**Oocyte collection in the mare; efficiency of ovum pick-up, and techniques for shipping ovaries and/or oocytes**  
Tom Stout, Juan Cuervo-Arango, Marta De Ruijter-Villani, Anthony Claes  
Department of Equine Sciences, Utrecht University (The Netherlands)

**Introduction**  
Oocyte recovery from live mares has become an increasingly common procedure, primarily due to growing interest in *in vitro* production (IVP) of horse embryos via intracytoplasmic sperm injection (ICSI), but also for oocyte transfer (OT). Oocytes can also be recovered from recently deceased mares, either to attempt genetic salvage of a valuable animal by IVP, or to act as host cytoplasts for attempts to clone by somatic cell nuclear transfer (SCNT). Since ICSI, SCNT and IVP are intricate procedures requiring dedicated facilities and considerable experience to achieve acceptable results, protocols have been developed for shipping ovaries or aspirated oocytes to specialized labs. By contrast, while methods for cryopreserving horse oocytes and ovarian tissue have been described, neither currently maintains oocyte viability to levels acceptable for clinical use.

**Recovering oocytes from live mares**  
Two approaches have been described for recovering oocytes from live mares, flank aspiration and transvaginal ultrasound guided follicle aspiration (also known as ovum pick-up: OPU). Flank aspiration is relatively straightforward and can be performed blindly, or with ultrasound guidance, via a trocar introduced through the flank under local anesthesia; the ovary is manipulated *per rectum* to place the pre-ovulatory follicle against the trocar, and a 12-17 gauge needle is then used to puncture the follicle and aspirate its contents [1]. Flank aspiration is, however, only suitable for recovering *in vivo* matured oocytes from gonadotrophin-stimulated, pre-ovulatory follicles. By contrast, OPU can be used to recover both *in vivo* matured oocytes, and immature oocytes from small antral follicles.

Although OPU was first described in the early 1990s translation to equine clinical practice was slow, primarily because oocyte recovery from small follicles was initially disappointing (<25%: for review see [2]), and blastocyst production rates after ICSI (<10%) were too low to be of commercial interest. It transpired that the equine cumulus-oocyte complex (COC) is attached to the follicle wall by a broad granulosa cell hillock with extensive connections to the underlying theca, which explains why aspiration alone is insufficient to reliably dislodge the oocyte. Instead, repeated flushing of the follicle accompanied by vigorous scraping with the aspiration needle is required for high oocyte recoveries [3].

Equine OPU is generally performed using a 60cm 12-gauge, double lumen needle. Follicle contents are aspirated via the inner needle which is connected, via a warmed collection vessel, to a vacuum pump. Once the follicle is empty, it is flushed repeatedly via the outer needle using commercial embryo flushing medium supplemented with heparin (5-20 i.u. per ml), to prevent clotting of blood or gelatinous fluid from large follicles.

**Recovering immature oocytes**  
The advantage of recovering immature oocytes is that a large number (e.g. 8-15) can be collected without any hormonal priming of the donor, at any stage of the cycle or time of the year (excepting deep anestrus). After initial aspiration, the follicle is flushed 6-8 times with 0.5-10ml of fluid depending on follicle size; if follicle number is low, they are flushed more often to maximize the likelihood of oocyte recovery. In general, mares are suitable OPU candidates when they have > 12
follicles ≥8mm. However, some (e.g. older) mares never develop many follicles, and it may be necessary to perform OPU on fewer follicles.

The need to repeatedly flush follicles means that OPU can be a long procedure (20-45 minutes); epidural anaesthesia is therefore recommended to prevent the mare straining in response to the presence of the vaginal probe or manipulation of the ovary per rectum. Heavy sedation is also advisable, and rectal relaxants can facilitate ovarian manipulation. Non-steroidal anti-inflammatory drugs (NSAID) will help counter post-OPU discomfort, while peri-operative antibiotics reduces the risk of post-procedure peritonitis. In fact, OPU appears to be (surprisingly) well tolerated, even by inexperienced mares, and post-procedure complications are usually limited to mild pyrexia or abdominal discomfort of short duration (12h) that respond well to NSAIDs. We have, however, seen 3 significant post-OPU abdominal hemorrhages, presumably from a ruptured blood vessel in the vaginal wall, ovary or ovarian ligament. Others have reported needle puncture of the rectum, peritonitis, rectal tears and ovarian hematomas and abscesses; fortunately, serious complications are uncommon, and even repeating OPU at 2-week intervals over many months does not appear to affect subsequent cyclicity or fertility [2]. With an established team, mean oocyte recoveries from immature follicles of 50-70% can be achieved [3,4]. In our clinic, 597 commercial OPUs yielded means of 13.7 oocytes from 25 follicles (55% recovery); the yield per individual OPU, however, ranged from 3-56 oocytes (20-100% recovery).

**Harvesting in vivo matured oocytes**

In vivo matured oocytes can be recovered from pre-ovulatory follicles after gonadotrophin induction of maturation; this is the norm for OT, and is also used in some OPU-IVP programs because oocyte recovery rates from pre-ovulatory follicles are high (>70%) and in vivo matured oocytes are more likely to yield blastocysts [5]. However, since eFSH is not commercially available, there is usually only 1 (occasionally 2-3) pre-ovulatory follicle per cycle.

For OT, not only must the mature oocyte be recovered from the donor, but also from the pre-ovulatory follicle of a synchronized, inseminated recipient; the donor oocyte can then be transferred surgically to the recipient’s oviduct [1]. Mature oocyte recovery is performed 20-35 hours after ovulation induction with a GnRH analogue (e.g. deslorelin), hCG or both [1,5]. Waiting until 35 hours has the advantage of ensuring that the oocyte has reached MII and is less tightly attached to the follicle wall, but increases the risk of the mare ovulating before oocyte recovery. Performing OPU at 20-24 h, reduces the risk of the mare ovulating ‘too early’ but means that the oocyte will require 12-16 h of culture to complete maturation before OT or ICSI.

**Shipping ovaries and/or oocytes**

While only a few laboratories are currently able to offer commercially viable equine IVP, others can make use of the technique by shipping either ovaries (after death of a valuable mare) or oocytes. Ovaries should be removed as soon as possible after death or euthanasia of the mare, and shipped at room temperature (22 ± 2°C) with the aim of reaching the laboratory within 6-8 h of death [2]. It is sensible to warn the client that if the mare has been severely toxemic for more than a few hours before euthanasia, the likelihood of producing viable embryos is greatly reduced.

While ovaries can be transported, it is preferable to recover the oocytes prior to transport, since immature oocytes tolerate storage much better than ovaries. Oocyte recovery from excised follicles is best performed by opening the follicle with a scalpel and scraping the entire inner surface with a bone curette, washing the contents into a petri dish with embryo flushing medium; recovery should be close to 100%, whereas aspirating and scraping with a needle will yield a similar percentage to OPU.
Oocytes recovered post mortem or by OPU can be shipped at room temperature overnight (<28h) in a holding medium (H-SOF or M199-based) to a dedicated equine IVP laboratory; ensuring that the oocytes do not cool to <18 °C appears to be more critical than time of transport. Oocyte shipping is a successful way of offering ICSI to clients distant from an ICSI laboratory. Indeed, in 2015 we shipped the oocytes from 158 OPUs performed on Warmblood mares in Utrecht to Avantea (Cremona, Italy) and achieved very similar blastocyst production (1.15 versus 1.23 per OPU; 15% versus 17% of injected oocytes) and post-transfer pregnancy rates (61% versus 70%) as 202 OPUs performed on-site in Italy [4].

Conclusions
Commercial interest in OPU-ICSI has increased markedly in the last 5 years. With the right equipment, it is possible to achieve high oocyte recoveries (>50%) from immature follicles. Moreover, the oocytes can be shipped overnight to a dedicated equine ICSI lab, with little or no loss of fertility. Finally, embryo production (0.8-1.2 per OPU) and pregnancy after transfer of ICSI embryos (60-75%) are, respectively, better than or similar to conventional ET. On the other hand, pregnancy loss after transfer of ICSI embryos is currently higher (15-25%), such that only 40-50% of transferred ICSI embryos result in the birth of a live foal.

References:

Contact address:
Professor Tom Stout, Utrecht University, t.a.e.stout@uu.nl