and 37% per injected oocyte, respectively). These groups yielded significantly higher blastocyst production than did oocytes held at 37°C overnight in mEH (Group C; 5%, P<0.05). Oocytes held in mEH at room temperature overnight for 3 nights (Group E) and overnight in M199H at 37°C (Group B) yielded intermediate blastocyst production (14 and 22%, respectively). In the most effective immature Groups (A, D and G), the predicted overall blastocyst production per aspiration session was 1.25 (average 4.15 oocytes/mare x average 84% maturation x average 36% blastocyst production per mature oocyte).

**Discussion**

The results of this study help to define the conditions under which equine oocytes may be successfully transported for ICSI and should help to make this procedure a more practical clinical option in many practices.

The media used in this study, Medium 199 and DMEM/F12, are classic tissue-culture media. Medium 199 is commonly used to handle and culture bovine and equine oocytes. The Hanks’ salts formulation uses a phosphate buffer system to maintain pH in room atmosphere (−0.05% CO2). The Earle’s salts formulation includes a bicarbonate buffer system that maintains physiological pH in a 5% CO2 atmosphere. The Hanks’ buffer, HEPES (greater than 10 mmol/l) medium) has been successfully used as a holding medium overnight at room temperature and atmosphere for both bovine and equine oocytes (13,16) and for shipping immature oocytes for ICSI (4). This holding procedure allows scheduling the onset of oocyte maturation for ICSI and yields equivalent (nonsignificantly higher) blastocyst development to that for immediate culture (34 vs. 25%, respectively, [13]). Due to its success as a holding medium for immature oocytes, EH was chosen as the primary medium for comparison as a proposed shipping medium for both immature and DSF equine oocytes in this study. Since neither formulation of Medium 199 contains pyruvate, an important nutrient for oocytes and early embryos in other species, sodium pyruvate was added to modify the EH medium (mEH). Holding immature oocytes overnight at room temperature in mEH before maturation served as the control; this is the...
standard procedure for immature oocytes in our laboratory as it allows scheduling of maturation onset so that ICSI is conducted during the day. DMEM/F-12 medium was the first medium shown to support adequate (>30%) equine blastocyst development [17,18] and has been reported by one laboratory to increase blastocyst development when used for in vitro maturation of equine oocytes [19]. The main differences between DMEM/F-12 and M199 are that DMEM/F-12 has a lower bicarbonate concentration (15 mmol) and a higher glucose concentration (17 mmol) than does M199 (25 and 5 mmol/l, respectively). The maturation media incubated together with M199 and DMEM/F-12 used in this study were developed by our laboratory over 3 years of trials to deliver optimum maturation and blastocyst rates in our system.

The findings of the present study indicate that DSF oocytes can provide high blastocyst rates under appropriate conditions. These oocytes have resumed meiosis and are typically at metaphase I when recovered 24 h after gonadotropin stimulation [20]. Because of the temperature sensitivity of the mammalian meiotic spindle, which can depolymerise in as little as 1.5 min at just 32°C [9–11], DSF oocytes are extremely sensitive to damage during handling. This is reflected in the poor blastocyst production (1/10) in the Equitainer-held DSF oocytes in the present study, as this system cools quickly to about 30°C [21]. Although the oocytes were cultured in Control conditions for 4 h before ICSI to allow spindle reformation, this treatment was still associated with the lowest DSF blastocyst rate in the present study.

The control system for DSF oocytes (incubation in MatM in culture dishes under mixed gas) was associated with high blastocyst rates (7/10); however, this medium requires a 5% CO₂ atmosphere to maintain pH. The potential transport method used in Group 2, equilibration of MatM in tubes under mixed gas, then sealing the tubes to prevent de-gassing, yielded equivalent blastocyst development (7/10) without the need for CO₂. The measured pH of the MatM in Group 2 was 7.30 after equilibration prior to the addition of oocytes and 7.36 when oocytes were removed from the sealed tube prior to ICSI. An optimal pH has not been identified for culture of equine oocytes and early equine embryos, but in other species is generally considered to be approximately 7.30 [22].

In the present study, mEH was equilibrated before use, although in previous studies this medium has been used successfully for holding immature oocytes at room temperature without equilibration [13]. In the present study, the measured pH of nonequilibrated mEH was 7.67 while the pH of equilibrated medium was 7.28. The difference in pH appeared to affect DSF oocytes when this medium was used for overnight culture, as cleavage was 4/10 in nonequilibrated mEH (Group 5), compared with 8/10 in equilibrated mEH (Groups 3 and 7). The mEH, which contains a proportion of M199 with Hank’s salts and thus a relatively low bicarbonate concentration, did not appear to support DSF blastocyst production as well as did MatM.

The blastocyst rate achieved with DSF oocytes in Groups 1 and 2 is notable (14 blastocysts from 20 oocytes, 70%, essentially twice that per immature oocyte). To the best of our knowledge, this represents the highest blastocyst rate yet reported after equine ICSI. A previous study reported that blastocyst rates were similar after ICSI for immature and DSF oocytes [33 and 41%, respectively; [5]; these authors postulated that the blastocyst rate for DSF oocytes should be much higher, as DSF oocytes yield a ≥75% pregnancy rate if transferred to the oviduct [7,8,23]. In contrast, use of immature oocytes yields a low pregnancy rate after they are transferred to the oviduct (∼15%; [6,24]). Thus, in vitro and in vivo blastocyst production appeared to be similar for immature oocytes. The authors thus concluded that the in vitro system used supported viability of immature, but not DSF oocytes. The findings of the present study indicate that under appropriate conditions, DSF oocytes can yield blastocyst rates in vitro equivalent to those obtained after oocyte transfer with these oocytes. Further work is needed to identify the features of the system that optimise support of DSF oocytes.

In the present study, holding immature oocytes at room temperature overnight in either mEH or in EM, a commercial embryo holding medium, was associated with subsequent high rates of maturation (81–85%) and blastocyst development (35–37%) for this type of oocyte. The ability to use a readily available and commonly used commercial embryo holding medium (EM) will help to simplify oocyte shipment. The finding that holding of immature oocytes at room temperature in mEH for 2 nights (∼39 h) had no detrimental effect on maturation or blastocyst rates was notable. This extended holding period may provide flexibility in collection, shipping and ICSI schedules, and may accommodate for unscheduled delays in shipping. Holding of the immature oocytes in these conditions for 3 nights was associated with a normal maturation rate (83%) but a reduced blastocyst development rate [14]. It should be noted that blastocyst formation, while a positive indicator, does not necessarily correlate with the production of a normal pregnancy and offspring. However, blastocysts produced from oocytes held one night in EM or mEH appear to have normal pregnancy rates after transfer (R. Foss, unpublished data; K. Hinrichs, unpublished data) and 4 ongoing pregnancies have currently resulted from transfer of 4 blastocysts produced from oocytes held 2 nights in mEH (R. Foss, unpublished data). Further work is necessary to determine whether holding of oocytes in EM, or prolonged holding in either medium, before in vitro maturation affects the ability of the resulting blastocysts to maintain normal pregnancies.

In the present study, holding of immature oocytes overnight in mEH at a warmer temperature (37°C) was associated with reduced maturation and blastocyst rates. While holding at room temperature in EM has been shown to maintain equine oocytes in the germinal vesicle (immature) stage [13], it is likely that at 37°C, oocytes attempted to resume meiosis during holding, but without FSH support may have failed to do so normally; after a further 28 h culture for maturation, these oocytes may have been aged or were otherwise abnormal at the time of sperm injection.

An optimum temperature for holding immature oocytes has not been determined. It is possible to ship these oocytes overnight at room temperature in passive cooling devices such as the Equitainer and blastocyst rates of 14–27% have been reported after doing so [25]. However, these devices are designed to be used with frozen coolant cans to keep the contents at −4°C and temperature maintenance is dependent on the phase change of the coolant from solid to liquid. Without this heat sink, these devices are sensitive to environmental temperature variations, such as may occur on airport tarmac or in the cargo hold of an airplane at altitude. Until more work has been done on the effect of holding temperature on viability of immature oocytes, shipping in a portable heating/cooling incubator at −23°C may be preferable.

In conclusion, the results of the present study indicate that shipment of equine oocytes for ICSI is possible and could provide consistent results. Oocytes from DSF could be shipped at 37°C in a sealed vial of pre-equilibrated maturation medium and provide high rates of blastocyst development (70%) after ICSI. Placement of immature oocytes in embryo holding medium would allow for convenient packaging and overnight shipment at 23°C with maintenance of optimum blastocyst production for these oocytes (∼35%) after ICSI. The ability to ship oocytes to a laboratory for sperm injection and embryo culture should greatly simplify the incorporation of ICSI as a clinical breeding management tool for equine practitioners.

Authors’ declaration of interests

The authors (R.F. and K.H.) have separate commercial equine ICSI (intracytoplasmic sperm injection) programmes that could benefit financially from performing ICSI on oocytes shipped to their laboratories by referring veterinarians.

Ethical animal research

Mares used in this study are owned by R.F. and all experimental procedures were performed according to the United States Government Principles for the utilisation and Care of Vertebrate Animals Used in Testing, Research and Training.

Sources of funding

No external funding was used for this project.

Acknowledgements

We thank Patty Williams and Maren Fleer for excellent technical assistance.
**Authorship**

R. Foss contributed to study design, was largely responsible for study execution, participated in data analysis and interpretation, prepared the manuscript and agreed to final approval. H. Ortis contributed to study design and execution, preparation and final approval of the manuscript. K. Hinrichs was consulted on study design, assisted in data analysis and interpretation, preparation of the manuscript and final approval.

**Manufacturers’ addresses**

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*Sonosite, Bothell, Washington, USA.

*Minitube of America, Inc., Verona, Wisconsin, USA.

*Bioniche Animal Health USA Inc., Pullman, Washington, USA.

*Sagent Pharmaceuticals, Schaumburg, Illinois, USA.

*Sigma-Aldrich, St. Louis, Missouri, USA.

*Gibco, Life Technologies, Inc., Grand Island, New York, USA.

*Hamilton Research, Inc., Ipswich, Massachusetts, USA.


*LifeGlobal, LLC, Guilford, Connecticut, USA.

*ICPbio Reproduction, Timaru, New Zealand.

**Sioux Biochemical, Sioux Center, Iowa, USA.

**Equine Veterinary Journal 45, Suppl. 45 (2013) 39–43 © 2013 EVJ Ltd

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